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Biodegradation of Sorbed 2,4-Dinitrotoluene in a Clay-Rich, Aggregated Porous Medium

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The availability of clay-sorbed 2,4-dinitrotoluene (2,4-DNT) for degradation by *Burkholderia* sp. strain DNT was investigated in column experiments. Artificial aggregates of montmorillonite glued to glass spheres served as the sorbent. Sorption isotherms and bacterial kinetic parameters were determined in batches. Sorption of 2,4-DNT to clay aggregates gave reasonable fit to the Langmuir equation. The degradative activity of *Burkholderia* sp. strain DNT followed Michaelis−Menten kinetics. This allowed inferring bioavailable concentrations in the presence of clay from degradation rates. It appeared that montmorillonite-sorbed 2,4-DNT was readily available to *Burkholderia* sp. strain DNT. However, despite the accumulation of biomass in the columns due to filtration, absolute degradation rates remained constant, and specific rates continuously decreased toward the end of the experiments. Removal of suspended cells by miscible displacement led to a drastically reduced degradation rate that was not due to decreasing desorption, as 2,4-DNT concentrations in column effluents increased simultaneously. Decreasing degradation could be explained fairly well assuming that the specific activity of suspended cells remained at the initial value of 0.93 nmol mg dw^{-1} min^{-1}, whereas the specific activity of adhered bacteria steadily dropped to 0.12 nmol mg dw^{-1} min^{-1}. A likely explanation is the prolonged exposure (up to 6 h) to 2,4-DNT and nitrite for adhered cells, compared with a maximum exposure for suspended cells of 19.5 min, i.e., their residence time in the column. According to the Michaelis−Menten equation, the initial activity corresponded to a bioavailable concentration that exceeded the aqueous equilibrium concentration in the absence of bacteria by a factor of roughly two. The most probable explanation is a shift of the sorption equilibrium in the presence of cells, as direct accessibility of sorbed 2,4-DNT for suspended cells can be excluded.

Introduction

The use of nitroaromatic compounds (NAC) as explosives, pesticides, and dyes, and as intermediates in chemical syntheses, has led to their entrance in all major environmental compartments. NAC of natural origin are very rare, and virtually all incidences in aqueous systems (*1*, *2*), terrestrial systems (*3*, *4*), and the atmosphere (*5*, *6*) can be ascribed to human activities. 2,4-Dinitrotoluene (2,4-DNT) is formed as the major byproduct during the synthesis of the explosive 2,4,6-trinitrotoluene (TNT). Due to its abundance in the environment and its toxicity, it is treated as a priority pollutant in several countries.

One of the processes that control the transport, chemical transformation, and biological degradation of contaminants in the subsurface is sorption to the solid matrix of soils and aquifers. Sorption to mineral surfaces can be an important process in environments with low content in organic matter or with chemicals which specifically interact with mineral surfaces, leading to increased affinity. This applies to NAC, which may sorb to clay minerals by forming electron donor−acceptor complexes (*7*). A consequence of specific sorption to clay minerals in a sandy aquifer is the much slower movement of 2,4,6-trinitrotoluene (TNT) than of the aliphatic explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (*8*). As the two chemicals have similar hydrophobicity, the stronger retardation of TNT, corresponding to a much higher distribution coefficient, K_d (21500 versus 1.2 L/kg of RDX) (*7*), visualizes the effect of its specific sorption to clay. K_d of up to 60 000 L/kg between clay and water have been reported for other NAC (*7*, *9*). Generally, planar NAC with several electron-withdrawing groups exhibit highest sorption. An important characteristic of NAC sorption to clay is the competition between different NAC and with strongly hydrated exchangeable cations such Ca^{2+}, Na^+, or H^+.

Strong sorption of environmental chemicals may have consequences for their biological breakdown, as it has been suggested that only water-dissolved chemicals are available for biodegradation (*10*, *11*). In this study, we aimed at quantifying the bioavailability of clay-sorbed 2,4-DNT for bacterial degradation. Complete breakdown of 2,4-DNT by *Burkholderia* sp. strain DNT was shown first by Spanggord et al. (*12*). It is initiated by the oxidative release of both nitro groups as nitrite, followed by ring cleavage of the resulting 2,4,5-trihydroxytoluene (*13*).

We employed artificial aggregates of montmorillonite and polyvinyl alcohol (PVA) glued to glass spheres. This enabled us to perform column percolation experiments, which is hardly possible with pure clay because of its low hydraulic conductivity. With this model system we were able to show that montmorillonite-sorbed 2,4-DNT was readily, although not directly, available to *Burkholderia* sp. strain DNT. It appeared that 2,4-DNT had to desorb prior to being degraded. However, the sorption equilibrium seemed to be shifted in the presence of bacteria. It was furthermore apparent that bacteria adhered to clay aggregates were considerably less active than suspended bacteria.

Materials and Methods

Chemicals. 2,4-DNT was purchased from Aldrich (Buchs, Switzerland) and recrystallized from ethanol before use. Montmorillonite K 10 (<20 μm) and polyvinyl alcohol (100 000) (PVA) were obtained from Fluka AG (Buchs, Switzerland). Glass beads (diameter, 250−310 μm) were from Roth AG (Reinach, Switzerland).

Bacteria, Media, and Cultivation. *Burkholderia* sp. DNT was a gift of J. C. Spain (Tyndall Air Force Base, FL, U.S.A). This bacterium was used because it degrades 2,4-DNT with stoichiometric release of 2 mol of nitrite per mol of 2,4-DNT (*12*). For biodegradation experiments, the bacterium was

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grown for 2 days in 1 L of mineral salt medium (*12*) supplied with 200 mg/L yeast extract and 549 μM 2,4-DNT at 30 °C on a rotary shaker at 180 rpm. Cultures were washed twice, resuspended in 500 mL of mineral medium containing 200 μM 2,4-DNT, and incubated under the same conditions for another 48 h. This procedure served to yield high amounts of cells grown on 2,4-DNT as the sole C-source. Growth during this phase of cultivation was confirmed with optical density measurements. Direct cultivation with 2,4-DNT as the sole carbon source was not possible with dilute inocula due to the toxicity of this compound. Cells were harvested, washed twice in phosphate buffer (pH 7.5) containing 0.286 g/L of KH_2PO_4 and 1.184 g/L of K_2HPO_4, and resuspended in the same buffer. They were used for biodegradation experiments immediately after this procedure. Cells used to quantify biosorption of 2,4-DNT were grown overnight in mineral salt medium with 200 mg L^{-1} yeast extract, washed, and resuspended in phosphate buffer.

Sorbent. Montmorillonite aggregates attached to glass beads were used as the sorbent. PVA was used as aggregating agent. Sorption of NAC by PVA had been found to be negligible (*14*). Glass beads were cleaned with chromosulfuric acid, washed with 100 mM KCl and then with distilled water, and subsequently dried to constant weight. The aggregates were made as previously described (*15*) with certain modifications: 100 g of glass beads were added to a suspension of 0.5 g of PVA in 200 mL of distilled water. The mixture was shaken and kept at 40 °C for approximately 15 min. Then, a suspension of 10 g of montmorillonite in 50 mL of water was added, and most of the water in the mixture was removed with a rotation evaporator. The mixture was transferred to a glass beaker, further dried on a hot plate under continuous stirring, and sieved (500 μm mesh). Excess clay and PVA were removed by repeated washing with water. The aggregates were filled into glass columns connected to a peristaltic pump and rinsed thoroughly with 0.1 M KCl to obtain homoionic K^+-clay. After washing with distilled water, the sorbent was dried at 60 °C.

Analytical Methods. Nitrite and thiourea were measured colorimetrically (*12*, *16*). HPLC analysis of 2,4 DNT was performed on a Lichrospher 100, RP-8 (5 μm) column (Merck, Darmstadt, Germany). The conditions of analysis have been described elsewhere (*9*). Dry weight (dw) determinations were performed after filtering cultures through cellulose acetate filters (pore diameter <0.45 μm) and drying to constant weight. Scanning electron microscopy of gold-sputtered aggregates with and without bacteria was performed with a Philips XL 30 microscope (Philips, Eindhoven, The Netherlands).

Sorption. Batch sorption experiments with clay aggregates or bacteria as the sorbents were conducted in 1.8 mL-borosilicate glass vials fitted with aluminum seals. The vials contained either 20 mg of clay aggregates or 0.19 mg (dw) of yeast-grown bacteria and 1.5 mL of phosphate buffer with various concentrations of 2,4-DNT as the sorbate. Equilibration was achieved on an end-over-end shaker (2 rpm) for 3 h. Preliminary experiments showed that this time period was sufficient to achieve equilibrium. The sorbents were removed by centrifugation, the liquid was analyzed for 2,4-DNT, and sorbed amounts were calculated. Isotherms were fitted to the Langmuir relationship

$$C_s = \frac{K_L C_w}{1 + K_L C_w} C_{smax} \quad (1)$$

where C_s and C_w are sorbed (μmol/kg) and dissolved (μM) 2,4-DNT concentrations, respectively, K_L is the Langmuir constant (μM^{-1}), and C_{smax} (mol/kg) is the adsorption capacity, and to the Freundlich relationship

$$C_s = K_F C_w^{\,n} \quad (2)$$

where K_F is the Freundlich constant and n is the parameter indicative of the nonlinearity of the isotherm. The affinity of sorption was described by the distribution coefficient K_d (L/kg) in the linear range of the isotherm

$$K_d = \frac{C_s}{C_w} \quad (3)$$

Batch Degradation Experiments. The kinetic parameters of resting cells suspended in phosphate buffer were determined in a batch system. For this aim, 25 mL of buffer solutions with different concentrations of the nitroaromatic compound were placed in 100 mL Erlenmeyer flasks and inoculated with 2,4-DNT-grown bacteria (final $OD_{600} = 0.4$). The flasks were incubated at 25 °C on a rotary shaker operating at 180 rpm. At certain time intervals, samples were taken from the flasks and centrifuged for 2 min at 12000 rpm. The supernatants were analyzed for nitrite and 2,4-DNT. Under these conditions, nitrite release from 2,4-DNT was stoichiometric and occurred without a lag period. The specific activity remained constant for at least 4 h (data not shown). Therefore, nitrite release was generally taken as an easy measure for 2,4-DNT biodegradation. Specific activities at various 2,4-DNT concentrations (up to 200 μM) were fitted by nonlinear regression to the Michaelis−Menten equation

$$V_s = \frac{V_{max} C}{K_m + C} \quad (4)$$

where V_s is the specific rate of 2,4-DNT biodegradation (nmol mg dw^{-1} min^{-1}), V_{max} is the maximum specific activity (nmol mg dw^{-1} min^{-1}), C is the substrate concentration (μM), and K_m is the substrate concentration (μM) resulting in $V_s = {}^1/_2 V_{max.}$ V_{max} at 20 °C was 7.6 (± 0.25) nmol mg dw^{-1} min^{-1} and K_m was 60.1 (±8.1) μM.

Column Experiments. Biodegradation experiments were performed at 25 °C in percolated columns according to the method of Harms and Zehnder (*10*). Aggregates containing sorbed 2,4-DNT were prepared by equilibration with solutions of the nitroaromatic compound in 20-mL glass vials with aluminum-lined screw caps for at least 3 h. The aggregates were wet packed in glass columns of 5 cm length and 1 cm internal diameter. The amount of packing present in each column was 4.3 g. The gravimetrically estimated porosity of the packing was 0.40, corresponding to a pore volume of 1.56 mL. The columns were connected to a peristaltic pump, and suspensions of 2,4-DNT-grown bacteria were pumped through the columns at a constant flow rate of 0.25 cm/min. Under these conditions, one pore volume took approximately 20 min to pass through the columns. The amount of 2,4-DNT sorbed at this time was calculated from the aqueous equilibrium concentration before packing, using the sorption isotherm. This value was corrected for the dissolved 2,4-DNT removed during packing and removal of air from the tubing system. Calculations showed that the constant flow guaranteed a sufficient supply of oxygen for the aerobic biodegradation of the compound at the concentrations of substrate and cells tested. Column effluents were collected at time intervals, put on ice to stop biodegradation, and immediately centrifuged for 2 min at 12 000 rpm. The supernatants were analyzed for nitrite and 2,4-DNT. 2,4-DNT was also measured in the effluent of columns without bacteria. Sorption of nitrite to the column packing was checked and could be excluded since there was no loss of solute from a 100 μM nitrite solution percolated through the columns. Specific activity of the bacteria present in the column feed reservoir remained constant during the experi-

ments. This was confirmed by the nitrite release rate of aliquots that were routinely taken from the reservoir and incubated with 2,4-DNT.

Specific biodegradation rates V_s (nmol mg dw^{-1} min^{-1}) in columns were calculated as

$$V_s = \frac{^1/_2 C_n V_{sample}}{X_{sus} t_{res} + X_{att} t_{sample}} \quad (5)$$

where C_n is nitrite concentration in the effluent (μM), V_{sample} is the sample volume (mL), X_{sus} is the biomass of suspended bacteria transported with the sample volume (mg dw), t_{res} is the residence time in the column (min), X_{att} is the biomass attached to the column matrix (mg dw), and t_{sample} is the sampling interval (min). Equation 5 accounts for the fact that suspended cells can release nitrite from 2,4 DNT only during their passage through the column, the duration of which is t_{res}. Before they enter the column, 2,4-DNT is unavailable, and after they leave the column, cooling stops their activity. Adhered bacteria can release nitrite from 2,4-DNT during the whole sample interval. Column breakthrough of bacteria was followed photometrically and compared to the breakthrough of the conservative tracer thiourea (*14*). The efficiency of filtration of bacteria was expressed as OD in column effluents C divided by those in column influents C_0. C/C_0 values and percolated volumes were used to calculate biomass accumulation in the columns during the experiments. All column experiments were done at least in duplicate. Light microscopy confirmed the absence of clay particles in column effluents.

Results

Batch Sorption Experiments. Scanning electron micrographs of clay-coated glass beads showed that the glass surface was discontinuously covered by clay aggregates of up to 10 μm depth. Sorption experiments indicated that a significant clay surface area in the aggregates was available for sorption. The sorption isotherm (Figure 1A) was convex, which is typical for interactions with surfaces of limited sorption capacity (*7*). It gave a slightly better fit to the Langmuir equation than to the Freundlich equation, with $K_L = 0.0698\ \mu M^{-1}$ and $C_{smax} = 2194\ \mu$mol kg^{-1}. K_d calculated from the linear part of the isotherm comprising the first four values was 302 L/kg. The deviation from the Langmuir fit (Figure 1A), i.e., more sorption at low concentrations, suggests an affinity distribution of the sorption sites, which is consistent with previous observations on NAC sorption to clay (*7*).

2,4-DNT sorption to yeast-grown *Burkholderia* sp. cells also resulted in a nonlinear isotherm (Figure 1B), which gave a reasonable fit to the Freundlich equation with $n = 0.54$ reflecting the convex shape of the isotherm. The maximum K_d of 21 860 L/kg, calculated from the first value of the isotherm, was 800 times higher than expected from the octanol−water partition coefficient (K_{ow}) of 2,4-DNT (*17*) and an empirical relationship between K_{ow} and the K_d for sorption to bacteria (*18*). The nonlinearity of the isotherm further suggested that a mechanism other than physical partitioning contributed to the sorption. Nitrite release by these uninduced cells from 2,4-DNT was below the detection limit of 300 nM h^{-1}, indicating the absence of constitutive degradative activity.

Column Experiments. Cell suspensions pumped through columns packed with clay-coated glass beads broke through slightly behind the conservative tracer thiourea, indicating little retardation of bacteria by the solid phase (Figure 2). The plateau C/C_0 value of 0.75 after breakthrough indicated cell deposition on the sorbent. This was confirmed by electron microscopy of material from loaded columns, which showed bacteria attached to the clay-aggregates. Because adhesion

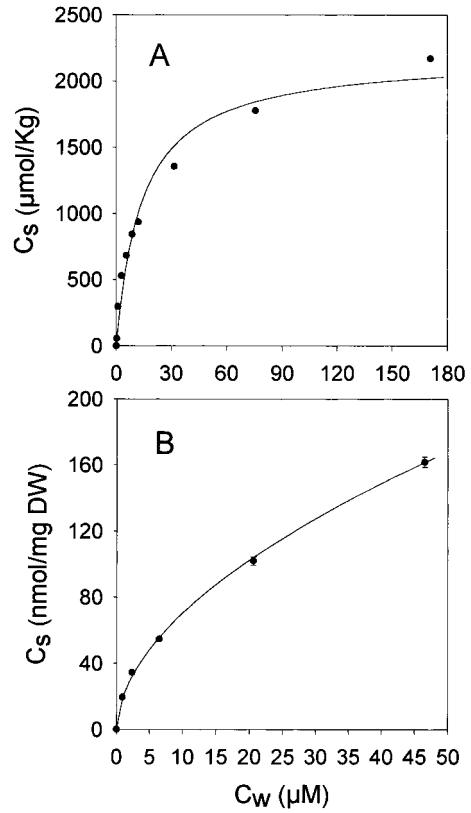

FIGURE 1. Isotherms for 2,4-dinitrotoluene sorption to montmorillonite aggregates (A) and *Burkholderia* sp. cells (B). The solid lines represent curve fittings to the Langmuir (A) and Freundlich equation (B), respectively.

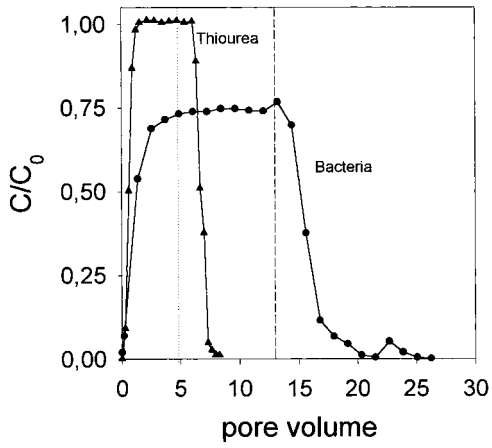

FIGURE 2. Breakthrough curves of thiourea and bacteria in columns packed with clay-coated glass beads. C_0 was 60 μM for thiourea and OD_{280} of 0.621 for bacteria. The dotted lines indicate the substitution of thiourea solution (left) and bacterial suspension (right) by buffer.

is a strict function of the concentration of suspended bacteria, that value also suggests the occurrence of a slight gradient in the concentration of adhered bacteria, with a probable difference of 25%, between the inlet and the outlet. The slight tailing of the biomass concentration in the effluent during subsequent flushing with buffer can be ascribed to reversible adhesion of a small fraction of the cells.

Percolation of columns containing sorbed 2,4-DNT either with phosphate buffer or with suspensions of 2,4-DNT-grown bacteria resulted in desorption or desorption/biodegradation of the compound, respectively. Figures 3 and 4 show the outcome of two experiments conducted with different sorbed

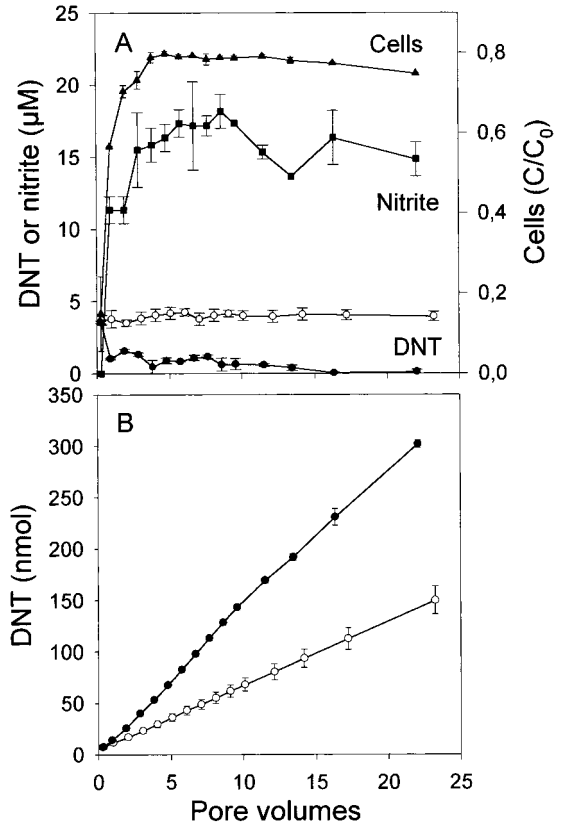

FIGURE 3. Biodegradation and leaching of 2,4-DNT from clay aggregates. Columns initially containing 1.93 μmol of sorbed 2,4-DNT were percolated at a rate of 0.25 cm min^{-1} either with a bacterial suspension of an OD_{600} of 0.606 or with buffer. Symbols represent effluent concentrations of cells (▲), nitrite (■), and 2,4-DNT (●) from columns percolated with cells and 2,4-DNT from cell free columns (○) (A). Cumulated amounts of leached (○) or leached and transformed (calculated from released nitrite) 2,4-DNT (●), in abiotic and biotic columns, respectively (B). Error bars represent standard deviations.

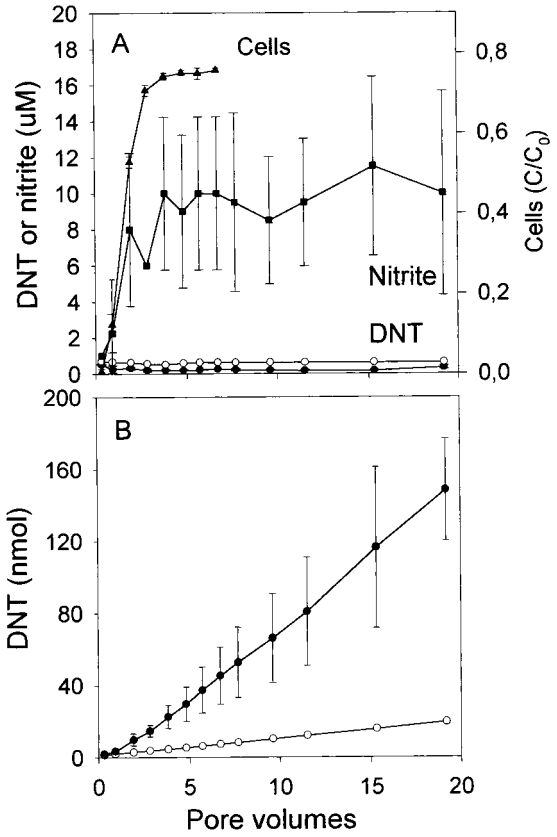

FIGURE 4. Biodegradation and leaching of 2,4-DNT from clay aggregates. Columns initially containing 0.99 μmol of sorbed 2,4-DNT were percolated at a rate of 0.25 cm min^{-1} either with a bacterial suspension of an OD_{600} of 0.621 or with buffer. Symbols represent effluent concentrations of cells (▲), nitrite (■), and 2,4-DNT (●) from columns percolated with cells and 2,4-DNT from cell free columns (○) (A). Cumulated amounts of leached (○) or leached and transformed (calculated from released nitrite) 2,4-DNT (●), in abiotic and biotic columns, respectively (B). Error bars represent standard deviations.

amounts of 2,4-DNT. When 1.93 (Figure 3) or 0.99 μmol 2,4-DNT (Figure 4) were initially sorbed to the column content, the 2,4-DNT concentrations in the effluent of columns without bacteria were constant at 3.9 ± 0.2 and 0.62 ± 0.04 μM, respectively (open symbols in Figures 3A and 4A). This corresponds to desorption rates of 0.40 and 0.056 nmol/min. These effluent concentrations differ from the initial equilibrium C_w of 5.0 or 2.19 μM, respectively, indicating that the residence time was too short for equilibration. The constancy of effluent concentrations during percolation indicated that the amount of sorbed 2,4-DNT directly exposed to the flowing liquid did not significantly decrease during the desorption experiment. This was probably because the transfer of 2,4-DNT from the inner parts of the aggregates to the bulk water was fast enough to replace 2,4-DNT desorbing from the exterior. This is no surprise, as during the experimental period only 150 or 20 nmol (7.8% and 2.0%) of the total 2,4-DNT desorbed (open circles in Figures 3B and 4B). The 2,4-DNT concentrations in the effluents from columns with bacteria decreased rapidly after two pore volumes, concomitantly with the appearance of nitrite, which reached steady-state effluent concentrations of 15.5 ± 2.1 and 9.3 ± 1.4 μM, respectively (closed symbols in Figures 3A and 4A). Taking into account the stoichiometry of nitrite production from 2,4-DNT, the total rates of 2,4-DNT desorption were 0.80 and 0.43 nmol/min. During the experimental period, 303 or 149 nmol (15.7% and 15.1%) of the total 2,4-DNT desorbed (closed circles in Figures 3B and 4B). Specific biodegradation rates V_s were 0.92 and 0.51 nmol mg dw^{-1} min^{-1} immediately after nitrite breakthrough. At this time, the fraction of adhered bacteria in the total biomass was small. According to eq 4, these specific activities correspond to 2,4-DNT concentrations of 8.6 and 4.5 μM. These values exceeded the equilibrium C_w by a factor of roughly two. Although there was considerable accumulation of biomass in the columns due to filtration, the absolute degradation rates remained relatively constant, and the specific rates V_s continuously decreased to 0.32 and 0.22 nmol mg dw^{-1} min^{-1} toward the end of the experiments.

We considered three hypotheses which could have explained the decrease of the specific activity during the experiment: (i) underestimation of desorption rates at later stages of the experiment because the increasing attached biomass in the columns sorbed more and more 2,4-DNT, (ii) reduced activity of adhered bacteria, and (iii) declining desorption rates during the experimental period.

(i) Sorption of 2,4-DNT by the biomass accumulating in the column was insufficient to explain the loss of specific activity. Theoretically, it could have resulted in lowered dissolved 2,4-DNT concentrations toward the end of the experiments. However, according to the relationship in eq 2, maximum final amounts sorbed to bacteria were 14 and 21 nmol for the experiments shown in Figures 3 and 4, respectively. This is insignificant compared with the 1627 and 840 nmol 2,4-DNT still sorbed to the clay aggregates at the end of the two experiments. Hence, 2,4-DNT depletion

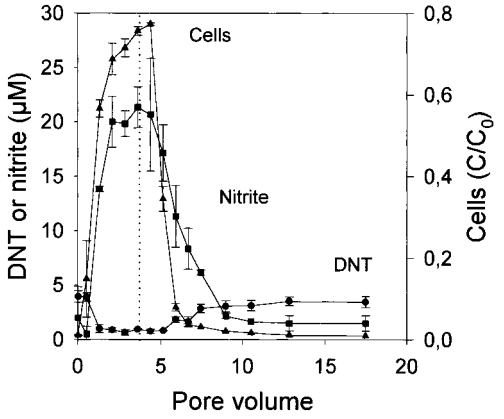

FIGURE 5. Effect of substitution of the cell suspension by buffer (indicated by the dotted line) on biodegradation and leaching of 2,4-DNT from clay aggregates. Columns containing 1.93 μmol of sorbed 2,4-DNT were initially percolated with a bacterial suspension of an OD_{600} of 0.606 at a rate of 0.25 cm min^{-1}. Symbols represent effluent concentrations of cells (▲), nitrite (■), and 2,4-DNT (●). Error bars represent standard deviations.

due to biosorption could have contributed only little to the observed decline in specific activity.

(ii) An experiment was conducted to assess the contribution of adhered bacteria to 2,4-DNT degradation. After a filtration period, nonadhered bacteria were eliminated from the columns by switching the influent from bacterial suspension to buffer (Figure 5). The initially sorbed amount of 2,4-DNT, the density of the cell suspension, and the flow rate were identical to those in the experiment shown in Figure 3. At the time of the switch, the suspended biomass contributed less than 50% to the total biomass in the column. The switch resulted, nevertheless, in a decrease of the effluent nitrite concentration from 20.0 μM, corresponding to V_s of 0.93 nmol mg dw^{-1} min^{-1}, to 0.8 μM, corresponding to V_s of 0.12 nmol mg dw^{-1} min^{-1}, in the absence of suspended cells at the end of the experiment. It is obvious that during the course of the experiment, adhered cells lost part of their activity. A likely explanation is their prolonged exposure to 2,4-DNT and nitrite of up to 6 h as compared with a maximum of 19.5 min for suspended cells, i.e., their residence time in the column.

(iii) After washout of the suspended cells (Figure 5), 2,4-DNT in the effluent rose to almost the initial concentration. This and the large amounts of 2,4-DNT still sorbed at the end of the experiments indicated that desorption was not the cause of decreasing specific degradation rates in later stages of the degradation experiments.

Discussion

Degradation experiments suggested that montmorillonite-sorbed 2,4-DNT was readily available to *Burkholderia* sp. strain DNT. This could be inferred from experimentally obtained specific activities, which were higher than expected from eq 4 when assuming access to only the equilibrium C_w of 2,4-DNT. It seemed that the presence of *Burkholderia* sp. strain DNT influenced the extent of sorption. The high activity of suspended bacteria indicates that 2,4-DNT desorbed prior to being degraded, since sorbed 2,4-DNT could have been directly available only to adhered cells. Theoretically, it can be conceived that the adsorption equilibrium is shifted in the presence of bacteria, i.e., bacteria close to the clay could have reduced the affinity of 2,4-DNT to clay. The K_d of NAC with homoionic K^+-clay is substantially higher than in the presence of highly hydrated cations such as Na^+ or H^+ (*7*). Locally increased concentrations of these ions, due to their excretion in the course of bacterial energy conservation or to local acidification as a result of CO_2 formation, may have promoted desorption. It is unlikely that the kinetic parameters of suspended bacteria changed upon addition to a column. Whereas a kind of sensing of the nearby surface may be conceived for adhered bacteria, suspended bacteria in columns were in a situation similar to the reference batch system used to characterize their kinetic parameters.

The high activity of suspended cells, caused by increased accessible substrate concentrations, contrasted with the reduced activity of adhered cells. It is not likely that the transfer of 2,4-DNT from clay to clay-adhered bacteria is less than to suspended bacteria. Smith et al. (*19*) attributed reduced degradation of acetate in the presence of montmorillonite and hectorite to the physical impairment of the substrate uptake by clay particles covering the cells. Figure 1B shows that this is not the case in our experiments. By intensively washing the material and using a flow-through system, we assured that there were no free clay particles available, which could have covered the cells. More likely is an inhibitory effect of 2,4-DNT and/or nitrite. Our findings deviate from earlier observations with *Sphingomonas* sp. HH19k degrading 3-chlorodibenzofuran (*10, 20*). When this organism was adhered to Teflon that released its sorbed substrate, its specific activity indicated unchanged kinetic parameters and exposure to the equilibrium C_w.

Desorption rates were considerably higher during percolation with bacterial suspension than with buffer. This can be explained by locations of the sinks for 2,4-DNT being different, namely the bacteria or the column outlet, respectively. Flushing with buffer creates both a longitudinal concentration gradient with increasing dissolved concentrations and decreasing desorption toward the column outlet and, at the same time, local concentration gradients between the sorbent's surface and the bulk liquid. The latter gradients extend from the glass surface through the layer of clay, far into the bulk liquid. Flow rates applied in our experiments caused laminar flow with stagnant layers >60 μm as could be calculated by the method described by Levich (*21*). Desorbing substrate molecules have to pass this zone by diffusion, i.e., local concentration gradients are formed. It has been shown before that both artificial clay aggregates and the stagnant zone around particles impede the transfer of molecules from the solid phase to the bulk water or vice versa (*14, 22*). Obviously, the residence time of the buffer was insufficient for complete equilibration. Biodegradation takes place over the whole column length, within the stagnant layer, and probably to some extent between the clay particles, resulting in much steeper concentration gradients which, in turn, promote desorption (*23*). In addition, locally increased concentrations are at least partly counterbalanced, as they prompt higher degradation rates according to eq 4. The occurrence of local concentration gradients close to the sorbent surface may have caused adhered bacteria to be exposed to inhibiting concentrations of 2,4-DNT and nitrite that led to their reduced activity.

Strong accumulation on clay a priori reduces the dissolved concentration of NAC as the factor controlling specific degradation rates. However, our experiments indicate that the exchange of 2,4-DNT between clay and adjacent water is fast, may be influenced by the presence of biomass, and does not limit the availability of the sorbed compound to suspended bacteria in the neighborhood of the sorbent. Considering the toxicity of NAC, reduced concentrations (due to sorption) in combination with ready availability (due to fast desorption) may be favorable, if not a prerequisite, for biological cleanup. Biodegradation technologies involving sorbents such as activated carbon make use of the detoxifying activity of the sorbent and the relatively fast transfer of desorbing substrate to nearby bacteria. The detoxifying effect of sorbents may also be used in new strategies for the selective

enrichment of new NAC-degrading bacteria from environmental samples. This study, however, may have limited relevance for remediation of aged contaminated material, where desorption rates may become very slow. From our findings, we can state that short-term sorption to clay does not prevent the degradation of 2,4-DNT, provided bacteria are present in the vicinity of the sorbent and active at the prevailing concentrations. The apparent deactivation of bacteria as a result of attachment to clay aggregates containing 2,4-DNT should be the subject of further investigation.

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