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Chemoeffectors De e the Deposition of Chen ic Bacteria during Transport in us Media

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Bacterial chemotaxis enables m to move along chemical gradients and to swim ptimal places for biodegradation. However, its po sitive effects on subsurface remediation rely iciency of bacterial movement in porous media, wh n restricted by high deposition rates and adhesion to ces. In well-controlled column systems, we assessed t ce of the chemoeffectors naphthalene, salicylate , and acetate on deposition of chemotactic, naphthalene-degrading *Pseudomonas putida* G7 in selected porous environments (sand, forest soil, and clay aggregates). Our data showed that the presence of naphthalene in the pore water decreased deposition of strain G7 (but not of a derivative strain, *P. putida* G7.C1 (pHG100), nonchemotactic to naphthalene) by 50% in sand-filled columns, as calculated by the relative adhesion efficiency (α_t). Similar effects were observed with *P. putida* G7 strain for the other chemoeffectors. Deposition, however, depended on the chemoeffector's chemical structure, its interaction with the column packing material, and concomitantly its pore-water concentration. As the presence of the chemoeffectors had no influence on the physicochemical surface properties of the bacteria, we suggest that chemotactic sensing, combined with changed swimming modes, is likely to influence the deposition of bacteria in the subsurface, provided that the chemoeffector is dissolved at sufficient concentration in the pore water.

Introduction

Hydrophobic organic contaminants (HOCs) tend to be distributed heterogeneously in the subsurface (*1*). At the microscale, i.e., the immediate environment of individual bacteria, HOC heterogeneity is caused by various soil-matrix-HOC interactions, such as adsorption, entrapment, or dissolution processes (*1*). Remediation technologies based on biological degradation face this situation by promoting the contact probability between degrader organisms and pollutants to increase bioavailability and therefore biodegradation rates (*2*). Bacterial chemotaxis, which enables motile cells to move along chemical gradients and to swim toward optimal places for biodegradation, has been proposed as a relevant mobilizing mechanism with potential in bioremediation of HOCs (*3*). Chemotaxis decreases the distance between the microorganisms and the pollutant source and, as a consequence, increases HOC mass transfer to the cells and concomitant HOC-biodegradation (*4*).

Bacterial chemotaxis in aqueous systems is well documented (*5–8*). The effect of chemotaxis on the bacterial degradation in a heterogeneous system was first shown by Marx and Aitken (*6*), who found enhanced naphthalene degradation by chemotactic *Pseudomonas putida* G7 as compared with nonchemotactic and nonmotile mutants. Degradation by the mutant strains was limited by the molecular diffusion of the polycyclic aromatic hydrocarbon (PAH), indicating that chemotaxis actively increased the bioavailability of naphthalene. Flagellated bacteria can swim through aqueous media at 1.7–3.5 m day^{-1} (*7, 8*) and exhibit a translocation velocity comparable to groundwater flow rates typically found in the subsurface (ca. 1 m day^{-1}). Chemotaxis may thus play an important role in bacterial transport at low flow rates. This is supported by earlier work demonstrating the presence of chemotaxis in porous media (*5*) and the chemotactic response of *Pseudomonas stutzeri* KC exposed to nitrate gradients in packed columns under various flow conditions (*9*). Using quantitative magnetic resonance imaging, Olson et al. (*10*) further demonstrated that bacterial chemotaxis has a statistically significant effect on bacterial migration through porous media in the presence of chemical concentration gradients. It may be because no attention has been given that indications for the effect of chemotaxis on biodegradation rate in natural matrices are still relatively scarce.

In this study, we assessed the influence of the chemoeffectors naphthalene, salicylate, acetate, and fumarate on the deposition and transport of the chemotactic, naphthalene-degrading *Pseudomonas putida* G7 in various porous media (sand, forest soil, and clay aggregates). A column percolation system was used as described earlier (*11, 12*) to assess bacterial transport under continuous flow conditions. Our data demonstrate that chemoeffectors can decrease the deposition of chemotactic bacteria in porous materials.

Experimental Section

Chemicals. Naphthalene was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Salicylate, fumarate, and acetate were obtained from Panreac Química SA (Barcelona, Spain). Polyvinyl alcohol (PVA) and glass beads (diameter, 212–300 μm) were from Sigma-Aldrich, Inc. (St. Louis, MO).

Bacteria, Media, and Cultivation. The naphthalene-degrading strains *Pseudomonas putida* G7 and its naphthalene-degradation plasmid-cured derivative *Pseudomonas putida* G7.C1 (pHG100) were kindly provided by C. S. Harwood (University of Iowa). Both strains are motile by means of polar flagella and able to use naphthalene as the sole source of carbon and energy. Whereas the parent strain G7 is known to be chemotactic toward naphthalene, the strain G7.C1 (pHG100) is deficient for chemotaxis to this compound (*13*). The bacteria were maintained on agar plates in the presence of an excess of naphthalene vapor. The plates contained solid minimal-salts medium (MSB), described elsewhere (*11*), supplemented with 16 g L^{-1} agar. Unless otherwise stated, all experiments were performed with bacteria previously grown at 30 °C on a rotary shaker at 150 rpm in Erlenmeyer flasks of 250 mL containing 100 mL of an inorganic-salts solution (MM; pH 5.7) (*14*), supplemented

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with 5 mM salicylate. Cells were harvested after 48 h (early stationary phase), separated from the cultivation medium by centrifugation at 1000*g* for 10 min, and resuspended for transport experiments in either MM or MM supplemented with 10 mM acetate, fumarate, or salicylate. This centrifugation speed was needed to prevent the breaking of flagella and the subsequent loss of motility. The ionic strength of the percolation media was, in mM, 7.7 (MM), 17.7 (MM-acetate), 37.7 (MM-fumarate), and 17.7 (MM-salicylate). Maximum growth rates (μ_{max}) for salicylate-pregrown *P. putida* G7 in MM supplemented with either 0.2% solid naphthalene or 5 mM of acetate, fumarate, or salicylate were derived from duplication periods (t_d) of optical density measurements at 600 nm (OD_{600}) according to $\mu_{max} = \ln 2/t_d$.

Determination of Physicochemical Cell Surface Properties and Bacterial Swimming Behavior. Cell surface hydrophobicities were derived from the static contact angles (θ_w) of small water droplets placed on filters covered either with layers of bacteria or finely powdered sand. Measurements were performed with a goniometer microscope (Krüss GmbH, Hamburg, Germany) as described before (*15*). Contact angles of at least 10 droplets of 1 μL were measured for each organism. The zeta potential (ζ) was approximated from the electrophoretic mobility, measured by a Doppler electrophoretic light-scattering analyzer (Zetamaster, Malvern Instruments, Malvern, UK) according to the method of Helmholtz-Smoluchowski (*16*). Mean effective cell radius (R) and swimming behavior of early stationary cells was determined in cell suspensions prepared as described above for transport experiments with an Axioskop 2 Carl Zeiss light microscope (Jena, Germany). Values for R were calculated as $R = 0.5\ (l \times w)^{1/2}$ with l and w being the length and width, respectively, of the bacteria. To obtain estimates for influence of the medium and the different chemoeffectors on the physicochemical surface properties of sand, the material was ground in a mill and the Zeta potential and the contact angle measured of the fraction remaining in suspension of the different percolation media after one hour of sedimentation.

Chemotaxis Assay. A modified version of the capillary tests described earlier (*17*) was used to quantify chemotaxis. In short, early stationary cells were harvested, centrifuged, and resuspended in MM to an OD_{600} of ca. 0.020 (corresponding to 10^6 cells mL^{-1}). Preliminary observations with an optical microscope had shown maximum motility of the cells in this growth phase. About 0.1 mL of this suspension was placed in a small chamber formed by placing two capillary tubes (1 μL) (Microcaps, Drummond, Broomall, PA) in parallel on a microscope slide. Another capillary tube (1 μL), heat-sealed at one end, containing the chemoeffector solution, was immersed in the cell suspension at its open end. The system was then closed with a glass coverslip, avoiding any formation of air bubbles in the chamber. The chemoeffector solution in the capillary contained MM supplemented with either saturating concentrations of naphthalene or 100 mM of salicylate, fumarate, or acetate. MM lacking any chemoeffectors was used as a control. The chambers were incubated for 2 h at room temperature. The number of bacterial cells accumulated in the test capillaries was quantified by colony-forming units on tryptic soil agar.

Mineralization Experiments. Naphthalene mineralization was determined at room temperature with shaking (100 rpm) in biometer flasks of 250 mL (Bellco glass, NJ) containing 32 mL of a cell suspension containing 10^8 cells mL^{-1}. Approximately 70 000 dpm of labeled naphthalene (^{14}C-UL-Naphthalene from Sigma Chemical Co., Steinheim, Germany; 31.3 mCi $mmol^{-1}$; radiochemical purity >98%) and unlabeled naphthalene to give a final concentration of 24 μM was added to the suspension in 0.1 mL acetone. Experiments were performed in duplicate. Maximum rates and extents of $^{14}CO_2$ production were estimated by the radioactivity appearing in the alkali traps as described earlier (*18*).

Column Deposition Experiments. *Packing Materials.* Sand was obtained from Panreac Quimica SA (Barcelona, Spain), and washed by gravity settling in deionized water. Its particle composition was 100% fine-grained sand (0.25–0.30 mm). Aggregates of montmorillonite clay (Swy-2, University of Missouri, Columbia, MO) were prepared by immobilizing the clay on glass beads, with polyvinyl alcohol (PVA) as aggregating agent as described earlier (*11*). A forest soil from Campo de Gibraltar, Cadiz, Spain (6.1% organic matter) was used in transport experiments with whole soil. Its particle-size distribution was 61.9% coarse-grained sand (2.0–0.2 mm), 21.4% fine-grained sand (0.20–0.05 mm), 11.2% silt (0.050–0.002 mm), and 5.5% clay (<0.002 mm). The soil was sieved (mesh 2 mm), preserved in sealed glass bottles at −80 °C, and dried at 70 °C immediately before use.

Column Experiments. Transport experiments were performed at 25 °C in vertical percolated columns (*19*). The test materials (sand, clay aggregates, and soil) were wet-packed in glass columns of 10 cm in length and 0.9 cm internal diameter. The dry amount of packing material present in each column was approximately 12 g. The columns were connected to a peristaltic pump, and suspensions of salicylate-grown bacteria, prepared as above ($OD_{600} = 0.3$, containing approximately 10^7 cells mL^{-1}), were pumped through the columns at constant flow rates. Identical empty-bed flow rates were used, leading to different porosity-dependent hydraulic flow rates. These were 0.37 cm min^{-1} for sand, which had a gravimetrically estimated porosity of 0.42, corresponding to a pore volume (PV) of 2.677 mL, 0.38 cm min^{-1} for clay aggregates (porosity, 0.42; PV, 2.654 mL), and 0.30 cm min^{-1} for soil (porosity, 0.52; PV, 3.309 mL). Column breakthrough of bacteria was followed photometrically at various time intervals. The efficiency of bacterial removal was expressed as optical density at 600 nm in column effluent (C) divided by that in column influent (C_0). All results are given as means of duplicate measurements.

All chemoeffectors except for naphthalene were introduced in the columns dissolved in the bacterial suspensions at a concentration of 10 mM. Due to its low solubility in water and its tendency to sorb to the tubing material, it was not possible with this procedure to generate a sufficiently high aqueous naphthalene concentration in the pore. Therefore, the columns with naphthalene contained 1.2 or 12.0 mg of solid PAH previously added (dissolved in 0.5 mL of acetone) to the upper 2 g of the packing material in the columns. The acetone that was left to evaporate completely under a nitrogen stream before packing. Experiments with the conservative tracer thiourea showed that solid naphthalene present in the columns did not affect the hydraulic properties. The columns were equilibrated in all transport experiments by pumping MM for 30 min (approximately 2.5 PV) before the addition of bacterial suspension.

Calculations. The adhesion efficiency (α_t) of bacteria is commonly defined as the ratio of the rate of attachment (η_t) to the rate of bacterial transport to the surfaces (η_{trans}) (*12*)

$$\alpha_t = \eta_t/\eta_{trans} \quad (1)$$

In the absence of repulsive forces, α_t reaches unity, whereas it falls below unity in the presence of repulsive forces. Unlike C/C_0, which is an overall measurement for cell removal, the calculation of α_t takes into account differential frequencies of contact with the column material for bacteria. Therefore, α_t represents the relative affinity of bacteria for the packing material. Values of η_{trans} were calculated taking into account the contributions of convection, diffusion, van der Waals attraction, and sedimentation. For the calculations, we assumed spheres of identical size (radius of 135 μm for sand grains and 355 μm for soil and clay aggregates) in their closest

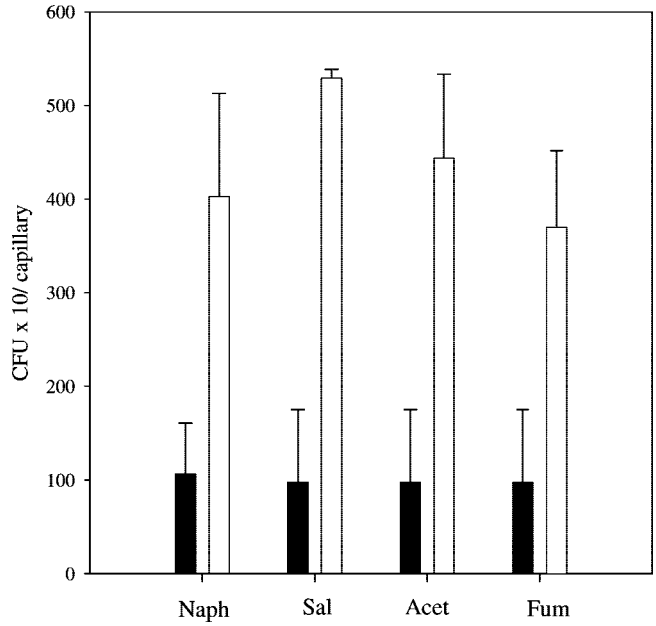

FIGURE 1. Quantitative chemotactic response of *Pseudomonas putida* G7 to different chemoeffectors as assessed by capillary assays. Relative to control (filled bars), the presence of a chemoeffector (MM inorganic-salts solution containing naphthalene or 100 mM of the organic acids, respectively) (open bars) resulted in a 4–5-fold increase of bacteria in the capillary, as detected by colony-forming units (CFU) on agar plates. Results represent the mean of three independent capillary assays, and error bars represent one standard deviation. As the chemoeffectors were tested successively, control data are reported for each experiment separately.

packing, and identical effective bacterial radius (R) of the bacteria. Values of η_t were calculated from C/C_0 values obtained in transport experiments. In some experiments, α_t values did not reach a constant value. For this reason, and to account for the dynamics of filter blocking, two representative values for α_t were calculated: an initial one, calculated at the point of change of slope in C/C_0 vs PV plots, corresponding to the end of the bacterial front, and a final one, corresponding to the end of the experimental period (*12*). A more detailed description of the calculation method and representative results obtained for transport of thiourea through sand can be found in the Supporting Information.

Chemical Naphthalene Analysis. Naphthalene concentrations were measured during transport experiments in the column effluent before (C_{n0}) and after (C_{nf}) the bacteria passed through the columns. Analysis was performed using a Waters HPLC system (2690 separations module and 996 photodiode array detector. column: Waters PAH columns, C_{18}, 5 μm, 4.6 $\times$ 250 mm; flow: 1 mL min^{-1}; mobile phase: 45% acetonitrile, 55% water). Samples containing bacteria were poisoned with 10 μL of the biocide formaldehyde (40%).

Results

Characteristics of the Bacteria. *Chemotaxis.* The chemotactic responses of strain *P. putida* G7 and nonchemotactic to naphthalene strain *P. putida* G7.C1 (pHG100) (*6, 13, 20, 21*) were tested using capillary chemotaxis assays. Whereas the nonchemotactic strain showed no response to naphthalene (data not shown), strain G7 exhibited a statistically significant (t test, $P = 0.01$) ca. 4-fold chemotactic attraction (*3*) by all of the chemoeffectors tested, i.e., naphthalene, salicylate, acetate, and fumarate (Figure 1).

Swimming behavior. The swimming behavior of individual cells in MM supplemented with excess of naphthalene was further analyzed by microscopic observation. In the absence of the chemoeffector, the two strains showed similar modes of swimming, characterized by short trajectories and abrupt changes in direction. As observed earlier for *P. putida* strains (*8*) *P. putida* G7 cells changed their swimming mode notably in the presence of naphthalene. In the presence of the chemoeffector, strain G7 exhibited smooth trajectories, often curved and forming counter-clockwise circles. However, no apparent effect of naphthalene on the motility of *P. putida* G7.C1 (pHG100) strain was observed.

Physicochemical Surface Properties. Water contact angles (θ_W) and zeta potentials (ζ) were used to describe the physicochemical surface properties of *P. putida* G7 and *P. putida* G7.C1 (pHG100). No influence of the growth phase was observed (data not shown); Both strains were negatively charged, exhibiting similar zeta potentials (ζ of ca. -39 mV) and water contact angles (θ_w) of 46–53° (Table 1), indicating intermediately hydrophilic cell surfaces (*22*). Furthermore, there was a small difference between their effective radii (Table 1). No significant changes of the physicochemical cell surface properties were observed when strain G7 was exposed to chemoeffector solutions as described above (Table 2). Likewise, no influence of the chemoeffectors on the physicochemical surface properties of sand ($\zeta = -33 \pm 3$ mV; θ_w = ca. 10°) was observed.

Biodegradation of Naphthalene. Growth and batch degradation experiments demonstrated the ability of both strains to grow on and to mineralize naphthalene. Radiorespirometry determinations with salicylate-grown bacteria further showed that the strains immediately mineralized naphthalene (24 μM), exhibiting similar (t test, $P = 0.05$) maximum mineralization rates of 18.8 $\pm$ 1.7% h^{-1} (strain G7) and 13.8 $\pm$ 0.8% h^{-1} (strain G7.C1 (pHG100)). The extent of mineralization was also similar (45.8 $\pm$ 1.9% for *P. putida* G7 and 48.7 $\pm$ 1.6% for *P. putida* G7.C1 (pHG100)), confirming earlier observations (*6*).

Effect of Naphthalene on Deposition. Cell suspensions pumped through sand-packed columns broke through (i.e., reached 0.5 $\times$ C/C_0 max) after one pore volume (Figure 2). Not unexpectedly, the two strains were transported through sand to a similar extent, and reached final C/C_0 values of approximately 0.4. The presence of dissolved naphthalene, however, resulted in C/C_0 values of 0.70 for strain G7 and 0.45 for strain G7.C1 (pHG100), indicating a ca. 50% decrease in the deposition of chemotactic strain *P. putida* G7 in sand columns not seen with the chemotaxis-negative strain (Figure

TABLE 1. Effect of Naphthalene on Deposition of Wild-Type *Pseudomonas putida* G7 and Nonchemotactic to Naphthalene *P. putida* G7.C1 (pHG100) Strains through Sand Columns

bacterium	cell width (μm)	cell length (μm)	mean radius[b]	θ_w[c,d] (deg)	ζ[d,e,f] (mV)	transport[a] chemoeffector[g]	C_{n0}[d,h] (μM)	C_{nf}[d,h] (μM)	C/C_0	α_t[i]	PV[j]
*Pseudomonas putida*G7	1.0	3.0	0.87	46 ± 4	−39 ± 2	none	NA[k]	NA[k]	0.39 (0.49)	o.172 (0.131)	1.83 (6.60)
						naphthalene	100 ± 5	124 ± 6	0.66 (0.72)	0.082 (0.065)	3.49 (7.54)
Pseudomonas putida G7.C1 (pHG100)	0.9	2.0	0.67	53 ± 3	−38 ± 2	none	NA[k]	NA[k]	0.42 (0.42)	0.142 (0.140)	1.84 (6.62)
						naphthalene	100 ± 0	109 ± 13	0.47 (0.50)	0.131 (0.120)	3.15 (7.78)

[a] Final experimental values are given in parentheses as an indication of the dynamics of filter blocking. [b] Radius derived from the average geometric mean of the cell width (w) and the cell length (l): $R = 0.5.\ (1 \times w)^{1/2}$. [c] θ_w, Contact angle without chemoeffector. [d] Values are reported as mean ± one standard deviation. [e] ζ, Zeta potential without chemoeffector. [f] Determinations were performed at pH 7.2 in KNO_3. [g] Applied as 12 mg solid naphthalene at the top of columns. [h] C_{n0} and C_{nf}, concentration of naphthalene in the effluent before and after pumping bacterial suspensions, respectively. [i] α_t, Adhesion efficiency. [j] PV, pore volume. [k] NA, not applicable.

TABLE 2. Influence of Chemoeffectors on Deposition of *Pseudomonas putida* G7 in Porous Media

chemoeffector[a]	θ_w[b,c] (deg)	ζ[c,d,e] (mV)	sand[f] C/C_0	sand α_t[g]	sand PV[h]	clay[f] C/C_0	clay α_t[g]	clay PV[h]	soil[f] C/C_0	soil α_t[g]	soil PV[h]
none	46 ± 4[i]	−39 ± 2[i]	0.39[i] (0.49)	0.172[i] (0.131)	1.83[i] (6.60)	0.11 (0.22)	2.159 (1.504)	1.56 (9.62)	0.43 (0.66)	1.404 (0.689)	1.98 (7.25)
naphthalene	33 ± 3	−35 ± 2	0.74 (0.80)	0.059 (0.045)	3.54 (7.63)	0.23 (0.35)	1.406 (1.018)	2.28 (9.19)	0.50 (0.53)	1.161 (1.052)	1.91 (7.02)
salicylate	36 ± 2	−36 ± 2	0.83 (0.84)	0.037 (0.036)	1.80 (7.91)	0.16 (0.20)	2.023 (1.794)	3.80 (10.78)	0.32 (0.37)	1.847 (1.597)	3.93 (6.72)
acetate	35 ± 3	−37 ± 2	0.61 (0.79)	0.105 (0.049)	1.43 (8.40)	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]
fumarate	34 ± 2	−36 ± 2	0.82 (0.83)	0.042 (0.039)	1.96 (8.51)	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]

[a] Applied, for naphthalene, as 1.2 mg solid compound at the top of columns or, for the rest of the chemoeffectors, dissolved in the bacterial suspensions at a concentration of 10 mM. [b] Contact angle. [c] Values are reported as mean ± one standard deviation. [d] Zeta potential. [e] Determinations were performed at pH 7.2 in KNO_3. [f] Final experimental values are given in parentheses as an indication of the dynamics of filter blocking. [g] Adhesion efficiency. [h] PV, pore volume. [i] For better comparison, data from Table 1 have been included. [j] ND, not determined.

2, Table 1). We therefore suggest that the chemotactic sensing of dissolved naphthalene is the probable cause of the lowered efficiency of adhesion of bacteria in porous media upon collision (α_t) of *P. putida* G7 (Table 1). Naphthalene was provided as slowly dissolving crystals (12 mg per column) placed in the influent at the top of the columns. Dissolved naphthalene concentration in the effluent was at 40% (100 μM) of the aqueous solubility of naphthalene ($c_{w,sat} = 242$ μM) (*23*) and remained constant throughout the experiment, as indicated by similar effluent concentrations before the addition of bacterial suspension (C_{n0}) and at the end of the experiment (C_{nf}) (Table 1). As shown by a doubling time of 18 h ($\mu_{max} = 0.04\ h^{-1}$) for strain G7, cell proliferation of the strain on naphthalene over the duration of the experiment (3 h) can be considered negligible.

The impact of naphthalene (1.2 mg per column) on the transport of strain G7 was additionally tested using clay-covered glass beads and an organic-matter (OM) -rich, sandy forest soil. Clay surfaces are prone to bind ionic compounds and to hamper bacterial transport (*11, 12*), OM-rich soil is likely to adsorb hydrophobic organic compounds (*12*). As reflected by the increasing α_t-values in sand, forest soil, and clay, strain G7 was retained most efficiently by clay aggregates (Table 2). As in the case of transport in sand, the presence of naphthalene in the pore water decreased the deposition of strain G7 on clay surfaces (Table 2). No effect of the PAH on transport through soil was observed, however. Moreover, no naphthalene was detected in the outflow (C_{n0} and $C_{nf} < 1$ μM), a reflection of the absence of dissolved naphthalene in the pore water, due to efficient sorption to the soil OM. The values for C_{n0} and C_{nf} in these experiments were, respectively, 82 ± 16 and 16 ± 2 μM for sand and 44 ± 6 and 1 ± 0 μM for clay. This led us to conclude that naphthalene-promoted transport was strongly influenced by the presence of dissolved chemoeffector in the pore water. However, as seen from the different naphthalene outflow concentrations in sand columns that contained 12.0 mg naphthalene (Table 1), it appears that the influence on deposition is not directly proportional to the concentration of the dissolved chemoeffector.

Effect of Salicylate, Acetate, and Fumarate on Deposition. We tested these chemoeffectors due to their high aqueous solubility and their likely dissimilar interactions with the collector surfaces, as compared to naphthalene. The presence of salicylate decreased deposition of strain G7 cells on sand surfaces, but exhibited no mobilizing effect in clay and soil (Figure 3). The calculation of α_b, in contrast, indicated an increased affinity toward soil surfaces in the presence of salicylate to levels close to those observed using highly retaining clay surfaces (Table 2). Transport experiments with acetate and fumarate likewise showed a clear promoting effect on transport through sand (Table 2). As in the naphthalene experiments, cell proliferation of strain G7 on fumarate, acetate, and salicylate could be considered negligible during

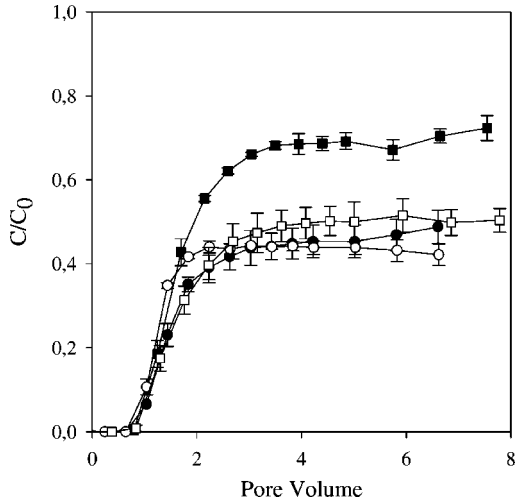

FIGURE 2. Breakthrough curves of wild-type *Pseudomonas putida* G7 (squares) and nonchemotactic to naphthalene *P. putida* G7.C1 (pHG100) (circles) strains transported through sand-filled columns in the presence (filled symbols) and absence (open symbols) of the chemoeffector naphthalene, applied as 12 mg of crystals at the top of columns. Error bars represent one standard deviation of duplicate columns.

the experiments (3 h), as shown by a lag period of ca. 18 h and low doubling times of 7.7 h ($\mu_{max} = 0.09\ h^{-1}$), 12.2 h ($\mu_{max} = 0.06\ h^{-1}$), and 2.7 h ($\mu_{max} = 0.26\ h^{-1}$) for growth on fumarate, acetate, and salicylate, respectively. Due to the specificity of the deficiency for chemotaxis toward naphthalene only, we did not test the percolation of *P. putida* G7.C1 (pHG100) strain in the presence of other chemoeffectors than naphthalene.

It should be noted that some of the α_t values obtained exceeded unity. This is in apparent contradiction with theory, which takes into account the contributions of convection, diffusion, sedimentation, and van der Waals attraction of the cells, assuming the collectors to be perfect spheres of uniform size. However, none of the collectors used in this study complied with this condition. Calculations of η_{trans}, and consequently of α_t, have to be considered approximations. Nevertheless, as we were interested in comparing the relative influence of various chemoeffectors on deposition in a given porous environment, we used eq 1, despite the potential overestimation of adhesion efficiency.

Discussion

Effect of Naphthalene on Bacterial Deposition. Our data demonstrate that naphthalene is able to promote the transport of chemotactic bacteria through porous materials. The reduced deposition of strain G7 on the surfaces tested is likely to be caused by chemotactic sensing in the pore scale as (i) naphthalene did not change the adhesion-relevant physicochemical cell surface properties of the bacteria, (ii) the physically similar, but nonchemotactic to naphthalene strain G7.C1 (pHG100) exhibited no increased transport in the presence of naphthalene, and (iii) the naphthalene-promoted transport depended on the presence of this chemoeffector in the pore water. The positive influence of chemotaxis on bacterial transport of strain G7, however, differed from literature data in long-term experiments using *Escherichia coli* (*24, 25*) and *P. putida* (*26*), which revealed no or clearly reduced subsurface dispersion of bacteria in the presence of chemoeffectors. According to this literature, the sand subsurface penetration rate of motile bacteria was regulated by the bacterial in situ growth rate rather than by chemotaxis, as the directed macro-scale bacterial transport may have been reduced by microscale chemotactic movement (*24*). The virtual absence of bacterial growth—due to the short-term of the experiments—and the application of a continuous flow regime in the columns allowed us to observe the per se influence of naphthalene and other chemoeffectors on the deposition rate of chemotactic bacteria through porous media. Furthermore, interference due to

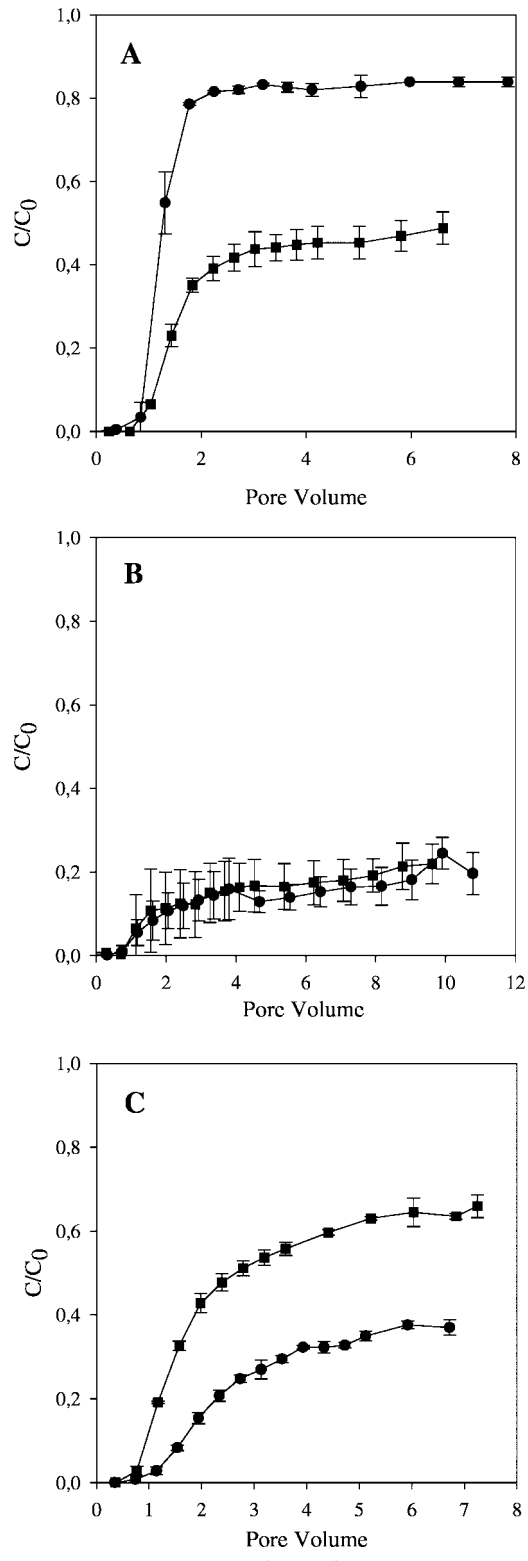

FIGURE 3. Effect of salicylate (10 mM) on *Pseudomonas putida* G7 transport through saturated porous columns of sand (A), clay (B), and soil (C). Circles and squares represent treatments with and without salicylate, respectively. Error bars represent one standard deviation of duplicate columns.

dissimilar growth rates and association phenomena typical of bacterial growth in static conditions, such as formation of symmetric patterns (*27*) and cell accumulation in closed compartments (*28*), was minimized.

Naphthalene reduced deposition through a mechanism other than that relying on chemotaxis-driven cell positioning at optimal places on macroscale chemical gradients. A decreasing gradient in aqueous naphthalene concentrations was expected to be formed within the columns from top to bottom, as a result of dissolution of solid naphthalene. Chemotactic attraction toward naphthalene (Figure 1) would have resulted in cell retention within the columns rather than in the opposite effect observed. Furthermore, the eventual effects of chemotactic repellence in the vicinity of naphthalene crystals (*3*) were unlikely to be detected due to cell retention in the underlying, solid-naphthalene-free column material amounting to 83% of the column length. The results are likely to be explained by different deposition mechanisms in the absence and presence of chemoeffectors. Similar to that proposed by Marshall et al. (*29*), the deposition of bacteria can be assumed to take place when the cells have a kinetic energy lower than the minimum in the interaction energy at the distance of closest possible approach. For chemotactic bacteria in the presence of chemoeffectors, this step is determined by the kinetic energies of both the Brownian motion at low flow rates and the directed movement along chemical gradients toward or away from the collector surface. As a consequence, the collision efficiency (α_t) is limited by the fraction of cells satisfying this condition. A likely explanation for changes in the interaction energies between cells and solid surfaces is the changed mode of swimming of strain G7 in the presence of a chemoeffector. Such effects have been observed by McClaine and Ford (*30*), who describe the influence of different motility patterns on bacterial attachment to glass. In that study, *E. coli* strains with different motility patterns were compared; two patterns are comparable to those exhibited in our study by strain G7. The authors observed that, at flow rates and ionic strengths similar to ours, bacterial attachment to glass for cells with a smoother and more-continuous movement was lower than for strains with more-abrupt movements. Differences in motility are also reflected as the different angles at which bacteria approach a surface (*7, 31*). It is thus likely that *P. putida* G7 in the presence of a chemoeffector moved more linearly and smoothly, exhibiting angles close to 0° relative to the surfaces. Accordingly, the rates of collision with the surfaces, and the deposition of the bacteria on the surfaces, may have been reduced in the presence of a chemoeffector.

Variations Among Chemoeffectors. The different effects of salicylate and naphthalene on deposition of *P. putida* G7 on the different collectors are likely to be due to the different sorption affinities of the chemoeffectors for the matrices tested. Due to its hydrophobic nature, naphthalene tends to absorb mainly to soil organic matter (*32*), whereas salicylate is more prone to interact with clay or other charged mineral oxide surfaces (*33*). Naphthalene, and very probably salicylate, was poorly retained by sand, leading to pore water concentrations that were recognized by the chemotactic bacteria and, concomitantly, their deposition decreased. By contrast, significant sorption of salicylate and naphthalene to clay surfaces and forest soil can be expected. Efficient sorption to the collector surface, however, reduces the chemoeffector's pore water concentration, and, therefore, its positive influence on the transport of chemotactic bacteria, as has been observed, for instance, in the unchanged deposition of strain G7 during transport through forest soil in the presence or absence of naphthalene (Table 2). The data for transport in soil in the presence of salicylate even suggest that adsorption of the chemoeffector to the collector surface may lead to a preferential accumulation of chemoeffector in areas close to aggregates, and concomitantly an increased cell deposition.

Implications for Bioremediation. Chemotaxis has been found to increase the biodegradation of chemicals in heterogeneous systems (*5, 6*). This observation was supported by model calculations showing that chemotaxis increases the rate of degradation of a discrete source of naphthalene. Those studies also indicated an important practical implication for bioaugmentation: as a consequence of the drastically reduced loss of chemotactic bacteria to regions free of separate-phase naphthalene, lower inoculum densities (1–0.1%) of chemotactic bacteria were needed to achieve rates of naphthalene degradation equal to those with metabolically equally active, but nonchemotactic, bacteria (*5*). Positive effects of chemotaxis on soil or groundwater remediation, however, equally rely on the efficiency of bacterial movement in porous media, which is often restricted by increased path lengths or surfaces for bacterial adhesion. Our study therefore indicates that, due to the increased subsurface mobility of chemotactic bacteria in the presence of chemoeffectors, the application of suitable combinations of catabolically active, chemotactic bacteria may be a valuable strategy to enhance efficient spreading of bacteria in the subsurface and to minimize potential inoculum densities in bioaugmentation regimes.

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Supporting Information Available

Additional description of the filtration model and experimental results with thiourea column experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Luthy, R. G.; Aiken, G. R.; Brusseau, M. L.; Cunningham, S. D.; Gschwend, P. M.; Pignatello, J. J.; Reinhard, M.; Traina, S. J.; Weber, W. J.; Westall, J. C. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.* **1997**, *31*, 3341–3347.

(2) Alexander, M. *Biodegradation and Bioremediation*; Academic Press: San Diego, CA, 1999.

(3) Ortega-Calvo, J. J.; Marchenko, A. I.; Vorobyov, A. V.; Borovick, R. V. Chemotaxis in polycyclic aromatic hydrocarbon-degrading bacteria isolated from coal-tar- and oil-polluted rhizospheres. *FEMS Microbiol. Ecol.* **2003**, *44*, 373–381.

(4) 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.

(5) Pedit, J. A.; Marx, R. B.; Miller, C. T.; Aitken, M. D. Quantitative analysis of experiments on bacterial chemotaxis to naphthalene. *Biotechnol. Bioeng.* **2002**, *78*, 626–634.

(6) Marx, R. B.; Aitken, M. D. Bacterial chemotaxis enhances naphthalene degradation in a heterogeneous aqueous system. *Environ. Sci. Technol.* **2000**, *34*, 3379–3383.

(7) Frymier, P. D.; Ford, R. M.; Berg, H. C.; Cummings, P. T. Three-dimensional tracking of motile bacteria near a solid planar surface. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 6195–6199.

(8) Harwood, C. S.; Fosnaugh, K.; Dispensa, M. Flagellation of *Pseudomonas putida* and analysis of its motile behavior. *J. Bacteriol.* **1989**, *171*, 4063–4066.

(9) Witt, M. E.; Dybas, M. J.; Worden, R. M.; Criddle, C. S. Motility-enhanced bioremediation of carbon tetrachloride-contaminated aquifer sediments. *Environ. Sci. Technol.* **1999**, *33*, 2958–2964.

(10) Olson, M. S.; Ford, R. M.; Smith, J. A.; Fernandez, E. J. Quantification of bacterial chemotaxis in porous media using magnetic resonance imaging. *Environ. Sci. Technol.* **2004**, *38*, 3864–3870.
(11) Ortega-Calvo, J. J.; Fesch, C.; Harms, H. Biodegradation of sorbed 2,4-dinitrotoluene in a clay-rich, aggregated porous medium. *Environ. Sci. Technol.* **1999**, *33*, 3737–3742.
(12) Lahlou, M.; Harms, H.; Springael, D.; Ortega-Calvo, J. J. Influence of soil components on the transport of polycyclic aromatic hydrocarbon-degrading bacteria through saturated porous media. *Environ. Sci. Technol.* **2000**, *34*, 3649–3656.
(13) Grimm, A. C.; Harwood, C. S. Chemotaxis of *Pseudomonas* spp. to the polyaromatic hydrocarbon naphthalene. *Appl. Environ. Microbiol.* **1997**, *63*, 4111–4115.
(14) Niqui-Arroyo, J. L.; Bueno-Montes, M.; Posada-Baquero, R.; Ortega-Calvo, J. J. Electrokinetic enhancement of phenanthrene biodegradation in creosote-polluted clay soil. *Environ. Pollut.* **2006**, *142*, 326–332.
(15) Wick, L. Y.; de Munain, A. R.; Springael, D.; Harms, H. Responses of *Mycobacterium* sp. LB501T to the low bioavailability of solid anthracene. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 378–385.
(16) Hiementz, P. C. *Principles of Colloid and Surface Chemistry*; Marcel Dekker Inc.: New York, 1986.
(17) Adler, J. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *J. Gen. Microbiol.* **1973**, *98*, 77–91.
(18) Garcia-Junco, M.; De Olmedo, E.; Ortega-Calvo, J. J. Bioavailability of solid and non-aqueous phase liquid (NAPL)-dissolved phenanthrene to the biosurfactant-producing bacterium *Pseudomonas aeruginosa* 19SJ. *Environ. Microbiol.* **2001**, *3*, 561–569.
(19) Rijnaarts, H. H. M.; Norde, W.; Bouwer, E. J.; Lyklema, J.; Zehnder, A. J. B. Bacterial adhesion under static and dynamic conditions. *Appl. Environ. Microbiol.* **1993**, *59*, 3255–3265.
(20) Law, A. M. J.; Aitken, M. D. Bacterial chemotaxis to naphthalene desorbing from a nonaqueous liquid. *Appl. Environ. Microbiol.* **2003**, *69*, 5968–5973.
(21) Law, A. M. J.; Aitken, M. D. Continuous-flow capillary assay for measuring bacterial chemotaxis. *Appl. Environ. Microbiol.* **2005**, *71*, 3137–3143.
(22) Bendinger, B.; Rijnaarts, H. H. M.; Altendorf, K.; Zehnder, A. J. B. Physicochemical cell surface and adhesive properties of coryneform bacteria related to the presence and chain length of mycolic acids. *Appl. Environ. Microbiol.* **1993**, *59*, 3973–3977.
(23) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, 2003.
(24) Reynolds, P. J.; Sharma, P.; Jenneman, G. E.; McInerney, M. J. Mechanisms of microbial movement in subsurface materials. *Appl. Environ. Microbiol.* **1989**, *55*, 2280–2286.
(25) Sharma, P. K.; McInerney, M. J.; Knapp, R. M. In situ growth and activity and modes of penetration of *Escherichia coli* in unconsolidated porous materials. *Appl. Environ. Microbiol.* **1993**, *59*, 3686–3694.
(26) Barton, J. W.; Ford, R. M. Determination of effective transport coefficients for bacterial migration in sand columns. *Appl. Environ. Microbiol.* **1995**, *61*, 3329–3335.
(27) Budrene, E. O.; Berg, H. C. Dynamics of formation of symmetrical patterns by chemotactic bacteria. *Nature* **1995**, *376*, 49–53.
(28) Park, S.; Wolanin, P. M.; Yuzbashyan, E. A.; Silberzan, P.; Stock, J. B.; Austin, R. H. Motion to form a quorum. *Science* **2003**, *301*, 188.
(29) Marshall, K. C.; Stout, R.; Mitchell, R. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* **1971**, *68*, 337–348.
(30) McClaine, J. W.; Ford, R. M. Reversal of flagellar rotation is important in initial attachment of *Escherichia coli* to glass in a dynamic system with high- and low-ionic-strength buffers. *Appl. Environ. Microbiol.* **2002**, *68*, 1280–1289.
(31) Ramia, M.; Tullock, D. L.; Phan-Thien, N. The role of hydrodynamic interaction in the locomotion of microorganisms. *Biophys. J.* **1993**, *65*, 755–778.
(32) Bayard, R.; Barna, L.; Mahjoub, B.; Gourdon, R. Investigation of naphthalene sorption in soils and soil fractions using batch and column assays. *Environ. Toxicol. Chem.* **1998**, *17*, 2383–2390.
(33) Celis, R.; Real, M.; Hermosin, M. C.; Cornejo, J. Sorption and leaching behaviour of polar aromatic acids in agricultural soils by batch and column leaching tests. *Eur. J. Soil Sci.* **2005**, *56*, 287–297.

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