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Biosurfactant (Rhamnolipid) Sorption and the Impact on Rhamnolipid-Facilitated Removal of Cadmium from Various Soils under Saturated Flow Conditions

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The efficiency of biosurfactant-facilitated removal of soil-bound metals is affected by biosurfactant sorption to soil. In this study, batch and column experiments were performed to minimize rhamnolipid biosurfactant sorption and to optimize rhamnolipid application for removal of cadmium from four soils. In batch studies, rhamnolipid sorption to a model coarse loamy soil was found to vary with applied rhamnolipid and K^+ concentration of the rhamnolipid matrix. The presence of solution-phase biosurfactant was correlated to the release into solution of a soil-bound metal (cadmium). A series of column experiments was performed to evaluate whether rhamnolipid could remove cadmium from soil under saturated flow conditions. Four different soils were contaminated with cadmium and treated first with an KNO_3 electrolyte solution (3.5 or 7 mM K^+) and then with a rhamnolipid-containing solution (5 or 10 mM). Results showed that between 15 and 36% of the cadmium was removed by the initial electrolyte treatment and an additional 8−54% of the cadmium was removed by rhamnolipid treatment. Rhamnolipid treatment was very effective for three of the soils tested, but for the soil with the highest clay content, rhamnolipid application caused soil dispersion and column plugging.

Introduction

Remediation of recalcitrant or sorbed contaminants from soil may necessitate the addition of chemical agents, such as surfactants, which act to mobilize or increase the availability of such contaminants (*1*−*3*). Microbially produced surfactants offer the advantage of being potentially less toxic and more biodegradable than some synthetic surfactants; the diversity of chemical forms produced by a variety of microbial species may allow the selection of microbial products with a high specificity for certain applications. An anionic rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* has been shown to promote the biodegradation of low-solubility hydrocarbons (*4*−*7*) and to remove metal contaminants from soil (*8*). The efficiency of biosurfactant application in soil remediation will be dependent, in part, on its interaction with the soil physicochemical environment. A decrease in the solution-phase availability of anionic synthetic surfactants (*9*−*11*) and biosurfactants (*8, 12*) can occur due to sorption and precipitation. For example, cations within the soil matrix interact with and affect the availability of anionic surfactants. A potential additional source of cations is those introduced during the preparation of surfactant solutions. In the case of rhamnolipid biosurfactant, the purified material is weakly acidic, and in order to dissolve rhamnolipid in solution to a neutral pH the addition of a base, such as KOH, is required.

The overall objective of this research was to determine whether rhamnolipids can enhance the removal of a model metal (cadmium) from various soils under saturated flow conditions. This paper describes an initial series of experiments on the sorption of a rhamnolipid biosurfactant by a model soil, with specific regard to how changes in K^+ concentration can alter the degree of rhamnolipid sorption. The information on rhamnolipid sorption was then used to optimize rhamnolipid application for metal removal from a variety of soils in both batch and column experiments.

Materials and Methods

Soil. Properties of the four soils used in this study are shown in Table 1. These were selected to vary clay, total organic carbon (TOC), and iron oxide content. Prior to the experiments, all four soils were air-dried, sieved through a no. 10 standard sieve (2 mm openings), autoclaved once, and then oven-dried.

Rhamnolipid Production and Quantification. Two types of rhamnolipid were used in this study (Figure 1). Monorhamnolipid was used to optimize conditions for rhamnolipid application and was produced under phosphate-limited growth conditions by *Pseudomonas aeruginosa* ATCC 9027 (*13*). A mono- and dirhamnolipid mixture, produced by *P. aeruginosa* IGB83, was used for column studies because it could be produced at high yields (2−3 g L^{-1}). IGB83 rhamnolipid was produced in 10-L batches in a 13-L New Brunswick biofermentor in a mineral salts medium (*14*) with glucose (20 g L^{-1}) added as the carbon source. The biofermentor was inoculated with 100 mL of an IGB83 preculture grown in Kay's minimal medium with shaking (200 rpm) for 24 h at 37 °C and was run for 3 days at 34 °C and 120 rpm. Both types of rhamnolipid were extracted and purified by silica gel chromatography as described previously (*6, 15*).

Both monorhamnolipid and the IGB83 rhamnolipid mixture have a critical micelle concentration (cmc) of 0.1 mM (*7*). The molecular weight of monorhamnolipid is 504, and the average molecular weight of the IGB83 rhamnolipid mixture is 577. Rhamnolipid concentration in solution was determined by surface tension analysis using a surface tensiomat (Model 21, Fisher Scientific) that employs the Du Nouy ring method. For this measurement, each sample was diluted appropriately to ensure that the rhamnolipid concentration was less than cmc. A calibration curve was constructed using rhamnolipid standards and relating rhamnolipid concentration (mg L^{-1}) to surface tension (dyn cm^{-1}).

Preparation of Rhamnolipid Solutions. To prepare a rhamnolipid solution, a known mass of purified rhamnolipid was dissolved in purified water (Barnstead, NanoPure water system, Dubuque, IA), and the solution was neutralized to a pH of 6.8 by titration with a 1 M KOH solution. As an example, a 14 mM rhamnolipid solution required the addition of KOH to a final concentration of 10 mM K^+. The K^+ concentration of the rhamnolipid solutions used in this study was calculated from the amount of base added and then adjusted to a predetermined concentration (10 mM for most

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TABLE 1. Physical and Chemical Properties of Soils Used

soil	% sand	% silt	% clay	CEC[a]	TOC %	Fe %[b]	pH
Vinton	95.9	1.5	3.0	4.85	0.1	ND[c]	7.7
Hayhook	86	4.7	8.8	7.04	0.11	0.19	7.5
Bonify	91.2	5.5	3.3	7.5	0.32	0.47	5.5
Comoro	83.9	10.8	5.3	6.3	1.27	1.6	6.7−7.2

[a] Mequiv 100 g^{-1}. [b] As iron oxides. [c] ND, not determined.

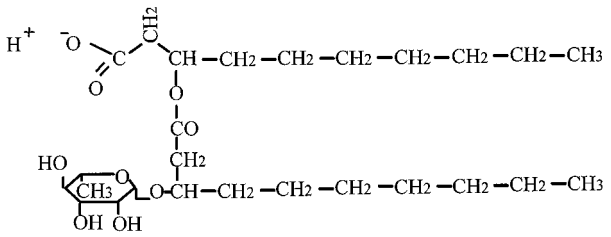

FIGURE 1. Structure of the rhamnolipids used in this study. For monorhamnolipid R = H and for dirhamnolipid R = rhamnose.

batch experiments and 3.5 or 7 mM for column experiments) using KNO_3. For batch (but not for column) experiments, rhamnolipid solutions were filter sterilized using a 0.22-μm cellulose acetate filter (Falcon Easy-Flow filters, Benton Dickinson Labware, Lincoln Park, NJ).

Rhamnolipid Sorption Isotherms. Hayhook soil (2 g) was transferred into 40-mL polypropylene centrifuge bottles and conditioned by washing with 4 mL of 10 mM KNO_3 for 2 days at room temperature on a shaker (100 rpm). Samples were centrifuged (40000*g*, 20 min), the supernatant was removed, and the soil pellets were autoclaved. Triplicate pellets were suspended in 4 mL of 0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 11.0, 14.0 mM rhamnolipid in a 10 mM K^+ matrix. The suspended soil pellets were shaken (100 rpm) for 3 days at room temperature and then centrifuged to collect the supernatant for surface tension analysis.

The effect of increasing K^+ concentration on rhamnolipid sorption by Hayhook soil was determined in the following set of experiments. Hayhook soil (2 g) was first conditioned in 10 mM KNO_3, and then triplicate soil samples were suspended in 4 mL of a rhamnolipid solution (11 mM) in which the K^+ concentration of the matrix solution had been adjusted to 10, 20, 50, 75, or 100 mM (trial 1) or to 10, 15, 20, 25, or 50 mM K^+ (trial 2). After 3 days of being shaken at room temperature, the tubes were centrifuged, and the rhamnolipid concentration in the supernatant was determined by surface tension analysis.

Effect of Rhamnolipid on Metal Removal—Batch Experiments. The batch procedure used to investigate rhamnolipid-facilitated desorption of soil-bound cadmium has been described previously (*8*). Briefly, Hayhook soil (2.5 g) was conditioned with 10 mM KNO_3 (5 mL) in 40-mL polypropylene centrifuge bottles. The soil was then mixed with 5 mL of 10 mM KNO_3 containing 1 mM $Cd(NO_3)_2$. After 3 days of shaking, the soil was separated from the supernatant by centrifugation, and the supernatant was diluted into 1% HNO_3 for atomic absorption analysis (Instrument Laboratory Video 12 aa/ae spectrophotometer, Allied Analytical Systems, Waltham MA). The amount of Cd^{2+} sorbed by the soil was determined from the reduction in Cd^{2+} concentration within the bulk solution and was found to range from 1.7 to 1.8 mmol kg^{-1}.

An experiment was performed to determine the relationship between rhamnolipid sorption and the removal of soil-bound metal. The cadmium-loaded soil pellets were suspended in a 5-mL solution containing 0, 1.6, 3.1, 6.3, and 12.6 mM rhamnolipid, with all solutions adjusted to a final concentration of 10 mM K^+. Triplicate soil pellets were exposed to each treatment. After 3 days of being shaken, the soil slurries were centrifuged, and the supernatant was collected for AA analysis to determine cadmium concentration and for surface tension analysis to determine rhamnolipid concentration.

In a second experiment, the effect of an increase in K^+ concentration on rhamnolipid mobilization of soil-bound cadmium was determined directly. A single rhamnolipid concentration (14 mM in 10 mM K^+) was prepared in solutions of increasing K^+ (10, 15, 20, 25, or 50 mM K^+) adjusted by the addition of KNO_3. Conditioned, cadmium-loaded Hayhook soil was exposed in triplicate to rhamnolipid at each K^+ concentration. After 3 days of being shaken, the soil was separated from the supernatant by centrifugation, and both cadmium concentration and surface tension in the supernatant were determined.

Effect of Rhamnolipid on Metal Removal—column Experiments. A plexiglass column (10 cm in length × 2.8 cm in diameter) was obtained from Soil Measurement Systems (Tucson, AZ). The columns for each experiment were packed and saturated as follows: soil was added to the column in roughly 1 g increments and tapped into place using a wooden dowel; prior to the addition of a new increment of soil, the soil surface was scored using a stainless steel spatula. The column was saturated with de-aired 10 mM $Ca(NO_3)_2$ from the bottom at an initial flow rate of 0.25 mL min^{-1} using an FMI Lab Pump, Model QG 6 (Oyster Bay, NY). Flow was subsequently increased daily by 0.25 mL min^{-1} to a maximum flow rate of 1.0 mL min^{-1}. Complete saturation of the column was assumed when the column exhibited a stable mass. Bulk density and pore volume values for each column are listed in Table 2. For each column, the hydrodynamic properties of the column were determined by examining the breakthrough of a conservative tracer, pentafluorobenzoic acid (PFBA).

Following characterization with PFBA, each column was loaded with a 1 mM $Cd(NO_3)_2$ solution prepared in 10 mM $Ca(NO_3)_2$ for approximately 150 pore volumes, at which point the concentration of cadmium in the influent (C_o) was equal to the concentration in the effluent (C) (relative concentration, $C/C_o = 1$). Following saturation with cadmium, each column was washed with 100 pore volumes of a KNO_3 electrolyte solution (3.5 mM or 7 mM K^+) until the relative concentration of cadmium in the effluent was approximately zero ($C/C_o = 0$). KNO_3 treatment was followed by between 20 and 100 pore volumes of a rhamnolipid solution (5 or 10 mM) in which the K^+ concentration in the matrix was set to 3.5 or 7.0 mM. The final stage of the experiment was to flush the column one more time with KNO_3 (3.5 or 7 mM). The effluent from the column was collected in borosilicate glass test tubes in 15-mL fractions. Fractions from each column were analyzed for cadmium and rhamnolipid and in some cases pH. For the analysis of cadmium, one drop of concentrated HNO_3 was added to a fraction, and the sample was diluted as necessary for AA analysis. For fractions containing rhamnolipid, an aliquot of the fraction was removed before acidification, and the rhamnolipid content was determined by surface tension analysis. The remaining fraction was acidified, centrifuged for 20 min at 6000 rpm to pellet the rhamnolipid, and then diluted for AA analysis.

Mass Balance Determination. To achieve a final mass balance, soil-bound cadmium not removed by the flushing solutions was determined. The soil was removed from the column and mixed thoroughly, and three 0.5-g samples were removed, oven-dried overnight, and weighed. The soil samples were washed three times with 7.1% HNO_3 using the following volumes and time schedule: (1) 10 mL for 24 h, (2) 10 mL for 15 min, and (3) 5 mL for 15 min. Between each washing, the samples were centrifuged at 10 000 rpm for 5 min, and the supernatant was collected for analysis. The

TABLE 2. Column Treatments

column	soil	bulk density (g cm^{-3})	pore vol (mL)	$Cd(NO_3)_2$ [a] (mL)	flushing treatments: KNO_3 [b] (mM)	flushing treatments: Rhamnolipid (mM, PV)
1	Vinton	1.63	24	1	3.5	5, 50
2	Vinton	1.74	21	1	7	10, 25
3	Vinton[c]	1.64	19−25[e]	1	7	10 (50, 25)[f]
4	Vinton[d]	1.61	21.2	1	7	10, 100
5	Bonify	1.92	17	1	7	10, 75
6	Comoro	1.79	22.4	1	7	10, 60
7	Hayhook	1.87	18	1	7[i]	10, 16

[a] Solution made in 10 mM $Ca(NO_3)_2$ and loaded for approximately 130−150 pore volumes. [b] KNO_3 treatment was applied for 100 pore volumes before rhamnolipid addition and for 50 pore volumes after rhamnolipid addition. [c] Column experiment was aged for 1 month. [d] No KNO_3 treatment prior to rhamnolipid treatment. [e] Pore volume fluctuated during the experiment between these values. [f] Two pulses of rhamnolipid; first for approximately 50 pore volumes, second for approximately 25 pore volumes.

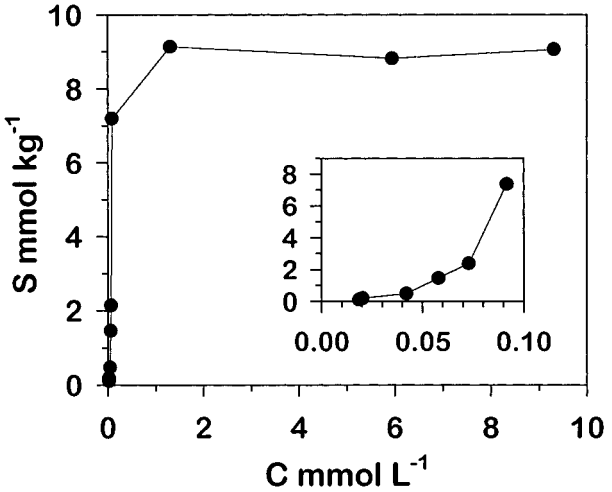

FIGURE 2. Sorption isotherm showing the relationship between sorbed [S mg (kg of Hayhook soil)$^{-1}$] and dissolved (C mg L^{-1}) rhamnolipid at equilibrium. The rhamnolipid concentration applied to the soil varied from 0.125 to 14 mM. The insert shows the sorption of low rhamnolipid concentrations (0.125−4 mM) on an expanded scale.

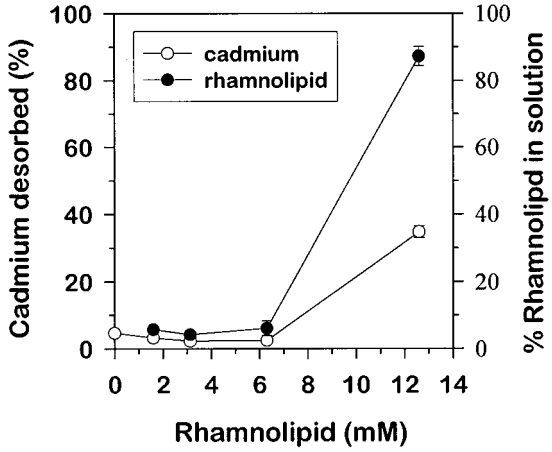

FIGURE 3. Desorption of soil-bound cadmium by rhamnolipid treatment, the relationship between the amount of rhamnolipid present in solution (●), and the removal of soil-bound cadmium (○) when rhamnolipid application is increased from 0 to 12.6 mM in a 10 mM K^+ matrix. Controls showed that 4% of the soil-bound cadmium was removed by 10 mM K^+ in the absence of rhamnolipid.

total volume for all three acid washes was 25 mL. Cadmium in the supernatant was determined by AA.

Results

Rhamnolipid Sorption and Metal Removal—Batch Studies. Rhamnolipid sorption to Hayhook soil was determined over more than 2 orders of magnitude from 0.125 to 14 mM rhamnolipid. Over this range, the isotherm shown in Figure 2 reveals two distinct phases in rhamnolipid sorption. The first phase was between 0.5 and 4 mM rhamnolipid (see inset) in which sorption of the biosurfactant was found to rapidly increase with an increase in the applied rhamnolipid concentration. In the second phase (between 4 and 14 mM applied rhamnolipid), sorption increased only slightly as the rhamnolipid concentration was increased. A plateau in rhamnolipid sorption was reached at the 8 mM treatment. This plateau represents an increase in the proportion of applied rhamnolipid that would be present in solution phase. For example, at 4 mM applied rhamnolipid, the amount of rhamnolipid in solution was 2%. As the applied rhamnolipid concentration was increased to 8, 11, and 14 mM, the percent of rhamnolipid in solution was increased to between 23% and 67%.

The impact of rhamnolipid sorption on the removal of soil-bound cadmium is illustrated in Figure 3. At rhamnolipid concentrations between 1.6 and 6.2 mM, more than 95% of the biosurfactant was sorbed to the soil, and less than 4% of the soil-bound cadmium was removed. At 12.6 mM rhamnolipid, only 15% of the biosurfactant was sorbed, and 35% of the soil-bound cadmium was removed from the soil. These results show that removal of soil-bound cadmium is dependent on the amount of rhamnolipid present in the solution phase.

Having illustrated the importance of rhamnolipid sorption for metal removal, attention was turned to factors that control the sorption of an anionic biosurfactant. The effect of increasing the concentration of K^+ in the rhamnolipid solution matrix on the sorption of rhamnolipid (11 mM) is illustrated in Figure 4. At the lowest K^+ concentration (10 mM), 67% and 94% of the rhamnolipid were present in solution, depending on the trial. As K^+ was increased, there was a rapid increase in rhamnolipid sorption. Consequently, at concentrations greater than 20 mM K^+, no more than 2% of the rhamnolipid was present in solution. Figure 5 confirms the importance of maintaining a low K^+ concentration with regard to rhamnolipid-facilitated removal of soil-bound cadmium. Rhamnolipid (14 mM) was dissolved in a K^+ matrix between 10 and 50 mM. The K^+ matrix alone removed between 5.3 and 9.7% of the cadmium. The presence of rhamnolipid in a 10 mM K^+ matrix increased cadmium removal to 33%. However, when the K^+ concentration within the rhamnolipid matrix was increased, a reduction in cadmium removal was evident. At 20 mM K^+ and greater, rhamnolipid treatment removed no more than 2% of the soil-bound cadmium. In fact, cadmium removal by the rhamnolipid treatments was less than what was found for

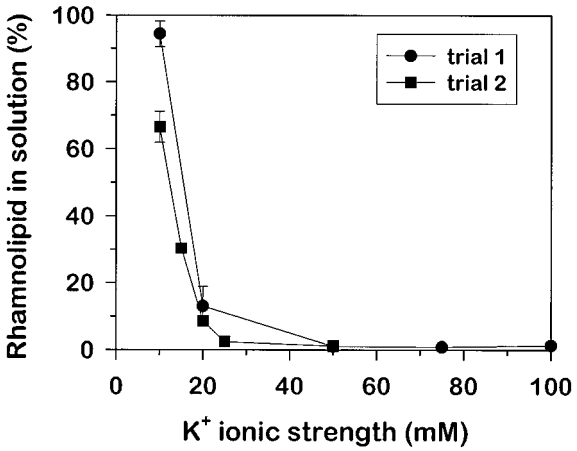

FIGURE 4. Effect of K^+ concentration on the percentage of rhamnolipid remaining in solution. The figure shows the results of two separate trials in which the sorption of rhamnolipid (11 mM) was determined for increasing K^+ concentrations in the matrix.

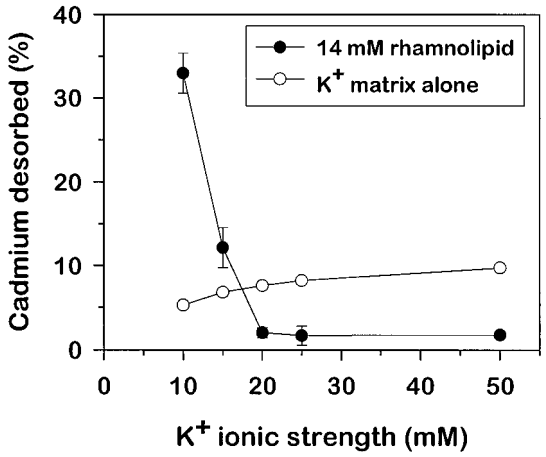

FIGURE 5. Desorption of soil-bound cadmium by rhamnolipid treatment and the effect of increasing K^+ concentration on the removal of soil-bound cadmium by 14 mM rhamnolipid.

the K^+ matrix alone. Complexation of K^+ or Cd^{2+} by rhamnolipid at the soil particle surface may account for the decreased removal of Cd^{2+} by ion exchange.

Metal Removal—Column Studies. A typical cadmium removal column experiment is shown in Figure 6. The breakthrough curve in Figure 6 can be divided into four stages (see arrows) each representing a new treatment including: I, cadmium loading; II, treatment with KNO_3 (3.5 or 7 mM depending on the rhamnolipid concentration); III, treatment with rhamnolipid (5 to 10 mM); and IV, a final treatment with KNO_3 (3.5 or 7 mM).

Cadmium removal from Vinton soil columns was examined using four different protocols (Table 2). Cadmium loading (stage I) in the sandy loam Vinton soil showed some variability, up to 23%, even though column packing characteristics were quite consistent. The mass of loaded cadmium was adjusted for liquid phase (nonsorbed) Cd^{2+} present in the pore volume before the flushing treatment. This mass represents approximately 4% of the cadmium loaded into each column. Results from columns 1−3 show that electrolyte flushing (stage II) resulted in a significant amount of cadmium being removed due to ion exchange processes (Table 3). Cadmium removal was dependent on the ionic strength of the KNO_3 treatment. For example, 26% of the Cd^{2+} was removed from column 1, which was treated with 3.5 mM KNO_3, but 36% of the Cd^{2+} was removed from column 2, which was treated with 7 mM KNO_3. Column 3,

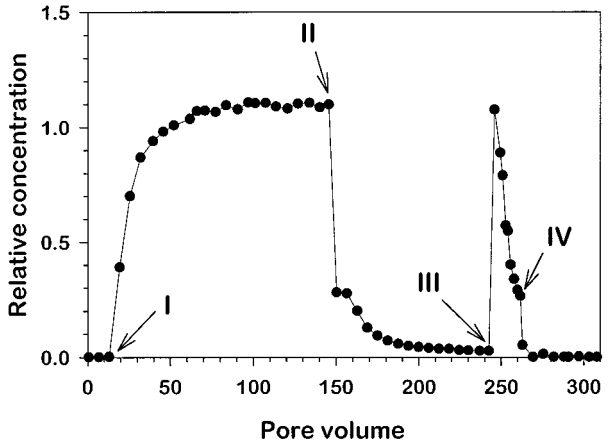

FIGURE 6. Breakthrough curve for a Vinton soil column treated with 10 mM rhamnolipid. The arrows indicate a switch of fluid reservoirs, and the numerals indicate the new fluid. I = 1 mM $Cd(NO_3)_2$ in 10 mM $Ca(NO_3)_2$; cadmium loading. II = 7 mM KNO_3; cadmium removal by electrolyte washing. III = 10 mM rhamnolipid in 7 mM KNO_3; rhamnolipid-enhanced cadmium removal. IV = 7 mM KNO_3; final electrolyte washing.

which was aged for 1 month after stage I, was also treated with 7 mM KNO_3 but was less affected by electrolyte flushing than column 2. Following KNO_3 treatment, each column was flushed with a rhamnolipid solution (stage III), as shown in Figure 7. A 5 mM rhamnolipid treatment for 50 pore volumes (column 1) removed 54% of the bound cadmium. A 10 mM rhamnolipid treatment for 25 pore volumes (column 2) removed 41% of the cadmium, although cadmium was still being removed when the flushing solution was switched from rhamnolipid to KNO_3 (Figure 6, stage IV, and Figure 7B), indicating that more cadmium would have been removed if rhamnolipid treatment had been continued. Thus, the total amount of cadmium removed from columns 1 and 2, which were treated with the same mass but different concentrations of rhamnolipid, was approximately the same, 79%. The aged soil (column 3) was treated with two 10 mM rhamnolipid pulses totaling 75 pore volumes. The first pulse removed 47% of the cadmium, while the second pulse removed only 3%. In comparing columns 2 and column 3 (aged), it can be seen that aging of the soil reduced the effectiveness of ion exchange removal but did not affect rhamnolipid complexation of soil-bound metals. In column 4, which was not pretreated with electrolyte solution, rhamnolipid treatment was the most effective, removing 96% of the cadmium.

The removal of soil-bound cadmium from the remaining three soil types is summarized in Table 3 and Figure 7. The KNO_3 treatment (stage II) removed between 15% and 36% of the cadmium from the column, depending on soil type. Rhamnolipid treatment (10 mM) was found to remove a further 8−45%. Rhamnolipid treatment was not successful for the column packed with Hayhook soil. Although initial batch studies performed with Hayhook revealed the potential to remove soil-bound cadmium (see Figures 3 and 5), under saturated flow conditions rhamnolipid addition caused dispersion of the soil and column plugging, which prevented cadmium removal. The removal of cadmium from the Bonify soil totaled 73%, similar to what had been observed for Vinton soil. However, cadmium removal did not tail off as rapidly as was evident for the Vinton soil, and presumably, further rhamnolipid treatment would have removed more cadmium (Figure 7C). For the Comoro soil, total cadmium removal was only 47%, although further treatment with rhamnolipid would likely have removed additional cadmium (Figure 7D).

The pH of occasional effluent samples was measured for each column. Effluent sample pH values ranged from 6.6 to

TABLE 3. Cadmium Loading and Removal for Soil Column Experiments

column	Cd loading (mg kg^{-1}) (stage 1)	Cd removal (%) KNO_3 (stage 2)	Cd removal (%) rhamnolipid (stage 3)	Cd removal (%) KNO_3 (stage 4)	Cd removal (%) total	Cd remaining[a] (%)	Cd recovery (%)
1 (Vinton)	592	23	54	3	80	ND[b]	ND
2 (Vinton)	658	36	41	2	79	28	107
3 (Vinton)	765	26	50[c]	2	78	22	100
4 (Vinton)	736	ND	96	6	102	11	113
5 (Bonify)	310	26	45	2	73	25	98
6 (Comoro)	938	15	31	1	47	47	94
7 (Hayhook)	469	36	8	ND	44	19	63

[a] Determined by acid digestion of soil in column after flushing treatment. [b] ND, not determined [c] Two pulses of rhamnolipid; first for approximately 50 pore volumes, second for approximately 25 pore volumes.

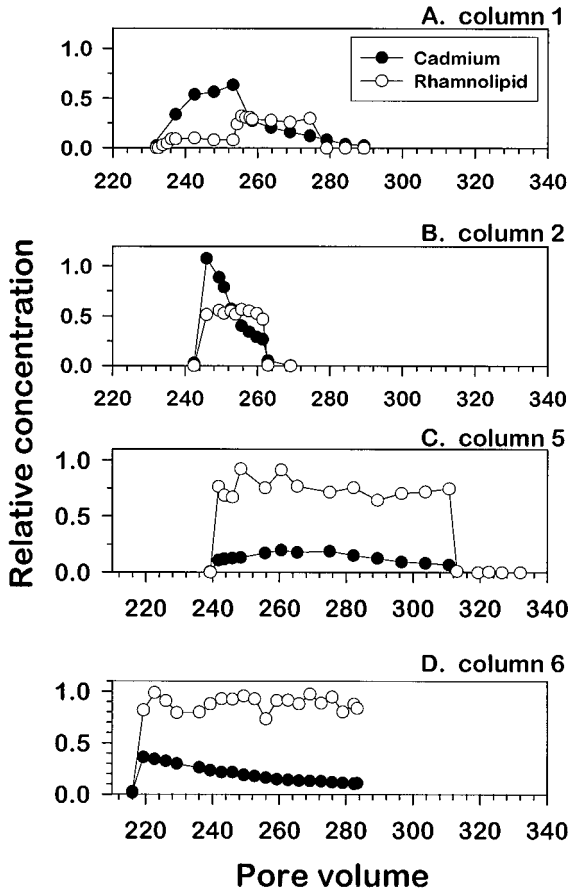

FIGURE 7. Breakthrough curves of cadmium and rhamnolipid during stage III of treatment of two Vinton column experiments (columns 1 and 2) and for the Bonify and Comoro soil column experiments (columns 5 and 6).

7.0, indicating no great fluctuation in pH in the soil solution during any of the experiments even though the soil pH values ranged from 5.5 for the Bonify soil to 7.7 for the Vinton soil (Table 1).

Discussion

Metal contamination of soil represents a potential environmental hazard in terms of toxicity to animals (*16*) and inhibition of microbial processes (*17,18*). Attempts to remediate metal-contaminated soil have involved soil washing strategies or pump-and-treat strategies for subsurface environments. However, slow desorption kinetics necessitate extended washing or pumping periods in order to displace soil-bound metals (*19*). Washing strategies can be greatly enhanced by the use of an agent that can increase the desorption of soil-bound metals and facilitate their transport through the soil matrix. An ideal complexing agent is one that is soluble in water, chemically stable under environmental conditions, not strongly bound to soil particles, and has a high affinity for complexing metals (*20*). Chen et al. (*20*) described the use of water-soluble bacterial exopolymers (MW $\sim 10^6$) to mobilize soil-bound metals in sand materials. Recent work in our laboratory has shown that rhamnolipid biosurfactant (MW $\sim$500) can complex heavy metals and is effective in removing soil-bound cadmium, zinc, and lead (*8, 21*). Rhamnolipid aggregates are predominantly small vesicles, <50 nm in diameter, and micelles are less than 5 nm in diameter at pH > 6.0 (*22*) and, therefore, should not be subject to physical straining within the soil matrix. However, the availability of rhamnolipid to remove metals from soil will be influenced by the degree of soil sorption.

The results of this research suggest that rhamnolipid sorption is strongly dependent on the amount of rhamnolipid applied to the soil and on the ionic strength of the rhamnolipid matrix solution. The triphasic nature of rhamnolipid sorption is similar to previously reported work on the sorption of synthetic anionic surfactants onto mineral surfaces (*23, 24*). The sorption mechanism described in this previous work showed that electrostatic and hydrophobic sorption forces can combine to create a characteristic s-shaped isotherm for the sorption of anionic surfactant. The first stage of the s-shaped isotherm sorption is controlled by electrostatic attraction between individual anionic surfactant ions and positively charged sites on a mineral surface. As the surfactant concentration is increased, there is an increased tendency for a self-association of surfactant ions due to electrostatic and hydrophobic forces. Self-aggregation can occur by a process that is analogous to micelle formation and is characterized by the formation of hemimicelles. Hemimicelle formation is the second stage of sorption, characterized by a rapid increase in surfactant sorption to particle surfaces. Hemimicellation will eventually result in the neutralizing of particle surface charge at which point the rate of further surfactant sorption will slow. As surfactant concentration is increased further, true micelle formation occurs and will result in the reversal of the surface charge. This will hinder further accumulation of surfactant at the particle surface by charge repulsion. Thus, the third stage in sorption is a plateau in which further surfactant sorption is inhibited and a greater proportion of added surfactant will be present in solution.

Rhamnolipid sorption to Hayhook soil was shown to have three distinct stages (Figure 2), and the mechanism described above provides a good fit to our observations. An important point to make is that the inflection point between the second and third stage of rhamnolipid sorption occurred when the solution-phase equilibrium concentration of rhamnolipid exceeded the cmc (50 mg/L). This pattern conforms to the idea that rhamnolipid saturation of a soil surface can modify

the surface such that further sorption is limited, and any further increase in rhamnolipid application will increase the proportion of rhamnolipid present in solution.

In terms of ionic strength, our results revealed that a slight increase in K^+ concentration can have a strong impact on rhamnolipid sorption. An increase in ionic strength can influence rhamnolipid sorption by reducing the electrostatic repulsion at the surfactant/particle interface. This will allow a greater density of surfactant ions to accumulate near the particle surface and will increase the capacity of the soil to sorb rhamnolipid ions. In a similar manner, increased ionic strength can reduce the electrostatic repulsion between surfactant ions allowing for a greater density of surfactant ions in the hemimicelle formations. The phenomenon of increased micelle aggregation number in the presence of divalent cations has been investigated by Bai et al. (*25*).

Minimizing ionic strength within the rhamnolipid solution matrix can help maximize rhamnolipid efficiency in terms of removing soil-bound metals. For example, a previous study showed that in a high ionic strength matrix (>100 mM K^+), 50 mM rhamnolipid was required to detect any removal of soil-bound cadmium or lead from Hayhook soil (*8*). In the present study, significant cadmium removal was detected using 12.6 mM rhamnolipid in a 10 mM K^+ matrix, which is a 4-fold decrease in the amount of rhamnolipid required.

For column experiments, cadmium loading in the four soils tested ranged from 310 to 938 mg kg^{-1} but did not correlate to CEC, as might have been expected. The CEC represents the total potential sites cations may occupy on the soil surface but does not predict the affinity of a soil for a particular cation. During the cadmium loading stage of the column experiments, there was a 10:1 ratio between the competing cations Ca^{2+} and Cd^{2+}. It is perhaps more useful to look at the percentage of the CEC occupied by cadmium. Vinton soil had the lowest CEC, and cadmium was found to occupy between 23 and 28% of the total cation exchange capacity in the four column studies. In contrast, Hayhook and Bonify soils had the highest CEC, and only 11% and 6.5%, respectively, of the cation exchange sites were occupied by cadmium. The Comoro soil exhibited loading of cadmium that occupied 29% of its CEC. In light of the competition between calcium and cadmium, a 6.5−29% loading of the CEC with cadmium does not seem unreasonable.

Rhamnolipid treatment of soil columns resulted in greatly improved metal recovery for three of the soils tested. For Vinton, Bonify, and Comoro soils, the effectiveness of rhamnolipid treatment was impacted by the interaction of cadmium with the soil. This is illustrated in Figure 7, which shows that there were significant amounts of free rhamnolipid available within the columns for metal complexation. Metal removal was most rapid in the Vinton soil, which had the lowest clay, TOC, and iron oxide content. The presence of increased organic matter and iron oxide content in the Bonify and Comoro soils resulted in a slower but more prolonged release of metal from the soil-suggesting that such soils will require more extended periods of treatment.

Results with Hayhook soil (column 7) show that rhamnolipid treatment was not effective under saturated flow conditions even though initial batch experiments were promising (see Figures 3 and 5). In this case, rhamnolipid addition caused dispersion of the soil, resulting in column plugging and an unstable and reduced flow. The problems with Hayhook soil may be attributed to its higher clay content and the dispersing effect of the K^+ present in the rhamnolipid treatment. Clearly, for such soils, rhamnolipid should be prepared in a matrix containing an electrolyte other than K^+. Calcium is an obvious alternative, but use of calcium can cause rhamnolipid precipitation (data not shown).

In summary, this study demonstrates that rhamnolipids are an effective agent for enhancing metal removal from some soils under laboratory conditions. Results also reveal the necessity for understanding the factors that affect rhamnolipid sorption to soil, such as ionic strength. Manipulation of the ionic strength within the rhamnolipid matrix provides an opportunity to maximize the efficiency of rhamnolipid use, thus minimizing the cost of rhamnolipid treatment. The efficiency of rhamnolipid treatment will also be affected by the mineral composition and pore water chemistry within metal-contaminated soils. Further research in our lab will evaluate the importance of soil mineral chemistry and of dominant soil electrolytes, such as calcium, on rhamnolipid sorption using a variety of soil types. The aim of this research will be to predict the rhamnolipid dose required to maximize rhamnolipid-facilitated removal of soil-bound metals.

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