Biodegradation **12:** 419–432, 2001.

Degradation of dissolved and sorbed 2,4-dichlorophenol in soil columns by suspended and sorbed bacteria

Göran Bengtsson* & Christel Carlsson
*Department of Ecology, Lund University, Sölvegatan 37, SE-223 62 Lund, Sweden (*author for correspondence; e-mail: goran.bengtsson@ekol.lu.se)*

Accepted 21 November 2001

Key words: aquifer, bioremediation, breakthrough, diffusion, Pseudomonas, sorption

Abstract

The influence of sorption of bacteria, as well as 2,4-dichlorophenol (2,4-DCP), on the mineralization of 100 μg l^{-1} of the organic compound was examined in an aquifer material under advective flow conditions (column displacement technique). The study was designed to distinguish the rates and extent of biodegradation of the sorbed and the dissolved trace organic and the contribution of sorbed and suspended bacteria to the degradation. The degradation of dissolved 2,4-DCP was significantly faster than the degradation of the same compound sorbed to the solids, and suspended bacteria degraded the dissolved compound at a higher rate than sorbed bacteria, also on a per cell basis. The suspended bacteria degraded 8–12% of the added dissolved 2,4-DCP, while sorbed bacteria made a smaller contribution by degrading about 5% of sorbed 2,4-DCP. No degradation was seen with sorbed 2,4-DCP and suspended bacteria, and a marginal contribution was made by sorbed bacteria on the degradation of dissolved 2,4-DCP (<0.4%).

Introduction

Bearing in mind the high volumetric content of solids in soils and sediments, it seems conceivable that interactions between the solids and bacteria are important in controlling the fate of toxic organic compounds. If a compound becomes available to a cell only through uptake from the aqueous phase, sorption of the chemical to the soil may decrease the degradation rate at low concentrations (Smith et al. 1992) or limit the degradation rate to the desorption rate (Robinson et al. 1990; Mihelcic & Luthy 1991; Harms & Zehnder 1995), which may be controlled by slow diffusion within the sorbent and in the pore water and by chemical reactions at the sorbent surface. Sorption may even prevent biodegradation of soil contaminants (Ogram et al. 1985; Weber & Coble 1968), especially in soils with high concentrations of soil organic carbon (Weissenfels et al. 1992).

The limitations of the mass transfer of sorbate to the cell surface by diffusive transport call attention to the influence of fluid mechanics in the interstitial pore environment. Increased advective flow across the cells could promote the uptake rates of low-molecular-weight compounds (Logan & Dettmer 1990), a phenomenon that has implications for the prediction of contaminant transport in soils with preferential flow in macropores and channels, in which the biodegradation rates tend to be faster than in the surrounding diffusion-limited soil matrix (Pivetz et al. 1996). Since sorption also tends to be facilitated by a reasonably high advective flow (Akratanakul et al. 1983b), through its compression of the hydrodynamic boundary layer, sorption and biodegradation may interact in concert in macropores and channels to prevent contaminants from spreading in subsurface, water-saturated soil environments.

The subsurface is normally an oligotrophic environment, encompassing soils with less than 0.1% of organic carbon and less than 10 mg carbon (DOC) per litre in their pore water. Bacterial densities in pristine subsurface sediments and groundwater normally range between 10^5 and 10^7 per gram of sediment or per litre of groundwater (Ghiorse & Wilson 1988), which re-

flect the oligotrophic conditions of pristine aquifers. Only 1 to 10% of the cells are metabolically active, and their activities and growth rates are lower than in bacteria from surface soils and waters (Gehlen et al. 1985). Despite their limited metabolic activity, subsurface bacteria are known to degrade xenobiotics at concentrations below 0.1 μg g^{-1} (Hutchins et al. 1991; Swindoll et al. 1988a). Mineralisation represents often less than 10% of the total metabolism, and the remaining is incorporated into biomass. By contrast, up to 90% of the carbon of added xenobiotics are converted to CO_2 in surface-water environments (Subba-Rao et al. 1982). The presence of xenobiotics in subsurface environments (Bhatt 1997), is of major environmental concern, and strategies to prevent their contamination of drinking water reservoirs and to facilitate bioremediation require accurate estimates of transport and fate under subsurface conditions.

The aim of this work was to examine the interaction between sorption and biodegradation in an aquifer material under advective flow conditions (column displacement technique). We used 2,4-dichlorophenol to estimate the contribution of sorbed compound to the total biodegradation in the system. The major sources of contamination by phenols have been industrial effluents, agricultural runoff, chlorination of wastewater, and as transformation products from other chemicals. Numerous reports describe the biodegradation transformation of phenols by a suite of monooxygenases and dioxygenases (Steiert & Crawford 1985), even in aquifer material (Smith & Novak 1987). The specific purpose of the study was to determine the rates and extent of utilization of adsorbed and dissolved 2,4-dichlorophenol by adsorbed and suspended bacteria in stainless-steel columns containing aseptically treated aquifer sand and sterile filtered groundwater. Closely related was the goal of estimating the contribution of adsorbed 2,4-dichlorophenol to the total biodegradation in the aquifer material. Our hypotheses to be tested were that (1) adsorbed 2,4-dichlorophenol would primarily be metabolized by suspended bacteria as a result of an increased desorption caused by the metabolism of dissolved 2,4-dichlorophenol by suspended cells; (2) adsorbed bacteria would mainly influence the metabolism of dissolved 2,4-dichlorophenol by adsorption and subsequent cell uptake, in contrast to adsorbed 2,4-dichlorophenol, which should be largely unavailable for the cells as long as it is bound to the sorbent.

Materials and methods

Organism

The soil bacteria *Pseudomonas putida EST4021* (Mäe et al. 1993) was used. It carries the plasmid pEST4011 encoding enzymes for the catabolism of 2,4-dichlorophenol (2,4-DCP).

Culturing and labelling of bacteria

The strain was precultured at room temperature over night on an orbital-shaker at 150 rpm in 20 ml of 20% Luria-Bertani (LB) medium. A volume sufficient to give an initial cell density of about 10^5 cells per ml (direct count in a Bürker chamber) was transferred to 200 ml of fresh 20% LB spiked with 50 μCi l^{-1} of [2,8,5-^{3}H]-adenosine (DuPont, 61.5 Ci $mmol^{-1}$). When the cells had taken up sufficiently high activity of ^{3}H-adenosine to allow their detection in the column experiments (i.e. after two to three days of incubation), they were centrifuged at 9,000 $\times$ g, 4 °C for 10 min on a Sorvall RC 5B Plus centrifuge (DuPont, Newtown) and resuspended in filter-sterilized groundwater (0.2 μm Millipore GS filter) collected from a well at the Vomb water treatment plant, which provides drinking water for the cities of Lund and Malmö from Lake Vombsjön. The centrifugation was repeated, and the bacteria were resuspended in sterile groundwater to working densities. Labelled bacteria were stored at groundwater temperature (12 °C) for less than 24 hours before used in sorption or degradation experiments.

Preparation of sand

The sand was collected from the same infiltration field at Lake Vombsjön as above and had a total organic carbon content (TOC) of about 0.2%. The sand was dried (2 days, 105 °C) and sieved to give a particle size <630 μm and then sterilized by autoclaving (121 °C, 20 min).

Batch sorption experiments

Bacterial sorption isotherm

The labelled bacterial stock solution was diluted with filter sterilized groundwater to six different cell densities ranging from 5.0 $\times$ 10^7 to 7.5 $\times$ 10^8 cells ml^{-1}. Nine ml of the bacterial solutions were added to 10-ml

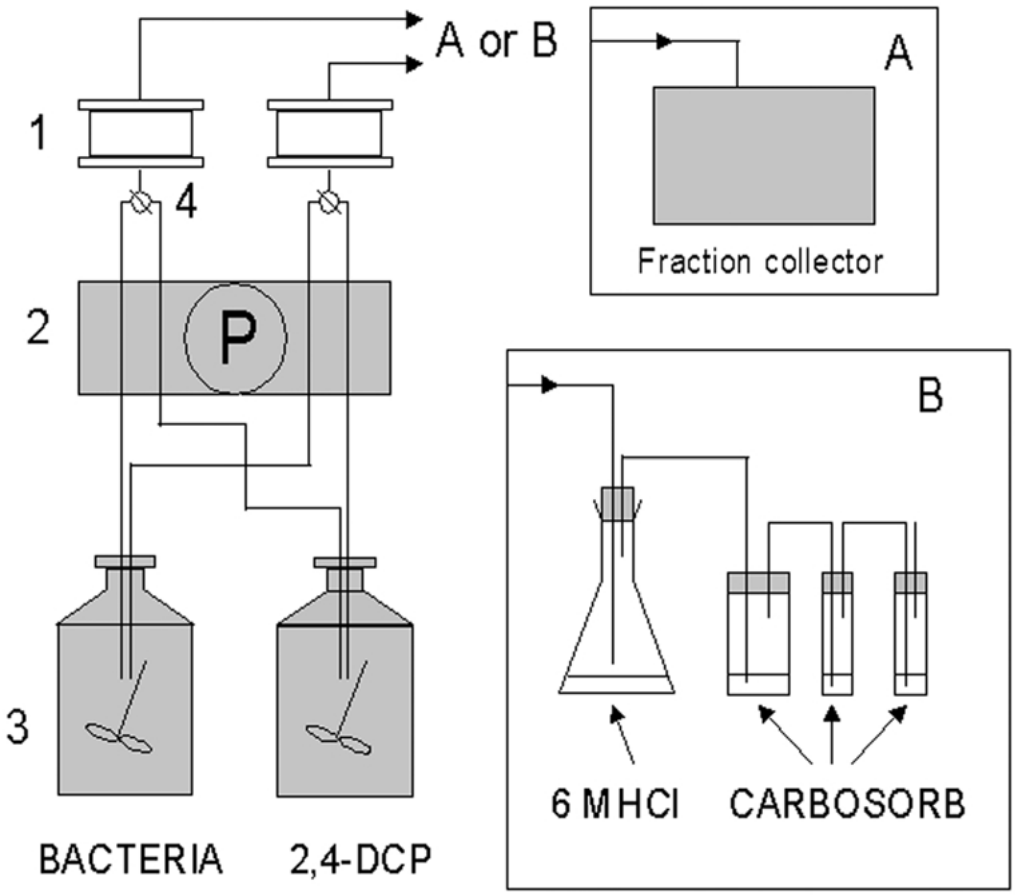

Figure 1. Schematic representation of the experimental set up for the continuous flow experiment. (1) Stainless steel columns. (2) Peristaltic pump. (3) Feed solutions with ^{3}H-bacteria and ^{14}C-2,4-DCP. (4) T-piece. Samples were collected either with an automatic fraction collector (A) or in an Erlenmeyer flask connected in a series with three scintillation vials (B).

Pyrex test tubes with screw caps containing one gram of sand. After equilibration for 64 h on an end-over-end shaker at 12 °C, sand and sorbed bacteria were allowed to settle for 30 minutes, followed by a gentle centrifugation at 200 × *g* for 2.5 minutes on a Hermle centrifuge model Z252MK. The ^{3}H-activity of the labelled cells was quantified in five replicates by withdrawing one ml of the aqueous phase from the vials, centrifuging and washing the cells twice as described before, and resuspending them in one ml of groundwater. Thereafter, they were mixed with 4 ml of Beckman Ready Safe scintillation cocktail, and the ^{3}H-activity was measured on a Beckman LS 1801 liquid scintillator. The activity in the sorbed cells was calculated as the difference between the total activity added to the vials and the activity of suspended cells and supernatant of the aqueous phase after centrifugation of cells. The partitioning coefficient was calculated from the slope of the linear sorption isotherm, which is a plot of the mass of cells in the aqueous phase versus the mass of cells on the solid phase. Controls without sand were included to measure sorption of bacteria to the glass surface.

The equilibrium sorption isotherm for the bacteria to the sand was linear in the density range used (5.0×10^{7} to 7.5×10^{8}, $r = 0.99$, Figure 2). The sorption coefficient, K_b, was calculated as 1.3 ml g^{-1}.

2,4-DCP sorption isotherm

Six different concentrations of U-^{14}C-2,4-DCP (American Radiolabelled Chemicals Inc., 10 mCi mmol^{-1}), ranging from 8.15 to 97.8 μg l^{-1}, were equilibrated on an end-over-end shaker with one gram of sterilized sand in 10 ml Pyrex test tubes at 12 °C for 21 hours. The tubes were centrifuged at 200 × *g*

for 20 minutes before the ^{14}C-activity in one ml of the supernatant was analysed by scintillation counting as described above. Controls without sand were included in the study to calculate the loss due to sorption to the glass surface.

The sorption isotherm for 2,4-DCP was linear in the investigated concentration range (r = 0.99), and the K_d was calculated as 0.33 ml g^{-1} (Figure 2).

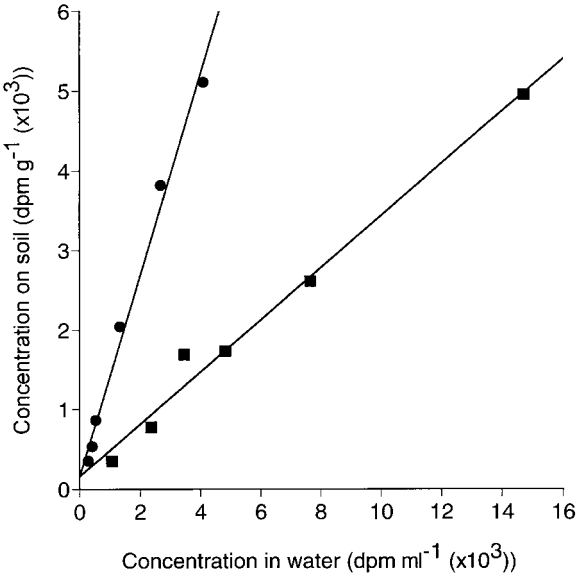

Figure 2. Sorption isotherms for 2,4-DCP (■) (units dpm ml $\times 10^3$) and 2,4-DCP degrader (●) to Vomb sand after 21 and 64 hours of equilibration, respectively. The sorption coefficient, K_d, was calculated to 0.33 for 2,4-DCP (r = 0.99), and K_b for the degrader to 1.3 (r = 0.99). Each data point is the mean value of five replicates. All standard errors were below 3% and typically less than 1%.

Column experiments

Column design

A schematic drawing of the experimental set up for the continuous flow experiment is shown in Figure 1. The stainless steel columns were 5 cm in diameter and 3 cm in length and packed with about 80 gram of dried and sieved sand of the same origin and size distribution as in the batch experiment. The columns were sealed at the ends with sintered stainless steel plates (pore size 200–300 μm) milled into stainless steel end plates with a short pipe in the middle for tubing connections. The bottom of the plate had an on/off valve welded to the pipe. The bacteria were labelled with ^{3}H as described above and diluted to a cell density of 1.8×10^8 cells ml^{-1}, corresponding to 1000 dpm ml^{-1}. The 2,4-DCP solution was made with labelled and unlabelled 2,4-DCP. It had a 2,4-DCP concentration of 100 ng ml^{-1} and an activity of 1000 dpm ml^{-1}. The bacterial and 2,4-DCP feed solutions were kept in separate 5-l flasks placed on magnetic stirrers and had the same density of cells and concentration of chemical and activity in all sorption and degradation experiments.

The flasks were connected by 1.6-mm i.d. Teflon tubings to a peristaltic pump (Ismatec Reglo-Digital MS-4/8-100) with 0.51- or 0.38-mm i.d. autoclavable Ismaprene tubings (Ismatec), which in turn were connected to the columns with Teflon tubings joint in a T-piece one cm ahead of the column inlet. The experimental arrangement allowed the complete set up to be autoclaved (121 °C, 20 min) in one piece before the addition of the different solutions to the flasks and before saturation of the sand. The columns were saturated by pumping sterilized groundwater through them from underneath at 0.5 ml min^{-1}. The flasks with the feeding solutions were placed above the pump to give an hydraulic head of about 20 cm at the top of the soil column. The homogeneity of the column packing was evaluated from breakthrough curves (BTCs) of a conservative tracer ($^{36}Cl^-$ or ^3H_2O). The columns were weighed empty, with added sand, and with added groundwater so that the mass of sand and the pore volume (≈30 ml) for each column could be calculated. Two columns were run in parallel for each treatment at 12 °C. Column data for the different experiments are listed in Table 1.

Column sorption-desorption experiments

BTCs for ^{14}C-2,4-DCP and ^{3}H-bacteria were generated in separate columns to estimate the number of pore volumes required to achieve effluent concentrations (C) from columns equal to influent concentrations (C_0) from stock solutions. The influent concentrations and activities of bacteria and 2,4-DCP were measured in the 5-L flasks before the start of each experiment. The peristaltic pump was set to deliver about 0.12 ml min^{-1} of 2,4-DCP solution and 0.05 ml min^{-1} of solution with ^{3}H-bacteria. This corresponded to a residence time of about 300 minutes and a Darcy velocity of 0.37 cm h^{-1} in the experiment with 2,4-DCP (560 minutes, 0.15 cm h^{-1} in the experiment with ^{3}H-bacteria). The effluent from two columns was sampled simultaneously in 1- to 2-ml portions in scintillation vials with an ISCO retriever II fraction collector modified for dual sampling, and the volume was measured

Table 1. Selected data for the different columns used in the study

Experiment	Mass of sand (g)		Pore volume (ml)		Flow (ml min^{-1})	
	col 1	col 2	col 1	col 2	col 1	col 2
BTC 2,4-DCP	77	82	36	35	0.12	0.13
BTC bacteria[a]	76	77	28	28	0.05	0.05
Degradation exp. 1[b]	106	106	21	22	0.05	0.05
Degradation exp. 2[a]	76	77	28	28	0.05	0.05
Degradation exp. 3[a]	76	77	28	28	0.05	0.05
Degradation exp. 4[b]	94	91	23	23	0.06	0.06

[a]These experiments used the same columns.
[b]Columns were packed with sand presorbed with 2,4-DCP.

by weighing the vials. The ^3H and ^{14}C activity in the vials was measured in the scintillator after the addition of 4 ml of Ready Safe scintillation cocktail.

Once loading of bacteria and 2,4-DCP was terminated, their desorption was determined during a period equivalent to the time needed for a degradation experiment. Filter-sterilized groundwater without 2,4-DCP and bacteria was pumped through the columns, and 1- to 2-ml aliquots of effluent water were collected and analyzed as before. When the cell density and the 2,4-DCP concentration of the effluent had dropped to levels below $C/C_0 = 0.1$, larger sample sizes were collected. Samples of 200 to 600 ml of desorbed 2,4-DCP were collected in sterile, rubber-sealed Erlenmeyer flasks and concentrated on LiChrolut EN 200 mg extraction columns (Merck). The extraction columns were initially conditioned by rinsing with 3 ml of ethyl acetate and then with 3 ml of sterilized water adjusted to pH 2.0 with HCl. The columns were loaded with the sample by pumping the sample through a Teflon tubing (i.d. 1.6 mm) connected to a peristaltic pump (Ismatech model MV-CA/8) supported with a Tygone pump tubing (Ismatech) at a flow rate of 5 ml min^{-1}. After washing with 1 ml of water (pH 2.0) the column was dried by N_2 (5 min, 2–3 bar). The 2,4-DCP was eluted with 2 × 1 ml of ethyl acetate, and the ethyl acetate remaining in the pores was forced through the column with N_2 (2–3 bar). The concentrate was analyzed for ^{14}C-2,4-DCP by scintillation after addition of 4 ml of Permafluor (Packard Instrument B.V.). Desorbed cells were collected in 30 to 200 ml portions and concentrated by centrifugation at 9000 rpm, 4 °C for 20 min on the Sorvall centrifuge before they were resuspended in about 1 ml filter-sterilized groundwater and analyzed by scintillation counting as above. First-order desorption rate coefficients, k (h^{-1}), for 2,4-DCP and cells sorbed to the sand were calculated using the expression $C_t = C_0 \times e^{-k \times t}$, where C_0 (dpm) is the initial amount of cells or 2,4-DCP sorbed to the sand and C_t (dpm) is the amount sorbed at time t (h).

Parameter evaluation

The transport of 2,4-DCP and bacteria through the columns was described with the advection-dispersion-sorption model (ADS-model) with or without a sink term to account for irreversible sorption. The ADS model with a sink term assumes linear, fast reversible and irreversible sorption:

$$\left(1 + \frac{\rho \times K_b}{\theta}\right) \times \frac{\partial C}{\partial t} = D \times \frac{\partial^2 C}{\partial x^2} - v \times \frac{\partial C}{\partial x} - \varphi C, \quad (1)$$

where ρ is the bulk density of the soil (g cm^{-3}), K_b the partitioning coefficient (g g^{-1}), θ the soil porosity (cm^3 cm^{-3}), C the concentration of the solute in the aqueous phase (μg ml^{-1}), D the longitudinal dispersion coefficient (cm^2 h^{-1}), x the coordinate in the flow direction (cm), and v the mean interstitial water velocity (cm h^{-1}) and φ is a first-order rate coefficient for irreversible loss (Bengtsson & Lindqvist 1991). The parameters ρ, θ, and v were calculated from column data, and D was estimated from curve-fitting of the conservative tracer breakthrough (K_b = 0) by the software package RS/1 using the Marquard–Levenberg non-linear least squares regression procedure (MRQFIT). The value of D was then used to obtain the retardation factor, R, defined by the expression within the parenthesis in Eq. 1, and φ for 2,4-DCP and the bacteria by curve-fitting with the ADS-model as before.

Column sorption-desorption results

BTCs for the bacteria and for 2,4-DCP in the sand columns were generated to estimate (i) the number of pore volumes required for a complete breakthrough, (ii) sorption and retardation, and (iii) the degree of desorption of cells and 2,4-DCP after saturation. The cells were slightly retarded compared with the conservative tracer ^{36}Cl, and about 90% of them passed through the columns, indicating that the cells were subject to irreversible retention reactions (Figure 3). The sorption coefficient, K_b, was calculated as 0.003–0.03 ml g^{-1}, which was 40–400 times smaller than in the batch sorption experiment. A smaller K_b in column studies has been observed before and attributed to differences in depth of the hydrodynamic boundary layer and in contact time between cells and the solid surface (Bengtsson & Lindqvist 1991, Lindqvist & Bengtsson 1995). The irreversible loss coefficient, φ, for the cells was calculated as 0.01 h^{-1} by fitting the ADS-model with a sink term to the breakthrough data (Table 2). The desorption of cells was measured when C/C_0 had decreased to 0.1 (indicated with an arrow in Figure 3) and during the following 14 pore volumes. Thirteen percent of the cells initially sorbed to the sand in column 1 and 51% in column 2 desorbed in this time. This corresponds to overall first-order desorption rate coefficients of 0.001 h^{-1} (column 1) and 0.006 h^{-1} (column 2). The net amount of cells initially sorbed to the sand was calculated from the differences in the dose of ^3H activity loaded and collected in the effluent during the loading period.

The relative retardation as well as the irreversible loss of 2,4-DCP in the sand columns was substantial (Figure 4, closed circles). The retardation coefficient was calculated as 1.6 and 1.7 in the two columns and the irreversible loss coefficients, φ, to 0.13 and 0.14 h^{-1} (Table 2). The sorption coefficient, K_d, obtained from curve-fitting with the ADS-model with a first-order sink term was 0.3 ml g^{-1} (Table 2) and close to the K_d calculated from the batch sorption experiment (Figure 2). After seven pore volumes of loading of the sand columns, C/C_0 had not reached 0.6. In fact, on another set of columns, C/C_0 stayed at about 0.65 during a loading period of 200 pore volumes and the number of pore volumes required for a complete breakthrough ($C/C_0 = 1$) could thus not be measured. The total accumulated amount of sorbed 2,4-DCP during this loading period, calculated from the difference in ^{14}C-activity loaded and collected in the effluent, was 280 μg in column 1 and 270 μg in column 2. The sand collected from the column cores after the sorption and degradation experiments was pooled in about 5 g portions and expected to contain 0.1–3.6 μg g^{-1} of 2,4-DCP. However, no 2,4-DCP was detected in the extracted samples by gas chromatography. Since aqueous samples spiked with the same concentration of 2,4-DCP gave 100% recovery, we are tempted to suggest that 2,4-DCP had become inextractable from the sand. Desorption of 2,4-DCP was measured by scintillation after C/C_0 had decreased to 0.1 and during the following 45 pore volumes and calculated to 0.1% (0.4 μg) of the sorbed amounts of 2,4-DCP in both columns. The overall first-order desorption rate coefficient was calculated to 2×10^{-5} h^{-1} in both columns.

Column degradation experiments

The mineralization of ^{14}C-2,4-DCP in the columns was determined by collecting sequences of aliquots of about 60 ml of column effluent in rubber sealed Erlenmeyer flasks containing 1 ml of 6 M HCl. The acid terminated the microbial activity and acidified the solution so that CO_2 evolved into the gaseous phase. The pump was set to deliver 0.05 ml min^{-1}, corresponding to a residence time in the column of about 560 minutes and a Darcy velocity of 0.15 cm h^{-1}. The Erlenmeyer flask was connected in a series with three scintillation vials (Figure 1) containing 4, 1, and 1 ml of Carbosorb (Packard Instrument Company, Inc.), respectively. The whole sampling setup was disconnected in one piece after sampling, and the remaining CO_2 in the gaseous phase of the Erlenmeyer flask was transferred into the Carbosorb solution by gently purging the collected water with N_2. After addition of 16, 4, and 4 ml, respectively, of Permafluor to the vials with Carbosorb solutions, the samples were analyzed by scintillation as above. A one ml aqueous sample was withdrawn from the Erlenmeyer flask, and the ^{14}C and ^3H activity was measured as described before.

At the end of the experiments on the BTC of 2,4-DCP and the degradation experiments 1, 3, and 4, samples of sand were collected in three replicates from three different heights in the columns, and 2,4-DCP sorbed to the sand was extracted as described by Knuutinen et al. (1990). The 2,4-DCP concentration was determined after adjustment to pH 6, derivatizing with acetic anhydride, and extraction with hexane (Bengtsson 1985), using a Hewlett Packard model 6890 gas chromatograph equipped with a flame ionisa-

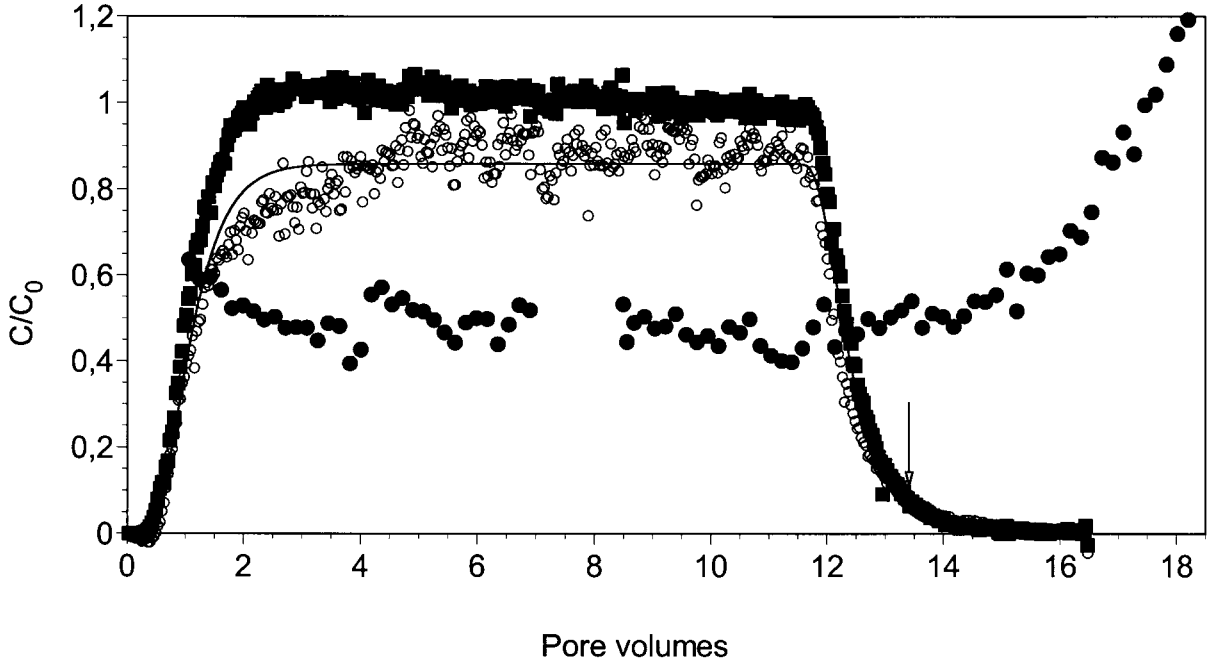

Figure 3. Observed BTC for ^{36}Cl (■) and ^{3}H-labelled bacteria (○) in a column packed with Vomb sand and for ^{3}H-labelled bacteria (●) in another column saturated with 2,4-DCP. The ADS-model with a first-order sink term to account for irreversible loss was used to describe transport of bacteria through the sand column without sorbed 2,4-DCP (—). The model parameters (porosity $\theta = 0.48$ cm^3 cm^{-3}, bulk density, $\rho = 1.29$ g cm^{-1}, and interstitial water velocity, $v = 0.32$ cm h^{-1}) were calculated from column data. Dispersivity, $D = 0.33$, $K_b = 0.032$, and the irreversible loss coefficient ,$\varphi = 0.01$ were derived from curve fitting with MRQFIT. The arrow indicates the start of measurement of cell desorption at $C/C_0 = 0.1$.

Table 2. Coefficients obtained from curve-fitting with the ADS-model with a first-order sink term for irreversible loss to the BTCs for bacteria and 2,4-DCP, and the corresponding parameters characterising the columns

		Porosity, θ (cm^3 cm^{-3})	Soil bulk density, ρ (g cm^{-3})	Interstitial velocity, v (cm h^{-1})	Dispersivity, D_w (cm^2 h^{-1})	Partitioning coefficient, K_b (ml g^{-1})	Irreversible loss coeff., φ (h^{-1})	Retardation factor, R
2,4-DCP	column 1	0.54	1.26	0.73	0.67	0.31	0.14	1.73
	column 2	0.54	1.26	0.73	0.71	0.26	0.13	1.6
Bacteria	column 1	0.48	1.29	0.32	0.33	0.032	0.01	1.09
	column 2	0.48	1.29	0.32	0.14	0.0025	0.01	1.01

tion detector and a 30 m fused silica capillary column (HP-5, film thickness 0.25 μm, i.d. 0.32 mm).

Four different experiments were performed to distinguish degradation of sorbed from dissolved DCP and contribution of sorbed from suspended bacteria. The methodology for the calculation of the degradation of 2,4-DCP in the different experiments is summarized in appendix A, with the assumption that the influence of cells desorbing from the solid phase could be disregarded. Mean values of column 1 and 2 were used in all calculations of degradation in experiment 2 through 4. The calculations are explicitly demonstrated in footnotes to each experiment. The overall first-order degradation (mineralisation) rate constant, k, was calculated from $C_0 - C_{CO_2} = C_0 \times e^{-k \times t}$, where C_0 (dpm) is the total amount of ^{14}C-2,4-DCP sorbed and dissolved during the measurement of degradation, and C_{CO_2} (dpm) is the amount of $^{14}CO_2$ produced from degradation of ^{14}C-2,4-DCP in the column after time t (h).

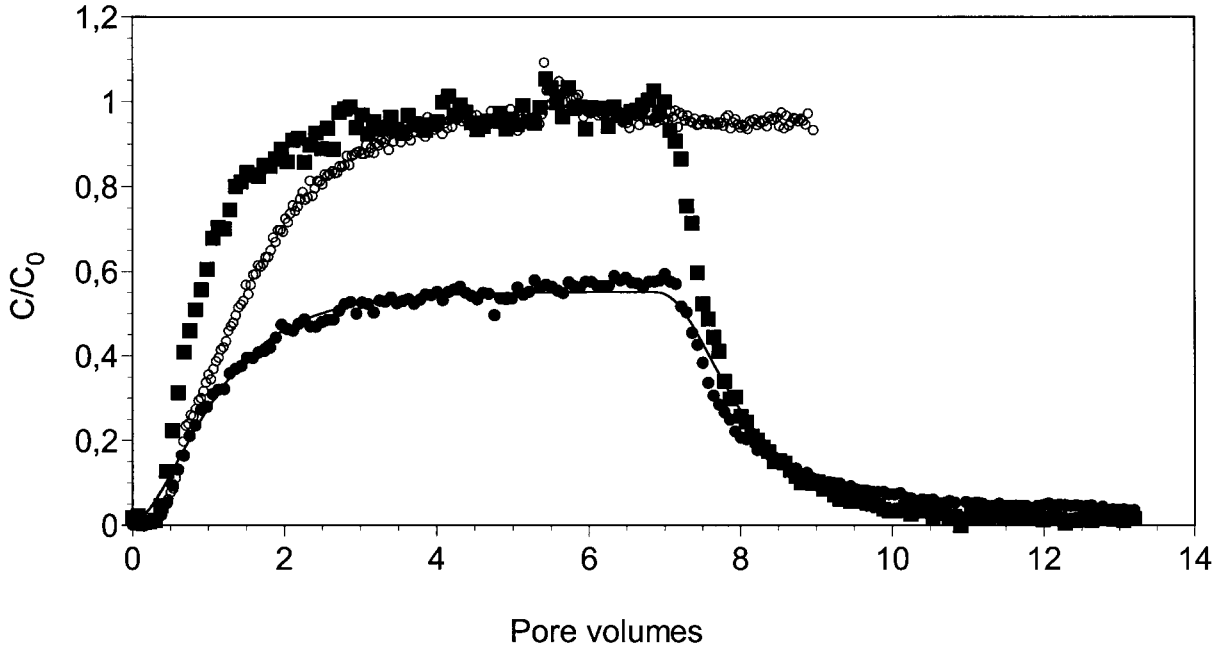

Figure 4. BTCs for ^3H_2O (■) and for 2,4-DCP with (○) and without (●) sorbed bacteria in a column packed with Vomb sand. The ADS model with sink term was used to obtain the curve fit for 2,4-DCP (solid line) from θ = 0.54 cm^3 cm^{-3}, ρ = 1.26 g cm^{-3}, v =0 .73 cm h^{-1}, resulting in D_w = 0.71 cm^2 min^{-1}, φ =0.13 and K_d = 0.26.

1. Degradation of dissolved 2,4-DCP by sorbed bacteria. (Exp.1)

In this experiment, the sand was first saturated with unlabelled 2,4-DCP by mixing 500 g of sieved and autoclaved sand with 600 ml of filter sterilized 2,4-DCP-solution (40 μg ml^{-1}) at room temperature over night in an Erlenmeyer flask on a magnetic stirrer. The concentration of 2,4-DCP remaining in the aqueous phase after equilibration was analyzed with GC according to the procedure described above. The sand was then dried (105 °C) before it was packed with a spoon in the columns and autoclaved at 121 °C, 20 min. The columns were connected to the tubings as described above, and the whole set up was autoclaved before the sand was saturated with ^{3}H-labelled bacteria by pumping a cell solution through a column until the effluent and influent concentrations were equal ($C/C_0 = 1$). Effluent concentrations were measured by collecting samples of 1 to 2 ml and analysing them by scintillation as described before. Finally, the delivery of bacteria was interrupted, and ^{14}C-labelled 2,4-DCP was added by pumping a solution through the columns at 0.05 ml min^{-1}. Its degradation was measured in 60 ml portions, collected in Erlenmeyer flasks as described above, during about 30 pore volumes. The observed degradation was considered to largely reflect degradation of dissolved 2,4-DCP by sorbed bacteria (unless desorption of bacteria was shown to be substantial, see below).

The mixing of sand over night with unlabelled 2,4-DCP followed by drying and autoclaving left 2.4 μg 2,4-DCP sorbed per g sand, calculated from the 2,4-DCP remaining in the aqueous phase after equilibration and a 90% loss of 2,4-DCP during the sterilization procedure (data not shown). The number of ^{3}H labelled cells retained in the columns was about three times higher when 2,4-DCP was previously sorbed in the columns than in its absence (Figure 3), most likely because 2,4-DCP covered some negatively charged sites of the sand and reduced their interaction with the net negatively charged cells (Lindqvist & Bengtsson 1995). After 18 pore volumes of loading of cells, about 2×10^{10} had been sorbed to each 2,4-DCP presorbed column, corresponding to about 2×10^8 cells g^{-1} of sand. The sudden breakthrough of cells after 15 pore volumes may have followed upon saturation of the 2,4-DCP presorbed sand. The amount of ^{14}C-labelled 2,4-DCP trapped as $^{14}CO_2$ during 32–34 pore volumes was about 0.2 μg, corresponding to 0.2–0.3%[1] of added ^{14}C-labelled 2,4-DCP (Table 3).

[1] Calculated as described in Appendix A with data from Table 3, column 1: $P^{C_D B_S} = (0.23/(0.1 \times 725)) \times 100 = 0.32\%$.

2. Degradation of sorbed 2,4-DCP by sorbed bacteria. (Exp. 2)

In this experiment, we continued to use the same columns as used in the column sorption-desorption experiment generating the BTC of bacteria. The sand was again saturated with ^{3}H-labelled bacteria by pumping about 11 pore volumes of a cell solution through a column until $C/C_0 = 1$. The T connecting the column inlet with the 5 L flasks containing the feed solutions was then switched to ^{14}C-labelled 2,4-DCP, which was pumped through the column during 9 pore volumes, and a BTC was generated. A ^{14}C-labelled 2,4-DCP solution was then pumped through the columns during another 50 pore volumes in column 1 and 46 pore volumes in column 2, so that ^{14}C-labelled 2,4-DCP was present both in the aqueous and solid phase. The degradation of ^{14}C-2,4-DCP was then measured in 60-ml portions, collected in an Erlenmeyer flask as described before. Since the bacteria were largely sorbed to the sand, degradation should here reflect degradation of sorbed and dissolved 2,4-DCP by sorbed bacteria. By subtracting the amount of dissolved 2,4-DCP degraded in the experiment on degradation of dissolved 2,4-DCP by sorbed bacteria (Exp. 1) from the observed degradation in this experiment, the degradation of sorbed 2,4-DCP by sorbed bacteria could be calculated. If desorption of cells was substantial (see below), adjustments had to be made for the possibility that desorbed bacteria could have degraded dissolved 2,4-DCP.

Four pore volumes was sufficient for a complete breakthrough of ^{14}C-labelled 2,4-DCP in columns that were saturated with 4–6 $\times$ 10^7 cells g^{-1} of sand (Figure 4, open circles). Very little irreversible loss of 2,4-DCP was seen in presence of presorbed bacteria as opposed to their absence on the sand (Figure 4, closed circles), most likely because the presorbed cells occupied the most favourable sorption sites of 2,4-DCP (Lindqvist and Enfield 1992). The collection of $^{14}CO_2$ started after an initial ^{14}C-2,4-DCP loading period of nine pore volumes, and continued until 50 pore volumes had elapsed. During this period about 0.6 μg of dissolved and sorbed 2,4-DCP were degraded in the columns. The evolved $^{14}CO_2$ representing mineralisation of only sorbed 2,4-DCP was about 0.5 μg, corresponding to a little more than 5%[2] of the total amount of 8.7 and 10.2 μg 2,4-DCP initially sorbed in the two columns (Figure 5, Table 3).

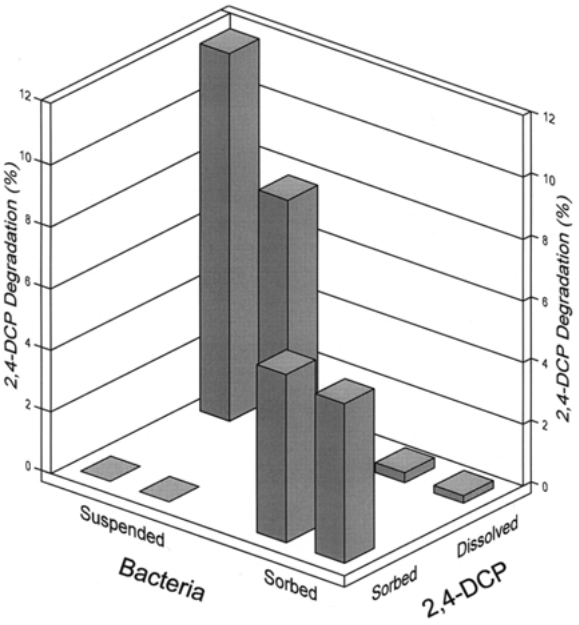

Figure 5. Partitioning of microbial degradation of 2,4-DCP in columns packed with the Vomb sand. Data are from the four different column degradation experiments and shows the degradation in per cent of the total amount of 2,4-DCP in the specific phase for different combinations of sorbed and dissolved 2,4-DCP by sorbed and suspended bacteria.

3. Degradation of sorbed 2,4-DCP by suspended bacteria. (Exp. 3)

In this experiment we continued to use the same columns as in Exp. 2. ^{3}H-labelled bacteria were added again and moved through the column largely in the aqueous phase. The degradation of the sorbed ^{14}C-labelled 2,4-DCP was then measured when bacteria were pumped through the column during 43 pore volumes, so that sorbed and suspended cells were present to degrade the compound. The contribution to the degradation of sorbed 2,4-DCP by suspended bacteria was calculated by subtracting the degradation of sorbed 2,4-DCP by sorbed bacteria in Exp. 2 from the observed degradation in this experiment.

Degradation of sorbed ^{14}C-2,4-DCP was measured during a period of 43 pore volumes in the same columns as used in Exp. 2 (above). The $^{14}CO_2$ collected corresponded to about 0.3 μg of degraded ^{14}C-2,4-DCP, equivalent to 3–4% of the sorbed amount of ^{14}C-2,4-DCP. When the numbers for the degradation of sorbed 2,4-DCP by sorbed bacteria in Exp. 2 were subtracted from the numbers for the degradation

[2] Calculated as described in Appendix A with data from Table 3, column 1 and 2: $P^{C_S B_S} = ((0.57/(1405 \times 4.5 \times 10^9) - ((0.23/(725 \times 2.9 \times 10^{10})) + (0.17/(711 \times 1.2 \times 10^{10}))/2)) \times 1405 \times 4.5 \times 10^9/8.7) \times 100 = 5.44\%$.

Table 3. Biodegradadation data for 2,4-DCP in the different column experiments. The calculation protocol is described in Appendix A

		Degradation experiment							
		1. Dissolved 2,4-DCP + sorbed bacteria		2. Sorbed 2,4-DCP + sorbed bacteria		3. Sorbed 2,4-DCP + suspended bacteria		4. Dissolved2,4-DCP + suspended bacteria	
		column 1	column 2	column 1	column 2	column 1	column 2	column 1	column 2
Sorbed 2,4-DCP[a]	(μg)	254.4	254.4	8.7	10.2	8.7	10.2	225.6	218.4
	(μg g^{-1})	2.4	2.4	0.11	0.13	0.11	0.13	2.4	2.4
Sorbed bacteria[a]	(cells)	2.9×10^{10}	1.2×10^{10}	4.5×10^{9}	2.8×10^{9}	2.8×10^{8} [b]	2.2×10^{8} [b]	6.3×10^{10}	14.2×10^{10}
	(cells g^{-1})	2.7×10^{8}	1.1×10^{8}	5.9×10^{7}	3.6×10^{7}	3.7×10^{6}	2.8×10^{6}	6.7×10^{8}	15.6×10^{8}
Suspended bacteria[c]	(cells)	0	0	0	0	1.8×10^{11}	1.9×10^{11}	1.7×10^{11}	1.7×10^{11}
Total volume during which degradation was measured	(ml)	725	711	1405	1282	1191	1222	918	884
Dissolved 2,4-DCP added during degradation	(μg)	72.4	71.0	140.5	128.1	0	0	91.7	88.4
Degradation of dissolved 2,4-DCP by sorbed bacteria	(μg)	0.23[g]	0.17[g]	–	–	–	–	–	–
	(%)	0.32[d]	0.24[d]						
	(μg ml^{-1} cell^{-1})	1.1×10^{-14}	2.0×10^{-14}						
Degradation of dissolved + sorbed 2,4-DCP by sorbed bacteria	(μg)	–	–	0.57	0.58	–	–	–	–
Degradation of sorbed 2,4-DCP by sorbed bacteria	(μg)	–	–	0.47[g]	0.53[g]	–	–	–	–
	(%)	–	–	5.44[e]	5.16[e]				
	(μg ml^{-1} cell^{-1})			7.5×10^{-14}	14.9×10^{-14}				
Degradation of sorbed 2,4-DCP by sorbed and suspended bacteria	(μg)	–	–	–	–	0.35	0.27	–	–
Degradation of sorbed 2,4-DCP by suspended bacteria	(μg)	–	–	–	–	0[g]	0[g]	–	–
	(%)					0[e]	0[e]		
	(μg ml^{-1} cell^{-1})					0	0		
Degradation of dissolved 2,4-DCP by suspended and sorbed bacteria	(μg)	–	–	–	–	–	–	11.8	8.9
Degradation of dissolved 2,4-DCP by suspended bacteria	(μg)	–	–	–	–	–	–	10.9[g]	6.9[g]
	(%)							11.9[d]	7.8[d]
	(μg ml^{-1} cell^{-1})							6.8×10^{-14}	4.7×10^{-14}
Overall first-order degradation rate constant, k [f]	(h^{-1})	1.3×10^{-5}	1.0×10^{-5}	0.8×10^{-5}	1.0×10^{-5}	10.5×10^{-5}	6.5×10^{-5}	54.1×10^{-5}	43.1×10^{-5}
Half life time, $t_{1/2}$	(h)	5.3×10^{4}	6.8×10^{4}	8.5×10^{4}	7.0×10^{4}	0.7×10^{4}	1.1×10^{4}	0.1×10^{4}	0.2×10^{4}

[a]Calculated from the difference in influent and effluent ^{14}C or ^{3}H activity.
[b]Sorbed cells left after cell desorption in experiment 2. Calculated with a desorption coefficient, $k = 0.006$ h^{-1}.
[c]Calculated from the influent ^{3}H-activity multiplied with the volume during degradation.
[d]Calculated in per cent of total dissolved 2,4-DCP.
[e]Calculated in per cent of total sorbed 2,4-DCP.
[f]Describes the overall degradation rate in the columns of ^{14}C-2,4-DCP, dissolved and/or sorbed according to treatment.
[g]Calculated from the numerator of the equations in Appendix A.

of sorbed 2,4-DCP in this experiment, the suspended bacteria were found to have no influence on the degradation of sorbed 2,4-DCP[3] (Figure 5, Table 3).

4. Degradation of dissolved 2,4-DCP by suspended bacteria. (Exp. 4)

The sand was first saturated with unlabelled 2,4-DCP and bacteria following the same procedure as in Exp. 1. A ^{14}C-labelled 2,4-DCP solution made from labelled and unlabelled 2,4-DCP at a concentration of 200 ng ml^{-1} and an activity of 1000 dpm ml^{-1} and a labelled bacterial solution (1.9×10^8 cells ml^{-1}, 1000 dpm ml^{-1}) were then added simultaneously from separate supplies during 41 pore volumes in column 1 and 38 pore volumes in column 2 at the same flow rate (0.03 ml min^{-1}). The tubings from the 2,4-DCP solution and the bacterial solution were not connected until in the T piece one cm ahead of the column inlet. The net flow rate through a column then became 0.06 ml min^{-1}, the concentration of 2,4-DCP in the pore water 100 ng ml^{-1}, and the cell density about 1.0×10^8 cells ml^{-1}. Since the columns initially had been saturated with unlabelled 2,4-DCP, the ^{14}C-labelled 2,4-DCP pumped through a column should be mainly in the aqueous phase. Samples of about 60 ml were collected and analyzed for $^{14}CO_2$ (and ^{1}C-2,4-DCP). The production of $^{14}CO_2$ should come from degradation of aqueous 2,4-DCP by suspended and sorbed bacteria, and the amount attributable to the suspended bacteria was calculated by subtracting the contribution from sorbed bacteria (Exp. 1).

The columns were initially sorbed with unlabelled 2,4-DCP at a concentration of 2.4 μg g^{-1} sand in both columns, and bacteria at 7–16 $\times$ 10^8 cells g^{-1}. The amount of degraded ^{14}C-2,4-DCP, measured as evolved $^{14}CO_2$ during about 40 pore volumes, was 9–12 μg, of which 7–11 μg - was the result of degradation by suspended cells, corresponding to a degradation of 8–12%[4] of dissolved 2,4-DCP by suspended bacteria (Figure 5, Table 3).

Since the column sorption-desorption experiment with bacteria demonstrated an appreciable desorption of cells when the columns were eluted with groundwater, the potential influence of unintentionally desorbing cells on degradation was also addressed. When the highest cell desorption rate coefficient derived from the column sorption-desorption experiment was used (k = 0.006 h^{-1}), as much as 77% of the degraded dissolved 2,4-DCP (corresponding to 0.2% of the total dissolved compound) in Exp. 1 might have been due to the activity of desorbed rather than sorbed cells. In Exp. 2, up to 93% of the bacteria initially sorbed to the sand might have desorbed during the experiment. However, since suspended cells, including those that might have desorbed, were not degrading sorbed 2,4-DCP in Exp. 3, desorbed cells had most likely no influence on the degradation of sorbed 2,4-DCP in Exp. 2. In Exp. 4, suspended and desorbed cells together might have been responsible for the degradation of 10–13% of the dissolved 2,4-DCP, slightly exceeding the 8–12% calculated when cell desorption was disregarded.

Summary of column degradation experiments

In summary, the column experiment demonstrated that both sorbed and suspended bacteria were metabolising 2,4-DCP, with dissolved 2,4-DCP and suspended bacteria being the most efficient combination. Although sorption of 2,4-DCP was substantial, it did not protect the compound from degradation. Suspended bacteria degraded 8–12% of the added dissolved 2,4-DCP, and adsorbed bacteria degraded about 5% of sorbed 2,4-DCP (Figure 5). Very little degradation was associated with the combinations of dissolved 2,4-DCP and sorbed bacteria (<0.4%), and with sorbed 2,4-DCP and suspended bacteria (0%).

The specific degradation activities expressed in μg 2,4-DCP degraded ml^{-1} cell^{-1} varied between the degradation experiments (Table 3). The highest specific degradation activity, 7.5–14.9 $\times$ 10^{-14} μg ml^{-1} cell^{-1}, was seen with the combination of sorbed 2,4-DCP and sorbed bacteria. This was about twice as high as the specific degradation activity for the combination of dissolved 2,4-DCP and suspended bacteria, and almost 10 times higher than the combination of dissolved 2,4-DCP and sorbed bacteria. The overall first-order degradation rate constants in the soil-water systems were about 10^{-5} to 10^{-4}, with the highest rates in experiment 4 (Table 3).

The desorption of 2,4-DCP was slow, and curve-fitting with the ADS-model indicated that sorption was partly irreversible. Desorption of cells was relatively fast when the columns were eluted with groundwater,

[3] Calculated as described in Appendix A with data from Table 3, column 1 and 2: $P^{C_S B_D}$ = ((((0.35/(1191 $\times$ (2.8 $\times$ 10^8 + 1.8 $\times$ 10^{11}))) − ((((0.57/(1405 $\times$ 4.5 $\times$ 10^9)) + (0.58/(1282 $\times$ 2.8 $\times$ 10^9)))/2) − (((0.23/(725 $\times$ 2.9 $\times$ 10^{10})) + (0.17/(711 $\times$ 1.2 $\times$ 10^{10})))/2))) $\times$ 1191 $\times$ 1.8 $\times$ 10^{11}/8.7) $\times$ 100 = Neg. value.

[4] Calculated as described in Appendix A with data from Table 3, column 1 and 2: $P^{C_D B_D}$ = ((11.8 − (((0.23/(725 $\times$ 2.9 $\times$ 10^{10})) + (0.17/(711 $\times$ 1.2 $\times$ 10^{10})))/2) $\times$ 918 $\times$ 6.3 $\times$ 10^{10}))/(0.1 $\times$ 918) $\times$ 100 = 11.9%.

unless the sand was presorbed with 2,4-DCP, which caused irreversible sorption or straining. The inclusion of an unintentional cell desorption had a marginal effect on the degradation results.

Discussion

Suspended bacteria effectively degraded dissolved 2,4-DCP, while sorption of the substance seemed to make it unavailable for the suspended cells (Figure 5). Sorption of substituted phenols occurs primarily by hydrophobic sorption and hydrogen bonding (Boyd 1982), but the high persistence and low extractability of 2,4-DCP in the solid aquifer material may also have been caused by polymerization into phenolic polymers. What mechanisms that were responsible for the irreversible loss of 2,4-DCP in the columns remain unknown, but the phenomenon has been observed in other sandy soils and sediments and referred to as irreversible sorption and inextractability (Isaacson & Frink 1984, Lagas 1988, Bhandari et al. 1997).

However, sorbed 2,4-DCP was readily available for degradation by sorbed bacteria (Figure 5), and sorbed cells were even twice as efficient, on a per cell basis, in degrading sorbed 2,4-DCP as suspended cells were in degrading the dissolved compound. The sorbed cells may have promoted the degradation of sorbed 2,4-DCP by various mechanisms. It is known that sorbed cells readily assimilate and synthesize other substrates, such as amino acids, proteins, and phosphorus (Paerl & Merkel 1982; Bright & Fletcher 1983), which has been explained as an effect of enhanced concentration of energy and nutrients at the surface. For example, surfaces induce denaturation and unfolding of proteins, which is thought to facilitate the exposure of peptide bonds to enzymatic hydrolysis and the anisotropic diffusion of the hydrolysis products away from the surface to the cells associated with the surface (Taylor 1995). Sorbed cells may scavenge the surface by exoenzymes but also trap ions and molecules by absorption (Baughman & Paris 1981, Salkinoja-Salonen et al. 1983, Lindqvist & Enfield 1992).

The more extensive degradation of dissolved 2,4-DCP by suspended compared with sorbed bacteria (Figure 5) was reflected also on a per cell basis, with suspended cells about three times faster than sorbed (Table 3). It is likely that the slower diffusion of the molecules through the hydrodynamic boundary layer limits their availability to the cells at the solid surface compared with the more extensive mixing of cells and molecules in the interstitial pores where advective movement of water may override constraints imposed by diffusion (Rijnaarts et al. 1990).

The overall degradation rate of 2,4-DCP agrees reasonably well with rates calculated by Smith and Novak (1987), $k = 0.0004\ \mathrm{h}^{-1}$, for slurries of aquifer sediments from pristine areas, when the anticipated rate differences between flow and slurry systems are considered. These comparisons indicate that rate coefficients are dependent on the hydrodynamics as well as on the temperature and nutrient status of the test system.

Knowledge of the influence of sorption on biodegradation is important for risk and bioremediation assessment of polluted soils and groundwater. Our results demonstrate that the solid phase of a subsurface environment may make a significant contribution to the total degradation of trace organics due to the large bulk density compared with porosity. For instance, if we assume a degradation rate ten times higher of dissolved than sorbed compound in an aquifer with a porosity of 25%, the contribution to the total amount degraded by the sorbed contaminant with a K_d of 10 ml g^{-1} would be three times greater than the contribution by the same, evenly distributed, dissolved compound.

Acknowledgements

The work was supported by grants from the Swedish Environmental Protection Agency, Crafoordska Stiftelsen, Magnus Bergvalls Stiftelse and Kungliga Fysiografiska Sällskapet i Lund. We would like to thank Vladimir Cvetkovic for improving the quality of the manuscript, and Sydvatten AB for allowing access to the groundwater infiltration area at Vomb. The *Pseudomonas putida* strain EST4021 was a gift from Dr. Maia Kivisaar at Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre.

Appendix A

The degradation of 2,4-DCP in the continuous flow system during the described four treatments was calculated in the following way:

$$P^{C_D B_S} = \frac{Q^{C_D B_S}_{C(CO_2)}}{C_0^{C_D B_S} \times V^{C_D B_S}},$$

$$P^{C_S B_S} = \frac{\left(\frac{Q^{C_S B_S}_{T(CO_2)}}{V^{C_S B_S} \times B_S^{C_S B_S}} - \frac{Q^{C_D B_S}_{C(CO_2)}}{V^{C_D B_S} \times B_S^{C_D B_S}}\right) \times V^{C_S B_S} \times B_S^{C_S B_S}}{S_0^{C_S B_S}} \times 100,$$

$$P^{C_S B_D} = \frac{\left[\frac{Q^{C_S B_D}_{S(CO_2)}}{V^{C_S B_D} \times (B_S^{C_S B_D} + B_D^{C_S B_D})} - \left(\frac{Q^{C_S B_S}_{T(CO_2)}}{V^{C_S B_S} \times B_S^{C_S B_S}} - \frac{Q^{C_D B_S}_{C(CO_2)}}{V^{C_D B_S} \times B_S^{C_D B_S}}\right)\right]}{S_0^{C_S B_D}} \times V^{C_S B_D} \times B_D^{C_S B_D} \times 100,$$

$$P^{C_D B_D} = \frac{Q^{C_D B_D}_{C(CO_2)} - \frac{Q^{C_D B_S}_{C(CO_2)}}{V^{C_D B_S} \times B_S^{C_D B_S}} \times B_S^{C_D B_D} \times V^{C_D B_D}}{C_0^{C_D B_D} \times V^{C_D B_D}} \times 100,$$

where $P^{C_D B_S}$, $P^{C_S B_S}$, $P^{C_S B_D}$, and $P^{C_D B_D}$ are the degraded amounts (μg) of ^{14}C labelled 2,4-DCP in per cent of added dissolved or sorbed compound when the dissolved (C_D) or sorbed (C_S) compound was degraded by sorbed (B_S) or suspended bacteria (B_D); $Q^{C_D B_S}_{C(CO_2)}$ and $Q^{C_D B_D}_{C(CO_2)}$ are the amounts (μg) of dissolved 2,4-DCP detected as $^{14}CO_2$ when the dissolved C_D) compound was degraded by sorbed (B_S) or suspended (B_D) bacteria; $Q^{C_S B_S}_{T(CO_2)}$ is the amount (μg) of sorbed and dissolved 2,4-DCP detected as $^{14}CO_2$ when the sorbed (C_S) compound was degraded by sorbed (B_S) bacteria; $Q^{C_S B_D}_{S(CO_2)}$ is the amount (μg) of sorbed 2,4-DCP detected as $^{14}CO_2$ when the sorbed (C_S) compound was degraded by suspended (B_D) bacteria; $C_0^{C_D B_S}$ and $C_0^{C_D B_D}$ are the concentrations 0.1 μg ml^{-1}) of dissolved 2,4-DCP added at the beginning of the experiment when the dissolved (C_D) compound was degraded by sorbed (B_S) or suspended (B_D) bacteria; $S_0^{C_S B_S}$ and $S_0^{C_S B_D}$ are the amounts (μg) of sorbed 2,4-DCP when the sorbed (C_S) compound was degraded by sorbed (B_S) or suspended (B_D) bacteria; $V^{C_D B_S}$, $V^{C_S B_S}$, $V^{C_S B_D}$, and $V^{C_D B_D}$ are the total eluted volumes (ml) during which degradation of 2,4-DCP was measured as $^{14}CO_2$ when the dissolved (C_D) or sorbed (C_S) compound was degraded by sorbed (B_S) or suspended bacteria (B_D); $B_S^{C_S B_S}$, $B_S^{C_D B_S}$, $B_S^{C_S B_D}$, and $B_S^{C_D B_D}$ are the number of sorbed cells when the dissolved (C_D) or sorbed (C_S) compound was degraded by sorbed (B_S) and/or suspended (B_D) bacteria; $B_D^{C_S B_D}$ is the number of suspended (B_D) cells when the sorbed (C_S) compound was degraded by suspended bacteria.

References

Akratanakul S, Boersma L & Klock GO (1983b) Sorption processes in soils as influenced by pore water velocity. 2. Experimental results. Soil Science 135: 331–341

Baughman GL & Paris DF (1981) Microbial bioconcentration of organic pollutants from aquatic systems – a critical review. Crit. Rev. Microbiol. 8: 205–228

Bengtsson G & Lindqvist R (1991) Dispersal dynamics of groundwater bacteria. Microb. Ecol. 21: 49–72

Bengtsson G (1985) A gas chromatographic micromethod for trace determinations of phenols. J. Chrom. Sci. 23: 397–401

Bhandari A, Novak JT, Burgos WD & Berry DF (1997) Irreversible binding of chlorophenols to soil and its impact on bioavailability. J. Environ. Engn.-ASCE 123: 506–513

Bhatt K (1997) Occurrence and distribution of nitrate and pesticides in Bowdle aquifer, South Dakota. Environm. Monit. Assessm. 47: 223–237

Boyd SA (1982) Adsorption of substituted phenols by soil. Soil Sci. 134: 337–343

Bright JJ & Fletcher M (1983) Amino acid assimilation and respiration by attached and free-living populations of a marine Pseudomonas sp.. Microb. Ecol. 9: 215–226

Gehlen M, Trampish HJ, & Dott W (1985) Physiological characterization of heterotrophic bacterial communities from selected aquatic environments. Microb. Ecol. 11: 205–219

Ghiorse WC & Wilson JT (1988). Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. 33: 107–172

Harms H & Zehnder AJB (1995) Bioavailability of sorbed 3-chlorodibenzofuran. Appl. Environ. Microbiol. 61: 27–33

Hutchins S.R, Sewell GW, Kovacs DA & Smith GA (1991) Biodegradation of aromatic hydrocarbons by aquifer microorganisms under denitrifying conditions. Environ. Sci. Technol. 25: 68–76

Isaacson PJ & Frink CR (1984) Nonreversible sorption of phenolic compounds by sediment fractions: the role of sediment organic matter. Environ. Sci. Technol. 18: 43–48

Knuutinen J, Palm H, Hakala H, Haimi J, Huhta V & Salminen J (1990) Polychlorinated phenols and their metabolites in soil and earthworms of sawmill environment. Chemosphere. 20: 609–623

Lagas P (1988) Sorption of chlorophenols in the soil. Chemosphere 17: 205–216

Lindqvist R & Enfield CG (1992) Biosorption of dichlorodiphenyltrichloroethane and hexachlorobenzene in groundwater and its implications for facilitated transport. Appl. Environ. Microbiol. 58: 2211–2218

Lindqvist R & Enfield CG (1992) Cell density and nonequilibrium sorption effects on bacterial dispersal in ground water microcosms. Microb. Ecol. 24: 25–41

Lindqvist R & Bengtsson G. (1995) Diffusion limited and chemical-interaction-dependent sorption of soil bacteria and microspheres. Soil Biol. Biochem. 27: 941–948

Liu S-Y, Zheng Z, Zhang R, & Bollag J-M (1989) Sorption and metabolism of metolachlor by a bacterial community. Appl. Environ. Microbiol. 55: 733–740

Logan BE & Dettmer JW (1990) Increased mass transfer to microorganisms with fluid motion. Biotechnol. Bioeng. 35: 1135–1144

Mäe A, Marits R, Ausmees N, Koiv V & Heinaru A (1993) Characterization of a new 2,4-dichlorophenoxyacetic acid degrading

plasmid pEST4011: physical map and localization of catabolic genes. J. Gen. Microbiol. 139: 3165–3170

Mihelcic JR & Luthy RG (1991) Sorption and microbial degradation of naphtalene in soil-water suspensions under denitrification conditions. Environ. Sci. Technol. 25: 169–177

Nergaard Jacobsen B, Arvin E & Reinders M (1996) Factors affecting sorption of pentachlorophenol to suspended microbial biomass. Wat. Res. 30: 13–20

Ogram AV, Jessup RE, Ou LT & Rao PSC (1985) Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. 49: 582–587

Paerl HW & Merkel SM (1982) Differential phosphorous assimilation in attached vs. unattached microorganisms. Arch. Hydrobiol. 93: 125–134

Pivetz BE, Kelsey JW, Steenhuis TS & Alexander M (1996) A procedure to calculate biodegradation during preferential flow through heterogeneous soil columns. Soil Sci. Soc. Am. J. 60: 381–388

Poglazova M N & Meisel MN (1971) Localization of benz(a)pyrene in bacterial cells. Mikkrobiologija 40: 1050–1053

Rijnaarts HHM, Bachmann A, Jumelet JC & Zehnder AJB (1990) Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. 24: 1349–1354

Robinson KG, Farmer WS & Novak JT (1990) Availability of sorbed toluene in soils for biodegradation by acclimated bacteria. Water. Res. 24: 345–350

Salkinoja-Salonen MS, Hakulinen R, Valo R & Apajalahn J (1983) Biodegradation of recalcitrant organochlorine compounds in fixed film reactors. Water Sci. Technol. 15: 309–319

Smith J A & Novak JT (1987) Biodegradation of chlorinated phenols in subsurface soils. Water, Air, and Soil Pollution. 33: 29–42

Smith SC, Ainsworth CC, Traina SJ & Hicks RJ (1992) Effect of sorption on the biodegradation of quinoline. Soil Sci. Soc. Am. J. 56: 737–746

Steiert JG & Crawford RL (1985) Microbial degradation of chlorinated phenols. Trends in Biotechnology. 3: 300–305

Subba-Rao RV, Rubin HE & Alexander M (1982) Kinetics and extent of mineralization of organic chemicals at trace levels in fresh water and sewage. Appl. Environ. Microbiol. 43:1139–1150

Swindoll CM, Aelion CM, Dobbins DC, Jiang O, Long SC & Pfaender FK (1988a) Aerobic biodegradation of natural and xenobiotic organic compounds by subsurface microbial communities. Environ. Toxicol. Chem. 7: 291–299

Taylor GT (1995) Microbial degradation of sorbed and dissolved protein in seawater. Limnol. Oceanogr. 40: 875–885

Weber JB & Coble HD (1968) Microbial decomposition of diquat adsorbed on montmorillonite and kaolinite clays. J. Agric. Food Chem. 16: 475–478

Weissenfels WD, Klewer HJ & Langhoff J (1992) Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles – Influence on Biodegradability and Biotoxicity. Appl. Microbiol. Biotechnol. 36: 689–696