

Bacterial Pore Sealing as a Tool for Chemical Risk Reduction

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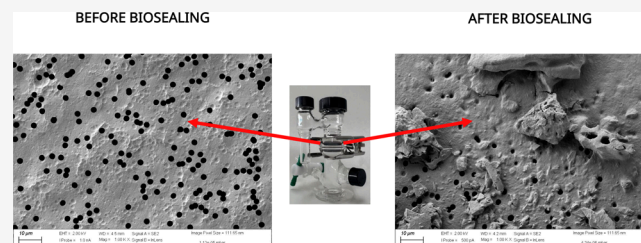
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Supporting Information

ABSTRACT: Hydrophobic organic compounds (HOCs) such as naphthalene are persistent environmental contaminants whose mobility and bioavailability challenge conventional remediation. Sorptive amendments such as biochar can reduce HOC mobility but may introduce pollutants and lose sorption capacity over time. In this work, we investigated whether the bacterium *Bacillus subtilis*, biochar, and mixtures of bacteria and biochar could seal pores and impede naphthalene diffusion. We employed two experimental approaches: (i) centrifugation-compacted layers over solid naphthalene (simulating source-zone conditions) and (ii) bioreactors with 3 and 12 μm membranes representing distal transport. *B. subtilis* exhibited a higher sorption affinity than biochar alone (Freundlich capacity or $\log K_F$ of 4.33 vs 3.47) and reduced naphthalene mobilization 3-fold, releasing only 2% of the applied mass. The mixture of biochar and bacteria achieved the greatest reductions in naphthalene flux (70% and 50% for 3 and 12 μm membranes, respectively), with effective diffusivities 2 orders of magnitude lower than molecular diffusion through water. Electron microscopy confirmed pore occlusion by bacterial extracellular polymeric substances and biochar aggregation. These findings demonstrate microbial pore sealing as a promising strategy to limit HOC diffusion in porous media, with potential applications for source-zone and plume containment.

KEYWORDS: naphthalene diffusion, *Bacillus subtilis*, biochar, bioremediation, microbial pore sealing, contaminant immobilization



1. INTRODUCTION

The presence of hydrophobic organic compounds (HOCs) in soil and sediment poses significant environmental risks due to their toxicity, high mobility, and persistence. Sorptive amendments such as biochar have been widely employed to immobilize these contaminants and reduce their bioavailability.^{1–4} However, this remediation approach faces limitations as biochars may themselves contain pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, and their sorption capacity often decreases over time due to aging.⁵

Recent advances in biologically mediated soil improvement suggest that microorganisms may offer alternative mechanisms for contaminant immobilization.⁶ *Bacillus subtilis*, a Gram-positive, rod-shaped, motile, and chemotactic bacterium common in soil and plant rhizospheres, thrives under aerobic and facultative anaerobic conditions and forms highly resistant endospores.⁷ Its capacity to develop robust biofilms⁸ and precipitate calcium carbonate⁹ makes *B. subtilis* a promising agent for physically obstructing pore structures and inhibiting the diffusion of contaminants through microbial pore sealing. In this study, we evaluated the ability of *B. subtilis* and wheat straw-derived biochar to reduce naphthalene diffusion using two complementary experimental settings: (i) centrifugation-compacted layers and (ii) micrometer-scale pore systems. The first setting comprised a solid naphthalene coating overlaid with centrifuged layers of bacteria, biochar, or a mixture of

bacteria and biochar, simulating source-zone conditions with maximum concentration gradients driven by solid crystals. The second utilized a bioreactor where membranes of defined pore size separated these biomaterials from saturated aqueous naphthalene, representing the distal transport of dissolved pollutants. In both systems, diffusing naphthalene was continuously trapped in a headspace cyclodextrin solution, preventing the disturbance of both concentration gradients and the integrity of the sealing biolayer during sampling. Our results demonstrate a substantial reduction in naphthalene flux, highlighting a novel biosealing strategy for limiting contaminant transport in porous environmental media.

2. MATERIALS AND METHODS

2.1. Materials. ¹⁴C-labeled naphthalene (58 mCi/mmol, radiochemical purity of 99%) was purchased from Moravek, Inc. Laboratory-grade hydroxypropyl- β -cyclodextrin (HPCD, >95% purity) was purchased from Thermo Fisher Scientific. Biochar was sourced from the UK Biochar Research Center

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(UKBRC) and produced from pyrolysis at 550 °C using wheat straw pellets (WSP550).¹⁰ Wheat straw biochar (WSP550) combines sustainability (given its abundance as an agricultural residue), optimal physicochemical properties (large surface area, well-developed porosity, and stable aromatic structures), and practical advantages. It offers lower ash content than manure biochars and higher reactivity than wood biochars, enhancing pollutant adsorption and soil amendment efficacy.^{1,11,12} The biochar pellets were ground, homogenized with a mortar and pestle to afford fine particles, and sterilized by being autoclaved.

2.2. Bacterial Strain and Culture Conditions. *B. subtilis* DSM10 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Its average cell dimensions are 2.5 μm $\times$ 1.2 μm . Bacterial suspensions were prepared following the procedure described elsewhere¹³ with minor modifications, as described in the [Supporting Information](#). The final resuspension medium was MQT medium (0.01 mM potassium phosphate (pH 7), 0.3 mM $(\text{NH}_4)_2\text{SO}_4$, 0.14 mM CaCl_2 , 0.1 mM EDTA, 5 mM sodium lactate, and 0.05% (v/v) glycerol) or mineral medium,¹⁴ which is indicated below for each type of experiment.

The optical density (OD), dry biomass, organic carbon content, and colony-forming units (CFU) in bacterial suspensions were determined via serial dilutions of a high-OD culture. For each dilution, the OD was measured and a known volume was filtered; filters were dried at 60 °C for 72 h, and the weight difference provided the dry weight (DW) of biomass. Organic carbon (OC) was quantified by using a Shimadzu TOC autoanalyzer. CFU were determined by plating on TSA agar, incubating at 30 °C for 24 h, and counting colonies. A bacterial suspension with an OD of 2.11 had a DW of 0.64 mg mL^{-1} , an OC of 0.33 mg mL^{-1} , and 7 $\times 10^{10}$ CFU mL^{-1} .

2.3. Sorption Experiments. Sorption was measured in sterilized 50 mL glass bottles containing 50 mL of MQT medium. Three treatments were tested: abiotic control, 20 mg of biochar, and 3 mL of a *B. subtilis* suspension ($\text{OD}_{600} = 1.35$). Duplicate bottles were sealed and prewarmed to 40 °C to dissolve naphthalene and prevent crystallization and then spiked with [^{14}C]naphthalene in 100 μL of acetone to four concentrations (up to 75% solubility), yielding ~ 400 dpm mL^{-1} . After incubation at 22 °C for 48 h with shaking (150 rpm), samples were centrifuged at 2570 rpm, and 1 mL of each supernatant was mixed with scintillation cocktail for radioactivity quantification with a model LS6500 liquid scintillation counter (LSC) (Beckman Instruments, Fullerton, CA). Sorbed naphthalene was calculated from the decrease in the aqueous concentration, with volatilization losses considered negligible. Isotherms were fitted to the logarithmic form of the Freundlich relationship:¹⁵

$$C_s = K_F C_w^n \quad (1)$$

$$\log C_s = n \log C_w + \log K_F \quad (2)$$

where C_s and C_w are sorbed (mg kg^{-1}) and dissolved (mg L^{-1}) naphthalene concentrations, respectively, K_F (in (mg $\text{kg}^{-1})/(\text{mg } \text{L}^{-1})^n$) is the Freundlich or capacity constant, and n is the parameter indicating the heterogeneity of sorption energies on the material surface. The maximum distribution coefficient, K_d (liters per kilogram), was calculated from the first isotherm point by dividing C_s and C_w . The theoretical aqueous fraction of naphthalene at equilibrium (F_w) was calculated for each

diffusion experiment by using the fitted K_F and n values from sorption experiments (see the [Supporting Information](#) for details).

2.4. Batch Diffusion Experiments. Conical glass tubes (120 mm $\times$ 15 mm) equipped with headspace traps were used for the diffusion experiments. A 50 μL aliquot of acetone containing 1 mg of unlabeled naphthalene and approximately 100 000 dpm of [^{14}C]naphthalene was deposited at the bottom of each duplicate tube. After acetone evaporation, a uniform coating of solid naphthalene remained. Biomaterial suspensions (biochar, bacteria, or a mixture of biochar and bacteria in mineral medium to maintain resting cell conditions) were then added and centrifuged at 8000 rpm for 15 min. The preparation procedure varied by biomaterial type (explained in detail in the [Supporting Information](#)). Tubes were then sealed with silicone stoppers. Headspace trap solutions (0.5 mL of 100 mM cyclodextrin) were collected for radioactivity quantification and replaced periodically. The naphthalene flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$) was calculated from the linear regression of the naphthalene concentration in the suspensions over the period of maximum passage (3 days) normalized to the tube area section (in cm^2). Statistical comparison of mean values was performed with analysis of variance with the Tukey post hoc test at the $P = 0.05$ level.

2.5. Bioreactor Experiments. The bioreactors employed were those previously described,¹⁶ equipped with membranes with two pore sizes (3 and 12 μm). The setup was adapted to measure the diffusion of naphthalene through membranes with biomaterial deposition above membranes occurring by gravitational settling. In the lower chamber (LC), 50 μL of the ^{14}C -labeled naphthalene solution was deposited as described above; after acetone evaporation, the chamber was filled with 15 mL of MQT medium. Four bioreactor configurations were established in duplicate, differing in the biomaterial dispensed into the 3 mL upper chamber (UC) volume: (1) 20 mg of biochar in MQT, (2) concentrated bacterial suspension (OD of 1.5–2.0) in MQT, (3) a mixture of both biomaterials at the concentrations specified above (in this case, the biochar was introduced into the UC homogenized with the bacterial suspension), and (4) control with MQT only. These biomaterial loadings were selected based on sorption results demonstrating greater naphthalene affinity for bacteria than biochar on a DW basis and preliminary bioreactor trials across biochar loadings ranging from 3 to 70 mg mL^{-1} .

Organic constituents in MQT were retained in all bioreactor configurations to maintain physiological conditions during incubation while minimizing sorption interference from dissolved organic carbon ($\log K_{oc} = 2.98$ for naphthalene¹⁷) and preventing substantial growth given the high initial inoculum. Preliminary bacterial translocation experiments ([Figure S2](#)) confirmed that 3 μm membranes were more restrictive than 12 μm membranes for *B. subtilis* cell passage. These results are consistent with previous observations on *Pseudomonas putida* G7 mobilization through micrometer-size pores, which has similar cell dimensions ($\sim 2 \mu\text{m} \times 1 \mu\text{m}$).¹⁴ Deposition of the material onto membranes by settling was indirectly assessed by monitoring the loss of optical density (OD) in 3 mL suspensions prepared in glass vials with a geometry similar to that of the upper chamber (2 cm diameter, 10 mL volume), maintained under the same static conditions as the bioreactors. After 3 days, the supernatant OD decreased substantially to 14%, 17%, and 13% of the initial values for biochar, bacteria, and the mixture of biochar and bacteria,

respectively, confirming effective biomaterial sedimentation occurred.

Each bioreactor trap was filled with 0.5 mL of 200 mM cyclodextrin. The trap contents were collected daily and combined with a scintillation cocktail to measure radioactivity via liquid scintillation counting. After sampling, the trap was replenished with 0.5 mL of a fresh cyclodextrin solution. The accumulated naphthalene, expressed in micrograms, was calculated based on the recorded dpm. All results are provided as the mean values of duplicate experiments. To confirm that radioactivity measurements corresponded to ^{14}C -labeled naphthalene, selected trap solutions were analyzed by HPLC, adapting previously described procedures.^{18,19} Analytical details are provided in the Supporting Information (Figure S1). The naphthalene flux ($\mu\text{g cm}^{-2} \text{h}^{-1}$) was calculated from the linear regression of the naphthalene concentration in the UC over the period of maximum passage (3 days) normalized to the total available pore area (in cm^2) of each membrane. The effective diffusion coefficient (D_{eff}) was calculated using the equations for a diaphragm cell,^{14,20} as detailed in the Supporting Information. Statistical comparison of mean values was performed with analysis of variance with the Tukey post hoc test at the $P = 0.05$ and $P = 0.10$ levels.

At the conclusion of select bioreactor experiments, air-dried membranes were studied by scanning electron microscopy on a Zeiss Crossbeam 550 instrument. Samples underwent dehydration in a graded ethanol series, air drying, and sputter coating with a 15 nm gold/palladium layer using a Leica ACE600 instrument. Three membrane sectors per condition were prepared and examined separately via SEM to confirm the reproducibility of microstructural features.

3. RESULTS AND DISCUSSION

3.1. Batch Sorption and Diffusion Experiments.

Naphthalene sorption isotherms for *B. subtilis* cells and biochar were well described by eq 2, with $n < 1$ in both cases (0.72 and 0.76, respectively), indicating progressively reduced sorption efficiency at higher sorbate concentrations (Figure S2). The log K_F value of the biochar sorption isotherm (3.47) was intermediate within the range reported for the sorption of naphthalene to biochars produced at 150–700 °C with varying degrees of carbonization.²¹ The higher log K_F value for bacteria (4.33) demonstrated a greater sorption affinity of *B. subtilis* cells compared to biochar. The maximum K_d was 19 444 L kg^{-1} for bacteria and 3020 L kg^{-1} for biochar. For bacteria, this value was 40 times higher than predicted by the empirical log $K_{\text{ow}} - K_d$ relationship for bacterial sorption,²² which suggests that mechanisms beyond physical partitioning contributed to the sorption of naphthalene by *B. subtilis* cells.

Centrifugation-compacted biomaterial layers substantially reduced the level of diffusion of the pollutant from solid naphthalene (Figure 1A). Although calculated F_w values indicated similar high dissolved naphthalene sorption (>95% sorbed) across treatments (Table 1), the naphthalene maximum flux through the bacterial layers was significantly lower (about 3-fold) than through biochar. After 15 days, bacterial treatments released only 2% of the total naphthalene mass, while mixtures of biochar and bacteria produced intermediate effects. These results demonstrate that bacterial layers impeded diffusion more effectively than did biochar alone.

3.2. Diffusion through Micrometer-Size Pores. The diffusion of naphthalene through membranes with two pore

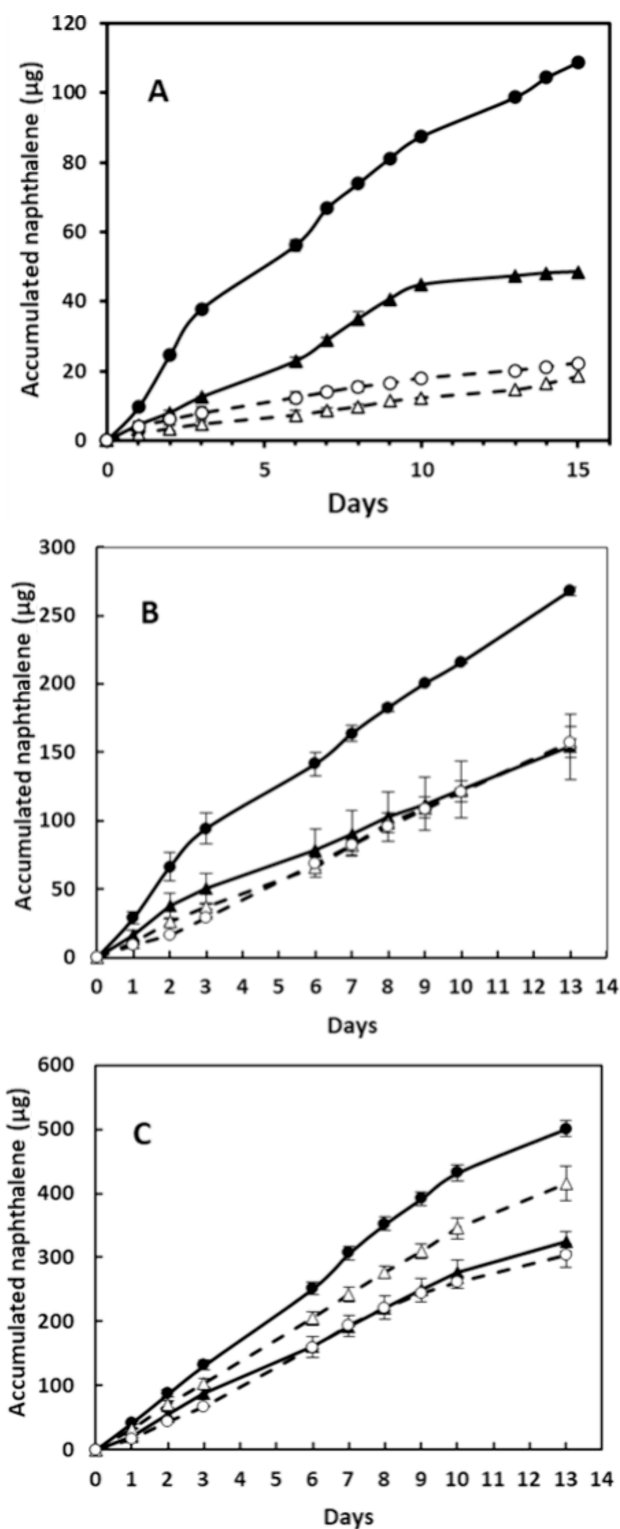


Figure 1. Influence of biomaterial layers on naphthalene diffusion: (A) solid naphthalene in centrifugation-compacted biomaterial layers, (B) saturated aqueous solutions in 3 μm membrane bioreactors, and (C) saturated aqueous solutions in 12 μm membrane bioreactors. Symbols indicate control without a biolayer (●), biochar (▲), *B. subtilis* (△), and biochar with *B. subtilis* (○). Data points represent means of duplicate tubes or bioreactors; error bars show one standard deviation. Error bars that are not visible are smaller than the symbols. The different y-axis maxima across the three settings reflect the distinct pollutant flux magnitudes observed under each condition.

Table 1. Influence of Biomaterial Layers on Naphthalene Diffusion^{a,b}

conditions ^c		control	biochar	bacteria	biochar and bacteria
tubes, centrifugation	flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$)	0.35 A	0.11 B	0.04 C	0.07 BC
10 mg mL ⁻¹ biochar	extent _{3d} (%) ^c	3.8 A	1.2 B	0.5 B	0.8 B
0.8 mg mL ⁻¹ bacteria	extent _{max} (%) ^c	10.9 A	4.8 B	1.8 B	2.2 B
	F_w (%) ^e	NA ^f	2.4	4.3	1.6
3 μm bioreactors, gravitational settling	flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$)	2.90 A	1.56 A	1.15 A	0.86 B
6.7 mg mL ⁻¹ biochar	D_{eff} ($\times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$)	2.49 A ^b	1.21 AB ^b	0.85 B ^b	0.63 B ^b
0.6 mg mL ⁻¹ bacteria	extent _{3d} (%) ^d	9.4 A	5.0 AB	3.7 AB	2.9 B
	extent _{max} (%) ^d	26.8 A	15.4 B	15.7 B	15.7 B
	F_w (%) ^e	NA ^f	3.6	5.3	2.2
12 μm bioreactors, gravitational settling	flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$)	6.10 A	4.13 AB	4.82 AB	3.17 B
6.7 mg mL ⁻¹ biochar	D_{eff} ($\times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$)	5.85 A	3.49 AB	4.23 AB	2.54 B
0.5 mg mL ⁻¹ bacteria	extent _{3d} (%) ^d	13.2 A	8.8 AB	10.3 AB	6.7 B
	extent _{max} (%) ^d	50.1 A	32.5 B	41.6 AB	30.3 B
	F_w (%) ^e	NA ^f	3.6	7.1	2.4

^aValues in a row followed by the same uppercase letter are not significantly different ($P = 0.05$). ^bValues in this row followed by the same uppercase letter are not significantly different ($P = 0.10$). ^cKey experimental conditions for diffusion experiments, including the setup configuration, consolidation process, and biomaterial dry weight concentration. ^dextent_{3d} and extent_{max} indicate percentages of naphthalene mass recovered at the end of the period (3 days) for flux and D_{eff} calculations and at the end of the experimental period, respectively. ^e F_w is the theoretical aqueous fraction of naphthalene at equilibrium, assuming a homogeneous biomaterial distribution. ^fNA, not applicable.

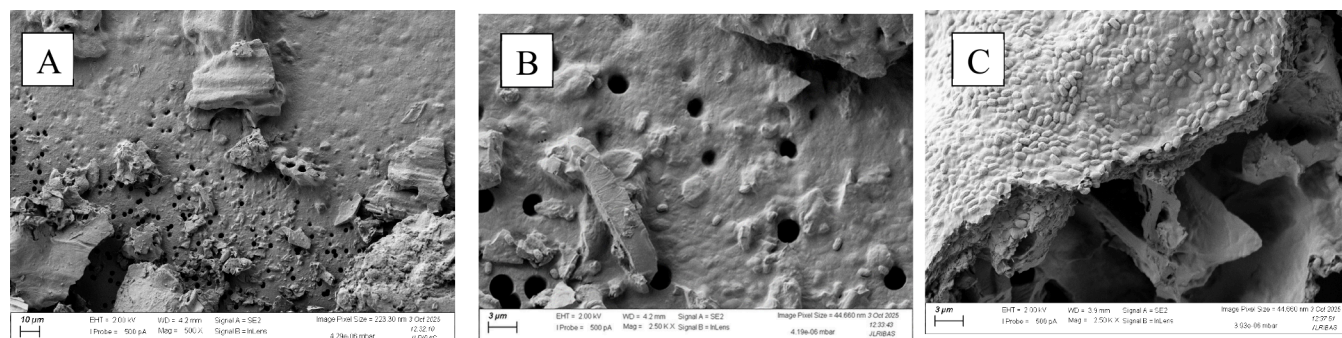


Figure 2. Scanning electron micrographs of 3 μm membranes after incubation for 40 days in bioreactors inoculated with *B. subtilis* in the presence of biochar. (A) Edge of the sealing layer. (B) Detail of pore occlusion. (C) Biochar–bacterium layer developed on the membrane with biochar particle aggregation by *B. subtilis* cells and extracellular polymeric substances.

sizes (3 and 12 μm) was evaluated in bioreactors containing biochar, bacteria, or their combination. Figure 1B shows a representative experiment depicting naphthalene recovery in cyclodextrin traps over time for 3 μm membranes. All treatments reduced the naphthalene passage relative to the controls. Maximum fluxes through 3 μm pores (Table 1) were 10-fold higher than those measured with solid naphthalene in centrifugation-compacted biomaterial layers. The mixture of biochar and bacteria achieved a statistically significant flux reduction (70% decrease vs the control). In reactors with 12 μm membranes (Figure 1C), overall naphthalene recovery increased, likely reflecting the larger effective diffusion surface area in these membranes compared with that of 3 μm membranes. The mixture of biochar and bacteria again proved to be most effective at impeding flux significantly, achieving 50% of control values. However, at this pore size, the bacterial treatment alone caused intermediate effects, possibly because some cells traversed the pores into the lower chamber, as indicated by preliminary translocation experiments (Figure S3), thereby reducing their effectiveness as diffusive barriers at the membrane. To exclude the possibility of weaker biofilm formation in larger pores, a separate bioreactor experiment was conducted under the same conditions as the diffusion experiments, confirming substantially higher bacterial trans-

location across 12 μm versus 3 μm pore-size membranes (Supporting Information).

The diffusive barrier effect was quantified by calculating the effective diffusivity (D_{eff}) based on the total pore area (Table 1). The resulting values ranged from 10^{-8} to $10^{-7} \text{ cm}^2 \text{ s}^{-1}$, approximately 2 orders of magnitude lower than the molecular diffusivity of naphthalene in water ($7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ at 25 $^{\circ}\text{C}$).²³ The mixture of biochar and bacteria produced statistically significant reductions in D_{eff} across both 3 and 12 μm pore-size membranes (75% and 57% reductions, respectively) compared to the control. In contrast, biochar-only treatments did not significantly alter the D_{eff} . The effective diffusivity in this system encompasses multiple sequential processes: naphthalene transport through membrane pores (and biomaterial layers, where present), partitioning into the headspace, and uptake by the cyclodextrin trap. This multistep mechanism accounts for the significantly lower D_{eff} values compared to previous studies on naphthalene diffusion in porous media (water, air, and agar), where phase volume variations increased D_{eff} from 1.0×10^{-5} to $1.0 \times 10^{-2} \text{ cm}^2 \text{ s}^{-1}$.²⁴ Similarly, biofilm development has been shown to reduce naphthalene dissolution rates by 90%, versus only a 20% reduction in the aqueous diffusion coefficient.²⁵ To the best of our knowledge, quantitative estimates of naphthalene D_{eff} in

biochar remain unavailable. However, diffusivity differences between carbonized and uncarbonized phases are known to influence the kinetics of sorption of naphthalene to biochar.²¹ Fast sorption typically occurs via partitioning in uncarbonized matter (<150 °C) or surface adsorption on high-temperature chars (>700 °C), whereas condensed phases formed at intermediate temperatures (250–350 °C) cause slow matrix diffusion. We propose that bacterial biomass functions as a partitioning medium similar to uncarbonized organic matter, effectively promoting fast sorption and thereby reducing the bulk D_{eff} . Notably, however, bacterial batch sorption data deviated from K_{ow} predictions, pointing to additional sorption mechanisms beyond physical partitioning that will be the subject of future research.

Separate bioreactor experiments were conducted to examine incubated membranes by SEM and further elucidate the role of biomaterial layers in restricting pore diffusion. Focus was placed on bacterial treatments, which proved to be the most effective diffusive barriers. Key conditions included 7.6 mg mL⁻¹ biochar, 0.32 mg mL⁻¹ bacteria, and 3 μm pore-size membranes, with no ¹⁴C-labeled naphthalene added to the lower chamber. The incubation period was extended to 40 days to ensure thorough membrane colonization. SEM images provided clear evidence of pore occlusion by bacterial extracellular polymeric substances both with (Figure 2A–C) and without (Figure S4) biochar. In addition, *B. subtilis* extracellular polymeric substances mediated the aggregation of biochar particles among themselves and on the membrane surface, likely enhancing their adherence and facilitating SEM sample preparation (Figure 2B and Figure S4). Although dense biofilms were visible macroscopically above membranes in bacterium-only treatments, SEM imaging did not capture their full extent, probably due to limited membrane adherence.

Batch diffusion experiments demonstrated that *B. subtilis* layers were more effective than biochar in reducing naphthalene mobility, suggesting that physical blockage and biofilm formation play key roles in impeding contaminant transport. In bioreactor assays, the substantial reduction in naphthalene flux reflected progressive pore colonization and obstruction, as confirmed by SEM observations. The combination of biochar and bacteria proved to be the most promising, integrating both pore sealing and material aggregation. Collectively, these findings indicate that naphthalene bioavailability can be diminished through application of biochar and *B. subtilis* layers. When used together, the observed decrease in naphthalene diffusion appears to be attributable to bacterial–biochar aggregate formation, which enhances barrier effects and further restricts diffusion.

This study demonstrates, for the first time, that bacterial layers can act as highly effective diffusive barriers, surpassing conventional biochar in limiting the mobility of naphthalene through microporous media. The synergistic combination of bacterial biofilms and biochar enhances contaminant immobilization via aggregate formation and pore occlusion, integrating microbial activity with sorptive material functionality. The underlying mechanisms are supported by well-documented phenomena. Biofilm growth in porous media can reduce permeability by narrowing or blocking pore throats, with reductions of ~70% reported in sand due to bioclogging, directly supporting the role of microbial layers as diffusive/advective barriers.²⁶ The pore-size distribution and architecture of biochar will also influence microbial attachment and colonization, with optimal bacterial colonization occurring in

5–15 μm pores, highlighting the synergistic interplay between biochar and biofilms for contaminant sequestration.²⁷ Soil texture will further modulate the barrier efficacy. Coarse, sandy soils with pore diameters comparable to the 3 μm membranes used in this study support richer biofilm coverage and greater flux reduction, whereas fine-textured, clay-rich soils with narrower or more tortuous pores may constrain microbial growth and alter transport pathways.^{28,29} Finally, contaminant transport in biochar-amended porous media is influenced by pyrolysis temperature and biochar aging, with higher-temperature biochars providing greater surface area and sorptive capacity to inhibit naphthalene mobility.³⁰ Together, these findings indicate that combining microbial pore sealing with biochar sorption can effectively limit hydrophobic contaminant flux, particularly in soils where pore-scale dimensions align with the barrier mechanism and highlight the need for soil texture-aware strategies to translate laboratory flux reductions into field-scale remediation outcomes. While this study demonstrates the efficacy of microbe–biochar barriers under controlled laboratory conditions, the barrier durability under variable environmental conditions (pH, temperature, and nutrient availability) warrants further investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.estlett.5c01220>.

Additional information about the experimental procedures and calculation methods, including the culture conditions for *B. subtilis*, compaction methods for batch diffusion experiments, HPLC analysis, sorption isotherms, calculation of the effective diffusion coefficient, *B. subtilis* translocation in the bioreactor, and scanning electron micrographs of colonized membranes (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Anae, J.; Ahmad, N.; Kumar, V.; Thakur, V. K.; Gutierrez, T.; Yang, X. J.; Cai, C.; Yang, Z. G.; Coulon, F. Recent advances in biochar engineering for soil contaminated with complex chemical mixtures: Remediation strategies and future perspectives. *Sci. Total Environ.* **2021**, 767, 144351.
- (2) Estoppey, N.; Knight, E. R.; Allan, I. J.; Ndungu, K.; Slinde, G. A.; Rundberget, J. T.; Ylivainio, K.; Hernandez-Mora, A.; Sormo, E.; Arp, H. P. H.; Cornelissen, G. PFAS, PCBs, PCDD/Fs, PAHs and extractable organic fluorine in bio-based fertilizers, amended soils and plants: Exposure assessment and temporal trends. *Sci. Total Environ.* **2024**, 957, 177347.
- (3) Malheiro, C.; Cardoso, D. N.; Neves, J.; Lima, D. L. D.; Esteves, V. L.; Soares, A.; Loureiro, S. Biochar in soil mitigates dimethoate hazard to soil pore water exposed biota. *J. Hazard. Mater.* **2020**, 400, 123304.
- (4) Gomez-Eyles, J. L.; Ghosh, U. Enhanced biochars can match activated carbon performance in sediments with high native bioavailability and low final porewater PCB concentrations. *Chemosphere* **2018**, 203, 179–187.
- (5) Nguyen, T. B.; Sherpa, K.; Bui, X. T.; Nguyen, V.; Vo, T. D. H.; Ho, H. T. T.; Chen, C. W.; Dong, C. D. Biochar for soil remediation: A comprehensive review of current research on pollutant removal. *Environ. Pollut.* **2023**, 337, 122571.
- (6) DeJong, J. T.; Mortensen, B. M.; Martinez, B. C.; Nelson, D. C. Bio-mediated soil improvement. *Ecol. Eng.* **2010**, 36, 197–210.
- (7) Losick, R. *Bacillus subtilis*: A bacterium for all seasons. *Curr. Biol.* **2020**, 30, R1146–R1150.
- (8) Arnaouteli, S.; Bamford, N. C.; Stanley-Wall, N. R.; Kovacs, A. T. *Bacillus subtilis* biofilm formation and social interactions. *Nature Reviews Microbiology* **2021**, 19, 600–614.
- (9) Feng, J.; Chen, B. C.; Sun, W. W.; Wang, Y. Microbial induced calcium carbonate precipitation study using *Bacillus subtilis* with application to self-healing concrete preparation and characterization. *Construction and Building Materials* **2021**, 280, 122460.
- (10) Atai, E.; Jumbo, R. B.; Andrews, R.; Cowley, T.; Azuazu, I.; Coulon, F.; Pawlett, M. Bioengineering remediation of former industrial sites contaminated with chemical mixtures. *Journal of Hazardous Materials Advances* **2023**, 10, 100319.
- (11) Settimi, C.; Conti, B.; Verginelli, I.; Zingaretti, D.; Bortone, I.; Coulon, F.; Baciocchi, R. Use of biochar and coal ash as passive sorbent barriers for long-term mitigation of chlorinated solvent vapours. *J. Environ. Manage.* **2025**, 391, 126501.
- (12) Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J. L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, 159, 3269–3282.
- (13) Ordal, G. W.; Goldman, D. J. Chemotaxis away from uncouplers of oxidative-phosphorylation in *Bacillus subtilis*. *Science* **1975**, 189, 802–805.
- (14) Castilla-Alcantara, J. C.; Akbari, A.; Ghoshal, S.; Ortega-Calvo, J. J. Role of tactic response on the mobilization of motile bacteria through micrometer-sized pores. *Sci. Total Environ.* **2022**, 832, 154938.
- (15) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*, 3rd ed.; John Wiley & Sons: Hoboken, NJ, 2017.
- (16) Balseiro-Romero, M.; Prieto-Fernández, A.; Shor, L. M.; Ghoshal, S.; Baveye, P. C.; Ortega-Calvo, J. J. Chemotactic Bacteria Facilitate the Dispersion of Nonmotile Bacteria through Micrometer-Sized Pores in Engineered Porous Media. *Environ. Sci. Technol.* **2022**, 56, 13975–13984.
- (17) Sungthong, R.; van West, P.; Cantos, M.; Ortega-Calvo, J. J. Development of eukaryotic zoospores within polycyclic aromatic hydrocarbon (PAH)-polluted environments: A set of behaviors that are relevant for bioremediation. *Sci. Total Environ.* **2015**, 511, 767–776.
- (18) Posada-Baquero, R.; Fernandez-Lopez, C.; Hennecke, D.; Ortega-Calvo, J. J. Integrating bioavailability measurements in persistence testing of partially biodegradable organic chemicals in soil. *Sci. Total Environ.* **2024**, 909, 168460.
- (19) Posada-Baquero, R.; Semple, K. T.; Ternero, M.; Ortega-Calvo, J. J. Determining the bioavailability of benzo(a)pyrene through standardized desorption extraction in a certified reference contaminated soil. *Sci. Total Environ.* **2022**, 803, 150025.
- (20) Cussler, E. L. Diffusion: mass transfer in fluid systems. *Cambridge Series in Chemical Engineering*, 3rd ed.; Cambridge University Press: New York, 2009.
- (21) Chen, Z. M.; Chen, B. L.; Chiou, C. T. Fast and Slow Rates of Naphthalene Sorption to Biochars Produced at Different Temperatures. *Environ. Sci. Technol.* **2012**, 46, 11104–11111.
- (22) Baughman, G. L.; Paris, D. F.; Hodson, R. E. Microbial bioconcentration of organic pollutants from aquatic systems- A critical review. *CRC Crit. Rev. Microbiol.* **1981**, 8, 205–228.
- (23) Gustafson, K. E.; Dickhut, R. M. Molecular diffusivity of polycyclic aromatic hydrocarbons in aqueous solution. *J. Chem. Eng. Data* **1994**, 39, 281–285.
- (24) Harms, H. Bacterial growth on distant naphthalene diffusing through water, air, and water-saturated and nonsaturated porous media. *Appl. Environ. Microbiol.* **1996**, 62, 2286–2293.
- (25) Mulder, H.; Breure, A. M.; van Honschooten, D.; Grotenhuis, J. T. C.; van Andel, J. G.; Rulkens, W. H. Effect of biofilm formation by *Pseudomonas* 8909N on the bioavailability of solid naphthalene. *Appl. Microbiol. Biotechnol.* **1998**, 50, 277–283.
- (26) Bateman, K.; Coombs, P.; Harrison, H.; Milodowski, A. E.; Noy, D.; Vane, C. H.; Wagner, D.; West, J. M. Microbial transport and microbial indicators of mass transport through geological media - A literature survey. British Geological Survey Internal Report IR/06/029; 2006. <https://nora.nerc.ac.uk/id/eprint/7179/1/IR06029.pdf>.
- (27) Yang, C. D.; Liu, J. J.; Ying, H. C.; Lu, S. G. Soil pore structure changes induced by biochar affect microbial diversity and community structure in an Ultisol. *Soil & Tillage Research* **2022**, 224, 105505.
- (28) Acharya, B. S.; Dodla, S.; Wang, J. J.; Pavuluri, K.; Darapuneni, M.; Dattamudi, S.; Maharjan, B.; Kharel, G. Biochar impacts on soil water dynamics: knowns, unknowns, and research directions. *Biochar* **2024**, 6, 34.
- (29) Fu, C.; Wu, Y.; Sørensen, S. J.; Zhang, M.; Dai, K.; Gao, C.; Qu, C.; Huang, Q.; Cai, P. The mitigation of spatial constraint in porous environments enhances biofilm phylogenetic and functional diversity. *Microbiome* **2025**, 13, 84.
- (30) Ma, P.; Qi, Z.; Wu, X.; Ji, R.; Chen, W. Biochar nanoparticles-mediated transport of organic contaminants in porous media: dependency on contaminant properties and effects of biochar aging. *Carbon Res.* **2023**, 2, 4.



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