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Mass transfer limitation of microbial growth and pollutant degradation

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Organic pollutants in soil can be removed by biotechnological treatment. A limitation of this technology is the efficiency of biodegradation. In many cases, the bulk of the pollution can be removed but residual pollutants remain and biodegradation rates are slower than expected from laboratory trials. Low biodegradation rates are often a result of limited accessibility of the pollutants. Major reasons for the reduced bioavailability are the unequal spatial distribution of microorganisms and pollutants and the retardation of substrate diffusion by the soil matrix. Mechanical mixing and the addition of surfactants are possible approaches to improve the bioavailability of pollutants during bioremediation. The application of flow-stop-flow techniques may be of help to overcome the limitations resulting from advective-diffusive transport mechanisms during pump-and-treat remediation of contaminant plumes.

Keywords: bioavailability; bioremediation; effective diffusivity; homogenization; mass transfer; surfactants

Introduction

Scope of the problem

The production and use of organic chemicals for more than one hundred years has caused the pollution of virtually all compartments of the environment. Nowadays, pollutants severely affect essential human activities such as food production and recreation, and may even threaten human health and reproductivity [7,28]. Many synthetic compounds accumulate in nature because the release rates exceed the rates of microbial and chemical degradation. Two major reasons for low biodegradation rates have been identified. (i) The biochemical potential to degrade a certain compound is limited. This is more likely the less the chemical resembles natural compounds [5,89,116]. (ii) The pollutant or other substances, eg appropriate electron acceptors, are unavailable to the microflora [19,73,79]. In this short review we discuss biological and environmental factors that govern the mass transfer of pollutants to microbes which possess the capacity to degrade them. Since limited mass transfer has not only been observed during bioremediation of polluted soil, this review also aims at making results from fields such as cell physiology, fermentation technology, and theoretical biology available to microbiologists and engineers.

Mass transfer limitation of cells and populations

Chemicals seem to be available for microbes only when they are dissolved in water [31,51,84,109,114,124,125]. Consequently, nonaqueous phase liquids (NAPL) and solid compounds have to dissolve [117] and sorbed substrates have to desorb to become available [42,51,75,93,106,110] as shown in Figure 1. Degradation of the rate-limiting chemical, which is usually the carbon source [6], by a single cell (microscopic scale) or a population of a given three-dimensional array (macroscopic scale) can be described by Michaelis–Menten kinetics [25]. The flux of a chemical through the membranes of cells Q_c (microscopic: mass per time; macroscopic: mass per volume per time) is

$$Q_c = Q_{\max} \frac{C_c}{K_m + C_c} \tag{1}$$

where $Q_{\max}$ is the maximum flux, C_c (mass per volume) is the concentration of chemical exposed to the cell surfaces and K_m (mass per volume) is the cell surface concentration yielding $\frac{1}{2}Q_{\max}$. When the chemical is a carbon source, part

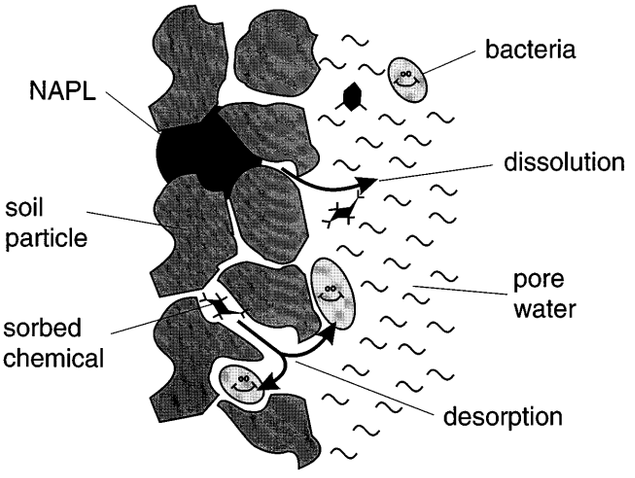

Figure 1 Distribution of bacteria and contaminants in a contaminated soil. Desorption and dissolution are indicated by arrows. NAPL, nonaqueous phase liquids.

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of the substrate transported into the cells is used to synthesize biomass. This can be accounted for by extending Equation 1

$$Q_c Y = Q_{\max} Y \frac{C_c}{K_m + C_c} - b \qquad (2)$$

where Y (numbers per mass) is the cell yield, $Q_c Y$ equals the growth rate μ, whereas $Q_{\max} Y$ equals the maximum growth rate $\mu_{\max}$ [82]. The actual growth is biased by the rate constant b (per time) that accounts for the fraction of substrate used to maintain the population by compensating for cell decay [55].

Equation 1 describes the flux into cells as a function of the concentration at the cell surface. However, the uptake of substrate reduces the concentration at the cell surface. C_c is therefore determined by both the substrate uptake and the substrate transfer to the cells. For substrate diffusion, the flux to the cells Q_d is

$$Q_d = k(C_d - C_c) \qquad (3)$$

where C_d is the distant aqueous concentration of the chemical and the mass transfer coefficient k (microscopic: volume per time; macroscopic: per time) accounts for the capacity of the medium to let the chemical diffuse through. Appropriate terms for k on the microscopic scale are: (i) $D_{\text{eff}}A/x$ for linear diffusion [64]; (ii) $D_{\text{eff}}4\pi R$ for radial diffusion [64]; and (iii) $k_d A_{sw}$ for the dissolution of solids or separate phase liquids [117]. D_{eff} (area per time) is the effective diffusivity which may include effects of physical restriction and sorption on diffusion, A is the area through which the diffusion takes place, x is the distance, R is the diameter of a cell, k_d (distance per time) is the rate constant of dissolution, and A_{sw} is the contact area between the chemical and water. Macroscopic k values are obtained by dividing microscopic k values by the volume of the living space occupied by the population. Equations 1 and 3 can be combined when steady state $Q_d = Q_c$ is assumed:

$$Q_c = Q_{\max}(C_d + K_m + J)\left(1 - \sqrt{\frac{1-4C_d J}{(C_d+K_m+J)^2}}\right)/2J \qquad (4)$$
$$J = Q_{\max}/k$$

Equation 4 was derived by Best [12]. The factor J (mass per volume) equals the ratio of the degradative capacity of the cells to the transport capacity of the medium around the cells. Equation 4 represents a general concept for the consumption of substrate by microorganisms as a function of a distant substrate concentration. Figure 2 illustrates how the parameters included in Equation 4 determine the actual substrate flux. First, the mass transfer rate is a function of the cell parameters $Q_{\max}$ and K_m which, in combination, influence C_c (Figure 2a). Second, the distance between the substrate source and the microorganisms determines the slope of the diffusion gradient (Figure 2b). Third, properties of the chemical determine the distant substrate concentration (Figure 2c). Fourth, the geometry and the type of the medium between the substrate source and the microorganisms determines the effective diffusivity of the chemical (Figure 2d). In the following we want to discuss the importance of these factors for the availability of pollutants in nature.

Factors influencing mass transfer

Characteristics of the microorganisms

Many microbial transporters and catabolic enzymes are regulated, ie they are only synthesized in response to the presence of a certain concentration of their substrate [104,105]. A concentration of 0.1 μM induced the degradative pathway of *p*-hydroxybenzoate [36]. Enzymes for the catabolism of 3- and 4-chlorobenzoate by *Acinetobacter calcoaceticus* were induced at substrate concentrations above 1 μM [88], while 50 μM linuron was needed to induce the amidase that is necessary for a Gram-negative bacterium to cleave certain phenylurea herbicides [68]. In addition, microbes may need a continuous flux of substrate to keep enzyme synthesis turned on [54].

Mass transfer rates in non-sterile systems are faster than those in sterile systems [21]. This is because metabolically active microorganisms drive the diffusion by reducing the local concentrations of substrates, nutrients, and electron acceptors. Moreover, mass transfer rates seem to depend on the organism. Guerin and Boyd reported that *Pseudomonas putida* 17484 added to soil caused a flux of sorbed naphthalene that exceeded that driven by the strain NP-Alk or the desorption rate in a sterile control [48]. An explanation for these findings given by the authors was the attachment of *Pseudomonas putida* 17484 to the sorbent leading to the reduction of the naphthalene concentration close to the sorbent. Such an effect of the distance between cells and substrates has also been observed by others [50,51,90].

The ratio of $Q_{\max}$ to K_m determines how much a cell reduces the substrate concentrations at its surface [17,25,63,65,67]. The reduction is more efficient the higher the uptake rate at low substrate concentrations. Law and Button defined this so-called specific substrate affinity $a°_A$ (volume per biomass and time) as the slope of the first order part of the activity-versus-concentration plot [67]. Koch combined the specific affinity of *Escherichia coli* with the substrate diffusion to the cell by expressing the efficiency of the solute uptake as the volume of solution cleared of substrate per unit time and per cell [63]. However, the cleared volume has no physical meaning, since it does not reflect the dynamic substrate resupply. Equation 4 can be used to calculate the mass transfer as a function of specific affinities of the cells and their distance to the substrate source. Figure 3 shows a calculation of linear diffusion driven by the dibenzofuran uptake of *Sphingomonas* sp HH19k [50] and of imaginary cells with 10-fold and 100-fold lower $a°_A$ (due to 10- and 100-fold higher K_m). When $C_d >> K_m$, the substrate flux is insensitive to $a°_A$ and to the distance from the substrate (Figure 3a). The flux becomes sensitive to both parameters only at $C_d \approx K_m$ (Figure 3b).

Often only a few of the indigenous microorganisms are able to degrade a pollutant added to the environment for the first time and efficient biodegradation will only occur after multiplication of these specialists [2,4,102]. However, growth of specialized organisms will only be favored in zones close to the contamination, where the flux of sub-

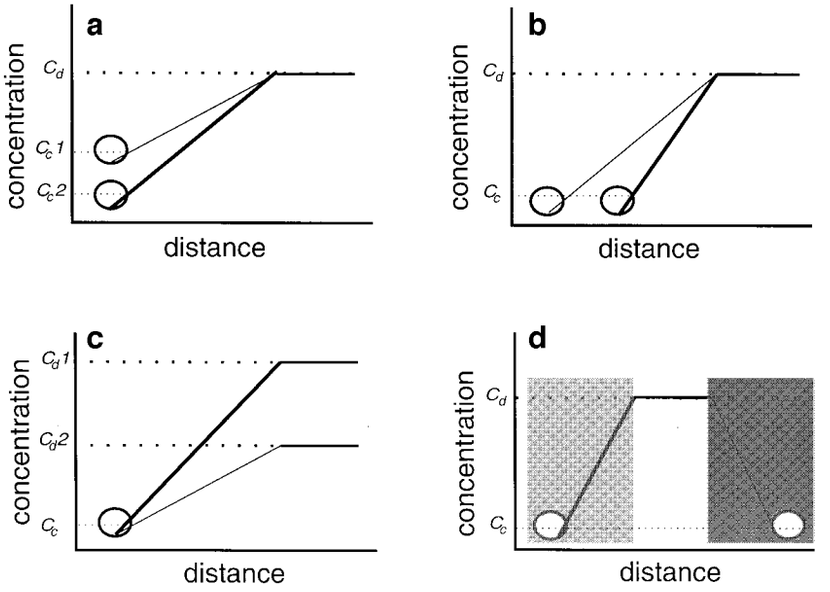

Figure 2 Schematic representation of factors governing the diffusive mass transfer of chemicals. More mass transfer is indicated by thicker lines. The circles represent bacteria. More mass transfer may result from the activity of cells with higher specific affinity, ie a lower cell surface concentration (a), from a shorter distance between substrate and cells (b), from higher distant substrate concentrations (c), and from less diffusion retardation (lighter background) by the medium between the substrate and the cells (d).

strate is sufficient to provide a nutritional advantage. Accordingly, limited substrate availability leads to a longer acclimation time by slowing the growth rate of the microbial population [2,69,112]. The rate constant b is a measure of substrate needed to maintain a viable population. A microbial culture will grow until it reaches a density at which the substrate provision only fulfills its maintenance needs. The closer a culture comes to this density, the more of the substrate consumed will not form additional biomass. Alexander reported that under certain conditions in aqueous systems all the carbon was mineralized and little or none appeared in the biomass [6]. In one study, 93–98% of benzoate, benzylamine, aniline phenol, and 2,4 D added to lake water or sewage at concentrations below 300 μg per liter was converted to CO_2, and no carbon assimilation was observed during the mineralization of 24 ng to 250 μg of benzylamine per liter [111]. In another study, only 1.2% of the carbon source was converted to biomass [14].

In the 1960s, Jannasch postulated that the constant low concentration of carbon in the oceans was unavailable to support microbial growth and was therefore not mineralized [58]. Threshold concentrations observed during the remediation of subsurface environments were correlated with the maintenance needs of the microorganisms [22,92]. Theoretical considerations show that a drop of the substrate flux below the maintenance rate prompts the appearance of a threshold concentration below which the substrate is not further degraded. The value of this threshold is determined by k and the maintenance coefficient [20]. Observations in our laboratory indicate that cells die when the substrate flux is below their maintenance requirements (H Harms, unpublished results).

Spatial distribution of pollutants and microbes

The distribution of a chemical and the microorganisms enters Equation 4 as one of the factors determining k. A pollutant may enter the soil as crystals [8], as a liquid [6], dissolved in an organic solvent [41,72], or sorbed to a solid phase [35]. The compound will, depending on its properties, either keep its physical state, dissolve in the soil water, sorb to the soil matrix, or evaporate in the gas-phase. In any case, the pollutant will be distributed non-randomly. Soil microorganisms occur mainly in an attached state [34,108]. Moreover, it was found that 60% of the soil bacteria were attached to particles covered with organic matter although these particles contributed only 15% to the total particle surface [46]. Upon multiplication microorganisms may form microcolonies, fostered by the low mobility of bacteria in soil. As a result of predation, soil bacteria most of which range in size between 0.5 and 0.8 μm [30], occupy pores with a mean diameter of 2 μm, the so-called protected habitable space [60,87]. More than 50% of the total pore volume of a soil may consist of pores with diameters <2 μm [29] and 30% of the total volume of some soils consists of pores with diameters less than 0.2 μm [52]. Hence, 30–50% of the pores are not accessible, simply because they are too small for a bacterium to fit in. As a consequence, the mean distances between microcolonies and pollutants in soil may be relatively large.

Degradation of toxic compounds will occur only as the pollutant is transported away from the source with resultant dilution to subtoxic concentrations [6]. But even when the pollutant is non-toxic the degradation zone may not approach the pollutant source, since the diffusion of electron acceptors to the contaminant may be limiting [23]. Lyngkilde and Christensen identified a sequence of distinct

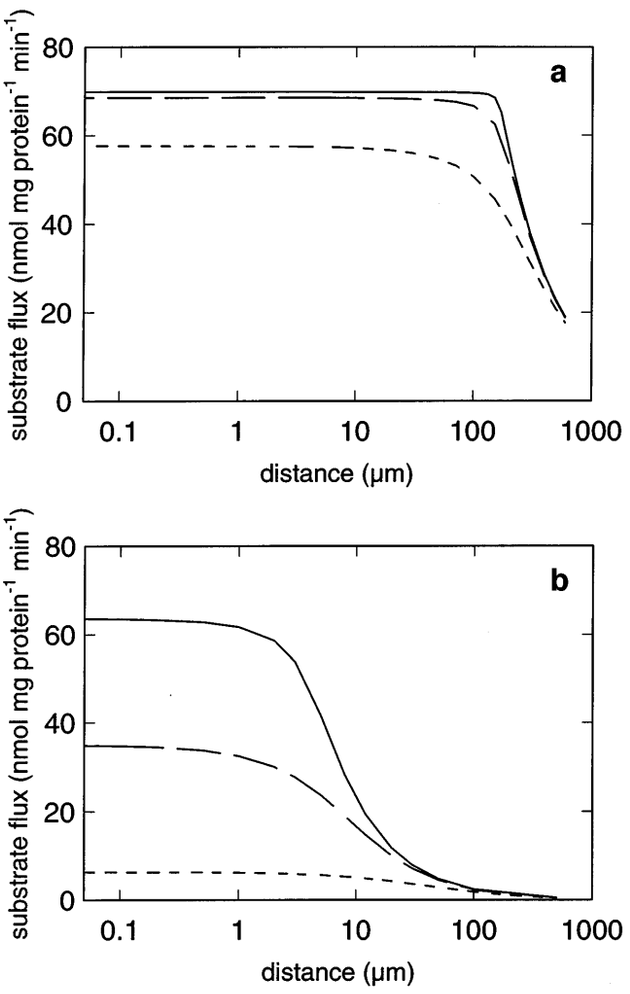

Figure 3 Calculations of the uptake-driven diffusive transfer of dibenzofuran to *Sphingomonas* sp HH19k (solid lines) and two imaginary bacterial strains only differing from HH19k by their ten-fold (long dashes) and hundred-fold (short dashes) lower specific affinities, as a function of the distance from the dibenzofuran source. Linear diffusion to the cells was assumed. C_d-values were set to the aqueous solubility of dibenzofuran at 28°C (32 μM, a) and to $10 \times K_m$ of HH19k (0.68 μM, b), respectively.

methanogenic, sulfidogenic, ferrogenic, nitrate-reducing and aerobic zones in the plume below a landfill [73]. The high organic content close to the landfill had resulted in the depletion of all electron acceptors except carbon dioxide, while during dilution of the leachate the other electron acceptors successively became available again. Pollutants which are not subject to anaerobic degradation will remain intact until they reach the oxic zone.

Many microorganisms reduce the distance to the substrate source by adhering to non-aqueous phase liquids, solid substrates, or organic matter containing their substrate. Marshall and Cruickshank observed the partitioning of bacteria into an oil phase followed by perpendicular orientation of the cells at the oil–water interface [76]. Bacteria that grow on hydrocarbons often adhere to their substrates [83]. Bacterial adhesion to hydrocarbons is governed by the cell surface hydrophobicity and is, therefore, commonly used to distinguish between hydrophilic and hydrophobic bacteria [94]. Bacteria degrading palmitic acid and sterol attached to their solid substrates after having degraded the dissolved fraction [44,113]. In contrast, no growth was observed on biphenyl and phenanthrene crystals [109]. The importance of a close contact to substrates becomes obvious when the contact is suppressed. A *Pseudomonas aeruginosa* strain which did not produce rhamnolipids could not attach to hexadecane and no growth was observed on hexadecane during 2 days of incubation [62]. Non-adhesive mutants of other alkane-degrading bacteria could not grow on pure alkane, however growth could be re-established by the addition of an emulsifier [95]. A similar result was observed with bacteria attaching to heptamethylnonane containing the growth substrate hexadecane, where adhesion and growth could be suppressed by the nontoxic surfactant Triton X-100 [40]. Preventing the adhesion of yeasts to hydrocarbons by a surfactant also suppressed their activity [3,81]. Association to substrates is even more important when their consumption requires exoenzymes. Here the enzyme has to diffuse from the cell to the substrate and the product has to diffuse back to the cells. Sorbed methyl-coumarinyl-amide leucin was therefore only used by surface-associated microorganisms but not by free ones [47]. Scow and Alexander studied the spatial separation of cells and substrates by providing the substrates inside artificial aggregates which excluded the bacteria [99]. An increase in the volume of solution retained inside the beads resulted in slower initial mineralization rates and lower residual substrate concentrations.

In soils bacteria are barely mobile. When applied to soil surfaces they penetrate only 3–5 cm [38,74]. Goldstein *et al* suggested that adding bacteria to soil did not stimulate the degradation of 2,4-dichlorophenol or *p*-nitrophenol because the cells were immobile in the porous matrix [43]. It seems therefore, that degradation of distant pollutants in soils relies mainly on the transfer of the chemicals rather than on movement of bacteria.

A special case exists under advective flow, when bacteria are attached on the surfaces of particles and the substrate is dissolved in the mobile water phase. This situation may arise in contaminant plumes in aquifers. It was shown experimentally that under advective flow the diffusion of substrate to the cells limits the biodegradation rate [26,50]. The mass transfer to individual cells on the particles is determined by the liquid flow, the particle diameter, the cell density, and the microbial rate constants [50,91,123].

Pollutant and matrix properties

Chlorinated phenols, benzoic acids, and anilines are similar to natural organic compounds and can be incorporated in humus via oxidative coupling [16]. This reaction is catalyzed by peroxidases and phenol mono-oxygenases [77] and by inorganic materials, such as oxides and oxyhydroxides of iron [97], allophane [66], and silica and clay [118]. Phenolic lignin derivatives, such as vanillic acid, vanillin, ferulic and syringic acid, and man-made organics such as chlorinated phenols, naphtholic compounds and halogenated anilines can be cross-coupled with natural phenols in soil [11]. Chemicals which are linked to organic matter lose their original chemical and biological activity, and hence, are less bioavailable than the free compounds [15].

While the diffusivity of low-molecular weight chemicals in water is generally in the range of 1×10^{-9} m^2 s^{-1}, diffusion in soils can be much slower. D_{eff} enters Equation 4 as one of the factors determining k. Table 1 summarizes D_{eff}-values of pollutants that were observed in soils and sediments. Reported D_{eff} up to 12 orders of magnitude lower than in pure water can be ascribed to the combined effects of: (i) physical barriers which restrict the diffusion to pores of small diameters and high tortuosities; and (ii) sorption, ie reversible physical or chemical interactions of the compounds with soil constituents. Mineral particles increase the diffusion path length and restrict the cross-sectional area through which the diffusion takes place. Sorption, in contrast, retards diffusion by transferring the solute from the aqueous phase into solid soil components. Sorption of hydrophobic contaminants in soil has been considered as partitioning between the water phase and the organic matter [32,59,98,121] or as a physical binding to the organic matter [27]. Polar solutes like nitroaromatic compounds may adsorb specifically to mineral surfaces [49]. Smith *et al* explained the reduced degradation of quinoline in the presence of montmorrilionite and hectorite by the sorption of the compound to these clay minerals [103]. However, reduced degradation of nonsorbing acetate in the same system could only be explained by the physical impairment of the substrate uptake by clay particles covering the cells. Ou and Alexander showed that the presence of glass beads between chitin and degrading organisms delayed the degradation [85]. Adu and Oades showed that starch in micropores of artificial soil was unavailable [1] and Weissenfels *et al* concluded that scarcely available polycyclic aromatic hydrocarbons (PAH) are located in inaccessible sites within the soil [122].

Slow diffusion through natural and artificial aggregates reduces the bioavailability of pollutants [51,80,90,100]. A sorption-retarded radial diffusion model [126] could explain the effect of aggregate size on α-hexachlorocyclohexane (α-HCH) desorption and bioconversion rate [90]. Intraparticle diffusion may be a slow process which does not reach equilibrium within years [9,10,24,45,90]. The longer certain compounds are in contact with soil, the more resistant they become to desorption and degradation. In soils this has been found with chlorophenol [96], TCE [86], picloram [78], simazine [101], 1,2-dibromoethane [107], hexachlorobenzene [13], nitrophenol, and phenanthrene [53]. Steinberg attributed this so-called contaminant aging to progressive entrapment of the chemicals in microscopic pores [107]. Contaminant aging could be simulated in short time scales with 3-chlorodibenzofuran that was sorbed by Teflon particles [51].

Another kind of physical barrier is pore space filled with gas. The diffusivity of small molecules in gas is generally in the range of 1×10^5 m^2 s^{-1} [119]. The effect of gas on D_{eff} will therefore depend mainly on the Henry's law constant for the distribution between both phases. The mass transfer of volatile compounds such as naphthalene through the gas phase is favored as compared to the water phase. Therefore, naphthalene-degrading bacteria grew denser when the water content of their porous environment was lower (H Harms, unpublished results). Air–water interfaces act as reactive surfaces which accumulate hydrophobic chemicals [56]. Before mass transfer through unsaturated zones is in a steady state, part of the chemical will be lost to the multitude of air–water interfaces.

Table 1 Effective diffusivities of low-molecular weight chemicals

Sorbate	System	Effective diffusivities (m^2 s^{-1})	Ref
Low molecular weight chemicals	pure water	5×10^{-10}–1×10^{-9}	[120]
Low molecular weight chemicals	air	5×10^{-6}–2×10^{-5}	[119]
α-HCH	mixed soil suspensions	5×10^{-17} [a]	[90]
Pentachlorobenzene	Charles River sediment	$<8.3 \times 10^{-15}$ [a]	[126]
Pentachlorobenzene	North River sediment	8.3×10^{-16} [a]	[126]
Pentachlorobenzene	soil	2.5×10^{-14} [a]	[126]
1,2,4-Trichlorobenzene	Charles River sediment	3.3×10^{-14} [a]	[126]
1,4-Dichlorobenzene	Charles River sediment	1×10^{-13} [a]	[126]
1,2,3,4-Tetrachlorobenzene	Charles River sediment	2.0×10^{-14} [a,b] 8.3×10^{-15} [a,c]	[126]
1,2,3,4-Tetrachlorobenzene	North River sediment	5.0×10^{-15} [a,b] 1.3×10^{-14} [a,c]	[126]
1,2,3,4-Tetrachlorobenzene	soil	1×10^{-13} [a]	[126]
1,2,4,5-Tetrachlorobenzene	sandy aquifer material	1.3×10^{-11} [a,d] 1.7×10^{-13}	[9]
Perchloroethene	sandy aquifer material	1.4×10^{-10} [a,d] 9.2×10^{-12}	[9]
1,2-Dibromoethane	soil	2–8×10^{-21} [a]	[107]
Kepone	salt marsh sediment	3.7×10^{-16} [a]	[33]

[a]Determined in soil slurries.
[b]Determined during sorption.
[c]Determined during desorption.
[d]Differences were observed with different particle size classes.

Measures to improve mass transfer in soil

Homogenization

Degradation rates can be enhanced by reducing the average distances between the cells and their substrates. This leads to faster substrate flux and allows maintenance of more cells. It is well accepted, that homogenization of soils promotes the degradation of sparsely mobile pollutants [18,90]. Zehnder and his co-workers studied the feasibility of bioremediation of a soil that had been contaminated with α-HCH for more than 20 years [8,37,57,90]. Figure 4 summarizes the results of this study. Biodegradation was stimulated after the soil was dug out and put into lysimeters. Addition of nutrients or oxygen did not increase biotransformation proving that nutrient availability was not limiting. Also, no biodegradation was observed at concentrations below 150 mg kg^{-1} [37]. In contrast, a rigorous mixing of the soil breaking up the large soil particles resulted in an almost complete degradation of the α-HCH within 2 weeks [90]. Biodegradation in these soil slurries was still desorption-limited since a pure culture isolated from the polluted soil completely mineralized the same amount of α-HCH within 2 days [57]. Only a pulverization of soil particles liberated the α-HCH trapped inside, increasing the biotransformation rates and decreasing the residual concentrations.

Vapor extraction

Soil vapor extraction (SVE) or bioventing makes use of the high Henry constants of pollutants such as chlorinated hydrocarbons. Air injection wells provide air to the subsurface and enhance air flow through the contaminated zone. The volatilized contaminant is removed through extraction wells. The efficiency of SVE arises from the high air flow rates which can be applied. Travis and MacInnes report on the fast removal of non-sorbed trichloroethylene (TCE) and perchloroethylene (PCE) from several sites [115]. However, when the pollutant had time to enter the interior of soil particles, SVE seems to lose its superiority over the water-based pump-and-treat technique. It was shown that for an aged TCE contamination, intraparticle transport limited the rate of TCE removal by SVE [45].

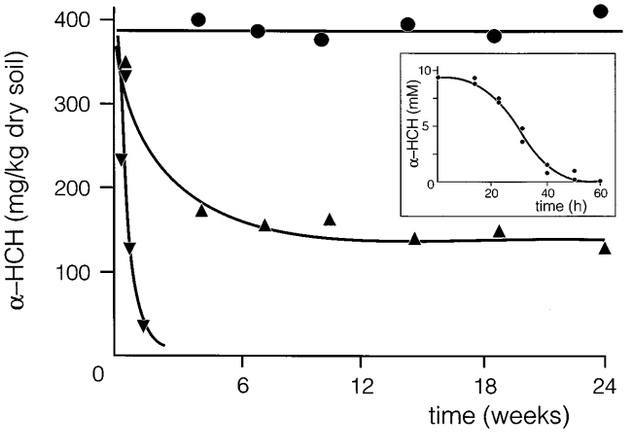

Figure 4 Biodegradation of α-HCH in the field (●), in lysimeters (▲), in laboratory slurries (▼) and in pure culture (insert) (data from references 37, 57, and 90).

Surfactants

There is an ongoing discussion about the effectiveness of surfactants in bioremediation. For a summary of positive and negative effects of surfactants, the reader is referred to Liu *et al* [70]. Enhancement of biodegradation has usually been ascribed to the surfactant-mediated solubilization or emulsification of sorbed, crystalline or separate phase pollutants. Detrimental effects have been attributed to the toxicity of the surfactant, the prevention of bacterial adhesion to the substrate, or the preferred degradation of the surfactants. Typically, surfactant concentrations must be greater than the critical micelle concentration (CMC) before solubilization occurs [39], especially when some of the surfactant sorbs to soil [71], but some surfactants may increase the water pseudo-solubility of hydrophobic molecules below the CMC [61]. Surfactants do not increase the aqueous concentration of a compound, but introduce the so-called micellar pseudophase [70], which accumulates the chemical and is mobile. Therefore, it may be argued that the effectiveness of surfactants is mainly due to the homogenization of hydrophobic pollutants.

In situ bioremediation

Residual concentrations of pollutants will always remain after bioremediation of polluted soil, due to sorption and incorporation in organic matter. It is imaginable to first remove the mobile fraction of pollutant via a biological treatment. During this phase, both homogenization and the addition of surfactants may stimulate the biodegradation rate. However, the effect of homogenization is minor in later stages of *in situ* bioremediation. The residual pollutant is trapped inside soil aggregates which cannot be broken up by mechanical mixing. Therefore, diffusion within the micropores, retarded by sorption, is the limiting factor for biodegradation. It should be sufficient to monitor pollutants that are slowly leaching from the soil and to stimulate biotransformation by the addition of nutrients when a critical concentration is reached. Bioremediation can be stopped when the risk associated with the soil pollution is below acceptable limits. A similar approach would be feasible for contaminated groundwater. The application of flow-stop-flow techniques would overcome the limitations resulting from advective-diffusive transport mechanisms during pump-and-treat remediation of contaminant plumes. New pollutions have to be treated biologically as soon as possible to achieve optimal results since long contact times between pollutants and soil have a negative effect on the success of bioremediation. However, priority should be given to the prevention of new pollutions.

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