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# Transport behavior of pathogenic microorganisms in porous media and remediation capability of biochar: A review

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## ABSTRACT

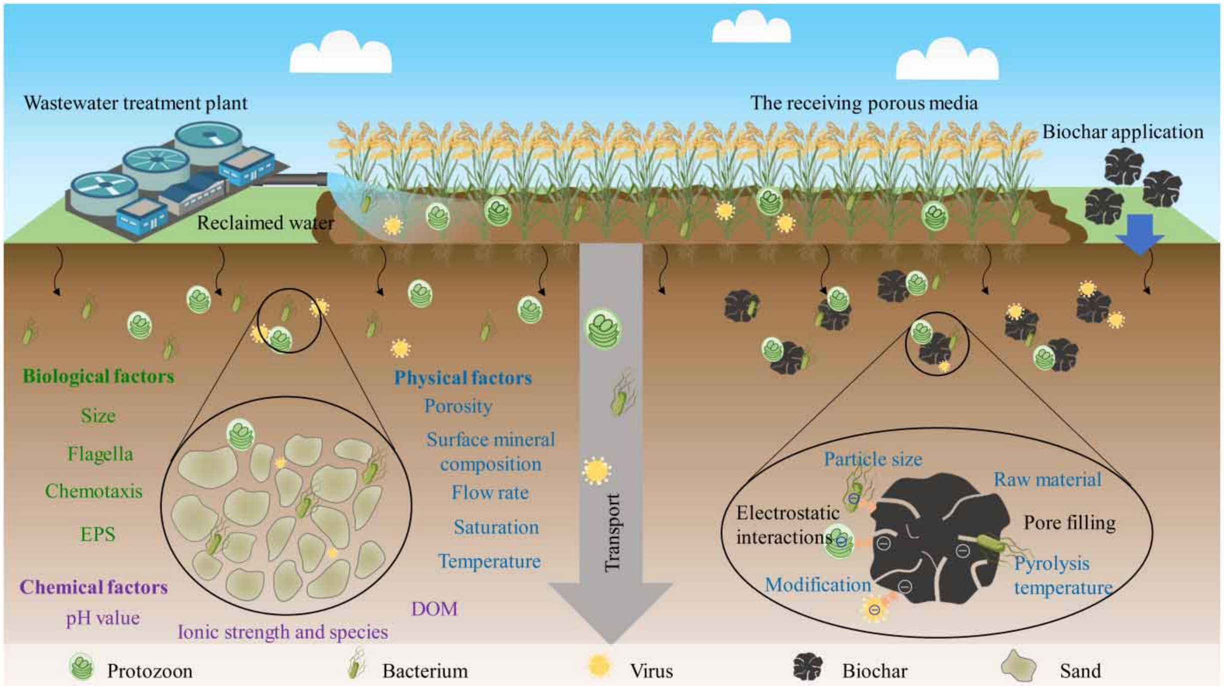
The reuse of reclaimed water is a cost-effective way to alleviate water resource scarcity, but the residual pathogenic microorganisms inevitably influence the safety of its reuse. The transport behavior of pathogenic microorganisms in receiving porous media varies under different environmental factors and could be harmful to the natural ecology and even human health if not well treated. Biochar is expected to be an effective, environmentally-friendly functional material to inhibit the transport of pathogenic microorganisms, with unreplaceable advantages of low price, simple preparation method, and strong adsorption capacity. In the present paper, we start from identifying the transport behavior of typical pathogenic microorganisms in porous media, including protozoa, bacteria, and viruses, and then analyzing the primary factors affecting the transport of pathogenic microorganisms from the aspects of biology, physics, and chemistry. Furthermore, the effects of types of raw materials, pyrolysis temperature, particle size, and functional modification methods on the remediation performance of biochar for the transport of pathogenic microorganisms are clearly reviewed. Finally, we aim to clarify the transport rules of pathogenic microorganisms in porous media and provide biochar-based technical means for effectively inhibiting the transport of pathogenic microorganisms, thereby improving the ecological and health safety of reclaimed water reuse.

**Key words:** bacteria, biochar, pathogenic microorganism, porous media, reclaimed water, transport

## HIGHLIGHTS

- Pathogenic microorganisms could spread widely through transport behavior in porous media.
- The biological, physical, and chemical factors affecting the transport of pathogenic microorganisms were reviewed.
- Parameters of biochar impacting the transport of pathogenic microorganisms were discussed.
- Biochar is expected to have practical applications in inhibiting the transport of pathogenic microorganisms.

## GRAPHICAL ABSTRACT



## 1. INTRODUCTION

With the global population explosion, environmental pollution and water resources crisis are becoming increasingly serious (Wang *et al.* 2022). Reclaimed water with large water quantity and stable water quality is an inevitable choice to deal with the problem of water shortage and water environmental pollution (Xu *et al.* 2020; Shan *et al.* 2023). Reclaimed water mainly refers to the secondary or tertiary effluent from urban wastewater treatment plants for reuse, in which there are still a variety of pathogenic microorganisms such as protozoa, bacteria, and viruses affecting the safety of its reuse (Caicedo *et al.* 2019; Chen *et al.* 2020; Lima *et al.* 2022; Mortula *et al.* 2023). However, the standards for the reuse of reclaimed water only take fecal coliform as the indicator of pathogenic microorganisms, which inevitably underestimates the ecological and health risks that residual pathogenic microorganisms may cause (Figure 1). The residual pathogenic microorganisms in reclaimed water will inevitably enter the porous media such as wetland, soil (Weidhaas *et al.* 2022) and underground aquifer (Fan *et al.* 2020), and transport on a large scale during the reuse process, which seriously affects the safety of reclaimed water reuse. Therefore, exploring the transport rules of pathogenic microorganisms in porous media can deeply understand the diffusion process and influencing factors of pathogenic microorganisms, which provides theoretical support for inhibiting the transport behavior of pathogenic microorganisms.

The transport behavior of pathogenic microorganisms is easily affected by their properties and the physical and chemical properties of porous media (Abudalo *et al.* 2010; Kim *et al.* 2010; Jin *et al.* 2021). Due to the complex structures and spatial heterogeneity of porous media in nature, the transport behavior of pathogenic microorganisms in the environment is affected by multiple factors and becomes more complex (Dong *et al.* 2014; Zhang *et al.* 2021a). At present, there are many studies on the transport behavior of pathogenic microorganisms in porous media, and some studies have paid attention to the effects of biological factors (Haznedaroglu *et al.* 2010; Chandrasena *et al.* 2017; Kim & Kwon 2022), physical factors (Abit *et al.* 2012; Jin *et al.* 2021; Zhang *et al.* 2022), and chemical factors (Mohanram *et al.* 2010; Weaver *et al.* 2013; He *et al.* 2019) on the transport behavior of pathogenic microorganisms in porous media. However, no review has systematically summarized the transport rules of pathogenic microorganisms in porous media. Therefore, summarizing the transport rules of pathogenic microorganisms in porous media and clarifying their influencing factors has certain theoretical guiding significance for inhibiting the pollution of pathogenic microorganisms and improving the utilization rate of reclaimed water.

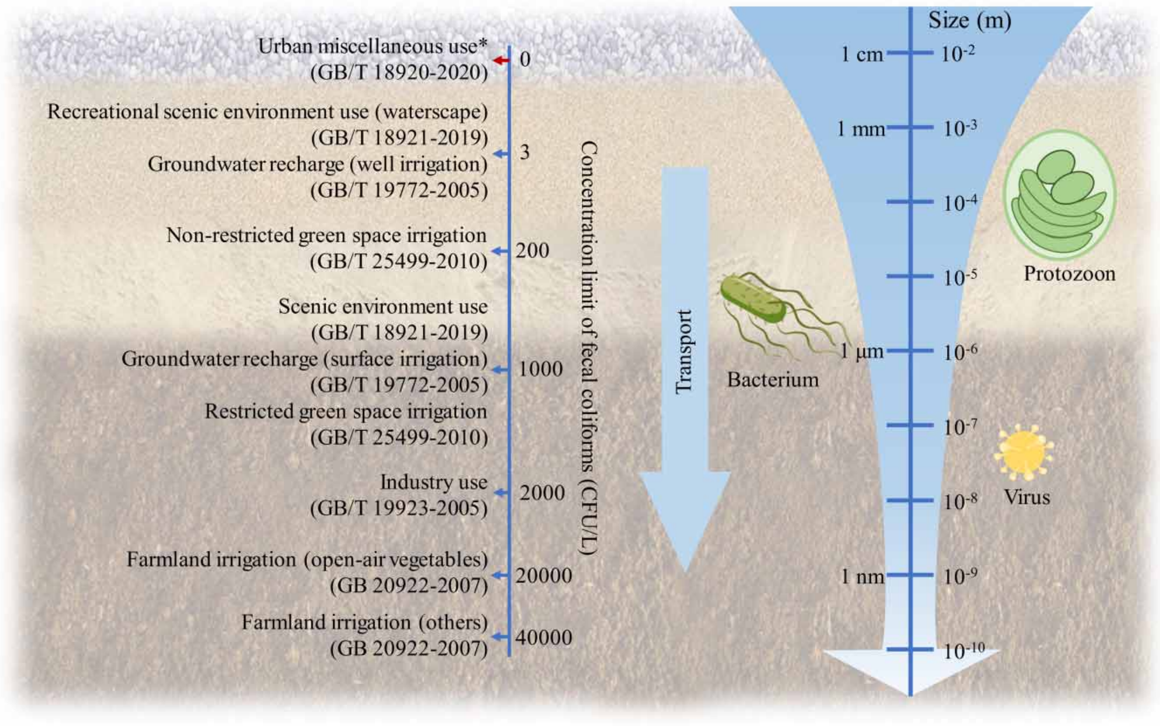


**Figure 1** | The concentration limits of pathogenic microorganisms in reclaimed water and their transport behavior in receiving porous media (* refers to *Escherichia coli* (*E. coli*) and unlabeled refers to fecal coliform).

At present, environmental functional materials such as organic polymer materials, nanoparticles, natural minerals, activated carbon, and biochar have been used to inhibit the transport behavior of pathogenic microorganisms in porous media due to their unique physical and chemical properties (Ngwenya *et al.* 2015; Sasidharan *et al.* 2016; He *et al.* 2019). Among them, biochar is a carbon-rich substance produced by the pyrolysis of biomass at high temperatures under anaerobic or anoxic conditions. It has active functional groups, excellent pore structure, and large specific surface area (SSA), and has been widely used in environmental treatment and restoration (Wang & Wang 2019). The application of biochar to the environment is bound to impact the transport behavior of pathogenic microorganisms. Studies currently have shown that adding biochar can inhibit the transport of pathogenic microorganisms, especially bacteria in porous media (Mohanty & Boehm 2014; Fernando Perez-Mercado *et al.* 2019). However, some studies also stated that the transport of pathogenic microorganisms such as bacteria and viruses was promoted after adding the low-temperature pyrolyzed biochar into sand media (Abit *et al.* 2012; Bolster & Abit 2012; Sasidharan *et al.* 2016). The conflicts among previous studies greatly limit the application of biochar in inhibiting the transport of pathogenic microorganisms.

Therefore, a literature review of studies reporting the transport behavior of pathogenic microorganisms in porous media and the remediation capability of biochar until February 2024 was performed. Publications were retrieved from databases including Web of Science, ScienceDirect, Springer, American Chemical Society, and Wiley. The following search strings were used: ‘transport’ AND ‘porous media’ AND (‘pathogenic microorganism’ OR ‘protozoa’ OR ‘bacteria’ OR ‘phages’ OR ‘cryptosporidium’ OR ‘giardia’ OR ‘amoeba’) and ‘transport’ AND ‘porous media’ AND ‘biochar’ AND (‘pathogenic microorganism’ OR ‘protozoa’ OR ‘bacteria’ OR ‘phages’ OR ‘cryptosporidium’ OR ‘giardia’ OR ‘amoeba’). Finally, we aim to provide information to reduce the risk of the transport of pathogenic microorganisms in the receiving porous media and improve the ecological and health safety of reclaimed water reuse.

## 2. INFLUENCING FACTORS OF THE TRANSPORT BEHAVIOR OF PATHOGENIC MICROORGANISMS IN POROUS MEDIA

As shown in Figure 2, a large number of previous laboratory studies on the transport behavior of pathogenic microorganisms in porous media were conducted through column experiments. The devices of column experiments generally include

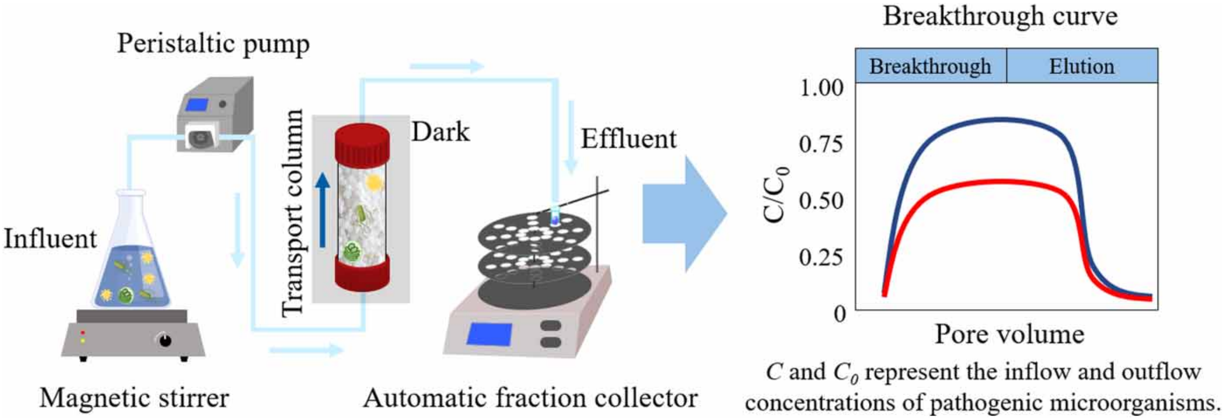


**Figure 2** | Experimental design of a column experiment for studying the transport behavior of pathogenic microorganisms in porous media.

magnetic stirrers, transport columns, automatic fraction collectors, and detection instruments. The experimental stage includes the pre-equilibration stage, the breakthrough stage, and the elution stage. The inflow and outflow concentrations of pathogenic microorganisms were defined as $C_0$ and $C$, respectively, and the breakthrough curve is generated using the $C/C_0$ during the breakthrough stage and the elution stage as parameters to calculate the breakthrough percentage.

According to previous studies using column experiments, the transport ability of typical pathogenic microorganisms (protozoa, bacteria, and viruses) in porous media was summarized, as shown in Table 1. The three pathogenic microorganisms have a certain transport ability in porous media, and there is a potential risk of large-scale transport and diffusion. It should be noted that most column experiments used quartz sand as the porous media, which ignored the heterogeneity and complexity of actual porous media in the natural environment to a certain extent. Therefore, whether the research results and rules obtained at the laboratory scale can reflect the transport behavior of pathogenic microorganisms in the actual environment requires further verification. The transport behavior of pathogenic microorganisms was strongly affected by biological factors (the size of pathogenic microorganisms, the flagella, and chemotaxis of bacteria, the extracellular polymeric substances (EPS) of pathogenic microorganisms), physical factors (the porosity and surface mineral composition of porous media, the flow rate, saturation, and temperature of the water), chemical factors (the pH value, ionic strength and species of the water, dissolved organic matter (DOM) in the water), etc. (Figure 3). It is important to understand the effect of different factors on the transport behavior of pathogenic microorganisms in porous media.

## 2.1. Biological factors

### 2.1.1. The size of pathogenic microorganisms

The size of typical pathogenic microorganisms is protozoa (1–100 μm) > bacteria (0.1–10 μm) > viruses (18–1,500 nm) (Figure 1), and their transport abilities in porous media gradually increase with decreasing size (Hijnen *et al.* 2005). For example, the effluent concentrations of *Giardia intestinalis*, *Cryptosporidium parvum*, *Clostridiumper fringens*, and *E. coli* in the soil column were decreased by 3.9–6.2 $\log_{10}$, while that of the MS2 phage was decreased by only 3.3 $\log_{10}$ (Hijnen *et al.* 2005). Chandrasena *et al.* (2017) also found that the effluent concentrations of *Cryptosporidium Oocysts*, *Clostridium perfringens*, and *E. coli* in the stormwater biofilters were reduced by about 1.7–2 $\log_{10}$, while the concentration of adenoviruses was only reduced by about 1 $\log_{10}$. However, it has also been shown that a smaller phage phiX174 might be trapped by the narrow pores of the porous media, resulting in a lower transport ability than *Bacillus subtilis* endospores (Oudega *et al.* 2021). Therefore, the size of pathogenic microorganisms and the characteristics of porous media should be considered comprehensively to judge the transport abilities of pathogenic microorganisms.

### 2.1.2. The flagella of bacteria

The flagella are the key motor organ of bacteria, giving them the ability to smooth swim, swim backward, and execute a tumble. Flagella can promote bacterial deposition on the surface of the porous media by increasing the number of contacts

**Table 1** | Transport abilities of typical pathogenic microorganisms in porous media

| **Pathogenic microorganism** | | **Initial concentration** | **Type of porous media** | **Breakthrough percentage** | **Reference** |
|---|---|---|---|---|---|
| Protozoon | *Cryptosporidium* (oocysts/mL) | $1.4 \times 10^3 \pm 7.5 \times 10^2$ | Castricum soil, roosteren soil | 0.6–3% | Hijnen *et al.* (2005) |
| | | $2 \times 10^6$ | Quartz sand, ottawa sand | 1.0–96.3% | Kim *et al.* (2010) |
| | | $2 \times 10^6$ | Soil | 1–71% | Mohanram *et al.* (2010) |
| | | 100 | Sandy soil | 0–18.7% | Santamaria *et al.* (2011) |
| | | $2 \times 10^6$ | Quartz sand | 1–69% | Bradford *et al.* (2016) |
| | *Amoeba* spores (spores/mL) | $2 \times 10^2$–$2 \times 10^3$ | Quartz sand | 29.9–89.5% | Jin *et al.* (2021) |
| | | $10^2$–$10^3$ | Quartz sand | 21.4–83.2% | Jin *et al.* (2022) |
| | *Giardia* (cysts/mL) | $1.6 \times 10^3 \pm 5.0 \times 10^2$ | Castricum soil, roosteren soil | 1.6–8.5% | Hijnen *et al.* (2005) |
| | | $8.23 \times 10^3$ | Ottawa aquifer sand | 0.4–1.8% | Bradford *et al.* (2006) |
| Bacterium (CFU/mL) | *E. coli* | $1.0 \times 10^8 \pm 10\%$ | Quartz sand | 4.0–85.0% | Yang *et al.* (2012b) |
| | | $1.5 \times 10^7 \pm 10\%$ | Quartz sand | 47.8–92.7% | Wu *et al.* (2016) |
| | | $2.5 \times 10^7 \pm 10\%$ | Quartz sand | 15.8–89.8% | Yang *et al.* (2016) |
| | | $1.5 \times 10^7 \pm 10\%$ | Quartz sand | 23.2–84.0% | Wu *et al.* (2018) |
| | | $1.25 \times 10^7 \pm 10\%$ | Quartz sand | 48.5–90.7% | He *et al.* (2018) |
| | | $1.5 \times 10^7 \pm 10\%$ | Quartz sand | 4.4–92.5% | He *et al.* (2019) |
| | | $5 \times 10^7$ | Quartz sand | 63.3–70.0% | Liu *et al.* (2021) |
| | | $1.35 \times 10^7 \pm 10\%$ or $1.35 \times 10^8 \pm 10\%$ | Quartz sand | 33.9–94.2% | Zhang *et al.* (2021a) |
| | | $1.6 \times 10^7 \pm 10\%$ | Quartz sand | 11.0–59.2% | He *et al.* (2022) |
| | *Bacillus subtilis* | $1.5 \times 10^7 \pm 10\%$ | Quartz sand | 63.3–93.8% | Wu *et al.* (2016) |
| | | $1.1 \times 10^9$ | Aquifer soil | 10.9–16.5% | Oudega *et al.* (2021) |
| | *Salmonella typhimurium* | $6.4 \times 10^8$––$1.93 \times 10^9$ | Fontainebleau sand | 33.1–52.0% | Zheng *et al.* (2022) |
| | *Pseudomonas putida* | $5 \times 10^7$ | Quartz sand | 65.2–70.0% | Liu *et al.* (2021) |
| | *Desulfovibrio* sp. | $5 \times 10^7$ | Quartz sand | 58.7–70.0% | Liu *et al.* (2021) |
| | *Shewanella oneidensis* MR1 | $5 \times 10^7$ | Quartz sand | 37.5–70.0% | Liu *et al.* (2021) |
| | *Shewanella putrefaciens* CN32 | $5 \times 10^7$ | Quartz sand | 43.2–70.0% | Liu *et al.* (2021) |
| Virus (PFU/mL) | *Φ*X174 phage | $(3.7+0.9) \times 10^3$ | Glass beads | 31.9–43.6% | Syngouna & Chrysikopoulos (2016) |
| | | $10^6$ | Sand | 1–93% | Xu *et al.* (2017) |
| | | $2.1 \times 10^9$ | Aquifer soil | 0.3–0.4% | Oudega *et al.* (2021) |
| | MS2 phage | $10^9$ | Sand, sand loaded with iron oxide | 0.1–50.1% | Bradley *et al.* (2011) |
| | | $10^8$–$10^{10}$ | Quartz sand | 82% | Ghanem *et al.* (2016) |
| | | $(11.6 \pm 4.4) \times 10^3$ | Glass beads | 29.9–51.6% | Syngouna & Chrysikopoulos (2016) |
| | vB_PSPS-H40/1 phage | $10^8$–$10^{10}$ | Quartz sand | 12% | Ghanem *et al.* (2016) |
| | | $10^8$–$10^{10}$ | Quartz sand | 25% | Ghanem *et al.* (2018) |
| | PSA-HM1 phage | $10^8$–$10^{10}$ | Quartz sand | 36% | Ghanem *et al.* (2016) |
| | | $10^8$–$10^{10}$ | Quartz sand | 80% | Ghanem *et al.* (2018) |
| | T4 phage | $10^8$–$10^{10}$ | Quartz sand | 4% | Ghanem *et al.* (2016) |
| | | $10^8$–$10^{10}$ | Quartz sand | 63% | Ghanem *et al.* (2018) |
| | vB_EcoM-ep3 phage | $10^6$ | Natural sand | 33.2–59.0% | Qin *et al.* (2020) |
| | | $10^6$ | Natural sand | 6.6–38.8% | Zhang *et al.* (2021b) |
| | PSA-HP1 phage | $10^8$–$10^{10}$ | Quartz sand | 75% | Ghanem *et al.* (2016) |
| | PSA-HS2 phage | $10^8$–$10^{10}$ | Quartz sand | 63% | Ghanem *et al.* (2016) |
| | vB_PSPS-H6/1 phage | $10^8$–$10^{10}$ | Quartz sand | 24% | Ghanem *et al.* (2016) |
| | H3/49 phage | $10^8$–$10^{10}$ | Quartz sand | $<1\%$ | Ghanem *et al.* (2016) |

The breakthrough percentage referred to the percentage of pathogenic microorganisms passing through columns, it was obtained by the following equation: Breakthrough percentage (%) = the total number of pathogenic microorganisms passing through columns/the total number of pathogenic microorganisms input × 100%.

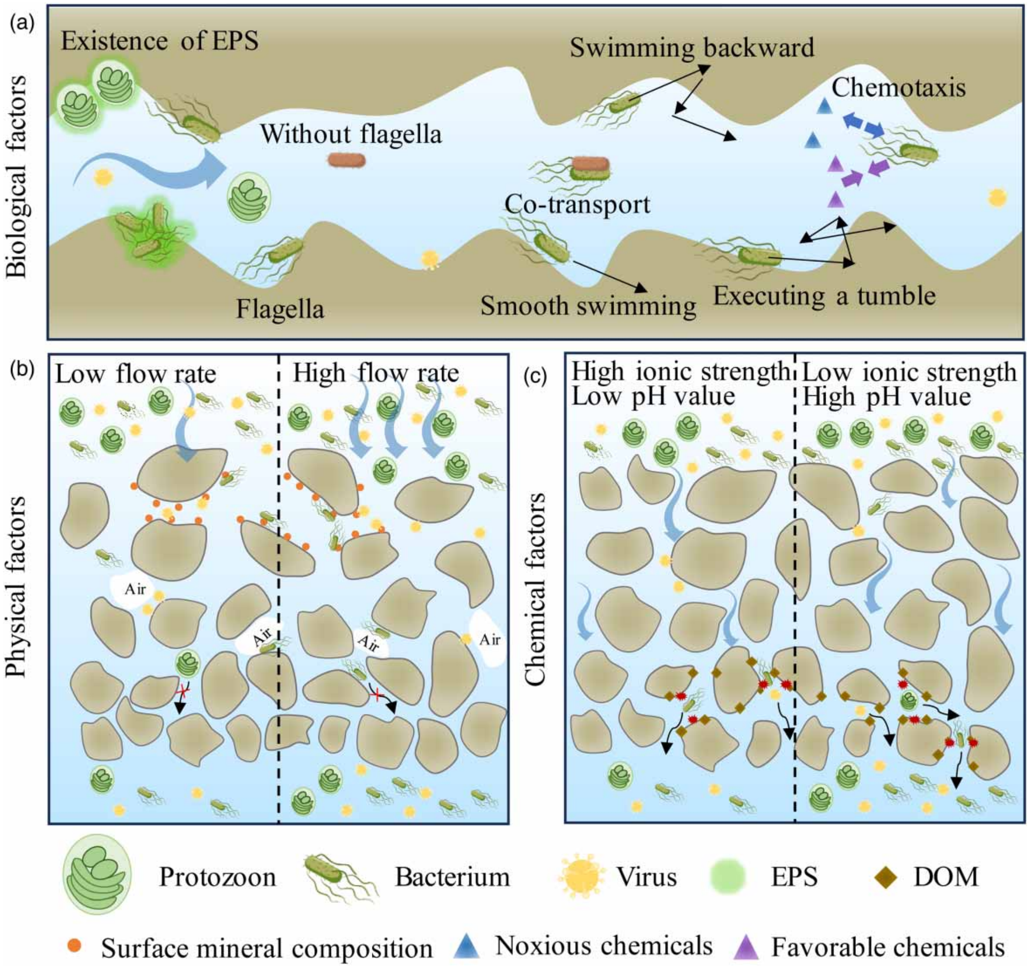


**Figure 3** | Influencing factors of the transport behavior of pathogenic microorganisms in porous media. (a) biological factors; (b) physical factors; and (c) chemical factors.

between bacteria and the porous media (Zhang *et al.* 2021a). Bacteria with sticky flagella have a lower energy barrier with porous media and can be more closely adsorbed on the surface of porous media (Haznedaroglu *et al.* 2010; Zhang *et al.* 2021a). For example, under the ionic condition of 10 mM KCl, the breakthrough percentage of *Salmonella enterica* SGSC 2478 without flagella in quartz sand columns was 81%, while that of *Salmonella enterica ST 5383* and *SGSC 1512* with flagella was only 36 and 37%, respectively (Haznedaroglu *et al.* 2010). Under the ionic condition of 25 mM NaCl, the breakthrough percentage of *E. coli* MG1655 ΔfliC without flagella, *E. coli* MG1655 with normal flagella, and *E. coli* RP437 with sticky flagella in quartz sand columns were 85.0, 65.9, and 42.1%, respectively (Zhang *et al.* 2021a). In addition, a few studies have focused on the interactions between flagellated bacteria and non-flagellated bacteria in porous media. When both coexist, non-flagellated bacteria can gain transport ability by hitchhiking on flagellated bacteria (Samad *et al.* 2017; Balseiro-Romero *et al.* 2022).

### 2.1.3. The chemotaxis of bacteria

Chemotaxis refers to the biased movement of bacteria when sensing coexisting chemicals. Bacteria can move toward favorable chemicals while escaping from noxious chemicals in porous media, thus affecting their transport behavior in porous media. For example, when favorable chemicals were added to the outlet of columns, the breakthrough percentage of bacteria was significantly increased (Balseiro-Romero *et al.* 2022), whereas when favorable chemicals were added into porous media, two-thirds of bacteria were retained in the column (Gao *et al.* 2022). Wang *et al.* (2023) found that the breakthrough percentage of gentamicin-sensitive *E. coli* S17-1 in quartz sand columns increased by 13.48% when 5 μg/L gentamicin was added into the background solution due to its chemotaxis to gentamicin.

#### 2.1.4. The EPS of pathogenic microorganisms

The surface of pathogenic microorganisms is easily attached to the EPS secreted by themselves. The presence of EPS can enhance the surface hydrophobicity and roughness of protozoa and bacteria to form large aggregates, thus promoting their deposition on the surface of porous media (Liu *et al.* 2007; Jin *et al.* 2021). In addition, biological clogging caused by excessive EPS accumulation can further inhibit the transport ability of pathogenic microorganisms (Kim & Kwon 2022; Zheng *et al.* 2024). For example, the breakthrough percentage of *Dictyostelium discoideum* spores decreased by 13.59% in quartz sand columns due to the existence of EPS. The increase in the content of $\beta$-sheets, which represents hydrophobic proteins in EPS components, leads to the binding between spores through hydrophobic interaction, which is the main reason for the decrease in the breakthrough percentage (Jin *et al.* 2021). Liu *et al.* (2007) found the $C/C_0$ of the breakthrough plateau of *Pseudomonas aeruginosa* PDO300 (mucoid alginate-overproducing strain) was 30% lower than *Pseudomonas aeruginosa* PAO1 psl pel (mutant strain with a deficiency in exopolysaccharide production).

### 2.2. Physical factors

#### 2.2.1. The porosity of porous media

With the decrease of the particle size of the porous media, the porosity of the porous media decreased, while the SSA and roughness of the media increased, providing more deposition sites for pathogenic microorganisms. Therefore, the smaller the porosity of porous media, the lower the transport ability of pathogenic microorganisms tends to be (He *et al.* 2020; Jin *et al.* 2021; Eisfeld *et al.* 2022). For example, the breakthrough percentage of *Dictyostelium discoideum* spores in quartz sand columns with porosity of 0.47 and 0.42 was 45.17 and 39.49%, respectively (Jin *et al.* 2021). Zhang *et al.* (2022) showed that when the porosity of quartz sand columns decreased from $0.424 \pm 0.005$ to $0.414 \pm 0.003$, the breakthrough percentage of Gram-negative *E. coli* and Gram-positive *Bacillus subtilis* was reduced by 13.0 and 12.7%, respectively. Zhang *et al.* (2021a) showed that as the porosity of quartz sand columns decreased from 0.44 to 0.39, the breakthrough percentage of *E. coli* MG1655, *E. coli* MG1655 $\Delta$flic, *E. coli* RP437, and *E. coli* RP437 fliC$^{st}$ decreased by 25.1–32.9%.

#### 2.2.2. The surface mineral composition of porous media

Porous media in the environment are rich in types and complex in composition, and there are metal oxides, clay, and other minerals on the surface of the porous media (Guo *et al.* 2021a). The existence of these substances may decrease the electronegativity of the zeta potential of the porous media, thus inhibiting the transport of pathogenic microorganisms by reducing the electrostatic repulsion between pathogenic microorganisms and porous media (Dong *et al.* 2014). Dong *et al.* (2014) found that in 20 mM NaCl solution, the breakthrough percentage of *E. coli* BL21 in bare quartz sand columns was 71.1%, while the breakthrough percentage in iron mineral-loaded quartz sand columns was significantly reduced to 0.27%. Zhuang & Jin (2003) showed that the addition of aluminum oxide to the quartz sand columns resulted in an obvious hysteresis of the breakthrough plateau during the transport process of $\Phi$X174 and MS2 phages, and the $C/C_0$ of the breakthrough plateau in the aluminum oxide-loaded quartz sand columns was lower than that in the bare quartz sand columns.

#### 2.2.3. The flow rate of the water

Generally speaking, the increasing flow rate would increase hydrodynamic shear stress between the water flow and the media, which will lead to the desorption of pathogenic microorganisms attached to the porous media. Simultaneously, the high flow rate can reduce the hydraulic residence time of pathogenic microorganisms in porous media, thereby decreasing the number of collisions with the porous media. The aforementioned phenomenons together enhance the transport ability of pathogenic microorganisms (Zhang *et al.* 2022). For example, the breakthrough percentage of *Dictyostelium discoideum* spores (representative amoeba spores) in quartz sand columns increased significantly from 39.5–45.2 to 77.1–89.5% when the water flow rate increased from 0.5 to 2.5 cm/min (Jin *et al.* 2021). In 0.1 mM NaCl solution, when the water flow rate increased from 0.2 to 0.5 cm/min, the breakthrough percentage of *Cryptosporidium parvum* oocysts in quartz sand columns increased from 50.6 to 68.7% (Kim *et al.* 2010). Wang *et al.* (2023) found that when the flow rate increased from 0.07 to 0.28 cm/min, the breakthrough percentage of antibiotic-resistant *E. coli* and antibiotic-sensitive *E. coli* in quartz sand columns increased from 42.66–43.71 to 70.49–71.06%. Walshe *et al.* (2010) also showed that the breakthrough percentage of

MS2 phage in gravel columns containing kaoline increased with the increase of flow rate, which increased from 1–2 to 7–16% when the flow rate increased from 1.6 to 3.1 cm/min.

### 2.2.4. The saturation of the water

Compared with saturated porous media, unsaturated porous media are more likely to cause bacteria and viruses to deposit on the surface of the porous media due to the existence of air–water interfaces or solid–water interfaces, thus reducing their transport abilities (Abit *et al.* 2012). Madumathi *et al.* (2017) showed that the $C/C_0$ of the breakthrough plateau of *E. coli* BL21 in quartz sand columns decreased from 68% under saturated conditions to 46% under unsaturated conditions. Other studies showed that *E. coli* phages have a lower transport ability in unsaturated quartz sand mainly because of the presence of solid–water interfaces in porous media (Qin *et al.* 2020; Zhang *et al.* 2021b). The decrease of water saturation in porous media led to the decrease of water film thickness on the surface of the media, which increased the possibility of collisions between *E. coli* phages and porous media and made it easier to deposit on the surface of the media (Zhang *et al.* 2021b). However, Bai *et al.* (2016) and Bai & Lamy (2021) obtained opposite results. The transport abilities of *E. coli*, *Klebsiella* sp., and *Rhodococcus rhodochrous* in saturated and unsaturated media were similar, and unsaturated porous media was even beneficial for the transport of bacteria. That was probably caused by the loss of adsorption sites in part of the unsaturated zones, thus offsetting or even exceeding the inhibition of the air–water interface on the transport of bacteria.

### 2.2.5. The temperature of the water

Temperature may affect the transport ability of pathogenic microorganisms by influencing their growth states, activity, and surface properties (Kim & Walker 2009; Birgander *et al.* 2018). Kim & Walker (2009) found that *E. coli* D21g and XL1-Blue had the weakest transport ability at 10 °C compared with 5 and 25 °C. Sarkar *et al.* (1994) showed that compared with 25 °C, *Bacillus Liceniformis* JF-2 had lower Brownian motion at 4 °C and was more likely to form larger cell clusters, resulting in lower transport ability. Sasidharan *et al.* (2017) showed that in 10 mM NaCl solution, with the temperature rising from 4 to 20 °C, the $C/C_0$ of the breakthrough plateau of PRD1 and $\Phi$X174 viruses decreased by 1 $\log_{10}$. At present, the effect of temperature on the transport behavior of pathogenic microorganisms is still inconclusive, and the differences in the above studies may be caused by the differences in different types of pathogenic microorganisms.

## 2.3. Chemical factors

### 2.3.1. The pH value of the water

The pH value can affect the transport ability of pathogenic microorganisms by influencing the zeta potential of the surface of pathogenic microorganisms and porous media. Under natural conditions, the surface of pathogenic microorganisms and porous media are negatively charged. With the increase of pH value, the negative charge on the surface of pathogenic microorganisms and porous media increases, and the electrostatic repulsion between them gradually increases, resulting in the gradual enhancement of the transport ability of pathogenic microorganisms (Mohanram *et al.* 2010; He *et al.* 2019). For example, as the pH value increased from 3 to 9, the breakthrough percentage of *Cryptosporidium parvum* oocysts in the Drummer soil columns increased by 63% (Mohanram *et al.* 2010). Suliman *et al.* (2017) reported that as the pH value increased from 2.0 to 8.0, the negative zeta potential of *E. coli* pathogenic O157:H7 and *E. coli* nonpathogenic K12 increased by 15.4 and 68.7 eV, respectively, and the increase of electrostatic repulsion between bacteria and porous media promoted their transport. Zhang *et al.* (2021b) reported that the breakthrough percentage of *E. coli* phages in quartz sand columns (particle size in 0.425 mm) at pH 5.0, 7.4, and 9.0 was 6.52, 11.36, and 38.78%, respectively. In addition, pH value may also affect the transport ability of *E. coli* phages by affecting their stability. In the coarse sand column, when the pH value was neutral, the number of collisions between *E. coli* phages increased, resulting in the formation of aggregates that increased their particle sizes, ultimately reducing their breakthrough percentage (Zhang *et al.* 2021b).

### 2.3.2. The ionic strength and species of the water

According to the classical Derjagin-Landau-Verwey-Overbeek (DLVO) theory, with the increase of ionic strength, the double electric layer on pathogenic microorganisms and porous media surface will be compressed, thereby reducing the energy barrier of interaction and ultimately increasing the deposition of pathogenic microorganisms on the porous media surface (Walshe *et al.* 2010; He *et al.* 2019; Zhang *et al.* 2021a). For example, in quartz sand columns at a flow rate of 0.2 cm/min, the breakthrough percentage of *Cryptosporidium parvum* oocysts under NaCl concentrations of 0.1, 1, and 100 mM were 50.6, 26.2, and 1.1%, respectively (Kim *et al.* 2010). He *et al.* (2019) reported that in saturated quartz sand columns, when

NaCl concentration increased from 10 to 25 mM, the breakthrough percentage of Gram-negative *E. coli* BL21 decreased from 73.9 to 54.0%. Walshe *et al.* (2010) demonstrated that the breakthrough percentage of MS2 phage gradually decreased from 31 to 20% when $CaCl_2$ concentration increased from 0 to 0.72 mM. However, studies on *Dictyostelium discoideum* spores had an opposite conclusion (Jin *et al.* 2021, 2022). When KCl concentration increased from 1 to 100 mM, the breakthrough percentage of *Dictyostelium discoideum* spores in quartz sand columns increased from 21.4–33.4 to 78.6–83.2%. It might be because the EPS surrounding *Dictyostelium discoideum* spores might shrink and form a more condensed outer layer under high ionic strength conditions due to compression of the electrical double layer, thereby reducing the deposition of *Dictyostelium discoideum* spores on the surface of the media (Jin *et al.* 2021).

Compared with monovalent cations, multivalent cations have a stronger ability to neutralize the negative charges on the surface of pathogenic microorganisms and porous media due to the cationic bridges, which are more likely to cause the deposition of bacteria and viruses on the surface of porous media (Zhang *et al.* 2021b). For example, the breakthrough percentage of *E. coli* MG1655 was 69.5% in 1 mM $CaCl_2$ solution, which was significantly lower than the breakthrough percentage of 80.5% in 5 mM NaCl (Zhang *et al.* 2021a). He *et al.* (2019) reported that the breakthrough percentage of Gram-negative *E. coli* BL21 in 5 mM $CaCl_2$ solution was significantly lower than that in 10 mM NaCl solution. This might be because the negative zeta potential of porous media in 5 mM $CaCl_2$ solution ($-21.90 \pm 1.00$ eV) was less than that in 10 mM NaCl solution ($-35.48 \pm 2.01$ eV). Zhang *et al.* (2021b) reported that under the ionic condition of 10 mM $CaCl_2$ or 10 mM NaCl, the breakthrough percentage of *E. coli* phage was lower in $CaCl_2$ solution.

### 2.3.3. DOM in the water

The presence of DOM such as amino acid, lipid, protein, and humic acid can promote the transport of pathogenic microorganisms by competing with them for adsorption sites on porous media (Abudalo *et al.* 2010; Aiken *et al.* 2011; Zhou & Cheng 2018). Abudalo *et al.* (2010) found that the breakthrough percentage of *Cryptosporidium parvum* oocysts increased by 66% in quartz sand columns treated with fulvic acid compared with no treatment. The $C/C_0$ of the breakthrough plateau of *Rhodococcus* sp. QL2 and *E. coli* BL21 in quartz sand columns pretreated with Suwannee River humic acid increased by 40 and 20%, respectively (Yang *et al.* 2012a). Weaver *et al.* (2013) showed that the breakthrough percentage of *E. coli* J6-2 in quartz sand columns pretreated with DOM of wastewater source was increased by 69.5% compared with the untreated group. Walshe *et al.* (2010) showed that as the pretreatment concentration of fulvic acid increased from 0.1 to 30 mg/L, the breakthrough percentage of MS2 phage in aquifer sand columns increased from 26–31 to 61–67%.

## 3. INFLUENCING FACTORS OF BIOCHAR INHIBITING THE TRANSPORT OF PATHOGENIC MICROORGANISMS

Clarifying the transport rules and mechanisms of pathogenic microorganisms in porous media will help guide the application of biochar in inhibiting the transport of pathogenic microorganisms. Due to the large size of protozoa, it is easier to be trapped in porous media. Therefore, the current studies on inhibiting the transport of pathogenic microorganisms by biochar are more concerned with bacteria and viruses (Table 2). For example, Fernando Perez-Mercado *et al.* (2019) used *Saccharomyces cerevisiae* to simulate the transport of *Cryptosporidium parvum* oocysts. The study found that adding 1.4 or 2.8 mm of hardwood biochar in sand columns was able to remove about 90% of *Saccharomyces cerevisiae*. Several studies have reported that biochar can inhibit the transport of bacteria in different types of porous media, such as quartz sand (Suliman *et al.* 2017), biofilter (Mohanty & Boehm 2014), and soil (Sun *et al.* 2019). In addition, Fernando Perez-Mercado *et al.* (2019) also found that the addition of hardwood biochar in sand columns resulted in a decrease in the concentrations of $\Phi$X174 phages and MS2 phages in the effluent by 1–2.3 $\log_{10}$. However, some other studies also have shown that the addition of biochar had a limited ability to inhibit the transport of pathogenic microorganisms, and even promoted their transport (Bolster & Abit 2012). For example, Guan *et al.* (2020) found that the addition of wheat straw biochar or willow wood biochar increased the breakthrough percentage of *E. coli* by 4–7%. Sasidharan *et al.* (2016) added the plant-derived biochar into the quartz sand columns, which increased the breakthrough percentage of *E. coli* and PRD1 phages by 20.7 and 87.4%, respectively. These phenomena indicate that the ability of biochar to inhibit the transport of pathogenic microorganisms is controlled by its own diverse physical and chemical properties. Therefore, this section reviewed the effects of raw materials, pyrolysis temperature, particle size, and functionalized modification on the remediation capability of biochar (Figure 4).

**Table 2** | The ability of biochar to inhibit the transport of pathogenic microorganisms

| Pathogenic microorganism | | Raw material | Pyrolysis temperature (°C) | Particle size (mm) | Inhibition efficiency | Reference |
|---|---|---|---|---|---|---|
| Bacterium (CFU/mL) | *E. coli* | Pine chip | 350, 700 | / | 15–58% | Abit *et al.* (2012) |
| | | Forestry wood | 700 | / | 52.9–62.9% | Lau *et al.* (2017) |
| | | Softwood | 550–600 | 0.425 | 0.2–2.3 $\log_{10}$ | Bolster (2019) |
| | | Hardwood | / | 1.4, 2.8, 5 | / | Fernando Perez-Mercado *et al.* (2019) |
| | | Poultry litter feed stock | 300, 700 | / | −68 to 52% | Bolster & Abit (2012) |
| | | Poultry litter | 350, 700 | / | −27 to 45% | Abit *et al.* (2012) |
| | *E. coli* (NCM 4236) | Softwood | 815–1,315 | 0.125–1 or <1 | 27.2–60.6% | Mohanty & Boehm (2014) |
| | | | | <1 | / | Mohanty & Boehm (2015) |
| | | Mixed plant | 180–395 | 0.595 | / | Afrooz *et al.* (2018) |
| | *E. coli* K-12 | Wood chips | 350, 700 | / | 0.6–3.3 $\log_{10}$ | Mohanty *et al.* (2014) |
| | | Pine wood, pine bark | 350, 600 | / | / | Suliman *et al.* (2017) |
| | | Maize straw | 300–700 | / | 2.0–4.7 $\log_{10}$ | Sun *et al.* (2019) |
| | *E. coli* O157:H7 | Pine wood, pine bark | 350, 600 | / | / | Suliman *et al.* (2017) |
| | *E. coli* (ATCC® No. 8739) | Wheat straw, willow wood | 500–560 | 1–4 | −9 to −4% | Guan *et al.* (2020) |
| | *E. coli* 13706 | Plant | 450–650 | 0.06–2 | −20.7 to 55.0% | Sasidharan *et al.* (2016) |
| | *K. pneumonia* K-6 | Maize straw | 300–700 | / | 5.2–6.3 $\log_{10}$ | Sun *et al.* (2019) |
| | *Corynebacterium variabile* HRJ4 | Poplar sawdust, corn straw | 300, 600 | / | 2.2–45.2% | Guo *et al.* (2021b) |
| | *Salmonella* | Softwood | 550–600 | 0.425 | 0.2–4.4 $\log_{10}$ | Bolster (2019) |
| | *Enterococcus* spp. | Hardwood | / | 1.4, 2.8, 5 | / | Fernando Perez-Mercado *et al.* (2019) |
| | *Saccharomyces cerevisiae* | Hardwood | / | 1.4, 2.8, 5 | / | Fernando Perez-Mercado *et al.* (2019) |
| | *Salmonella enterica* | Mixed plant | 180–395 | 0.595 | / | Afrooz *et al.* (2018) |
| Virus (PFU/mL) | MS2 phages | Mixed plant | 180–395 | 0.595 | / | Afrooz *et al.* (2018) |
| | | Hardwood | / | 1.4, 2.8, 5 | / | Fernando Perez-Mercado *et al.* (2019) |
| | *Φ*X174 phages | Plant | 450–650 | 0.06–2 | −71.5 to −52.3% | Sasidharan *et al.* (2016) |
| | | Hardwood | / | 1.4, 2.8, 5 | / | Fernando Perez-Mercado *et al.* (2019) |
| | F + coliphage | Mixed plant | 394 | 0.595 | / | Kranner *et al.* (2019) |
| | PRD1 phages | Plant | 450–650 | 0.06–2 | −87.4 to −48.6% | Sasidharan *et al.* (2016) |

w/w, weight/weight; v/v, volume/volume. Inhibition efficiency was obtained by the following equation: Inhibition efficiency (%) = Breakthrough percentage of pathogenic microorganisms in columns without biochar – Breakthrough percentage of pathogenic microorganisms in biochar amended columns.

## 3.1. Raw materials

Raw materials such as rice straw, wood, corn stalk, sludge, livestock, and poultry manure have been widely used to produce biochar to inhibit the transport of pathogenic microorganisms in porous media (Yuan *et al.* 2019). Plant-derived biochar is often better than sewage sludge or manure-derived biochar in inhibiting the transport of pathogenic microorganisms due to its higher C/N ratio and larger SSA (Lei & Zhang 2013). Abit *et al.* (2012) showed that the breakthrough percentage of *E. coli* SP2BO7 and SP1HO1 in the 700 °C pine chip biochar amended sand columns with a mass ratio of 2% was 36 and 0%, respectively. However, the breakthrough percentage of these two bacteria in the poultry litter biochar amended sand columns was 60 and 23%, respectively. This might be because the abundant pores in the 5–10 μm range in pine chip biochar were very suitable for retaining bacteria through pore filling.

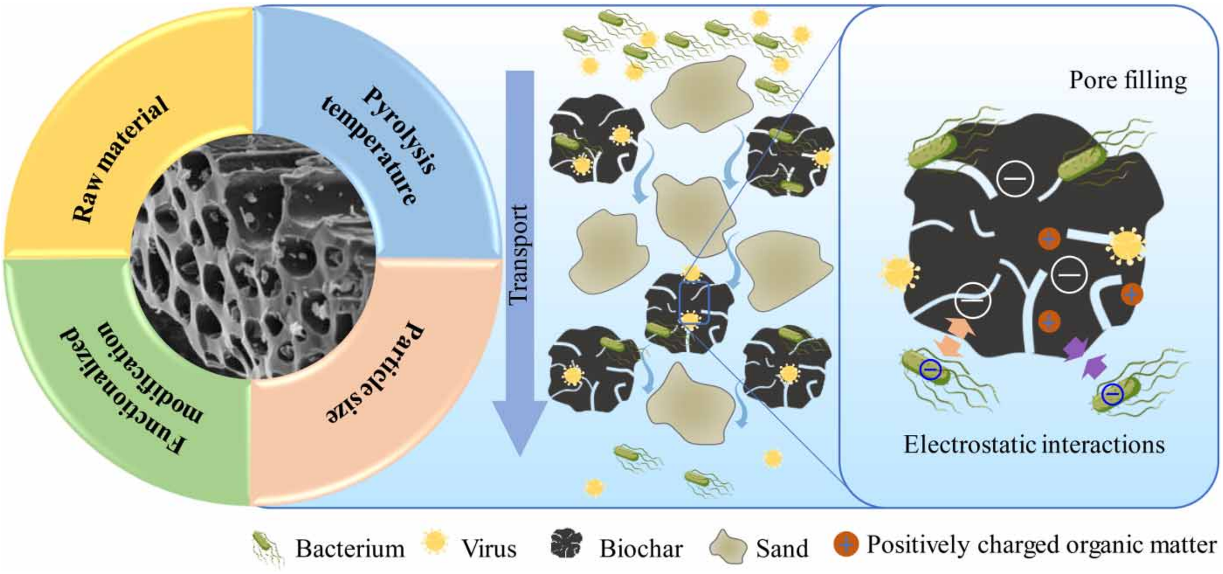


**Figure 4** | Influencing factors of biochar inhibiting the transport of pathogenic microorganisms in porous media.

### 3.2. Pyrolysis temperature

Pyrolysis temperature is a key factor affecting the physical and chemical properties of biochar. With the increase of pyrolysis temperature, properties such as carbon content, aromaticity, pH value, ash content, SSA, and stability of biochar increase, while properties such as yield, hydrogen content, oxygen content, H/C ratio, and O/C ratio decrease (Xiao *et al.* 2018). High-temperature biochar has a larger SSA and roughness, and fewer negatively charged oxygen-containing functional groups (such as –COOH), so it has stronger adsorption with pathogenic microorganisms and is more conducive to inhibiting their transport (Bolster & Abit 2012; Suliman *et al.* 2017). For example, the SSA of 700 °C pine chip biochar was 37 $m^2/g$ higher than that of 350 °C pine chip biochar. Moreover, the breakthrough percentage of *E. coli* SP2BO7 and SP1HO1 in the 700 °C pine chip biochar amended sand columns with a mass ratio of 1% was 33.0 and 32.8% lower than that in the 350 °C pine chip biochar amended sand columns, respectively (Abit *et al.* 2012). The negative zeta potential of 700 °C cellulose biochar was reduced by 13.0 mV due to the decrease of the surface negatively charged functional groups (e.g., –COOH). Therefore, the breakthrough percentage of *E. coli* and *Bacillus subtilis* in the 700 °C cellulose biochar amended sand columns was 16.7 and 17.4% lower than that in the 400 °C cellulose biochar amended sand columns (Zhang *et al.* 2022). Suliman *et al.* (2017) found that the isoelectric point of 700 °C pine biochar was 2.6 higher than that of 300 °C pine biochar, which resulted in a smaller electrostatic repulsion between *E. coli* K12 and 700 °C pine biochar, making it easier to adsorb on the surface of 700 °C pine biochar. Guo *et al.* (2021b) also showed that a typical Gram-positive petroleum degradation bacteria – *Corynebacterium variabile* HRJ4 – was more likely to be retained in large and abundant round pores of 600 °C poplar straw biochar through pore filling compared to 300 °C poplar straw biochar.

### 3.3. Particle size

In general, the smaller the particle size of biochar, the larger the SSA, the more adsorption sites are provided, and the stronger the inhibition ability on the transport of pathogenic microorganisms (Mohanty & Boehm 2014). For example, when the size of hardwood biochar was reduced from 2.8 to 1.4 mm, the removal rates of *Saccharomyces cerevisiae* (simulated *Cryptosporidium parvum* oocysts), bacteria (*E. coli* and *Enterococcus* spp.), and bacteriophages (*Φ*X174 and MS2 phages) in hardwood biochar amended sand columns were increased by 0.5–0.9 $\log_{10}$, 0.4–2.8 $\log_{10}$, and 0.3–1.3 $\log_{10}$, respectively (Fernando Perez-Mercado *et al.* 2019). Another study stated that *E. coli* had a breakthrough percentage of 4.8 ± 1.0% in softwood biochar amended sand columns containing a particle size range of less than 125 μm, and the breakthrough percentage significantly increased to 38.2 ± 4.9% when the part of biochar with a particle size range of less than 125 μm was removed (Mohanty & Boehm 2014).

### 3.4. Functionalized modification

Functionalized modification can change the SSA, surface functional groups, zeta potential, and other properties of biochar, which is expected to improve the ability of biochar to inhibit the transport of pathogenic microorganisms in porous media (Chu *et al.* 2018; Wang *et al.* 2019). However, research on inhibiting the transport of pathogenic microorganisms through functionally modified biochar is still in its infancy. Sporadic studies found that $H_2SO_4$ modification can increase the SSA of biochar by increasing the micropores on the surface of biochar through oxidation. Therefore, the breakthrough percentage of *E. coli* decreased from 3.4% in quartz sand columns to 1.3% in $H_2SO_4$-modified biochar amended sand columns (Lau *et al.* 2017). Modification of positively charged arginine decreased the negative zeta potential of biochar from $-19.8 \pm 2.0$ to $-8.9 \pm 3.6$ mV. The results showed that the breakthrough percentage of *E. coli* and *Bacillus subtilis* in the arginine-modified biochar amended sand columns was 26.9 and 9.8% lower than that in the original biochar amended sand columns, respectively (Zhang *et al.* 2022).

It should be noted that most studies on the application of biochar to inhibit the transport behavior of pathogenic microorganisms are short-term laboratory-scale experiments. Therefore, the impact of long-term changes in the physical and chemical properties of biochar on the transport inhibition ability and the possible environmental risks, such as the transport and diffusion risk of broken biochar colloid itself, have not been systematically explored (Zhao & Shang 2023). In addition, to improve the application performance of biochar, a variety of functionalized modification methods have been introduced, including treatment with acid, base solutions, or reducing agents, modification with minerals and nanoparticles, and functionalization with specific functional groups. However, the chemical modifiers (or loaded components) used for functionalized modification may pose secondary pollution risks to the environment, owing to their instability from pH changes, turbulence, and aging (Tan & Yu 2023). Therefore, when considering the application of original biochar or functionalized modified biochar to inhibit the transport behavior of pathogenic microorganisms, potential environmental risks need to be considered and appropriate risk management and careful evaluation need to be carried out.

## 4. CONCLUSION

In this review article, the transport rules and influencing factors of pathogenic microorganisms in porous media were carefully reviewed, as well as the capability of biochar with different properties for inhibiting the transport of pathogenic microorganisms was summarized. The following conclusions are obtained: (1) The transport behavior of pathogenic microorganisms in porous media is affected by biological, physical, and chemical factors. To evaluate the transport risk of pathogenic microorganisms in the process of reclaimed water reuse, it is necessary to comprehensively analyze the types and characteristics of pathogenic microorganisms and diverse environmental conditions; (2) Biochar, especially plant-derived biochar, can effectively inhibit the transport of pathogenic microorganisms because of its well-developed pore structure and huge SSA. Moreover, the higher the pyrolysis temperature, the smaller the particle size, the fewer negatively charged surface functional groups, and the stronger the ability of biochar to inhibit the transport of pathogenic microorganisms; (3) Although the research on inhibiting the transport of pathogenic microorganisms through functionalized modification of biochar (such as acid modification and organic modification) is still in its infancy, preliminary studies have proved its feasibility. Appropriate risk management and careful evaluation are also essential when considering the use of biochar and functionalized modified biochar. This review article is helpful to understand the transport rules and mechanisms of pathogenic microorganisms in porous media and biochar-based remediation technology, to improve the ecological and health safety of reclaimed water reuse.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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