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Sorption and Transport of Naphthalene and Phenanthrene in Silica Sand in the Presence of Rhamnolipid Biosurfactant

Gang Chen[a]; Mingqi Qiao[b]; Huiyun Zhang[c]; Honglong Zhu[d]
[a] Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering, Tallahassee, FL, USA [b] Scientific Research Office, Shandong University of Traditional Chinese Medicine, Jinan, P.R. China [c] Subdecanal of College of Chinese Herb, Shandong University of Traditional Chinese Medicine, Jinan, P.R. China [d] Qingdao Haichuan Biological Renovation Research Center of Natural Medicine, Qingdao, P.R. China

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Gang Chen
Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering, Tallahassee, FL, USA

Mingqi Qiao
Scientific Research Office, Shandong University of Traditional Chinese Medicine, Jinan, P.R. China

Huiyun Zhang
Subdecanal of College of Chinese Herb, Shandong University of Traditional Chinese Medicine, Jinan, P.R. China

Honglong Zhu
Qingdao Haichuan Biological Renovation Research Center of Natural Medicine, Qingdao, P.R. China

Abstract: Sorption and transport of naphthalene and phenanthrene in silica sand in the presence of rhamnolipid biosurfactant were investigated by batch and column experiments. Naphthalene and phenanthrene had linear sorption isotherms on silica sand with partition coefficients decreasing with increasing rhamnolipid biosurfactant concentrations until the critical micellar concentration (CMC). After the CMC was reached, naphthalene and phenanthrene partition coefficients on silica sand increased with the increase of rhamnolipid biosurfactant concentrations. It was believed that micelles were formed when rhamnolipid biosurfactant was supplied at concentrations higher than the CMC, to which naphthalene and phenanthrene had higher affinity than silica sand. In column experiments, both naphthalene and phenanthrene had early breakthroughs in the

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Address correspondence to Gang Chen, Department of Civil and Environmental Engineering, FAMU-FSU College of Engineering, 2525 Pottsdamer Street, Tallahassee, FL 32310, USA. Fax: (850) 4106142; E-mail: gchen@eng.fsu.edu

presence of rhamnolipid biosurfactant than in its absence. Estimated naphthalene and phenanthrene retardation using naphthalene and phenanthrene batch sorption data was consistent with column experimental observations. Nevertheless, naphthalene and phenanthrene recovery decreased in answer to the presence of rhamnolipid biosurfactant at concentrations greater than the CMC, which was attributed to the entrapment of naphthalene and phenanthrene associated rhamnolipid biosurfactant micelles.

Keywords: Napthalene, phenanthrene, silica sand, rhamnolipid biosurfactant, micelle, sorption, transport

INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) contaminations are results of underground gasoline tank leakage or spills, and in situ biodegradation has been well accepted as one of the effective ways of environmental restoration (1). In practice, the in situ PAH contaminant bioremediation in the subsurface is strictly restricted by PAH sorption on medium matrices (2). To account for the strong sorption of PAH on porous media, surfactant-enhanced remediation technology has been developed (3–6). Surfactants increase PAH desorption from medium matrices by lowering the interfacial surface tension. At concentrations higher than their critical micellar concentrations (CMCs), surfactants also form micelles to which PAH has high affinity, additionally increasing PAH desorption (7–9). Though applications of surfactants at concentrations higher than their CMCs have high potentials of PAH desorption, the consequent mobilization of PAH associated with the micelles is retarded since the micelles might be trapped in the media depending on their sizes and medium surface properties (9, 10). In the presence of surfactants, PAH sorption on porous media has been interpreted by means of equilibrium sorption isotherms through batch (11) and column experiments (12).

Among the PAHs, naphthalene (a double cyclic aromatic hydrocarbon) and phenanthrene (a tricyclic aromatic hydrocarbon) are the two simplest forms. Biodegradation of both naphthalene and phenanthrene can be achieved aerobically, which is strictly limited by their bioavailability owing to their low solubility (6). Naphthalene and phenanthrene biodegradation has been reported to be greatly enhanced in the presence of surfactants (13, 14). In this research, naphthalene and phenanthrene sorption and transport in silica sand in the presence of rhamnolipid biosurfactant, a biosurfactant produced by *Pseudomonas aeruginosa* during its late logarithmic growth phase, was investigated by batch and column experiments. In batch experiments, naphthalene and phenanthrene sorption on silica sand in the presence of rhamnolipid biosurfactant was quantified in terms of partition coefficient. For column experiments, transport of naphthalene and phenanthrene was simulated by the convection-dispersion model with appropriate sink terms to account for naphthalene and phenanthrene affinity to the porous media. Under the circumstances when rhamnolipid

biosurfactant was applied at concentrations higher than the CMC, additional sink terms were included to account for surfactant micelle entrapment. Batch results were compared with column observations against the relationship of partitioning of naphthalene and phenanthrene to porous media with the transport retardation. The objectives of this study were to characterize the effect of rhamnolipid biosurfactant on naphthalene and phenanthrene sorption and transport in silica sand. Especially, naphthalene and phenanthrene sorption and transport in silica sand in the presence of rhamnolipid biosurfactant at concentrations higher than the CMC were quantified because high surfactant concentrations are commonly used in practice to enhance the mobility of PAHs. Results of this research will lead to a better understanding of naphthalene and phenanthrene sorption in the subsurface and will provide guidelines for field PAH bioremediation applications.

MATERIALS AND METHODS

Naphthalene and Phenanthrene

Naphthalene and phenanthrene used in this research were obtained from Sigma, St. Louis, MO in powder form. Naphthalene is slightly soluble in water (31 mg/L, 1 atm, 25°C) and phenanthrene is barely soluble in water (1.2 mg/L, 1 atm, 25°C). Naphthalene and phenanthrene were analyzed using a HPLC (Water Associates, Milford, MA) equipped with an ODS hypersil column from Hewlett Packard, Palo Alto, CA. Quantification of naphthalene and phenanthrene was accomplished using a UV detector (Water Associates, Milford, MA). The mobile phase was 80% methanol (high-pressure liquid chromatography grade) mixed with 20% nanopure deionized water (v/v) (Barnstead Thermodyne, Debuque, IO). The flow rate was 1 mL/min and the injection was 25 μL. Naphthalene and phenanthrene concentrations were quantified against external standards.

Rhamnolipid Biosurfactant Production and Extraction

Rhamnolipid biosurfactant is a bacterial biosurfactant produced by several *Pseudomonas* species. It contains two covalently linked headgroups and two tails and has been reported to be an effective biosurfactant (15). When dispersed in water, hydrophilic headgroups have sufficient affinity to water to drag nonpolar tails into aqueous solutions (16). Whereas, at high concentrations, hydrophobic interactions between amphiphiles may contribute to the formation of micelles, which becomes appreciable at a well-defined concentration known as the CMC (17).

Rhamnolipid biosurfactant used in this research was produced by *Pseudomonas aeruginosa*, the most common gram-negative bacterium

during its late logarithmic growth phase (18, 19). After inoculated with 1 mL (1.0%, 5×10^8 cells/mL) stationary phase culture, *P. aeruginosa* (ATCC 9027) was grown in Kay's minimal medium, which was composed of 0.3 g $NH_4H_2PO_4$, 0.2 g K_2HPO_4, 0.2 g glucose, 0.5 mg $FeSO_4$, and 0.1 g $MgSO_4 \cdot 7H_2O$ at 37°C for 24 h. A total of 2 mL of the above culture was used to inoculate 200 mL of phosphate-limited proteose peptone-glucose-ammonium salt medium, which consisted of 1.0 g NH_4Cl, 1.5 g KCl, 19.0 g Tris-HCl, 5 g glucose, 1 g proteose peptone, and 0.4 g $MgSO_4 \cdot 7H_2O$, adjusted to pH 7.2 in a 1000 mL flask. This flask was placed on a Gyrotory Water Bath Shaker and shaken at 25°C at 250 rpm for 60 h.

The extraction of rhamnolipid biosurfactant followed the method modified from Zhang and Miller (18). The collected culture supernatant was first centrifuged at 7000 × g for 15 min to remove *P. aeruginosa* cells and then precipitated by acidification to pH 2.0. After centrifuged at 12100 × g for 20 min, the precipitate was extracted with chloroform–ethanol (2 : 1 V/V). Extract was then transferred to a round bottom flask connected to a rotoevaporator for evaporation and then freeze dried. Rhamnolipid biosurfactant was purified and analyzed by thin-layer chromatography. Rhamnolipid biosurfactant was then suspended in the sterilized deionized water to make rhamnolipid biosurfactant solutions.

Porous Medium

The porous medium used in this research was silica sand (Fisher Scientific, 8 mesh). Silica sand was first rinsed using deionized water and then treated with sodium acetate, hydrogen peroxide, sodium dithionate, and sodium citrate to remove organic matters. Silica sand was then extensively flushed with sterilized deionized water until the electrical conductivity was less than 1 dS/m. Before experiments, silica sand was sterilized at 121°C for 20 min.

Surface Tension Measurements

The surface tension was measured using a Kruss K10 tensiometer (Krüss GmbH, Hamburg, Germany) with a platinum plate. Each measurement was repeated three times and average results were reported. The temperature was held constant at 20.0°C by circulating thermostated water through a jacketed vessel containing the sample. The experimental uncertainty of these surface tension measurements was approximately 0.1 mN/m.

Batch Sorption Experiments

Batch sorption isotherms were used to determine naphthalene and phenanthrene affinity to silica sand. To determine the sorption of naphthalene on silica sand, a series of 25 mL vials containing naphthalene solutions (20 mL)

at the concentrations of 0.25, 0.5, 1, 5, 10, 15, 20, and 30 mg/L and 4 g porous media (including blank controls) (sealed with Teflon-lined screw caps) were agitated on a Wrist Action Shaker (Burrel Scientic, Model 75) for 24-h (pre-determined to be sufficient) to reach equilibrium. For phenanthrene sorption on silica sand, phenanthrene solutions (20 mL) at the concentrations of 0.001, 0.025, 0.05, 0.075, 0.1, 0.5, 0.75, and 1 mg/L and 1 g porous media (including blank controls) were used. The suspension was then centrifuged at 12,000 × g for 15 mins, after which naphthalene and phenanthrene concentrations in the supernatant were measured.

To quantify rhamnolipid biosurfactant micelle formation, 100 mL of rhamnolipid biosurfactant at concentrations of 200, 400, and 800 mg/L was centrifuged at 12,000 × g for 15 min. The solid phase was measured for the mass of rhamnolipid biosurfactant micelles.

Column Experiments

Column experiments of naphthalene and phenanthrene transport in porous media were conducted using an HPLC column (Ace 300C18, 1.0 cm × 5 cm). The column was oriented vertically and sealed at the bottom with end-fittings. The end-fitting at the bottom contained an ACE glass custom frit (porosity C) to permit the flow of water and retain the medium. A fresh column was packed with silica sand for each experiment through CO_2 solvation to eliminate air pockets. Prior to each experiment, the column was stabilized by extensive flushing with sterilized deionized water (prefiltered by a 0.22 μm cellulose acetate filter) at a flow rate of 0.1 mL/min for at least 100 pore volumes by an HPLC pump. Before naphthalene and phenanthrene column experiments, breakthrough experiments of a conservative tracer of nitrate (0.2 mM $NaNO_3$) were conducted. Nitrate was measured with a spectrophotometer at 204 nm wavelength. For each run, naphthalene (at a concentration of 10 mg/L) and phenanthrene (0.5 mg/L) solutions in the presence of rhamnolipid biosurfactant at concentrations of 0, 10, 20, 30, 200, 400, and 800 mg/L were introduced to the column inlet at a flow rate of 0.1 mL/min until flat naphthalene and phenanthrene breakthrough peaks were reached. The elution was collected by a fraction collector and analyzed for naphthalene and phenanthrene concentrations.

RESULTS AND DISCUSSION

Water Surface Tension in the Presence of Rhamnolipid Biosurfactant

Water surface tension dropped linearly with regard to rhamnolipid biosurfactant concentration increase until 30 mg/L (Fig. 1). When rhamnolipid

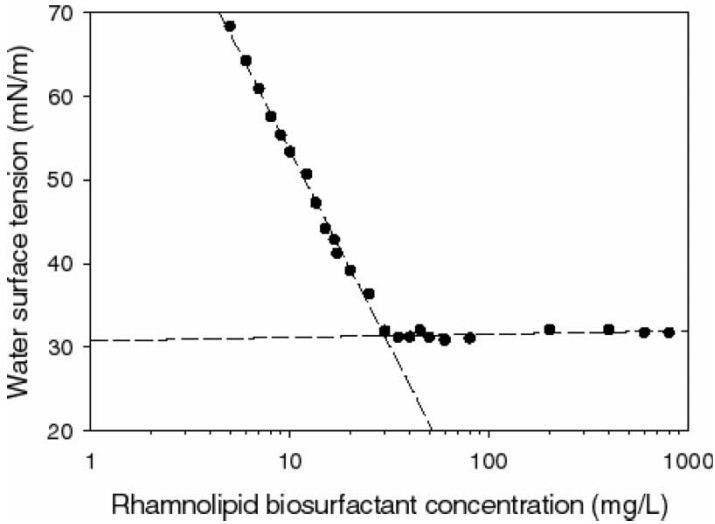

Figure 1. Water surface tension drop as a function of rhamnolipid biosurfactant concentration.

biosurfactant concentrations were greater than 30 mg/L, water surface tension did not drop any more; instead, it was maintained at 30.8 mN/m. Therefore, the CMC of rhamnolipid biosurfactant was assumed to be 30 mg/L. The surface tension of 30.8 mN/m was determined by interactions between rhamnolipid biosurfactant molecules and water molecules at the air-water interface (20).

Batch Sorption Isotherms

Based on five replicates, naphthalene and phenanthrene displayed linear sorption isotherms on silica sand (Fig. 2). In Fig. 2, average values of five replicates were reported with "$\pm$" denoting standard deviation. Naphthalene and phenanthrene partitioning to silica sand was calculated based on the following equation

$$q_s = \frac{(C_0 - C_{eq})V}{M_s} = K_s C_{eq} \tag{1}$$

where q_s is the naphthalene and phenanthrene concentration on silica sand (mg/kg), C_0 is the initial naphthalene and phenanthrene aqueous concentration (mg/L); C_{eq} is the naphthalene and phenanthrene aqueous equilibrium concentration (mg/L); V is the aqueous volume (mL); M_s is the mass of silica sand

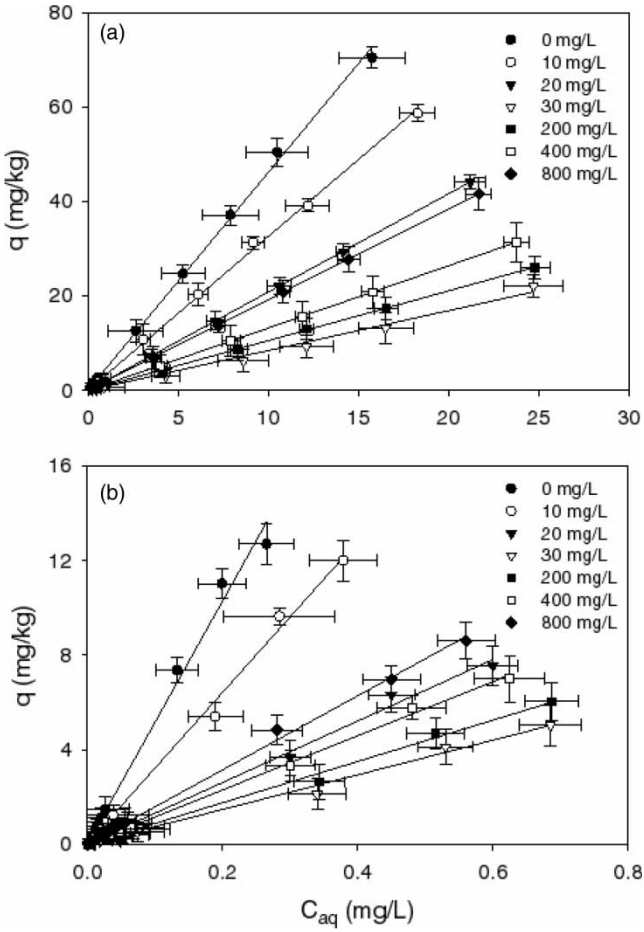

Figure 2. Batch sorption isotherms of (a) naphthalene and (b) phenanthrene on silica sand. Symbols are measured data and solid lines are linear isotherm simulations.

(kg); and K_s is the napthlene and phenanthrene partition coefficient between the aqueous phase and silica sand (L/kg).

Naphthalene and phenanthrene had a partition coefficient of 4.53 L/kg and 55.5 L/kg on silica sand, respectively (Table 1). In the presence of rhamnolipid biosurfactant, K_s decreased to 3.21, 2.08, 0.82, 1.05, 1.32, and 1.92 L/kg for naphthalene and 32.8, 13.3, 7.13, 9.07,11.2, and 15.8 L/kg for phenanthrene in answer to rhamnolipid biosurfactant concentrations of 10, 20, 30, 200, 400, and 800 mg/L, respectively. K_s decreased significantly with the rhamnolipid biosurfactant concentration increase until reaching the CMC, after which it increased with the increase of rhamnolipid biosurfactant concentrations (Fig. 3). It was believed that micelles were formed when rhamnolipid

Table 1. Naphthalene and phenanthrene sorption and transport parameters

Rhamnolipid concentration	K_s (L/kg)	Retardation factor (−)	Predicted retardation factor (−)	k_d (hr^{-1})
0	4.53	15.8	16.9	0.03
10	3.21	12.1	12.3	0.02
20	2.08	9.80	8.34	0.03
30	0.82	6.32	3.88	0.05
200	1.05	5.54	4.70	0.07
400	1.32	5.42	5.65	0.13
800	1.92	5.35	7.77	0.20
0	55.5	196	190	0.03
10	32.8	116	112	0.03
20	13.3	48.0	56.4	0.03
30	7.13	26.1	48.8	0.03
200	9.07	33.0	42.8	0.08
400	11.2	40.5	44.9	0.19
800	15.8	56.7	43.4	0.28

biosurfactant was supplied at concentrations higher than the CMC and naphthalene and phenanthrene had higher affinity to the rhamnolipid biosurfactant micelles than silica sand. As the formed micelles were segregated from the aqueous phase during centrifugation, naphthalene and phenanthrene sorbed on the micelles were included in the calculation of K_s, resulting in the increase of K_s with increasing rhamnolipid biosurfactant concentrations when they were greater than the CMC.

Assuming napthlene and phenanthrene partition coefficients between the aqueous phase and silica sand were not affected by the formation of the rhamnolipid biosurfactant micelles, the increase of K_s can be explained by napthlene and phenanthrene equilibrium partitioning to rhamnolipid biosurfactant micelles. To quantify naphthalene and phenanthrene affinity to the micelles, mass balance was performed to estimate the amount of naphthalene and phenanthrene sorbed to the rhamnolipid biosurfactant micelles

$$C_0 V = q_s M_s + q_m M_m + C_{eq} V \tag{2}$$

where M_m is the mass of rhamnolipid biosurfactant micelles (kg) and q_m is the naphthalene and phenanthrene concentration on rhamnolipid biosurfactant micelles (mg/kg). Mass of rhamnolipid biosurfactant micelles was determined during the sorption isotherm experiments, which linearly increased with increasing rhamnolipid biosurfactant concentrations (Fig. 4).

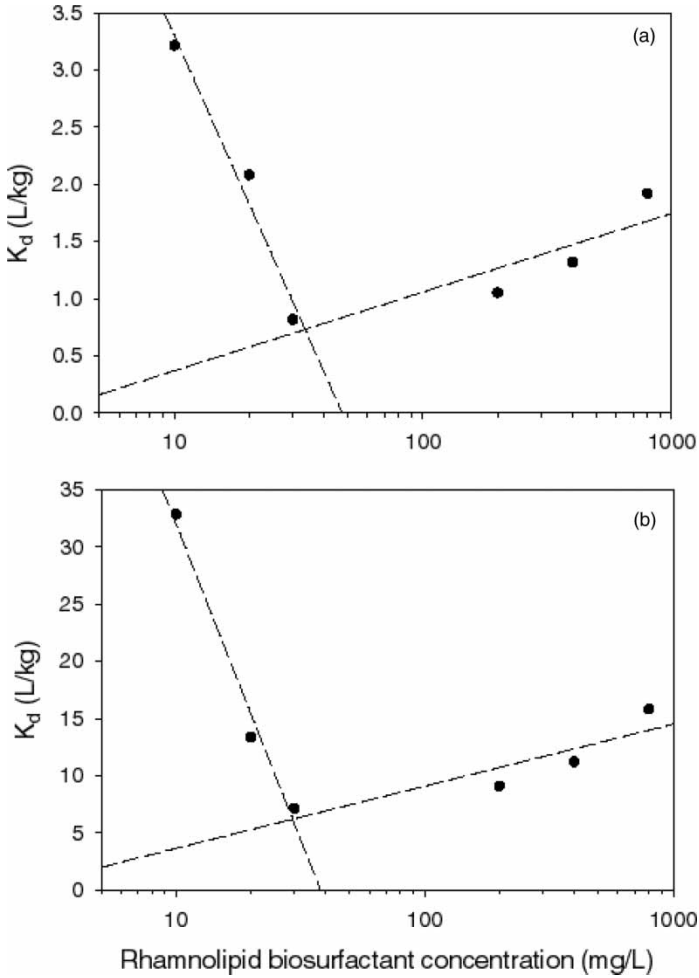

Figure 3. Partition coefficient as a function of rhamnolipid biosurfactant concentration for (a) naphthalene and (b) phenanthrene.

Based on Eqs. (1) and (2), napthlene and phenanthrene partition coefficients between the aqueous phase and rhamnolipid biosurfactant micelles, K_m, can be calculated by

$$K_m = \frac{C_0 V - C_{eq} V - K_s C_{eq} M_s}{C_{eq} M_m} \tag{3}$$

During the calculations, K_s of 0.82 L/kg and 7.13 L/kg were used for naphthalene and phenanthrene. The thus calculated K_m was 56.6 $\pm$ 1.3 L/kg for napthalene and 114.4 $\pm$ 3.3 L/kg for phenanthrene, which were consistent with the prediction that K_m should be much greater than K_s.

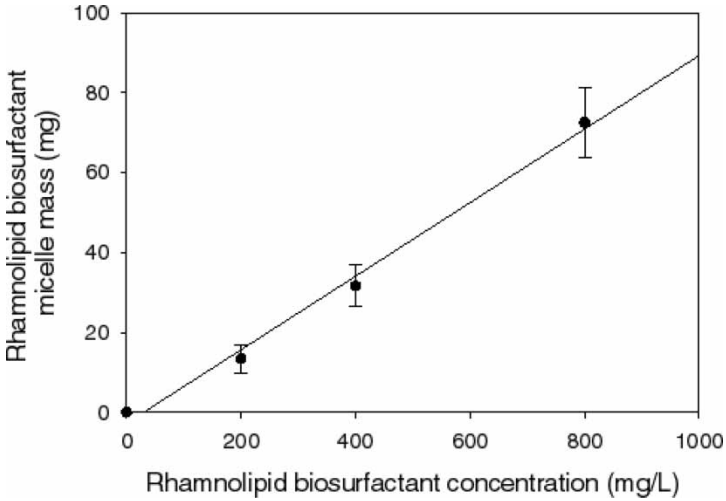

Figure 4. Rhamnolipid biosurfactant micelle formation as a function of rhamnolipid biosurfactant concentration.

Naphthalene and Phenanthrene Column Transport

Both naphthalene and phenanthrene breakthrough curves had a broad and diffuse infiltration front (Fig. 5). This behavior demonstrated that the retention of naphthalene and phenanthrene on silica sand increased with time. When steady state was reached, around 97.8% and 98.0% of naphthalene and phenanthrene were recovered from the silica sand column. Because no significant naphthalene and phenanthrene biodegradation was observed in preliminary experiments, naphthalene and phenanthrene loss due to biodegradation was assumed minimal. Therefore, 2.2% and 2.0% of naphthalene and phenanthrene not recovered in the elusion was assumed to be sorbed on sites or regions of silica sand that displayed slow desorption kinetics. Besides, retardation of naphthalene and phenanthrene was manifested by delayed breakthroughs. In the presence of rhamnolipid biosurfactant, both the naphthalene and phenanthrene had early breakthroughs. Nevertheless, naphthalene and phenanthrene recovery decreased in answer to the presence of rhamnolipid biosurfactant at concentrations greater than the CMC, i.e., 95.5%, 91.8%, and 87.7% of naphthalene and 95.1%, 88.4%, and 83.4% of phenanthrene were recovered in answer to the presence of rhamnolipid biosurfactant at concentrations of 200, 400, and 800 mg/L.

Column experiments were performed to simulate naphthalene and phenanthrene transport in field applications. Naphthalene and phenanthrene transport through silica sand was mathematically described using the one-dimensional transport model to account for the advective-dispersive

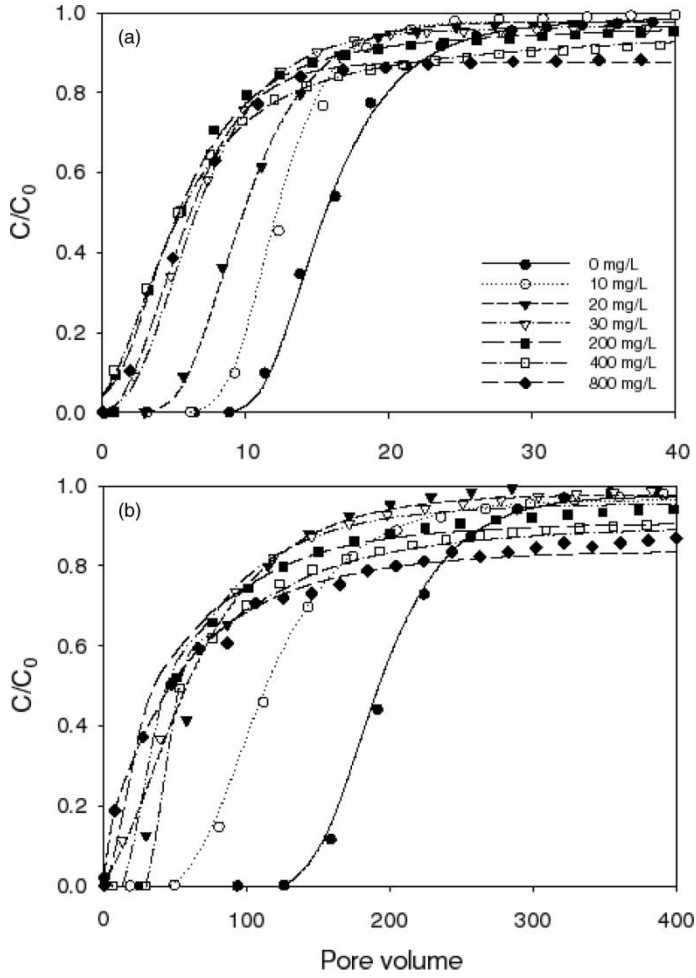

Figure 5. Breakthrough curves of for (a) naphthalene and (b) phenanthrene in the absence and presence rhamnolipid biosurfactant. Symbols are measured breakthrough data and lines are model fits.

movement and equilibrium reactions between the aqueous phase and the solid medium phase (21)

$$\left(1+\frac{\rho_b K_s}{\phi}\right)\frac{\partial C}{\partial t}=D\frac{\partial^2 C}{\partial x^2}-v\frac{\partial C}{\partial x}-k_d C \tag{4}$$

where C is the naphthalene and phenanthrene aqueous concentration (mg/L); ρ is the bulk density (kg/L); ϕ is the porosity (L/L); t is the elapsed time (hr); D is the longitudinal dispersion coefficient (cm^2/hr); v is the pore water

velocity (cm/hr); x is the coordinate parallel to the flow (cm); and k_d is the deposition coefficient (hr^{-1}). It was assumed that naphthalene and phenanthrene retention in silica sand was owing to their association with rhamnolipid biosurfactant micelles that were entrapped within the column. Thus, when rhamnolipid biosurfactant was applied at concentrations smaller than the CMC, $k_d = 0$. In Eq. (4), $(1 + \rho_b K_s/\phi)$ is the transport retardation factor. The simulation was proceeded by an implicit, finite-difference scheme and the simulation process was optimized by minimizing the sum of squared differences between observed and fitted concentrations using the nonlinear least-square method.

Both nitrate, naphthalene, and phenanthrene transport in silica sand can be well described by Eq. (4). The accuracy of the transport model was documented by similar sediment hydraulic properties from nitrate, naphthalene, and phenanthrene transport simulations. The migration of contaminants in the subsurface environment is described by the retardation factor, which is defined as the ratio of the solution velocity to the contaminant velocity. The retardation factor for any particular chemical is attributed to all of the interactions between the chemical species and the solid surfaces of the porous media. These interactions tend to retard the migration of the chemical relative to the water. To model or predict contaminant transport and plan remediation activities at a site, retardation factors need to be known. Retardation factor of naphthalene in silica sand was 15.8, 12.1, 9.80, 6.32, 5.54, 5.42, and 5.35 for naphthalene and 196, 116, 48.0, 26.1, 33.0, 40.5, and 56.7 for phenanthrene corresponding to the presence of rhamnolipid biosurfactant at concentrations of 0, 10, 20, 30, 200, 400, and 800 mg/L. Variations in retardation factor were more obvious at low rhamnolipid concentrations, but not significant at high rhamnolipid concentrations. Estimated naphthalene and phenanthrene retardation factors using naphthalene and phenanthrene partition coefficients obtained from batch sorption isotherms were consistent with simulated results from column experiments (Fig. 6).

Rhamnolipid Biosurfactant Micelle Entrapment

For naphthalene and phenanthrene transport in the presence of rhamnolipid biosurfactant at concentrations higher than the CMC, it was assumed that rhamnolipid biosurfactant micelles were formed and entrapped in silica sand. Consequently, naphthalene and phenanthrene associated with the micelles would be retained in the media. Based on batch experimental results, only around 0.9%, 2.1%, and 4.2% of naphthalene and 1.9%, 4.0%, and 8.1% of phenanthrene would associate with formed micelles. On the other hand, it was found that 4.5%, 8.2%, and 12.3% of naphthalene and 4.9%, 11.6%, and 16.6% of phenanthrene were retained in the column, corresponding to deposition factors of 0.07, 0.13, and 0.20 for naphthalene and 0.08, 0.19, and 0.28 for phenanthrene. This demonstrated that rhamnolipid

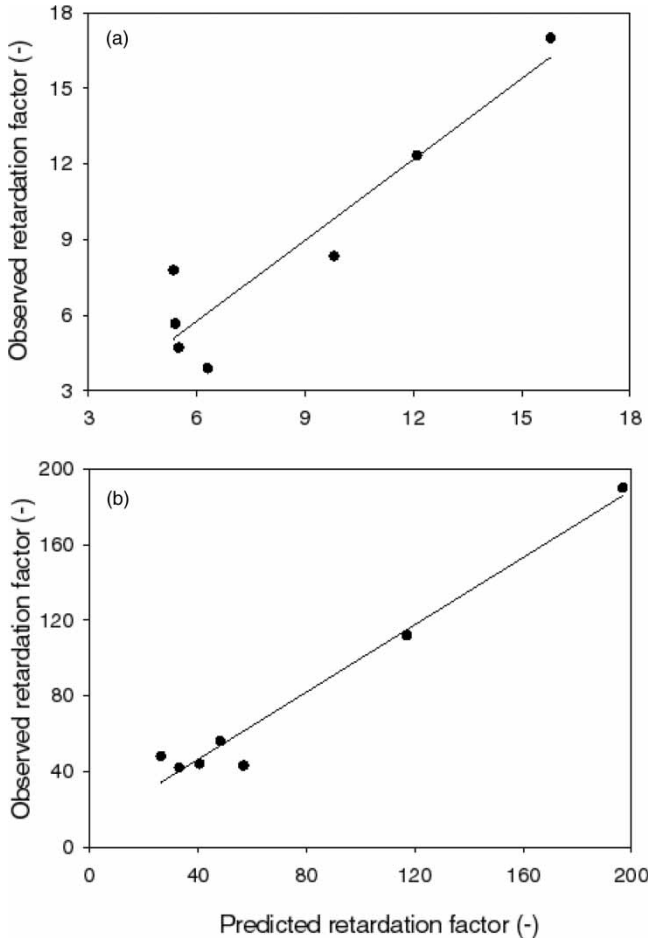

Figure 6. Observed retardation of (a) naphthalene and (b) phenanthrene as compared to predicted values from batch experiments. The lines show the trend.

biosurfactant micelles spread over silica sand surfaces, increasing contact areas between naphthalene and phenanthrene and rhamnolipid biosurfactant micelles.

IMPLICATIONS

Biosurfactants are biologically synthesized surface-active agents and have the advantages of being readily biodegradable over their chemical counterparts. They have a broad range of potential industrial and environmental applications from the point view of diversity, biodegradability, low toxicity, and

biocompatibility (22). Among available biosurfactants, rhamnolipid biosurfactant has been reported to be an effective biosurfactant (18, 19). When applied at concentrations lower than the CMC, rhamnolipid can enhance desorption or dissolution of contaminants, increase the rate of transport of contaminants in soil, and stimulate biodegradation. On the contrary, when applied at concentrations higher than the CMC, it may retard the transport of contaminants since rhamnolipid biosurfactant forms micelles that can be entrapped in the sediments. Extensive research has been done regarding rhamnolipid biosurfactant applications at low concentrations and little knowledge is available when it is applied at concentrations higher than the CMC. However, in practice, high concentrations of the surfactants (more than 10 times higher than the CMC) are commonly used to enhance the mobility of hydrophobic organic compounds from the soils, including PAHs (7, 23). Results of this research will provide a mechanistic understanding of rhamnolipid biosurfactant applications in the subsurface and will provide helpful guidelines for field PAH bioremediation applications.

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