SIMULATION OF SUBSURFACE BIOTRANSFORMATION

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Simulation of subsurface biotransformation

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Het in dit proefschrift beschreven onderzoek begon als 4-jarig projekt onder leiding van Gemeentewaterleidingen Amsterdam, gefinancierd door het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer. Toen het ministerie na 2 jaar de financiering plotseling had stopgezet, droeg het Speerpuntprogramma Bodemonderzoek er aan bij dat het onderzoek toch nog 2 jaar kon worden voortgezet na de vervulling van mijn vervangende dienst.

De steun van de vakgroep Organische Chemie was onontbeerlijk bij de totstandkoming van hoofdstuk 4. Hoofdstuk 6 is het resultaat van samenwerking met de vakgroep Wiskunde en het "Department of Geography and Environmental Engineering" van de Johns Hopkins University in Baltimore (USA).

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Stellingen behorende bij het proefschrift "Simulation of subsurface biotransformation", Tom N.P. Bosma, Wageningen, 14 maart 1994.

1. Een vereenvoudiging van het stelsel van sociale zekerheid door invoering van een basisinkomen voor iedereen in kombinatie met afschaffing van o.a. het wettelijk minimumloon, de bijstand en het studiefinancieringsstelsel, zal leiden tot een vergroting van de werkgelegenheid en voorkomt tegelijkertijd het ontstaan van een armoedige onderklasse.

2. De in de ekotoxikologie gangbare gedachte dat ekosystemen kunnen worden gerepresenteerd door één soort, berust op een conceptuele misvatting over struktuur en funktioneren van ekosystemen.

3. De herwaardering van reeds in gebruik zijnde chemikaliën kan beter worden gebaseerd op veldgegevens over persistentie en toxiciteit dan op gegevens verkregen met laboratoriumtoetsen.

4. Als beleidsmakers aan ekonomische modellen dezelfde eisen zouden stellen als aan milieumodellen, zouden de voorspellingen van het Centraal Planbureau niet meer worden gebruikt bij het opstellen van een nieuwe begroting.

5. In het bakteriënrijk heeft het onderscheiden van soorten weinig betekenis omdat bakteriën voor de voortplanting niet afhankelijk zijn van de uitwisseling van genetische informatie en omdat uitwisseling ook tussen verschillende "soorten" plaatsvindt.

6. Preciezie en bekrompenheid zijn uitingen van dezelfde karaktertrek, evenals tolerantie en onverschilligheid.

7. Aan stellingen behorende bij een proefschrift behoort, evenals aan wetenschappelijke hypothesen, niet als eis te worden gesteld dat zij verdedigbaar zijn, maar juist dat zij aanvechtbaar zijn.

8. Als S_5 "geestelijk of mentaal niet in staat tot het vervullen van aktieve dienst" betekent, moeten alle erkend gewetensbezwaarden worden afgekeurd en worden vrijgesteld van het vervullen van vervangende dienst.

9. Aangezien het nivo van universitaire opleidingen dat van hogere beroepsopleidingen niet ontstijgt, behoren beide te worden afgesloten met een "bachelors" diploma en moet er met spoed een wetenschappelijke tweede fase opleiding worden gekreëerd waarmee een titel op het nivo van "Master of Science" kan worden verworven.

10. Het feit dat emancipatoire stellingen vooral zijn te vinden bij proefschriften van promovendae is veelzeggend.

11. Het vervangen van ten minste één op de tien academici door twee leden van het ondersteunend personeel zou de produktiviteit van het wetenschappelijk werk aanzienlijk vergroten.

12. De gewoonte om slavinnenhandel aan te duiden als vrouwenhandel heeft uitsluitend tot doel de illusie in stand te houden dat de moderne westerse kultuur boven slavendrijverij verheven is.

13. Een volwaardige ontwikkeling van systeemwetenschappen als de ekologie en de ekotoxikologie is alleen mogelijk als de beoefenaars aan het credo "uitzonderingen bevestigen de regel" meer dan alleen een stochastische betekenis toekennen.

14. Europa is één in haar versplinterdheid.

15. Al is de mensheid nog zo snel, Gaia achterhaalt haar wel.

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CHAPTER 1

INTRODUCTION

ABSTRACT

Hydrophobic organic contaminants tend to accumulate in the atmosphere and the subsurface as a result of their physical and chemical properties. Their reactivities in these sinks mainly determine their persistence in the global environment. The research described in this thesis is intended to assess the potential of subsurface micro-organisms to transform organic contaminants and to assess the impact of the interaction between physical-chemical and microbial processes on their reactivities in the subsurface.

The extensive production and use of synthetic organic compounds in almost all human activities has led to a wide distribution of these compounds throughout the environment (Bouwer 1991). Synthetic organic compounds can be subdivided into several classes based on structural characteristics, like presence or absence of aromatic rings and their numbers, and the occurrence of halogen substituents. Table 1 lists some classes of organic contaminants with examples.

Table 1: Some classes of organic contaminants

Compound category	Description	Examples
Halogenated alkyl compounds	non-aromatic compounds with halogen substituents	chlorofluorocarbons (CFC's), bromoform, lindane, tetrachloroethene
Monocyclic aromatic hydrocarbons	non-halogenated compounds containing one ring with conjugated double bonds (the benzene ring)	benzene, toluene, phenol, benzoate
Polycyclic aromatic hydrocarbons (PAH's)	non-halogenated aromatic compounds containing more than one ring	naphthalene, fluorene, benz(a)pyrene
Halogenated aromatic hydrocarbons	compounds containing at least one aromatic ring plus halogenated substituents (not necessarily on the ring itself)	Alachlor, DDT, DDD, chlorobenzenes, chlorophenols, Polychlorinated biphenyls (PCB's)
Heterocyclic hydrocarbons	mono- and polycyclic hydrocarbon atoms in one of the nuclei	atrazin, bromacil, dibenzofuran, dioxins

Organic contaminants enter the environment via waste disposal, accidental spills, application as pesticide, and via losses during transport, storage, and use. It has been recognized already since the early sixties that environmental pollution with low concentrations of organic chemicals is world-wide. In the last decade, the danger of heavily polluted sites to nature and mankind has received increased attention.

Global exchange of organic chemicals mainly occurs via trade, the atmosphere , the oceans, and biota (Tanabe et al 1983, Krämer et al 1984, Eltzer and Hites 1989, Ballschmiter 1991, Kjeller et al 1991, Hoff et al 1992, Menzie et al 1992). The world-wide character of pollution is illustrated by the presence of man-made organics in Arctic snow and in the air of the Northern and Southern hemispheres (Tanabe et al 1983, Gregor and Gummer 1989). The spreading of these chemicals is caused by high production and release rates combined with their stability against biotic and abiotic transformation and their relative mobilities in air, water, soil, and biota (Ballschmiter 1991).

Contaminants like PCB's, dioxins and PAH's are released directly to the atmosphere in combustion processes and can exist there both unbound and bound to particles (Eltzer and Hites 1989, Kjeller et al 1991, Menzie et al 1992). Wet and dry deposition then lead to soil

and water pollution (Eltzer and Hites 1989, Kjeller et al 1991, Menzie et al 1992). Subsurface contamination results from infiltration of contaminated surface water into river borders, from deposition with settling particles onto the sediment in sedimentation areas of rivers and large water bodies and from seepage from the top-soil to deeper layers (Oliver and Nicol 1982, Schwarzenbach et al 1983, Carter and Hites 1992, Beurskens et al 1993). Thus, the atmosphere and subsurface are two major sinks where hydrophobic organic contaminants accumulate (Fig. 1.1). Volatile compounds accumulate more in the atmosphere while less volatile compounds accumulate more in the subsurface. Mineralization of these otherwise recalcitrant compounds in one of these sinks is the only pathway removing these compounds from our environment.

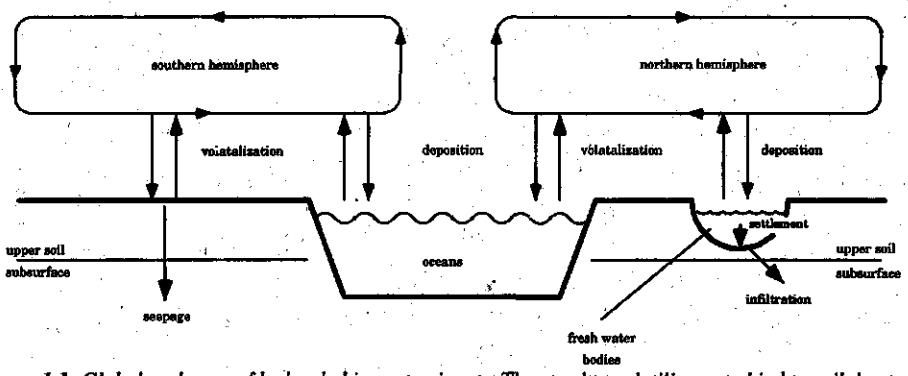

Figure 1.1: *Global exchange of hydrophobic contaminants. They tend to volatilize or to bind to soil due to their physical-chemical properties. As a consequence, the subsurface and the atmosphere are major sinks where these contaminants accumulate. Exchange between the northern and southern hemispheres is minor and occurs via human intervention (transport by air and sea) and biota (animals traveling over the equator).*

Transformation reactions in the atmosphere are almost exclusively (photo)chemical and may interfere with other atmospheric compounds. Volatile CFC's (chlorofluorocarbons) for instance, can survive unchanged for more than 100 years in the atmosphere due to their physical and chemical inertness. They diffuse eventually upward into the stratosphere where Cl-radicals are released by short-wavelength UV-radiation (Rowland 1991). This reaction initiates the well-known breakdown of the stratospheric ozone-layer and is the only removal mechanism of CFC's known to occur in the atmosphere (Rowland 1991).

Photochemical reactions are not possible in the subsurface, where microbially mediated transformations constitute the dominant removal mechanism of persistent organic compounds. The potential of micro-organisms to biotransform organic contaminants and the role of environmental parameters like redox potential and presence of a soil matrix is discussed in the following.

BIOTRANSFORMATION POTENTIAL

The biological and chemical reactivity of organic contaminants determine their level of persistence in soil and ground water. Microbial transformation is often required for eventual mineralization (Mackay and Cherry 1989, Bouwer 1991). However, transformation may also lead to the formation of (intermediary) products which can also be hazardous to the environment (Bouwer 1991). It was already recognized early in this century that bacteria are able to rapidly oxidize complex, chemically stable organic structures originating from petroleum, paraffin, and benzine (Söhngen 1913). Halogenated organic structures produced by marine algae, invertebrates, and terrestrial plants, are also rapidly transformed by micro-organisms (Faulkner 1980, Meyer et al 1990, Mohn and Tiedje 1992). The potential of micro-organisms from aquatic and subsurface habitats to metabolize and mineralize man-made halogenated and non-halogenated organic contaminants is well documented these days.

Environmental redox conditions play a major role in determining the kind of reactions that contaminants will undergo. Complete aerobic mineralization of non-halogenated mono- and poly-aromatic compounds like benzene, toluene, and naphthalene can proceed within hours or a few weeks, under optimal conditions (Atlas 1981, Bauer and Capone 1988, Mihelcic and Luthy 1988). Mineralization of such compounds is also possible under anoxic, denitrifying conditions (Berry et al 1987, Evans and Fuchs 1988, Kuhn et al 1988, Mihelcic and Luthy 1988, Grbic-Galic 1991). Biotransformation of mono-aromatics has even been demonstrated in sulfate reducing and methanogenic enrichments and in an anaerobic aquifer amended with sulfate (Wilson et al 1986, Evans and Fuchs 1988, Acton and Barker 1992, Edwards and Grbic-Galic 1992, Edwards et al 1992). Transformation rates of non-halogenated aromatic compounds generally become slower with increasing ring numbers in the molecule (Leahy and Colwell 1990).

Biodegradation of halogenated organic contaminants can occur under both aerobic and anaerobic conditions. Chlorinated compounds are readily mineralized by aerobic bacteria, provided that unsubstituted carbon atoms are available to bind with molecular oxygen (Abramowicz 1990, Commandeur and Parsons 1990, Leahy and Colwell 1990, Chaudhry and Chapalamadugu 1991). Chloro-, dichloro-, 1,2,3-trichloro-, 1,2,4-trichloro-, and 1,2,4,5-tetrachlorobenzenes for example can be mineralized by aerobic bacteria while aerobic conversion of penta- and hexachlorobenzene has never been demonstrated (Schraa et al 1986, Van der Meer et al 1987, Sander et al 1991). Highly chlorinated contaminants seem to resist aerobic transformation but they are susceptible to biological reductive dechlorination occurring under reducing conditions (Abramowicz 1990, Commandeur and Parsons 1990, Bosma et al 1991, Mohn and Tiedje 1992). Reductive dechlorination of hexa- and pentachlorobenzene can proceed at high rates in methanogenic samples from river sediment or activated sludge (Fathepure et al 1988, Holliger et al 1992). A similar pattern was found for aerobic and anaerobic metabolism of chlorinated biphenyls and chlorinated ethenes (Bedard et

al 1987a, Bedard et al 1987b, Barton and Crawford 1988, Bosma et al 1988, Fathepure et al 1988, Davis and Carpenter 1990, De Bruin et al 1992). Removal of all chloride substituents by reductive dechlorination appears to be necessary before the aromatic ring can be mineralized to methane and carbon dioxide in reduced environments. (Suflita et al 1982, Dolfing and Tiedje 1986, Mikesell and Boyd 1986).

Acclimation of microbial populations to organic contaminants

Biodegradation studies are normally done in the laboratory with enrichments obtained from various sources. Sometimes, acclimation periods of varying length are observed before transformation takes place (Spain and Van Veld 1983, Wilson et al 1986, Van der Meer et al 1987, Wiggins et al 1987, Swindoll et al 1988). Causes for the existence of such acclimation periods may be (i) the need to induce enzymes involved in contaminant breakdown, (ii) the multiplication of an initially small microbial population until levels are reached that allow observable removal, and (iii) the occurrence of genetic adaptation via mutation and gene transfer which results in the evolution of a microbial strain that is able to transform the contaminant (Spain and Van Veld 1983, Hutchins et al 1984, Wiggins et al 1987, Van der Meer et al 1992).

The length of the acclimation period depends not only on the underlying mechanism (genetic adaptation presumably requires more time than enzyme induction), but also on physical-chemical factors. Limited availability of organic and inorganic nutrients may lead to an increase in the acclimation time by slowing down the growth rate of the microbial population (Lewis et al 1986, Aelion et al 1987, Swindoll et al 1988). Prior (*in situ*) exposure to the contaminant decreases acclimation times while low temperature and predation by protozoa may increase acclimation times (Atlas and Bartha 1972, Spain et al 1980, Herbes 1981, Wiggins et al 1987). Finally, acclimation to complicated molecular structures requires more time than acclimation to simple ones (Spain et al 1980, Hutchins et al 1984, Leahy and Colwell 1990).

One may ask whether a contaminant which is broken down in the laboratory only after a long acclimation time, will also be transformed *in situ*, especially if genetic adaptation is the reason for the long lag-time. Long acclimation times are evidence for the absence of adapted micro-organisms on one hand but, on the other hand, they illustrate the potential of the microbial community to evolve adapted strains that are able to transform the contaminant.

The role of contaminant concentration

Biotransformation rates of contaminants are often described with a Michaelis-Menten equation assuming that the biomass is more or less constant in time:

$$V = V_{max} \frac{C}{K_m + C} \qquad (1)$$

where V is the biotransformation rate, C the contaminant concentration, V_{max} the maximum biotransformation rate, and K_m the half saturation constant (contaminant concentration where $V = ½V_{max}$). Biotransformation is first order in contaminant concentration when $C << K_m$. Michaelis-Menten kinetics predict decreasing first order rate constants with increasing contaminant concentration until biotransformation is zero order at high contaminant concentration with $V = V_{max}$. Concentrations of organic contaminants in the subsurface are often below the µg/l level and first order kinetics are therefore often used to describe biotransformation rates.

Mineralization rates of 4-chlorobenzoate and chloroacetate were proportional to concentration in surface water samples incubated with 50 ng/l and 50 mg/l of each compound, which fits with first order kinetics (Boethling and Alexander 1979). However, the kinetics of methyl parathion degradation by *Flavobacterium* species appeared to be multiphasic (Lewis et al 1985). Biotransformation involved a low-affinity, high capacity system (high K_m, high V_{max}) at concentrations about 4 mg/l and less, and a high-affinity, low capacity (low K_m, low V_{max}) system at concentrations about 20 µg/l and less. Simple first order kinetics did not apply in the concentration range studied. The ratios of V_{max} and K_m however, were of the same order of magnitude for both systems, which resulted in similar predictions of biotransformation rates at extremely low concentrations (<< 20 µg/l).

Lowered concentrations may slow down the acclimation of microbial populations to contaminants, because they cause decreased microbial growth rates (Hutchins et al 1984). Acclimation times may also increase due to increased induction times at lower concentrations (Koch and Coffman 1970). However, increased concentrations of toxic compounds may also increase acclimation times or even prevent acclimation due to inhibitory effects. High quinoline concentrations (155-775 µM) inhibited induction of starved *Pseudomonas cepacia* cells inoculated to a soil column (Truex et al 1992). Similarly, no biotransformation of 100 µg/l 1,2-dichlorobenzene occurred in a column packed with Rhine River sediment during 2 years of operation while biotransformation of 10 µg/l 1,2-dichlorobenzene in a parallel column experiment was preceded by an acclimation time of 3 months (Van der Meer et al 1987, Van der Meer and Bosma, unpublished results). Once biotransformation had started, effluent concentrations declined rapidly until a level close to the detection limit of 0.1 µg/l. The long acclimation time was ascribed to genetic adaptation.

BIOTRANSFORMATION AS AFFECTED BY TRANSPORT AND SORPTION

A picture of a versatile microbial community that is able to transform and mineralize a variety of complex organic structures, both of natural and anthropogenic sources, arises from the previous overview. However, many of these compounds can persist for decades or longer in

the subsurface environment. The reason for this may lie in the presence of a soil matrix in the subsurface environment.

Transport and sorption

The flow of water is responsible for migration of contaminants through aquifers. Dispersion and diffusion spread the contaminant both longitudinally and transversely while sorption onto the soil retards their movement. Sorption of hydrophobic contaminants in soil can be considered as a partitioning process between the water phase and the organic matter (Karickhoff et al 1979, Schwarzenbach and Westall 1981). Equilibrium partition coefficients of individual contaminants can be estimated from octanol-water partition coefficients using linear, free energy relationships (Karickhoff et al 1979, Schwarzenbach and Westall 1981). These relationships also hold in aquifer materials with organic carbon contents as low as 0.1% and for simultaneous sorption of several dilute non-polar compounds (Schwarzenbach and Westall 1981, Barber et al 1992). However, the presence of several non-polar compounds together can have a synergistic effect on sorption in aquifer material with extremely low carbon contents (<0.1%), resulting in stronger sorption than predicted from linear, free energy relationships (Brusseau 1991). Partitioning of hydrophobic organic contaminants between organic matter and water is a physical process reaching site equilibrium within milliseconds or, at most, seconds. Therefore, diffusion normally is the rate controlling process in sorption (Weber et al 1991). Intra-particle diffusion controlled sorption rates of tetrachloroethene and 1,2,4,5-tetrachlorobenzene on sandy aquifer material in batch experiments (Ball and Roberts 1991a). Effective pore diffusion coefficients of these compounds were 2 to 3 orders of magnitude lower than bulk aqueous diffusion coefficients (Ball and Roberts 1991b).

Many transport models assume sorption equilibrium to describe contaminant transport. However, the flow rate dependency of pseudo-equilibrium sorption constants observed in column experiments cannot be explained with equilibrium sorption. Limitation of effective sorption rates by microscopic mass transfer, the existence of immobile pore water in addition to mobile pore water , and binding of contaminants to colloidal particles in the water phase are possible causes for the observed anomalies in column experiments (Van Genuchten and Wierenga 1976, Bibby 1981, Schwarzenbach and Westall 1981, Valocchi 1985, Crittenden et al 1986, McCarthy and Zachara 1989, Brusseau 1992, Dunnivant et al 1992).

Biotransformation of contaminants present in infiltrating water

The biotransformation of organic contaminants can be studied by the use of columns packed with subsurface material and operated under saturated conditions (Kuhn et al 1985, Van der Meer et al 1987). Residual steady state concentrations are often detected in the effluents of such columns (Van der Meer et al 1987). These concentrations are generally too high to be

explained by uptake and growth kinetics of indigenous bacteria alone (Schmidt et al 1985a). The distribution of indigenous bacteria in natural soil (Fig. 1.2A) could provide an explanation for observed residual concentrations. Soil aggregates may serve as micro-habitats for micro-organisms, where they form micro-colonies which are protected from adverse environmental influences such as predation by protozoa and nematodes (Stotzky 1972, Hattori and Hattori 1976). The result is a patchy distribution of indigenous bacteria in natural soils. Subsurface bacteria are indeed mainly associated with the solid phase (Harvey et al 1984). Furthermore, bacteria occur in small colonies and are more susceptible to predation in the outer regions of soil aggregates and in relatively large pores (Bone and Balkwill 1986, Vargas and Hattori 1986, Postma et al 1990, Postma and Van Veen 1990). Thus, indigenous micro-organisms will only come in contact with contaminants present in infiltrating water after diffusion into soil particles (Fig. 1.2A) and a limitation of biotransformation rates by intra-particle diffusion may be expected. Unfortunately, data on the interaction between sorption and biotransformation of infiltrating, sorbing organic chemicals are scarce.

Biotransformation of contaminants present inside soil aggregates

Sorbed biodegradable contaminants in soil are often metabolized slower than in aqueous culture. Increasing attention is being given to the effects of sorption kinetics on biotransformation rates in contaminated soil. Sorbed substrates appear to be less available and biotransformation rates are limited by diffusion of the chemical from the inside of soil aggregates to the bulk liquid (Ogram et al 1985, Rijnaarts et al 1990). Biotransformation of non-sorbing organics present in synthetic aggregates is also limited by diffusion out of the aggregates (Scow and Alexander 1992).

The effects of entrapment of contaminants in intra-particle micropores is even evident for biodegradable, weakly sorbing, volatile contaminants in top-soil. 1,2-Dibromoethane was present in agricultural top-soils up to 19 years after its last known application (Steinberg et al 1987). An effective intra-particle radial diffusion coefficient of about 12 orders of magnitude lower than aqueous diffusivities was found in batch experiments. A similar value was found in a Dutch clayey soil for the radial diffusion coefficient of entrapped α-hexachlorocyclohexane (Rijnaarts et al 1990). These values strongly deviate from estimates for the adsorption of tetrachloroethene and 1,2,4,5-tetrachlorobenzene which were only 2 to 3 orders of magnitude smaller than aqueous diffusion coefficients (Ball and Roberts 1991b). The extremely small diffusivities of 1,2-dibromoethane and α-hexachlorocyclohexane can only be explained by assuming that the contaminants have entered particle regions with an extreme tortuosity and steric restriction.

At old waste sites, contaminants are often present inside soil aggregates, e.g. due to slow diffusion that has taken place during years or decades. Microbial activity is often stimulated by addition of nutrients during bioremediation. Sometimes, specialized bacteria

capable of degrading certain contaminants are also introduced.These bacteria are deposited from the flowing water onto the surface of soil particles (McDowell-Boyer et al 1986, Elimelech and O'Melia 1990, Harvey and Garabedian 1991, Martin et al 1992). Added nutrients will mainly stimulate microbial activity in the outer regions of soil aggregates (Priesack 1991). As a result, the active microbial mass will primarily accumulate on the surface and in the outer regions of soil aggregates, in contrast to indigenous bacteria in undisturbed soils (fig. 1.2). Therefore, overall biotransformation rates during bioremediation are presumably controlled by sorption retarded diffusion rates of contaminants and not by the activity of degrading micro-organisms.

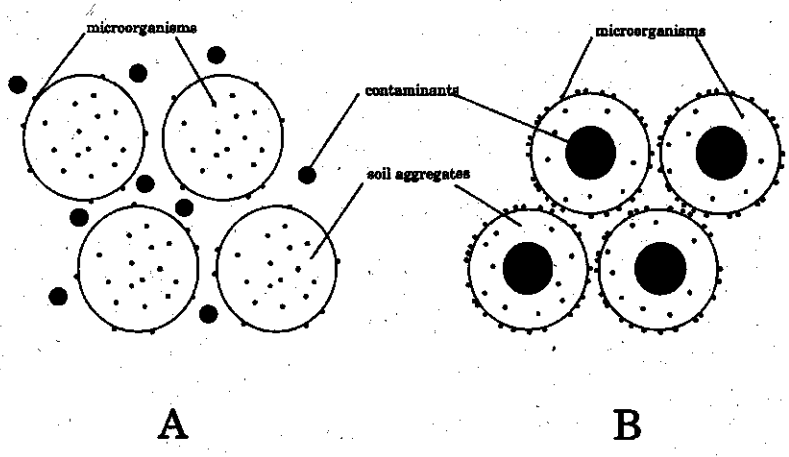

***Figure 1.2:** View of contaminants and bacteria in natural untreated soil (A) and in polluted soil (B).*

OUTLINE OF THIS THESIS

The research described in this thesis has two objectives. First, the biotransformation of organic contaminants under saturated flow conditions in two sandy sediments is simulated experimentally (Chapter 2-4). Chapter 2 describes results of column experiments performed under various redox conditions and temperature regimes. These experiments assess the potential of indigenous bacteria to biotransform a series of organic contaminants in sediment from the Rhine near Wageningen, The Netherlands, and from a dune infiltration area near Zandvoort, The Netherlands. The buffering capacity of the dune infiltration area with respect to temporal increases of contaminants in the infiltrating water is demonstrated by calculations with a simple transport model. Chapters 3 and 4 present pathways of reductive dechlorination of trichlorobenzenes and hexachloro-1,3-butadiene in Rhine River sediment columns operated under anaerobic conditions.

Then, experiments and computer simulations were carried out to investigate the interaction between physical-chemical and microbial processes which determine biotransformation rates under saturated flow conditions (Chapters 5-8). Chapter 5 demonstrates how chemotaxis may influence biotransformation. Chapter 6 shows how the interaction between the behavior of introduced bacteria and contaminant transport may affect biotransformation kinetics in saturated columns. A sensitivity analysis of a model of radial diffusion limited biotransformation of organic compounds in saturated columns is presented in chapter 7. Finally, chapter 8 discusses physical-chemical limitations of biotransformation kinetics which are imposed by the presence of a soil matrix.

CHAPTER 2

BIOTRANSFORMATION OF ORGANIC CONTAMINANTS IN SEDIMENT COLUMNS AND A DUNE INFILTRATION AREA

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ABSTRACT

Laboratory column experiments were performed to evaluate the fate of a series of chlorinated and non-chlorinated organic contaminants in Rhine sediment and sediment from the infiltration area of the Municipal Water Works of Amsterdam, near Zandvoort, The Netherlands. Columns were operated under aerobic, denitrifying and methanogenic conditions. All non-chlorinated and few chlorinated compounds were aerobically transformed. Of the compounds tested under denitrifying conditions, only 1,2-dichloro-4-nitrobenzene was partially transformed. Methanogenic conditions favored the transformation of chlorinated substances by reductive dechlorination. Toluene was the only non-halogenated compound that was transformed under methanogenic conditions. Residual concentrations after biotransformation were at least 10 times lower than the drinking water limit of 1 µg/l, except in the case of 1,2,4-trichlorobenzene which had a residual concentration of 2.6 µg/l. Residual concentrations did not depend on the influent concentration applied. Most transformations also proceeded at a temperature of 4°C, although the process of reductive dechlorination was slower than at 20°C. Hydrological calculations revealed that dispersion in the infiltration system can buffer 2 week pulses of contaminants resulting in concentration decreases in the water collected in the drainage system of at least 80 and 95% of polar and non-polar compounds respectively. There was a good qualitative agreement between removals observed in column experiments and the dune infiltration area.

INTRODUCTION

Chlorinated and non-chlorinated organic contaminants are present in many surface waters. Their concentrations are typically low, approximately 1 µg/l or less. However, temporarily increases resulting from accidental releases into surface water occur. Ground water quality can be affected by these contaminants via bank infiltration (Schwarzenbach et al 1983). Natural degradation processes may help to improve water quality during infiltration and are thus of importance for the protection of ground- and drinking water quality. These degradation processes may be biotic or abiotic, and the nature and rate of the reaction that can take place, are determined by the physical and chemical conditions at the infiltration site.

The present study was set up to investigate the fate of a range of organic contaminants in sandy sediments of the Rhine river and a dune infiltration site of the Municipal Water Works of Amsterdam. Rhine water is infiltrated in the latter to replenish the ground water resources for drinking water. Among the compounds studied were chlorinated and non-chlorinated benzenes and chlorinated aliphatic compounds, which have all been detected as contaminants in Rhine water. The behavior of these compounds in laboratory sediment columns was investigated under different environmental conditions. The role of redox potential and temperature were evaluated. Steady state effluent concentrations after biotransformation were determined and are discussed in view of drinking water quality requirements. Results are compared to field data to judge their relevance for prediction of the behavior of contaminants in the dune infiltration site. Finally, the buffering capacity of the dune infiltration area with respect to temporary increases of contaminants in the infiltrating water is demonstrated by calculations with a hydrological model.

MATERIALS AND METHODS

Description of the dune infiltration area

The dune infiltration area of the Municipal Water Works of Amsterdam is located near Zandvoort, The Netherlands. Rhine water is collected near Wijk bij Duurstede, The Netherlands, and pretreated to eliminate the high amount of particulate organic material present in Rhine water. Then, it is transported to the dune infiltration area via long distance tubing. The area harbors a system of infiltration ponds and drainage channels and tubes. A schematic drawing of one infiltration pond and its connection to the drainage system is presented in Fig. 2.1. The average residence time of the infiltrating water in the area is about 10 weeks. The water collected in the drainage system is subject to additional treatments in slow sand filters and an activated carbon installation before it is delivered to the drinking water system.

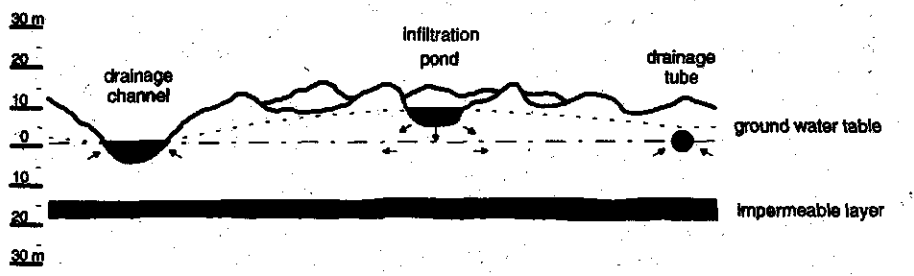

Figure 2.1: *Schematic drawing of the water flow between an infiltration pond and a drainage channel and tube in the dune infiltration area near Zandvoort, The Netherlands. The average distance between infiltration pond to drainage channel or tube is 80 m, and the average travel time is 10 weeks.*

A slime layer is deposited on the bottom of each infiltration pond. As a result, an anoxic zone develops just below the sediment surface where sulfate reduction and methanogenesis occurs. However, the lower part of the infiltration track becomes oxic due to air diffusion and input of oxygenated rain water from above. The extent of the anoxic zone in the area varies depending on the local organic carbon content of the dune sand which varies from less than 0.1% to 3-4%.

The pretreated infiltrating water and the drainage channels and tubes were sampled every two weeks and analyzed for organic contaminants in 1988. Techniques of sampling and analyses were described elsewhere (Smeenk et al 1993).

Experimental set-up

The column experiments were conducted in two different laboratories and each laboratory had its own set-up. Small PVC columns were used at the Department of Microbiology in Wageningen while large glass columns were used at the Municipal Water Works of Amsterdam in Heemstede. We refer to the experiments from the two laboratories as "small column experiments" and "large column experiments", respectively.

Small columns

Small columns were constructed of hard PVC (25 cm length, 5.5 cm i.d.) and were wet packed with sediment from the Rhine river near Wageningen, The Netherlands, or from the dune infiltration site of the Municipal Water Works of Amsterdam. The columns were percolated continuously at a flow rate of 1 cm/h in an up-flow mode, with a mineral medium prepared with highly purified milli-Q water (Millipore, USA) closely resembling the mineral composition of Rhine water (Van der Meer et al 1987). It contained NH_4Cl (27 mg/l), $MgCl_2 \cdot 6H_2O$ (102 mg/l), K_2HPO_4 (12 mg/l), $CaCl_2$ (222 mg/l), $NaHCO_3$ (215 mg/l), Na_2SO_4 (7 mg/l) and 0.15 ml/l of a trace element solution (Van der Meer et al 1987). In all experiments, the synthetic medium was continuously aerated in the presence of an excess of

granulated marble which served as carbonate buffer in combination with CO_2 in the air. In the anoxic columns, the originally aerated medium was depleted from oxygen by its continuous replacement with nitrogen gas amended with 0.5% CO_2 in a gas exchange chamber (Zeyer et al 1986). Reducing conditions were maintained by the addition of Na_2S (10 mg/l final concentration) via the solution of chlorinated hydrocarbons (Chapter 3). One anoxic column was operated without sulfide as reducing agent but with sodium nitrate (final concentration 47 mg/l) added as electron acceptor (Chapter 3). The columns were operated aseptically up to the influent port where a bacterial filter (cellulose-nitrate, 0.2 µm, Sartorius GmbH, Germany) prevented back-growth of micro-organisms from the sediment into the feeding lines. The medium was pumped into the columns by a peristaltic pump (Watson Marlow Ltd., U.K.) at a flow rate of 12.0 ml/h. Mixtures of chlorinated hydrocarbons were added continuously with a syringe pump (Perfusor VI, B. Braun Medical B.V., Germany) at a flow rate of 0.6 ml/h. These mixtures were prepared from water saturated stock-solutions, such that final concentrations in the influent were approximately 10 µg/l. The appropriate dilutions were autoclaved before use. Mixing of the chlorinated hydrocarbons with the mineral medium took place in a mixing chamber just before entering the sediment columns. The columns were operated in a climate room.

Large columns

Large columns were constructed of glass (60 cm length, 11 cm i.d.) and were wet packed with sediment from the dune infiltration site of the Municipal Water Works of Amsterdam. The columns were continuously percolated at a flow rate of 2.5 cm/h with infiltration water obtained from taps nearby the laboratory. On an average basis, the infiltration water contained (mg/l) HCO_3^- (154), Cl^- (163), PO_4^{3-}(0.035), SO_4^{2-} (69), NO_3^- (5), Na^+ (84), K^+ (5.9), Ca^{2+} (75), and Mg^{2+} (10.4) and had a pH of 7.7. The infiltration water was used without any further treatment. In the large column experiments 1 ml of a stock solution of the mixture of organic hydrocarbons in acetone, was directly dissolved in the influent vessel (which contained 10 L medium), which was kept under a constant air pressure. Influent vessels were never used for a period longer then two days. Concentrations of organic substances decreased less than 10% in this period. To investigate anoxic transformations, the influent medium was kept under nitrogen pressure and amended with ethanol (48 mg/l). Redox conditions were characterized by measurement of nitrate and sulfate reduction and methane production in the columns. The columns were kept at a constant temperature with a water mantle coupled to a water bath.

Sampling and analysis

Columns were sampled with glass syringes as previously described (Van der Meer et al 1987). Sample volumes varied from 1 to 50 ml, depending on the expected contaminant concentration. Samples were analyzed by hexane extraction followed by on-column injection

into a gas chromatograph (GC436, United Technologies, Delft, The Netherlands) equipped with an electron capture detector and a 25 m capillary column (Sil 5CB, 12 µm, Chrompack, Middelburg, The Netherlands) or by purge and trap injection (Chrompack, Middelburg, The Netherlands) · into a gas chromatograph (GC438, United Technologies, Delft, The Netherlands) equipped with an FID-detector and a 25 m capillary column (Sil 5CB, 12 µm, Chrompack, Middelburg, The Netherlands).

Chemicals

All organic compounds (analytical grade) were purchased from E. Merck (Darmstadt, Germany) and were used without further purification.

RESULTS AND DISCUSSION

Transformations under different redox conditions

Experiments were performed under aerobic, denitrifying, and methanogenic conditions to examine the potential of different microbial communities to transform organic contaminants. Aerobic, denitrifying and methanogenic conditions were created by the addition of molecular oxygen or nitrate or none of both in the influents of small columns, as the only major electron acceptor. The development of methanogenesis in the small columns was aided by the addition of sodium sulfide. The actual occurrence of aerobiosis, denitrification and methanogenesis in the large columns was tested by measuring oxygen and nitrate consumption and methane production. All columns were continuously operated for periods of 3 months to 4 years.

Observed transformations of chlorinated compounds in large and small columns packed with either Rhine or dune sediment, and operated at 20°C, are summarized in Table 2.1. Aerobic conditions turned out to be relatively unfavorable for transformation of the chlorinated compounds. In Rhine sediment, only chlorobenzene and 1,2-dichlorobenzene were transformed. In dune sediment, chlorobenzene and 1,2- and 1,4-dichlorobenzene were reduced by more than 90% while only a partial degradation of 1,3-dichlorobenzene (30%) and 1,2,4-trichlorobenzene (40%) was observed. Transformations in Rhine sediment were preceded by a lag-time of around 3 months after start-up of the column. Lag-times observed in dune sediment were 1-2 weeks for chlorobenzene and dichlorobenzenes and 2 years for 1,2,4-trichlorobenzene. The occurrence of lag-times before transformation took place, was taken as evidence that micro-organisms were involved. Different explanations can be given for these lag-times. They can be caused by the need to induce enzymes involved in contaminant breakdown (Spain and Van Veld 1983), or by growth of the microbial population until a level where transformation becomes measurable (Hutchins et al 1984, Wiggins et al 1987). Lag-times in the order of months or even years may be an indication that recombination of genetic

information takes place, leading to the appearance of microbes capable to metabolize chlorinated compounds (Van der Meer et al 1992). Thus the short lag-times observed with chlorobenzene and dichlorobenzenes in dune sediment indicate the existence of genetically adapted micro-organisms in the dune sediment while the longer lag-times for 1,2,4-trichlorobenzene in dune sediment and for chloro- and 1,2-dichlorobenzene in Rhine sediment may indicate their absence (Van der Meer et al 1987).

All chlorinated compounds tested except chlorobenzene, were transformed under methanogenic conditions, in both Rhine and dune sediments (Table 2.1). However, none of the observed reactions resulted in a complete detoxification of the parent compounds. Tetrachloroethene was dechlorinated to (*Z*)-1,2-dichloroethene and traces of chloroethene (< 5%) via trichloroethene as intermediary product in Rhine sediment. Hexachloro-1,3-butadiene was reductively dechlorinated via (*E*)-1,1,2,3,4-pentachloro-1,3-butadiene to (*E,E*)-tetrachloro-1,3-butadiene (Chapter 4). Trichlorobenzenes were reductively dechlorinated to chlorobenzene via 1,3-dichlorobenzene, while the 1,2,4-isomer was dechlorinated via 1,4-dichlorobenzene (Chapter 3).

Reductive dechlorination of hexachlorobenzene in dune sediment proceeded via 1,2,3,5-tetrachlorobenzene and 1,3,5-trichlorobenzene to yield 1,3-dichlorobenzene (90%) and via 1,2,4,5-tetrachlorobenzene and 1,2,4-trichlorobenzene to yield 1,4-dichlorobenzene (10%). This pathway is similar to pathways previously reported for hexachlorobenzene in microbial enrichments (Fathepure et al 1988, Holliger et al 1992), but it differs from the pathway reported for trichlorobenzene in Rhine sediment (Chapter 3).

Lag-times before reductive dechlorination took place, lasted longer than 2 months for all compounds in the small columns packed with Rhine sediment. In contrast, lag-times in the large dune sediment columns were 2 weeks or shorter. Like in the case of the aerobic transformations, this could be caused by the existence of an adapted population of bacteria in the dune sediment and its absence in the Rhine sediment. Alternative explanations are that the ethanol (48 mg/l) added to the influent medium of the dune sediment columns caused either a fast growth of the microbial population responsible for the reductive dechlorination or provided extra reducing equivalents needed for the reaction. No easily degradable carbon source was fed to the Rhine sediment column. Therefore, electrons for reductive dechlorination had to come from the organic matter (< 0.1%) present in the sediment already.

1,2-Dichloro-4-nitrobenzene was completely removed in all methanogenic columns without lag-phase. It was also partially degraded (50%) in the denitrifying column packed with Rhine sediment. The absence of a lag-time might be evidence that the reaction was purely chemical, but no attempt was made to prove the abiotic nature of the reaction. It has been demonstrated elsewhere that nitro-aromatic compounds can be reduced to corresponding anilines in presence of small concentrations of sulfides and mediators like quinone and iron porphyrin with half lives in the order of hours (Schwarzenbach et al 1990).

***Table 2.1:** Observed transformations (%) and products of chlorinated organic contaminants in columns with Rhine and dune sediment operated at 20°C with different electron acceptors.*

contaminant	condition			
	aerobic	denitrifying[1]	methanogenic	methanogenic products
tetrachloroethene	-	-	>99	(Z)-1,2-dichloroethene, chloroethene (< 5%)[2]
trichloroethene	-	-	>99	(Z)-1,2-dichloroethene, chloroethene (< 5%)[2]
hexachloro-1,3-butadiene	-	-	>99	(*E,E*)-1,2,3,4-tetrachlorobutadiene[2]
chlorobenzene	>99	NT	-	-
1,2-dichlorobenzene	90	-	>99	chlorobenzene[2]
1,3-dichlorobenzene	90	-	90	chlorobenzene[2]
1,4-dichlorobenzene	90	-	90	chlorobenzene[2]
1,2,3-trichlorobenzene	-	-	>99	chlorobenzene[2] 1,3-dichlorobenzene[4]
1,2,4-trichlorobenzene	40	-	>99	chlorobenzene[2] 1,4-dichlorobenzene[4]
1,3,5-trichlorobenzene	-	-	>99	chlorobenzene[2] 1,3-dichlorobenzene[4]
1,2,3,4-tetrachlorobenzene[3]	-	NT	>99	1,3- and 1,4-dichlorobenzene[4]
1,2,4,5-tetrachlorobenzene[3]	-	NT	>99	1,3- and 1,4-dichlorobenzene[4]
pentachlorobenzene[3]	-	NT	>99	1,3- and 1,4-dichlorobenzene[4]
hexachlorobenzene[3]		NT	>99	1,3- and 1,4-dichlorobenzene[4]
1,2-dichloro-4-nitrobenzene	-	50	>99	ND

-: no removal observed

NT not tested

ND not determined

1 only tested with Rhine sediment

2 only found in Rhine sediment

3 only tested in dune sediment

4 end product observed in dune sediment. In dune sediment, hexachlorobenzene was reductively dechlorinated to 1,3-dichlorobenzene via 1,2,3,5-tetrachlorobenzene and 1,3,5-trichlorobenzene (90%) and to 1,3- and 1,4-dichlorobenzene via 1,2,4,5-tetrachlorobenzene and 1,2,4-trichlorobenzene (10%).

Transformations of non-halogenated aromatics were studied in large columns packed with dune sediment and operated under both aerobic and methanogenic conditions and in the small Rhine sediment columns under aerobic conditions. All non-halogenated aromatics tested were aerobically transformed in both Rhine and dune sediment (Table 2.2). Under methanogenic conditions, only removal of methylbenzene (toluene) was observed (Table 2.2). These observations confirm existing data on the biotransformation of non-ionizable benzene

Table 2.2: Removal (%) of non-halogenated aromatics in columns packed with dune and Rhine sediment

	aerobic	methanogenic[1]
benzene	>95	-
methylbenzene	>95	>95
1,2-dimethylbenzene	>95	-
1,4-dimethylbenzene	>95	-
1,3,5-trimethylbenzene	>95	-
ethylbenzene	>95	-
naphthalene[2]	>95	-

-: no removal
1 only tested with dune sediment
2 not tested in Rhine sediment

Table 2.3: Residual concentrations (µg/l) observed in dune and Rhine sediment columns operated under aerobic conditions fed with low (0.5 µg/l) and high (20µg/l) concentrations of each compound.

	Dune sediment		Rhine sediment
	low	high	high
benzene	< DL	< DL	0.04
methylbenzene	< DL	< DL	0.03
ethylbenzene	< DL	< DL	0.01
1,2-dimethylbenzene	< DL	0.03	0.04
1,4-dimethylbenzene	< DL	< DL	0.02
1,3,5-trimethylbenzene	< DL	0.02	0.01
chlorobenzene	< DL	0.03	0.41
1,2-dichlorobenzene	0.06	0.05	1.0
1,3-dichlorobenzene	0.36	0.4	ND
1,4-dichlorobenzene	0.04	0.05	ND
1,2,4-trichlorobenzene	NT	2.6	ND

DL: Detection limit = 0.01 mg/l
ND: no degradation
NT: not tested

derivatives which show that they are readily biodegradable under aerobic conditions and tend to persist in anaerobic environments, except for toluene, which is also degradable under anaerobic conditions (Zeyer et al 1986, Grbic-Galic and Vogel 1987, Schocher et al 1991, Edwards et al 1992).

Residual concentrations

From the point of view of drinking water quality it is not only necessary to know whether a contaminant is degraded during infiltration, but also to which extent. There is uncertainty however, whether degradation by micro-organisms is still possible at very low concentrations or if threshold concentrations exist below which no transformation occurs. Residual concentrations can be the result of such threshold concentrations. Biotransformation will not

occur when the supply rate of a compound is lower than the rate needed for enzyme induction (Koch and Coffman 1970) or when the concentration of a substance that is used as carbon or energy source is too low to satisfy the maintenance requirements of a bacterial cell (Chapter 8). We determined residual concentrations of compounds that were degraded in oxic columns. Table 2.3 summarizes steady state residual concentrations in effluents of large dune sediment columns operated at low (0.5 µg/l) and high (20 µg/l) influent concentrations of the hydrocarbons. Residual concentrations observed in a small Rhine sediment column operated with high influent concentrations are also included in the table. Residual concentrations of non-halogenated aromatics in dune sediment are generally lower than those observed in Rhine sediment. Exceptions are 1,2-dimethylbenzene and 1,3,5-trimethylbenzene which have similar residual concentrations in both sediments. The residual concentrations of chloro- and 1,2-dichlorobenzene in the Rhine sediment column are more than 10 times higher than in dune sediment columns. The measured residual concentrations increase with increasing non-polarity of the compound. This suggests that sorption occurs before biotransformation takes place and therefore that mass transfer from the bulk solution via a sorbed phase to the transforming micro-organisms limits the biotransformation rates (Chapter 8).

No relation between residual concentrations and the influent concentration was found in a comparison of the results from dune sediment columns operated with high and low influent concentrations (Table 2.3). This confirms earlier results (Van der Meer et al 1987).

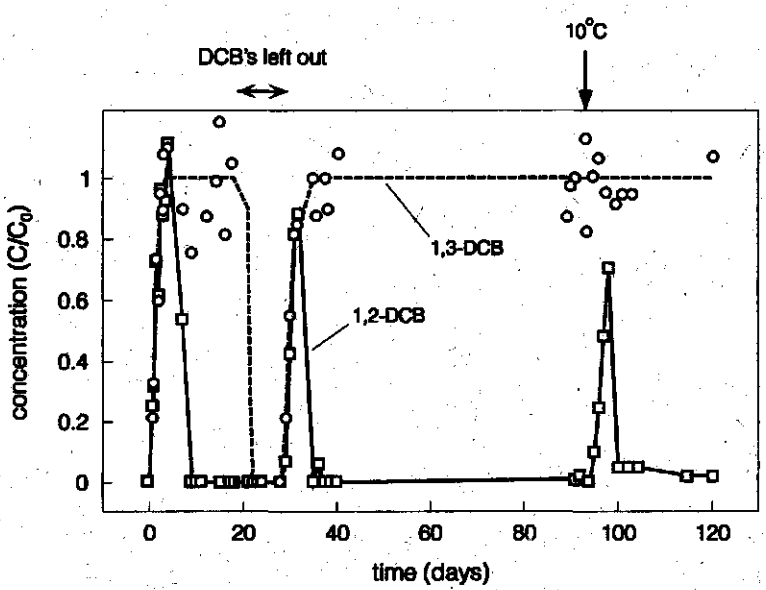

***Figure 2.2:** Behavior of dichlorobenzenes in a small dune sediment column at 20°C as the result of omitting dichlorobenzenes from day 23 to 28, and of a temperature decrease to 10°C at day 95. Effluent concentrations (C) are plotted as their ratio to the influent concentration (C_o). Only 1,2-dichlorobenzene was transformed, while 1,3-dichlorobenzene was not.*

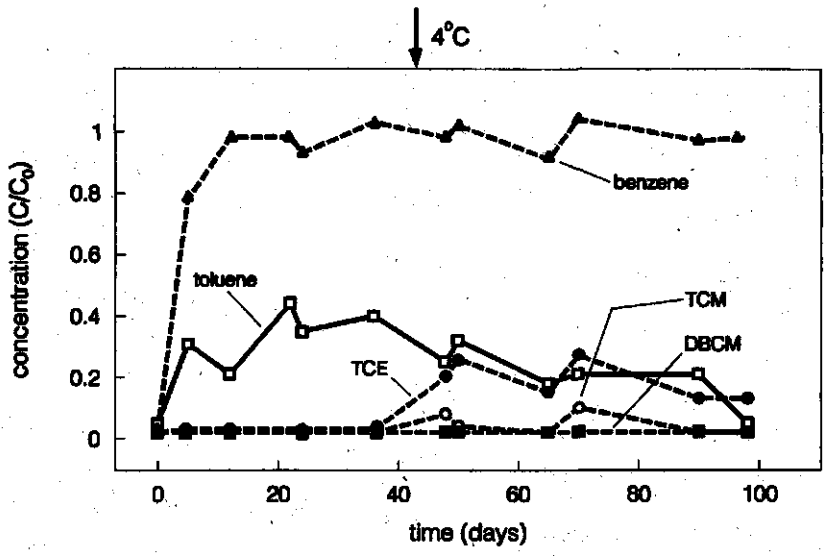

Figure 2.3: Behavior of benzene, tetrachloroethene (TCE), dibromochloromethane (DBCM), trichloromethane (TCM), and toluene in a large methanogenic dune sediment column at 20°C and the effect of a temperature decrease to 4°C. Effluent concentrations (C) are plotted as their ratio to the influent concentration (C_o).

Effect of temperature change and of omitting organic contaminants temporarily

After the initial experiments, the columns were percolated with medium without organic contaminants to prepare them for use in further experimentation. When a new experiment was started, we always observed an initial breakthrough of chlorinated hydrocarbons before biotransformation resumed. This is illustrated in Fig. 2.2 for the aerobic conversion of 1,2-dichlorobenzene at 20°C in a small column packed with dune sediment. It shows that 1,2-dichlorobenzene was completely removed after a breakthrough period which lasted for about 8 days. From day 23 to 28, no dichlorobenzenes were present in the influent. A 6 day breakthrough period of 1,2-dichlorobenzene was observed, followed by its complete disappearance when dichlorobenzene addition was resumed after this period. No removal of 1,3-dichlorobenzene was observed in this experiment.

At day 95, the temperature was decreased from 20 to 10°C. This resulted in a partial breakthrough of 1,2-dichlorobenzene followed by renewed removal to the same concentration as before (Fig. 2.2). No breakthrough of 1,2-dichlorobenzene in the column effluent was observed after an additional temperature decrease to 4°C at day 130 and a temperature increase to 20°C at day 150 (not shown). Profile measurements showed that steady state biotransformation rates were similar at all temperatures tested. Similar results were obtained with a small column packed with Rhine sediment (data not shown).

***Table 2.4:** Comparison of organic contaminant removal in the dune infiltration area and in columns packed with dune sediment and operated under aerobic and methanogenic conditions.*

contaminant	removal (%)		
	aerobic columns	methanogenic columns	infiltration area[1]
benzene	> 90	-	75-100
methylbenzene	> 90	> 90	75-100
1,2-dimethylbenzene	> 90	-	60-80
1,4-dimethylbenzene	> 90	-	60-80
ethylbenzene	> 90	-	40-60
1,2-dichlorobenzene	25-90	-	75-100
1,3-dichlorobenzene	25	-	75-100
1,4-dichlorobenzene	90	-	75-100
1,2-dichloro-4-nitrobenzene	-	> 90	75-100
trichloromethane	-	> 90	75-100
tribromomethane	> 90	> 90	75-100
atrazin	25-50	-	25-50
bentazon	25-50	-	-
tetrachloro-o-phthalic acid	-	> 90	40-60

ND: not determined

-: no removal observed

[1] data represent overall removal during infiltration of compounds which are always present in the infiltrated water and were taken from (Smeenk et al 1993)

The effect of a temperature decrease from 20°C to 4°C was also studied in large aerobic and methanogenic columns packed with dune sediment. Aerobic degradation was not affected by the temperature decrease (not shown). The decrease of temperature resulted in increased effluent concentrations of tetrachloroethene (0.5±0.1 µg/l) and trichloromethane under methanogenic conditions (0.25±0.05 µg/l, Fig. 2.3). In addition, breakthrough of trichloroethene - a degradation product of tetrachloroethene not present in the influent - to a concentration of 0.5 µg/l was observed (not shown). Toluene removal was not affected by the temperature decrease (Fig. 2.3).

Comparison of column and infiltration area data

An important question to ask is whether removals observed in column experiments also occur under field conditions, especially when these removals are preceded by long lag-times. Concentrations of a series of compounds in the infiltrating water and water collected from the drainage system were measured every two weeks in 1988. The water passed both oxic (residence time 1-7 weeks) and anoxic (residence time 3 weeks) zones during infiltration. Results for atrazin, bentazon and tetrachloro-o-phthalic acid were also included because they appeared to be present in Rhine water at 0.1-0.5 mg/l which is above the limit for drinking water (0.1 µg/l). The other compounds were present at concentrations below 0.05 mg/l. A good qualitative agreement exists between results from columns packed with dune sediment and overall removals observed in the dune infiltration area when both oxic and anoxic column experiments are included in the comparison (Table 2.4). Similarly, results from columns

packed with material from the Glatt River in Switzerland were in qualitative agreement with field data (Schwarzenbach et al 1983, Kuhn et al 1985). However, results are not always comparable on a quantitative basis. Removals of 1,2- and 1,4-dimethylbenzene, ethylbenzene, and tetrachloro-o-phthalic acid for example, are less in the infiltration area than in the column experiments. The contributions of anaerobic and aerobic processes to the overall transformation in the field are not known quantitatively, which complicates a quantitative comparison of column and field results. Removals of dichlorobenzenes in the columns are variable and generally smaller than in the infiltration area, maybe due to volatilization from the infiltration ponds.

Buffering of pulses of contaminants in the infiltration area

It is common practice to stop the intake of water from the Rhine when levels of halogenated and non-halogenated contaminants in the Rhine exceed 5 and 10 µg/l respectively, to prevent contamination of drinking water. These occurrences of high levels of contaminants normally result from upstream accidental spills and do not last longer than one or two weeks. However, longer lasting peaks do occur and sometimes pose problems in the management of the infiltration area. Intake is also halted in case of longer lasting pulses of biodegradable contaminants. These compounds could be allowed to enter the system when no temporary breakthrough resulting in a temporal appearance of a certain portion in the collected water,

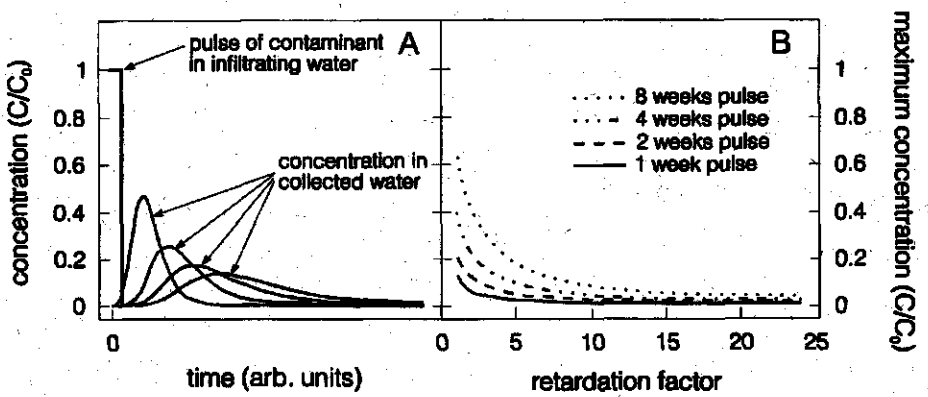

Figure 2.4: *Effect of dispersion on the breakthrough of a pulse of a contaminant in infiltrating water (A). Concentrations are plotted as their ratio to the concentration in the infiltrating water (C/C_O). The curves shifting to the right represent contaminants with increasing retardation factors. Total areas under the curves are equal and represent the total amount of contaminant added. The maximum concentration breaking through decreases with increasing retardation coefficient. The plot in B represents maximum concentrations breaking through from a temporary pulse of contaminant in infiltrating water as a function of the retardation factor. Concentrations are plotted as their ratio to the concentration in the infiltrating water (C/C_O). Increasing retardation factors represent compounds with decreasing polarity. The figure shows that the infiltration area buffers temporal contamination lasting for up to 2 weeks, efficiently. Longer lasting pulses of non-polar compounds are also buffered well, but in the case of polar compounds, breakthrough may be significant.*

would occur before biotransformation starts. There is a possibility however, that a greater dispersion in the field compared to the laboratory columns, will serve as a buffer for such temporary peaks. Dispersion causes a pulse of a chemical that is fed into a system, to spread in time. As a result, the maximum concentration in the water flowing out of the system is lower than the incoming concentration. The effect of dispersion increases with the residence time of the chemical in the system, so that non-polar compounds that are strongly sorbed, will be spread more in time and will reach lower maximum levels in the outflowing water than polar compounds which hardly sorb, as illustrated in Fig. 2.4A. The residence time in our columns was about 1 day, while the average residence time in the dune infiltration area is about 10 weeks. This difference alone, means that dispersion can have a greater impact in the field than in the laboratory columns. Calculations with a hydrological model (Olsthoorn 1991) were done to quantify the impact of dispersion on the behavior of organic contaminants in a part of the infiltration area with a relatively short residence time (7 weeks). Non-polar compounds will have longer residence times in the system due to sorption to the sediment. This can be expressed in a retardation factor that quantifies the attenuation compared to water. Typical values are 1-2 for polar compounds like bentazon and atrazin, 5-10 for dichlorobenzenes, and >15 for higher chlorinated benzenes or polycyclic aromatic compounds like pyrene or tetracene. Calculations were therefore made for compounds with retardation factors varying from 1 to 22, and for pulse times varying from 1 to 8 weeks. Typically, it takes 1 or 2 weeks for a contamination in the Rhine resulting from an accident, to pass at the location where infiltration water is taken in. The long-term appearance in infiltrating water of a new biodegradable contaminant to which the microbial population has to adapt can also be viewed as a short term pulse in the infiltrating water. If it takes 6 weeks for example to attain a complete steady state biotransformation, only the contaminant which was present in this period, will eventually reach the collected water. Therefore, calculations for longer pulse times were also included. Pulses with a duration of up to 2 weeks are very well buffered by the system, resulting in a decrease by 80% or more of the concentration in the water collected in the drainage system (Fig. 2.4B). The buffering effect of the system is even more pronounced for strongly sorbing species which are predicted to breakthrough at levels equal to 10% of the incoming concentration at most, even when the pulse lasts as long as 8 weeks. As a matter of fact, these are conservative estimates of the actual impact of dispersion in the area as a whole, since calculations were only made for a region with a low residence time (7 weeks).

CONCLUDING REMARKS

Many streamlines in the dune infiltration area show a sequence from oxic to anoxic and then oxic again (Chapter 3), which allows the complete mineralization of heavily chlorinated compounds via reductive dechlorination as the initial step, followed by aerobic mineralization

(Zitomer and Speece 1993). However, an accumulation of more toxic and more mobile lower chlorinated compounds may occur when reductive dechlorination is not followed by aerobic mineralization. This is a real risk in the case of the formation of (*Z*)-1,2-dichloroethene from tetra- and trichloroethene and of (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene from hexachloro-1,3-butadiene. Aerobic transformation of these products has not been observed yet.

The residual concentrations in our columns are well below standards for drinking water quality, except for 1,2,4-trichlorobenzene, which showed a residual concentration of 2.6 µg/l. One would expect that biotransformation of this compound will not occur in the field, since concentrations in the infiltrating water do not exceed this value normally. Moreover, we observed a lag-time of 2 years, indicating that no adapted bacteria were present in the dune sediment.

Many of the hydrocarbons studied can potentially be biodegraded at a temperature as low as 4°C, at rates which are comparable to those observed at 20°C. The process of reductive dechlorination however, appears to proceed slower at 4°C. As a consequence, the infiltration area will behave differently in summer and in winter. First of all, the oxic zone below the upper sediment layer in the infiltration ponds penetrates deeper in winter compared to the situation in summer, resulting in a decrease in the fraction of anoxic zones. In combination with the slower rates of the anaerobic reactions, the contribution of anaerobic processes to the overall performance of the area with respect to biodegradation of contaminants, is expected to be smaller in winter than in summer.

Temporal increases of levels of polar compounds that are not degradable, were shown to have a greater impact on the quality of the water collected in the drainage system than temporal increases of levels of non-polar compounds, due to the buffering capacity of the area caused by dispersion.

CHAPTER 3

REDUCTIVE DECHLORINATION OF ALL TRICHLORO- AND DICHLOROBENZENE ISOMERS

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ABSTRACT

All three isomers of trichlorobenzene were reductively dechlorinated to monochlorobenzene via dichlorobenzenes in anaerobic sediment columns. The dechlorination was specific: 1,2,3- and 1,3,5-trichlorobenzene were solely transformed to 1,3-dichlorobenzene, while 1,4-dichlorobenzene was the only product of 1,2,4-trichlorobenzene transformation. Micro-organisms were responsible for the observed transformations. Since monochlorobenzene and dichlorobenzene are mineralized by bacteria in the presence of oxygen, the process of reductive dechlorination may be an important initial step to obtain complete mineralization of otherwise recalcitrant trichlorobenzenes. This is especially true for the 1,3,5-isomer, which seems to resist biodegradation in oxic environments.

INTRODUCTION

Trichlorobenzenes are widely applied as intermediates and solvents in industry and agriculture (Verschueren 1983). As a result of their universal use, they are nearly ubiquitous pollutants in air, water, sediments, and soils (Pearson 1982). They are chemically stable in both aerobic and anaerobic environments. Therefore, it is of interest to know whether they might be subject to biotransformation. Information on the aerobic mineralization of trichlorobenzenes - especially the 1,3,5-isomer - is scarce. Ballschmiter and Scholz (1980) isolated a *Pseudomonas* sp. able to hydroxylate all isomers of trichlorobenzene through the action of a mono-oxygenase. Chlorinated phenols were the only detected transformation products. Marinucci and Bartha (1979) have demonstrated aerobic mineralization of 1,2,3- and 1,2,4-trichlorobenzene in soil samples, using ^{14}C-labelled substrate. However, the measured mineralization rates of 0.3-1.0 nmol/day per 20 g of soil sample are extremely low and only 10% of the initial amount of trichlorobenzene was recovered as $^{14}CO_2$. Van der Meer et al. (1987) isolated *Pseudomonas* sp. strain P51, which is able to mineralize 1,2,4-trichlorobenzene, from river Rhine sediment. In contrast, 1,2,4-trichlorobenzene was not removed in river Rhine sediment packed in columns which were continuously operated aerobically for two years.

Tiedje et al. (1987) reported the anaerobic reductive dechlorination of hexachlorobenzene to 1,3,5-trichlorobenzene as the sole end product. Anaerobic transformation of 1,2,4-trichlorobenzene to dichlorobenzenes has been shown in bacteria isolated from the intestinal content of rats (Tsuchiya and Yamaha 1984). A consortium able to mineralize 3-chlorobenzoate anaerobically has been isolated by Shelton and Tiedje (1984). It appeared that 3-chlorobenzoate degradation was initiated by its dechlorination to benzoate (Shelton and Tiedje 1984, Dolfing and Tiedje 1986). In this paper it is shown that biologically catalyzed reductive dechlorination in the absence of oxygen can serve as an initial step leading to the eventual mineralization of trichlorobenzenes. Anaerobic transformation was studied in columns packed with river Rhine sediment.

MATERIALS AND METHODS

Experimental set-up.

Details of the experimental set-up are given in van der Meer et al. (1987) and Zeyer et al. (1986). Columns, constructed of hard PVC (25 cm length, 5.5 cm i.d.), were wet-packed with anaerobic sediment from the river Rhine near Wageningen, Netherlands (Van der Meer et al 1987). The columns were percolated continuously at a flow rate of 1 cm/h in an up-flow mode, with an anaerobic mineral medium prepared with highly purified milli-Q water (Millipore, USA) closely resembling the mineral composition of Rhine water (Van der Meer et al 1987). The medium was depleted from oxygen by its continuous replacement with nitrogen

in a gas exchange chamber as described in Zeyer et al. (1986) and reducing conditions were maintained by the addition of Na_2S (10 mg/l final concentration). Chlorinated hydrocarbons were present as only source of carbon and energy and were constantly added with a syringe pump together with the Na_2S. Stock solutions were prepared as described in van der Meer et al. (1987). One column was operated without sulfide as reducing agent but with nitrate (final concentration 35 mg/l) added as electron acceptor.

Analytical methods.

Methods of sampling, extraction and GC/ECD analysis have been described in van der Meer et al. (1987). Analysis of the samples was done by hexane extraction followed by on-column injection into a gas chromatograph (United Technologies, Delft, Netherlands) equipped with an ECD detector and a 25 m capillary column (Sil 5CB, 12 µm, Chrompack, Netherlands). Since monochlorobenzene could not be detected with the GC technique, samples were also analyzed by HPLC (LKB 2150 pump and 2152 controller; 250 by 4 mm Ultra Pac column filled with Lichrosorb RP-8 and RP-18 with a diameter of 10 µm, preceded by a universal 75 by 2.1 mm Chrompack RP column, Schraa et al 1986). The mobile phase was acetonitrile-water (ratio by volume 55:45). The dechlorination products were identified by their mass spectrum and their retention times in the GC and HPLC analyses. The GC/MS (Finnigan 1050) was operated under the same conditions as the GC/ECD.

Chemicals.

1,2- 1,3- and 1,4-dichlorobenzene and 1,2,4-trichlorobenzene, hexachlorobutadiene and 1,2-dichloro-4-nitrobenzene were purchased from E. Merck, Darmstadt, Germany. 1,2,3- and 1,3,5-trichlorobenzene were kind gifts from the Organic Chemistry Department, Agricultural University, Wageningen, Netherlands. All chemicals were of analytical grade and used without further purification.

RESULTS AND DISCUSSION

In an initial experiment, a sediment column was continuously fed with a medium containing tetra- and trichloroethene, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, 1,2-, 1,3- and 1,4-dichlorobenzene, hexachlorobutadiene, and 1,2-dichloro-4-nitrobenzene. The concentration of each xenobiotic ranged from 30-50 nmol/l.

After an initial lag-phase of 2-6 months (depending on the compound), tetra- and trichloroethene, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene and hexachlorobutadiene were removed from the column. 1,2-Dichloro-4-nitrobenzene could never be detected in the column effluent. Both the breakthrough curves (Fig. 3.1A) and the concentration profiles (Fig. 3.1B) of the trichlorobenzenes show that the 1,2,3-isomer was first removed, followed by the 1,2,4-

and the 1,3,5-isomer. It has been concluded that the observed removal of the trichlorobenzenes was a biological process because (i) a long lag-phase preceded the disappearance of trichlorobenzenes, (ii) no elimination in anaerobic batch experiments with autoclaved sediment was observed, and (iii) a reduction potential of -1.962 to -2.440 V is necessary for their abiotic electrochemical reduction (Farwell et al 1975). Beland et al. (1976) have stated that reactions with a redox potential lower than -1.76 V will not proceed purely chemically in anaerobic environments.

To detect possible intermediary products of the anaerobic transformation of trichlorobenzenes, the concentration of each of these chemicals was raised to 300-500 nmol/l in the influent at day 250, while the other xenobiotics were omitted. Even at this tenfold higher

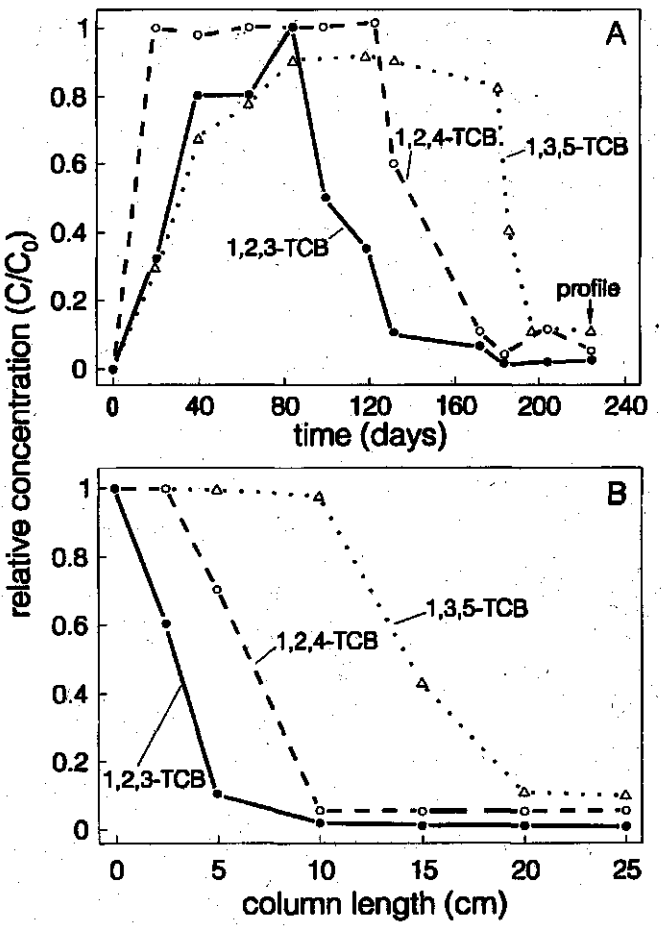

***Figure 3.1:** Breakthrough curves (A) and concentration profiles determined after 225 days of continuous operation (B) of 1,2,3- (●), 1,2,4- (○) and 1,3,5-trichlorobenzene (Δ) in anaerobic sediment columns. Concentrations are expressed as the measured concentration (C) divided by the influent concentration (C_0). The influent concentration of each compound was 30-50 nmol/l; the flow rate was 1 cm/h.*

concentration, all trichlorobenzene isomers disappeared within the first 5 cm of the sediment column. The concentration profiles in Fig. 3.2, measured at day 300, show that they were converted mainly to 1,3- and 1,4-dichlorobenzene with traces of 1,2-dichlorobenzene. In experiments with the individual trichlorobenzene isomers, 1,3-dichlorobenzene was formed from 1,2,3- and 1,3,5-trichlorobenzene and 1,4-dichlorobenzene from 1,2,4-trichlorobenzene. 1,2-Dichlorobenzene was not detected during these experiments. The dichlorobenzenes were identified by their mass spectrum and retention times in the GC and HPLC analyses. At the start of trichlorobenzene dechlorination its rate was so slow that 20 cm flow through the column was required for complete removal of all three isomers. In course of the experiments the flow distance needed for complete conversion of trichloro-benzenes to dichlorobenzenes decreased: from 20 cm at day 225 to 5 cm at day 300 and 2.5 cm at day 400. At the end of the experiments with the single isomers (450 days after start-up of the column with the complete mixture of chlorinated compounds) 1,2,4-trichlorobenzene was already removed entirely at 0.5 cm from the inlet.

In all experiments with individual trichlorobenzene isomers a slight decrease of the dichlorobenzene concentration in the last 10 cm of the column was observed, accompanied by monochlorobenzene formation. When after 450 days of continuous operation of the column the trichlorobenzenes were replaced by dichlorobenzenes in the influent, the dichlorobenzene isomers were dechlorinated after a lag of 7 days yielding monochlorobenzene (Fig. 3.3), which

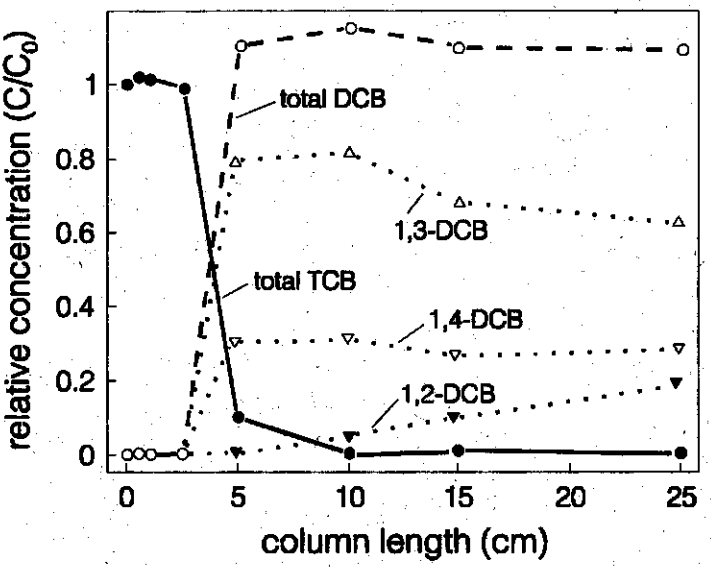

Figure 3.2: Concentration profile determined after 300 days of continuous operation, showing the anaerobic conversion of trichlorobenzenes to dichlorobenzenes. Concentrations (C) of both di- and trichlorobenzenes have been divided by the influent concentration (C_o) of trichlorobenzene (total) which was 1.0 µmol/l. The concentration of each trichlorobenzene isomer was 0.3-0.5 µmol/l; the flow rate was 1 cm/h.

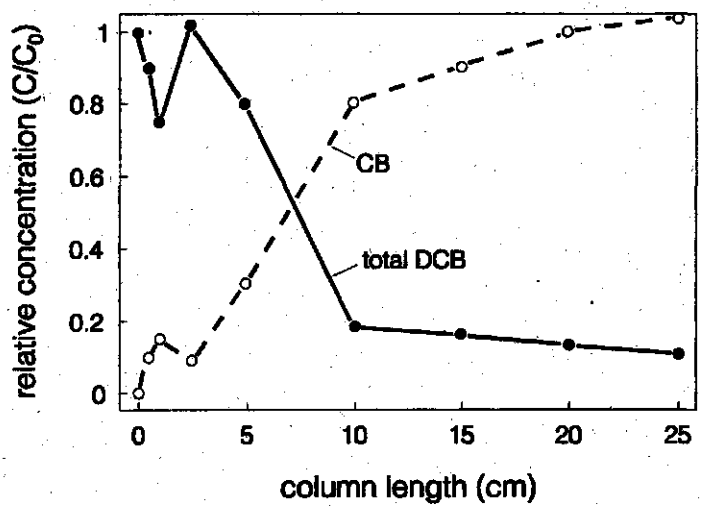

Figure 3.3: Concentration profile determined after 520 days of continuous operation, showing the anaerobic conversion of dichlorobenzene to monochlorobenzene. Concentrations are expressed as in Fig. 3.2. The influent concentration of each of the three dichlorobenzene isomers was 3-5 µmol/l; the flow rate was 1 cm/h.

was confirmed with GC/MS analysis. The dichlorobenzenes were fed to the column at high concentrations (3-5 µmol/l), and their transformation rates increased steadily reaching a maximum of 0.4 µM/h after eight weeks. 1,3- and 1,4-dichlorobenzene were transformed only after almost complete removal of 1,2-dichlorobenzene. The increase of the removal rate might be the result of (i) chemotaxis (Chapter 5), (ii) growth because of the increased influent concentrations, or (iii) a combination of both. Based on thermodynamic calculations, Vogel et al. (1987) showed that microbial reduction of chlorinated aliphatic compounds is favourable under methanogenic conditions and that energy can be obtained from the reductive dechlorination reaction. For the reductive dechlorination of 1,2-dichlorobenzene to monochlorobenzene a $DG_r^{0'}$ (aq) of -140.8 kJ/(mol Cl^- liberated) was calculated. Similar values can be obtained for the other di- and trichlorobenzene isomers. The free energies were calculated using the data given in Lange's Handbook of Chemistry (Lange 1973). Therefore, from a thermodynamical point of view it is possible that chlorinated aromatic compounds may act as terminal electron acceptors. The energy liberated during such a reduction process might then become useful for cell synthesis. Strong evidence for the occurrence of such a "halide respiration" mechanism was obtained by Dolfing and Tiedje (1987). They showed that an energy starved consortium accumulated twice as much ATP after the addition of 3-chlorobenzoate as after addition of benzoate. In addition, they observed a growth yield increase coupled to reductive dechlorination which could be fully accounted for by the increase in the numbers of the dechlorinating bacterium DCB-1.

The column results indicate that dechlorination of dichlorobenzene was inhibited in the presence of trichlorobenzene. Similar results were obtained by Suflita et al. (1983) who found

that 3-chlorobenzoate was reduced to benzoate only upon the complete dechlorination of 3,5-dichlorobenzoate. The dechlorination reactions observed in the anaerobic sediment columns appear to be determined by the electron withdrawing effect of the chlorine substituents on the aromatic ring, which supports the occurrence of a nucleophilic aromatic substitution mechanism: dechlorination proceeds via addition of an electron followed by the chemical binding of a proton to the resulting negatively charged intermediate followed by a second addition of an electron and release of a chlorine ion (Farwell et al 1975). Since the negative charges are balanced by neighbouring electronegative substituents, the occurrence of such a mechanism would predict that (i) dechlorination of trichlorobenzenes is more feasible than dechlorination of di- and monochlorobenzenes, (ii) dechlorination is easier when the chlorine substituents are closer to each other, and (iii) the chlorine substituents at the 2-position in 1,2,3- and 1,2,4-trichlorobenzene are preferably removed during dechlorination. These predictions are in agreement with the data presented here and also with results of electrochemical experiments (Farwell et al 1975), where the products of dechlorination at the 2-position are formed in relatively larger amounts than would be expected if dechlorination had occurred randomly at all possible positions. The fact that in the sediment column 1,2,3- and 1,2,4-trichlorobenzene are exclusively transformed to 1,3- and 1,4-dichlorobenzene respectively, indicates that specific enzymes and/or micro-organisms are responsible for the observed transformations.

Tsuchiya and Yamaha (1984) have found anaerobic dechlorination of 1,2,4-trichlorobenzene by bacteria isolated from the intestinal contents of rats. However, this transformation yielded all dichlorobenzene isomers as products and is probably not related to specific metabolic activities because only 6 nmol 1,2,4-trichlorobenzene per mg of dry cells (0.1-0.6% of the initial amount) was transformed during 24 hours of incubation. In addition, dechlorination mainly occurred during the stationary growth phase of the isolated bacteria. Parke and Williams (1960) investigated the detoxification of chlorinated benzenes in living rats and rat tissues and observed that 1-10% of the administered chlorinated benzenes were transformed to lower chlorinated benzenes.

So far, there is little evidence that chlorinated benzenes are transformed in anaerobic sediments. Tiedje et al. (1987) have found that hexachlorobenzene was transformed to 1,3,5-trichlorobenzene in anaerobic sludge from Jackson, Michigan. Analyses of sediment cores taken from the Great Lakes, Canada, revealed that in the top layers highly chlorinated benzenes are more dominant than in the lower and older layers, where lower chlorinated benzenes prevail (Oliver and Nicol 1982). It has been suggested that dechlorination of certain chlorinated benzenes has occurred in the course of time (Bailey 1983). The results presented in this paper support that such a mechanism may be responsible for the measured distribution of chlorinated benzenes in the Great Lakes sediment.

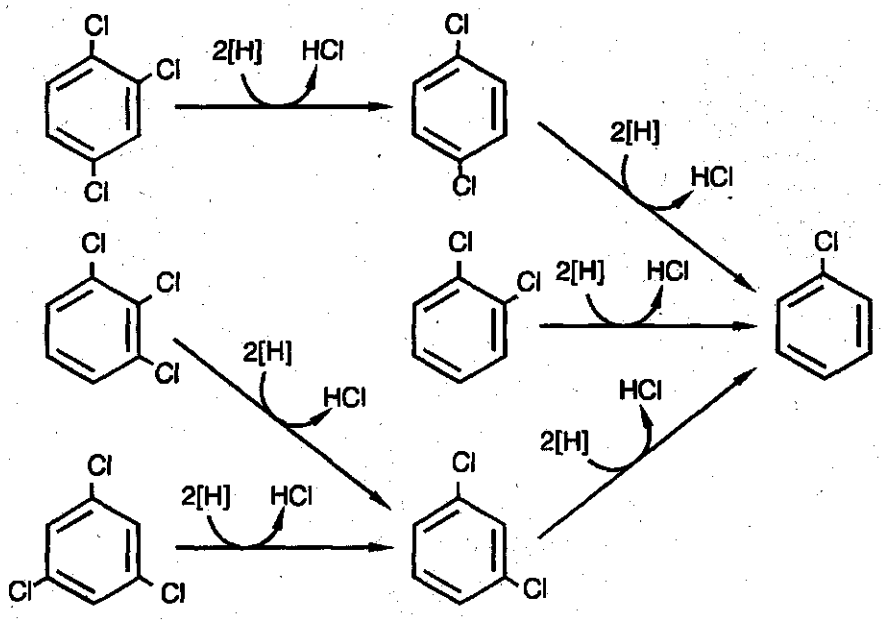

Figure 3.4: Summary of the dechlorination reactions observed in the anaerobic sediment column under reduced conditions. During each dechlorination step 2 electrons and two protons ([H]) are consumed yielding a lower chlorinated benzene and HCl. It is proposed that micro-organisms might use the energy liberated during reductive dechlorination for cell synthesis.

Based on the data presented here it is concluded that in reduced environments (sulfate up to 20 mmol/l did not inhibit the reaction; no dechlorination has been observed in the denitrifying column) all trichlorobenzene isomers can be dechlorinated, yielding dichlorobenzenes as intermediates and monochlorobenzene and chloride as sole end products (Fig. 3.4). Both dichlorobenzenes and monochlorobenzene are known to be mineralized aerobically by bacteria (De Bont et al 1986, Schraa et al 1986, Spain and Nishino 1987, Van der Meer et al 1987). Provided that after the initial passage of trichlorobenzenes through anaerobic sediment the monochlorobenzene formed has the possibility to enter an aerobic environment, mineralization of all trichlorobenzenes including the very recalcitrant 1,3,5-isomer could eventually occur. In a similar manner, Bedard et al. (1987) have shown that the degradation of chlorinated biphenyls is highly improved when they are first dechlorinated anaerobically. These authors isolated *Alcaligenes eutrophus* strain H850 which is able to oxidize aerobically 39 to 49 of the 60 congeners identified in the commercial mixture of Aroclor 2142. When Aroclor 2142 was pretreated anaerobicallly before incubating it with strain H850, nearly all of the congeners were degraded. Only two components resisted complete degradation in such an anoxic-oxic sequence. It appeared that the removal of chlorines from meta and para positions under anaerobic conditions made most of the otherwise

recalcitrant congeners biodegradable for strain H850. A place which allows a sequential anoxic-oxic mineralization *in situ*, exists at the dune infiltration site of the Amsterdam Water Works, Netherlands, where drinking water is produced from river Rhine water. At this site, pretreated river water is brought into small basins on the top of the dunes. The water then infiltrates into the dunes first through the sediments of these ponds and thereafter through about 60 meters of sand before it reaches lower situated collecting channels. The sediments are anoxic in summer because of the steady input of organic material from the river water. Due to air diffusion and input of oxygenated rain water, the lower part of the infiltration track becomes oxic. Studies with sand from the aerobic zone of the dunes revealed that in the presence of oxygen, monochlorobenzene and 1,2- and 1,4-dichlorobenzene are readily degraded (Chapter 2). By using columns which were filled with anaerobic sediments from the infiltration ponds and continuously fed with a mixture of 30-50 nmol/l of each of the trichlorobenzene isomers the same results were obtained as with the river Rhine sediment (Fig. 3.1). These findings indicate that a sequential degradation of trichlorobenzenes, i.e. anaerobic reductive dechlorination followed by aerobic mineralization, might be operative in such dune infiltration sites.

In conclusion, the biological process of anaerobic reductive dechlorination transforms otherwise very persistent trichlorobenzenes (especially the 1,3,5-isomer) and also higher chlorinated benzenes to intermediates which can easily be mineralized aerobically. It is therefore proposed to pretreat waste water, ground water or soil contaminated with highly chlorinated benzenes anaerobically before aerobic clean-up processes are applied.

ACKNOWLEDGEMENTS

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CHAPTER 4

REDUCTIVE DECHLORINATION OF HEXACHLORO-1,3-BUTADIENE

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ABSTRACT

Transformations of hexachloro-1,3-butadiene were studied in columns packed with Rhine River sediment and in batch incubations containing Ti(III) citrate and hydroxocobalamin. Columns were operated under various redox conditions. Transformation of hexachloro-1,3-butadiene was observed in a methanogenic column but not in columns where oxygen or nitrate were fed as terminal electron acceptors. It was reductively dechlorinated to (*E,E*)-1,2,3,4-tetrachlorobutadiene (>90%) and traces of a trichloro-1,3-butadiene isomer (<5%). (*E*)-1,1,2,3,4-pentachloro-1,3-butadiene was detected as intermediary product. The reductive dechlorination in the column was ascribed to the activity of anaerobic micro-organisms. In the batch experiments with Ti(III) citrate and hydroxocobalamin, hexachloro-1,3-butadiene was transformed to an isomer of pentachloro-1,3-butadiene and two compounds with molar masses of 154 and 52, tentatively identified as trichloro-1-buten-3-yn and 1-buten-3-yn, respectively.

INTRODUCTION

Several reports exist in which hexachloro-1,3-butadiene, which has been shown to be toxic to rats and humans (Yang 1988), is described as a pollutant present in sediment samples in Western Europe and North America (Li et al 1976, Durham and Oliver 1983, Rostad and Pereira 1989). Hexachloro-1,3-butadiene has been used as a heat transfer fluid in transformators, as an intermediate to produce lubricants, and as an intermediate in the manufacture of rubber compounds (Verschueren 1983, Yang 1988), while it is also formed as a by-product during the production of vinylchloride, trichloroethene, and tetrachloroethene. The latter may be the cause for the detection of high concentrations in soil near tetrachloroethene producing facilities (Li et al 1976, Milchert et al 1988). In the former Soviet Union hexachloro-1,3-butadiene has been applied as fungicide (Asriev 1970, Malama et al 1984).

No information has been reported thus far about transformations of hexachloro-1,3-butadiene in soil or ground water. The saturation of the molecule with chlorines may limit aerobic transformations but may be suitable for anaerobic reductive dechlorination reactions. In this study, we investigated the transformation of hexachloro-1,3-butadiene in columns packed with Rhine River sediment and operated under various redox conditions. Column experiments have been shown useful to assess the potential of sediments to transform organic contaminants (Kuhn et al 1985, Zeyer et al 1986, Van der Meer et al 1987, Van der Meer et al 1992, Chapter 2,3). Transition metal coenzymes are known to catalyze reductive dechlorination reactions and may provide a reference for comparison of dechlorination in environmental samples (Schanke and Wackett 1992). Therefore, we also studied the possible reduction of hexachloro-1,3-butadiene by Ti(III) citrate and hydroxocobalamin (vitamin B_{12a}).

MATERIALS AND METHODS

Chemicals

Hexachloro-1,3-butadiene (98% pure) was purchased from E. Merck (Amsterdam, The Netherlands). 1,1,4,4-Tetrachloro-1,3-butadiene (96% pure) was obtained from V.I. Potkin and R.V. Kaberdin of the "Institute of Physical Organic Chemistry" of the "Byelorussian Academy of Sciences", deuterated chloroform (99.8% D) from Janssen Chimica (Belgium), hydroxocobalamin from Fluka (Oud-Beijerland, The Netherlands).

Ti(III) citrate was prepared from $TiCl_3$ and sodium citrate (Zehnder and Wuhrmann 1976, Holliger et al 1992). In an anaerobic glove box (Coy Laboratories Products, U.S.A.), an ampoule of $TiCl_3$ (7,5 ml) was added to 25 ml of 0.6 M anaerobically prepared sodium citrate. The pH was adjusted to about 8 with solid $Na_2CO_3.10H_2O$, and subsequently to 9 with a

concentrated Na_2CO_3 solution. The volume was brought to 75 ml with anaerobic demineralized water resulting in a final concentration of 100 mM Ti(III) citrate.

Column experiments

Columns were constructed of hard PVC (25 cm length, 5.5 cm i.d.) and were equipped with stainless steel capillaries (2.0 mm in diameter) extending into the center of the column at various heights (Van der Meer et al 1987). They served as sampling ports for concentration profile measurements. Columns were wet packed with sediment from the Rhine River near Wageningen, The Netherlands and were percolated continuously at a flow rate of 1 cm/h in an upflow mode, with a mineral medium prepared with highly purified milli-Q water (Millipore, USA) closely resembling the mineral composition of Rhine water (Van der Meer et al 1987). It contained NH_4Cl (27 mg/l), $MgCl_2 \cdot 6H_2O$ (102 mg/l), K_2HPO_4 (12 mg/l), $CaCl_2$ (222 mg/l), $NaHCO_3$ (215 mg/l), Na_2SO_4 (7 mg/l) and 0.15 ml/l of a trace element solution (Zehnder et al 1980). The synthetic medium was continuously aerated with an excess of granulated marble which served as carbonate buffer in combination with CO_2 in the air (pH = 8.3±0.1). In the anaerobic experiments, the originally aerated medium was depleted from oxygen by its continuous replacement with nitrogen gas amended with 0.5% CO_2 in a gas exchange chamber (Zeyer et al 1986). Reducing conditions were maintained by the addition of Na_2S (10 mg/l final concentration) via the solution of chlorinated hydrocarbons (Chapter 3). One column was operated without sulfide as reducing agent but with nitrate (final concentration 35 mg/l) added as electron acceptor. The columns were operated aseptically up to the influent port where a bacterial filter (cellulose-nitrate, 0.2 µm, Sartorius GmbH, Germany) prevented back growth of micro-organisms from the sediment into the feeding lines. The medium was pumped into the columns by a peristaltic pump (Watson Marlow Ltd., U.K.) at a flow rate of 12.0 ml/h. A solution of hexachloro-1,3-butadiene was added continuously with a syringe pump (Perfusor VI, B. Braun Medical B.V., Germany) at a flow rate of 0.6 ml/h. This solution was prepared by adding 100 µl of a stock solution in methanol to 70 ml of milli-Q water. Final concentrations in the influent were 4 or 400 nmol/l, depending on the experiment. The solutions were autoclaved before use. Mixing of the hexachloro-1,3-butadiene with the mineral medium took place in a mixing chamber just before entering the sediment columns. The columns were operated in a climate room at a temperature of 20°C.

Column sampling and analyses

Columns were sampled with glass syringes as previously described (Van der Meer et al 1987, Chapter 3). Sample volumes varied from 1 to 50 ml. They were analyzed routinely by hexane extraction followed by on-column injection into a gas chromatograph (United Technologies, The Netherlands) equipped with an Electron Capture Detector (ECD) and a 25 m capillary column (Sil 5CB, 1.2 µm, Chrompack, The Netherlands).

To identify the various transformation products, samples of 50 ml were purged with nitrogen gas at a flow rate of 10 ml/min. during 30 minutes at a temperature of 90°C by means of a purge and trap system (Chrompack, The Netherlands). Components present in the outflowing gas were trapped in a glass tube packed with 90 mg Tenax TA. The components were released from the Tenax in a Thermodesorption Cold Trap (TCT) unit (Chrompack, The Netherlands) at 250°C for 10 minutes with a Helium flow of 10 ml/min. The desorbed compounds were cryofocused in a cold trap at -100°C. Fast heating of this cold trap gave a sharp injection of the compounds onto the analytical column (Supelcowax-10, 60m length, 0.25µm film thickness). After an initial oven temperature of 60°C during 4 minutes, the temperature was raised to 270°C at a rate of 4 °C/min. The GC was connected to a VG MM7070F mass spectrometer operating in the 70 eV EI ionization mode.

Samples for proton-NMR were prepared as follows. An aqueous sample (50 ml) was taken from the methanogenic column as described above. The sample was injected directly into a 2 ml aliquot of deuterated chloroform in a 60 ml extraction tube and shaken for five minutes. After settling, the aqueous phase was removed by syringe. Four additional samples were treated in a similar manner with the same aliquot of deuterated chloroform. Thus, 5 samples of 50 ml were extracted resulting in an increase in concentration by a factor of 125 at maximum. After extraction, 1 ml of deuterated chloroform could be separated from the aqueous sample and was measured directly in the NMR-apparatus (Bruker AC-E 200).

Reduction of hexachloro-1,3-butadiene by Ti(III) citrate

The assay was adapted from Holliger et al. (Holliger et al 1992) and carried out in a series of 13 ml serum bottles. The bottles were filled inside the anaerobic glovebox and sealed with viton stoppers (Maag Technic, Switzerland) and aluminium crimp caps. The reaction mixture contained 8.58 ml 100 mM TRIS/HCL (pH 9), 2.2 ml Ti(III) citrate (100 mM), and 220 µl hydroxocobalamin (5 mM). In addition, three control series were prepared, one without Ti(III) citrate, one without hydroxocobalamin, and one without both. Bottles were stored at 0°C before use. After the gas phase was changed with 100% N_2, 11 µl hexachloro-1,3-butadiene in ethanol (5 mM) was added by syringe. It was assured that ethanol was not transformed to ethene by GC/MSD-analysis (see below). The reaction was carried out at room temperature (22±1°C). After different time intervals, the reaction was stopped by the injection of 2 ml hexane into one bottle of each series. The bottles were stored at 4°C after extraction and analyzed within 48 hours by means of a Hewlett-Packard Mass Spectrometric Detector 5970B connected to an HP 5890 Gas Chromatograph (GC/MSD). The GC/MSD was equipped with a 25 m capillary column (Sil 5CB, 1.2 µm, Chrompack, The Netherlands). After an initial oven temperature of 40°C (5 min.) the oven temperature was raised to its final value of 200°C at a rate of 10°C/min.

A second assay was performed to determine transformation products. The reaction mixture contained 7.0 ml 100 mM TRIS/HCL (pH 9), 1.8 ml Ti(III) citrate (100 mM), 180 µl hydroxocobalamin (5 mM), and 350 µl 500 mM hexachloro-1,3-butadiene in ethanol. After one day of incubation, three bottles were analyzed via hexane extraction to determine non-gaseous transformation products. In addition, the head space of three other bottles was analyzed for possible gaseous dechlorination products by injection into the GC/MSD equipped with a 25 m capillary column (Sil 5CB, 1.2 µm, Chrompack, The Netherlands).

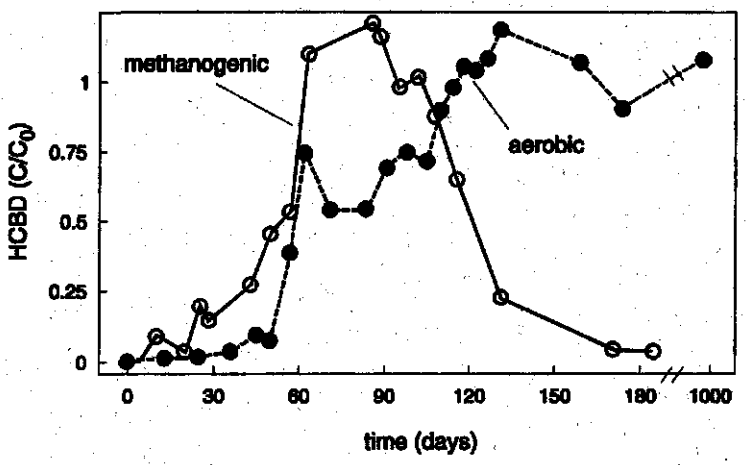

***Figure 4.1:** Breakthrough of hexachloro-1,3-butadiene (HCBD) in aerobic and methanogenic columns. Removal took place under methanogenic conditions only. Influent concentrations were 4 nmol/l.*

RESULTS

Column experiments

In an initial experiment, an aerobic column, a column fed with nitrate as terminal electron acceptor and a methanogenic column were continuously fed with a medium containing tetra- and trichloroethene, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, 1,2-, 1,3- and 1,4-dichlorobenzene, hexachloro-1,3-butadiene, and 1,2-dichloro-4-nitrobenzene. The concentration of each compound ranged from 4-50 nmol/l (1-10 µg/l). Results of previous experiments with these columns were reported in detail in earlier papers (Van der Meer et al 1987, Chapter 2,3). In the aerobic column only 1,2-dichlorobenzene was transformed after an acclimation period of 3 months (Van der Meer et al 1987). When nitrate was present as terminal electron acceptor, only 1,2-dichloro-4-nitrobenzene was partially removed (Chapter 2,3). In the methanogenic column, tetra- and trichloro-ethene, and 1,2,3-, 1,2,4- and

1,3,5-trichlorobenzene were transformed after initial acclimation times varying from 2-6 months (Chapter 2,3). Removal of hexachloro-1,3-butadiene under methanogenic conditions was observed after an acclimation time of approximately 4 months (Fig. 4.1). In the presence of nitrate and under aerobic conditions, no disappearance of hexachloro-1,3-butadiene could be detected within the experimental period of three years. The aerobic breakthrough curve of hexachloro-1,3-butadiene shows much more dispersion than the methanogenic curve. The reason for this is not known. A possible explanation would be the occurrence of channelling in the aerobic column in combination with slow sorption kinetics of hexachloro-1,3-butadiene.

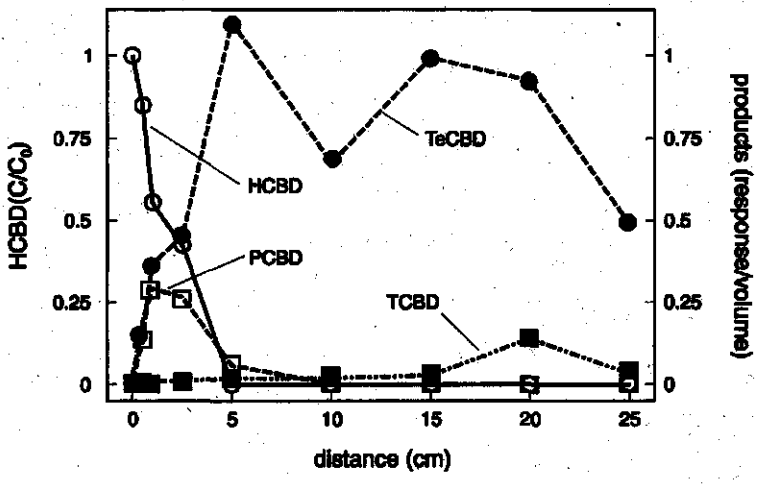

Figure 4.2: Disappearance of hexachloro-1,3-butadiene (HCBD) in a methanogenic column and the appearance of penta- (PCBD), tetra- (TeCBD) and trichloro-1,3-butadiene (TCBD). The influent concentration of hexachloro-1,3-butadiene was 400 nmol/l.

The methanogenic column was first used to assess the transformation pathway of trichloro- and dichlorobenzenes in detail (Chapter 3). No hexachloro-1,3-butadiene was fed to the column during the time of this study. After its completion, the chlorinated benzenes in the influent were replaced by hexachloro-1,3-butadiene as sole organic contaminant. To detect possible intermediary products of the anaerobic transformation, its influent concentration was raised to 400 nmol/l (≈100 µg/l). Hexachloro-1,3-butadiene was never detected in the effluent, despite its absence in the influent over a period of 1.5 year, in which the experiments with chlorinated benzenes were done. Profile measurements revealed that it disappeared within the first 5 cm of the column. A pseudo first order rate constant of 0.36 h^{-1} was calculated from the measurements at 0, 0.5, 1.0, and 2.5 cm from the inlet. Some unknown peaks appeared in the column (Fig. 4.2).

The mass spectra of these products (Fig. 4.3) show the characteristic pattern caused by the natural abundance of the chlorine isotopes. The molecular ions in the spectra a,b, and c of Fig. 4.3 indicate the presence of 5, 4, and 3 chlorine atoms respectively, in the corresponding compounds. They were identified as penta-, tetra-, and trichloro-1,3-butadiene, respectively. Judged from peak responses in the GC/MS-analyses, and taking into account the mass loss resulting from dechlorination, hexachloro-1,3-butadiene was completely converted to tetra- (>90%) and trichloro-1,3-butadiene (<5%).

There are 9 possible isomers of tetrachloro-1,3-butadiene (Kaberdin and Potkin 1991). Since mass spectrometry only yields information on the gross formulas of compounds, further identification was done by means of proton-NMR. A sample taken from the methanogenic column at a height of 20 cm, where tetrachloro-1,3-butadiene was the dominant transformation product (at least 90%, confirmed by GC/MS), gave a spectrum with a singlet at d=6.47 ppm (Table 4.1). This means that the tetrachloro-1,3-butadiene is symmetric, since a singlet can only result from the presence of two identical protons in one molecule. This

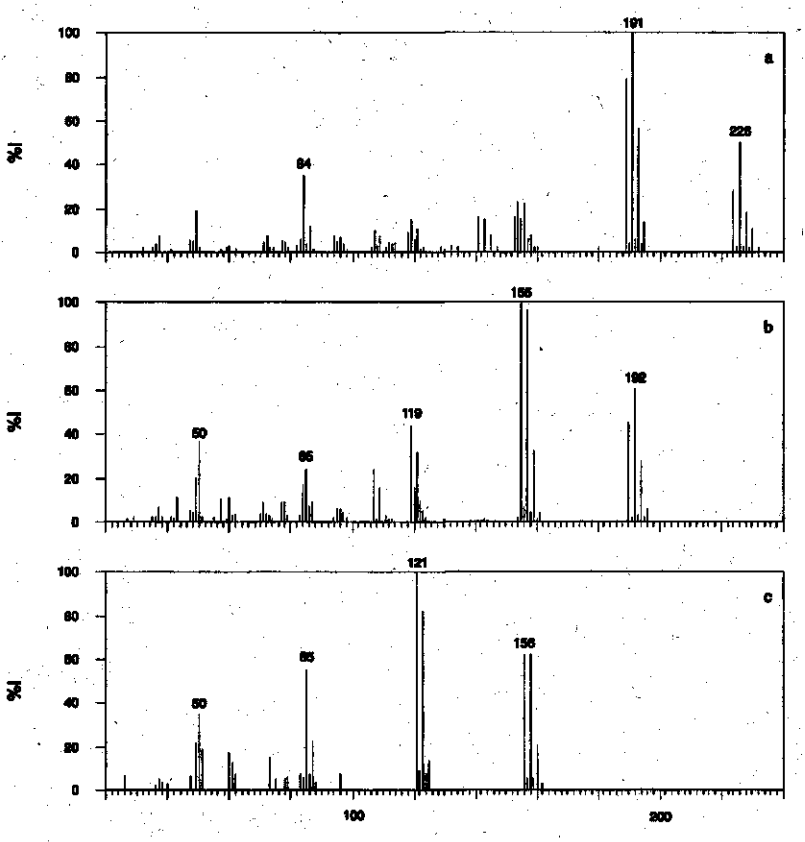

Figure 4.3: *Mass spectra of penta- (a), tetra- (b), and trichloro-1,3-butadiene (c) obtained with column samples taken at different distances from the influent port.*

restricted the number of possible structures to three symmetric isomers, which are listed in Table 4.1. By comparison of the measured chemical shift of 6.47 ppm to the values of the symmetric tetrachloro-1,3-butadienes (Table 4.1), we conclude that hexachloro-1,3-butadiene was reductively dechlorinated to (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene. The concentration of trichloro-1,3-butadiene in the extract was below the detection limit of the NMR-apparatus.

Reduction of hexachloro-1,3-butadiene by hydroxocobalamin and Ti(III) citrate

In the incubations with hydroxocobalamin and Ti(III) citrate, 5 mM hexachloro-1,3-butadiene was completely reduced within 2 hours (Fig. 4.4), with a first order rate constant of 2.5±0.5 h^{-1}. The low concentration prevented the detection of degradation products in this experiment. No reduction was observed in controls lacking hydroxocobalamin, Ti(III) citrate, or both. To detect and identify transformation products, a second experiment was done with an initial hexachloro-1,3-butadiene concentration of 0.5 mM. The GC/MSD-analysis revealed the

Table 4.1: NMR-data of symmetric tetrachlorobutadienes.

isomer	chemical shift (ppm)	reference
1,1,4,4-tetrachlorobutadiene	6.60	Prillwitz and Louw (1971)
	6.59	this study
(Z,Z)-1,2,3,4-tetrachlorobutadiene	6.93	Misra (1978)
	7.12	Otaka (1972)
	7.05	Avagyan et al (1984)
(E,E)-1,2,3,4-tetrachlorobutadiene	6.47	Köbrich and Buttner (1969b)
	6.47	Köbrich and Buttner (1969a)
	6.47	this study

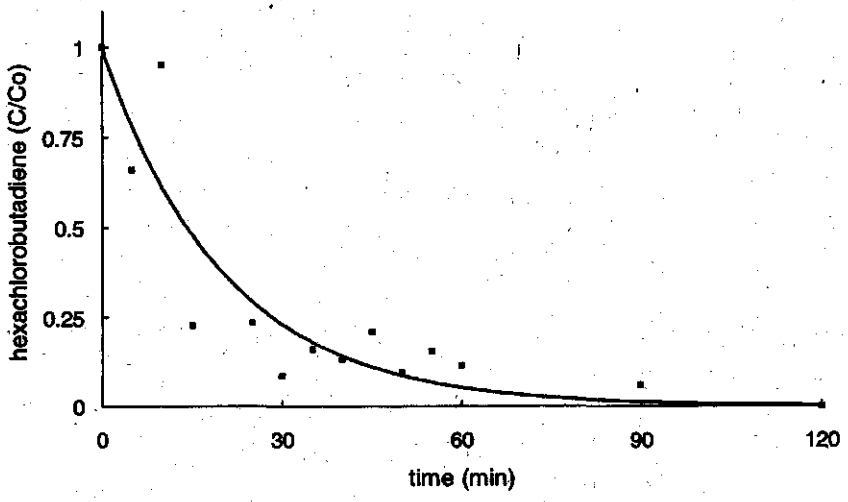

Figure 4.4: Reduction of hexachloro-1,3-butadiene (HCBD) in batch systems with Ti(III) citrate and hydroxocobalamin. The initial concentration was 5 mM.

appearance of two dechlorination products in the hexane extracts and a third in the head space. One product was identified as pentachloro-1,3-butadiene from its mass spectrum, which was similar to the spectrum in Fig. 4.3a. The second product in the hexane extract showed the characteristic pattern caused by the natural abundance of the chlorine isotopes (Fig. 4.5a). The molecular ions in spectrum "a" of Fig. 4.5 indicate the presence of 3 chlorine atoms in the compound. Furthermore, the molecular ion with a mass of 49, results from the presence of 4 carbons and 1 hydrogen in the molecule, and not from 1 carbon, 2 hydrogens, and 1 chloride, because a molecular ion with a mass of 51 is lacking. Thus, the compound was tentatively identified as trichloro-1-buten-3-yn. The product detected in the head space had a molar mass of 52 and showed the typical mass spectrum of 1-buten-3-yn (Fig. 4.5b), which was confirmed by comparison with a published reference spectrum (McLafferty and Stauffer 1989).

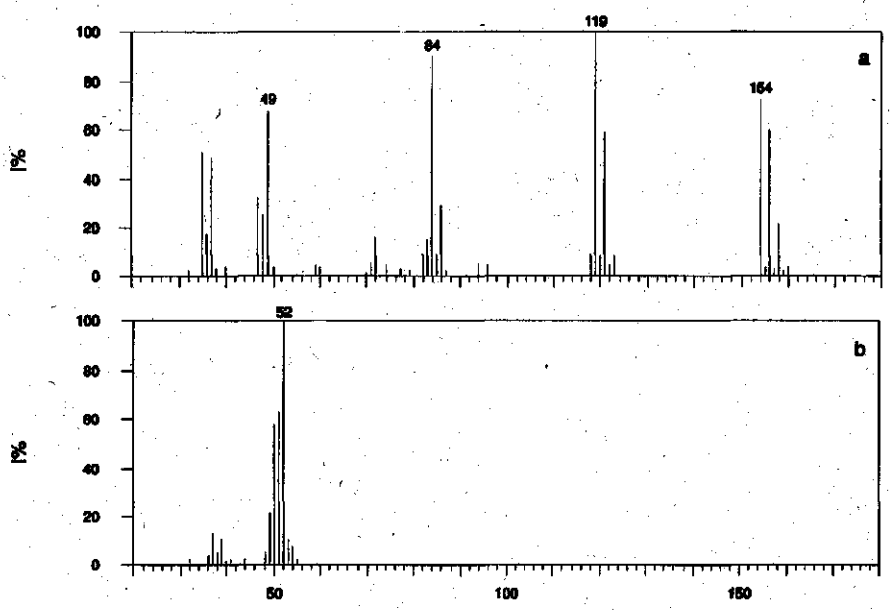

***Figure 4.5:** Mass spectra of trichloro-1-buten-3-yn (a) and 1-buten-3-yn (b) obtained with samples from batch incubations of hexachloro-1,3-butadiene with Ti(III) citrate and hydroxocobalamin.*

DISCUSSION

Results from the column experiments show that hexachloro-1,3-butadiene was only removed under methanogenic conditions, and not when oxygen or nitrate were present. It was concluded that the observed removal of hexachloro-1,3-butadiene under methanogenic conditions was a biological process because (i) a long acclimation time preceded its disappearance and (ii) elimination was also observed in anaerobic batch experiments

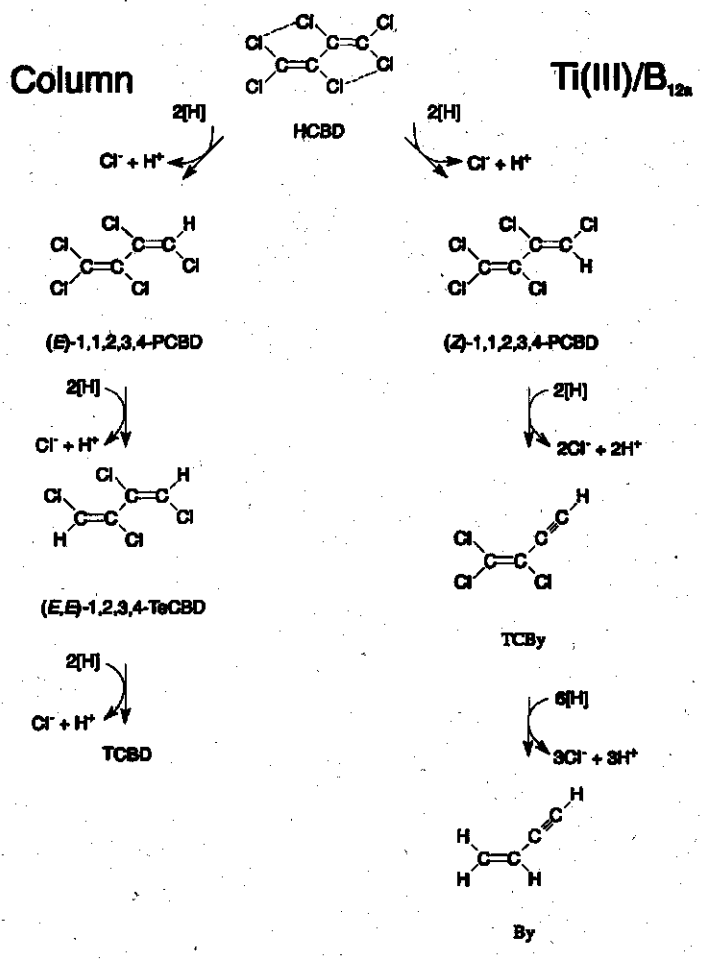

Figure 4.6: Tentative pathways of the reductive dechlorination of hexachloro-1,3-butadiene (HCBD) in a methanogenic sediment column and in batch systems with Ti(III) citrate and hydroxocobalamin (B_{12a}). (PCBD = pentachlorobutadiene, TeCBD = tetrachlorobutadiene, TCBD = trichlorobutadiene, TCBy = trichloro-1-buten-3-yn, By = 1-buten-3-yn). The steric interactions between 1- and 3- (2- and 4-) chlorines in hexachloro-1,3-butadiene are indicated by dashed lines.

inoculated with column material, but not in controls with autoclaved column material (not shown).

The transformation pathway of hexachloro-1,3-butadiene in the methanogenic column is depicted in Fig. 4.6. The end products were (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene (>90%) and an isomer of trichloro-1,3-butadiene. These products are known as antifungal agents (Malama et al 1984) which means that the dechlorination in the column does not lead to a complete detoxification. Small amounts of pentachloro-1,3-butadiene were detected as intermediary product. Assuming that no chlorine atoms are translocated in the molecule during

the dechlorination reaction, it follows that the observed pentachloro-1,3-butadiene was (*E*)-1,1,2,3,4-pentachloro-1,3-butadiene (Fig. 4.6). So, hexachloro-1,3-butadiene is reduced by consecutive steps in which one chlorine is substituted by one hydrogen (hydrogenolysis). A similar mechanism was found to be responsible for reductive dechlorination of other chlorinated hydrocarbons in sediment samples, such as tetrachloroethene (Bosma et al 1988, Bagley and Gossett 1990, Distefano et al 1991, De Bruin et al 1992), chlorinated benzenes (Fathepure et al 1988, Holliger et al 1992) and chlorophenols (Mikesell and Boyd 1986, Chapter 3). Electrochemical and biological reductive dechlorinations of chlorobenzenes probably occur via a nucleophilic substitution mechanism (Farwell et al 1975, Chapter 3). In hexachloro-1,3-butadiene, the two central carbon atoms have a net positive charge resulting from the electron withdrawing effect of the chlorine substituents while the carbons at the 1- and 4-positions have a net negative charge (Kokorev et al 1989). One would therefore expect that a nucleophilic attack would take place at the 2- (or 3-) position. However, dechlorinations in the methanogenic column actually occurred at the 1- and 4-positions, resulting in the formation of (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene. This may be due to steric interferences between the 1- and 3- and 2- and 4- substituents respectively (Fig. 4.6), resulting in a gauche configuration of the hexachloro-1,3-butadiene. Indeed, an angle (φ) of 78.1±1.1° in the single C-C bond has been measured by electron diffraction of gaseous hexachloro-1,3-butadiene (Gundersen 1975). Calculations with MINDO/3, a program that calculates the molecular structure and the enthalpy of formation of a molecule from a given initial structure by minimizing the total energy in the molecule (Dewar and Thiel 1977), predict a similar value (φ=95.8, Kokorev et al 1989). Furthermore, each chlorine substituent is forced out of the planar configuration of each double carbon bond, because their Van der Waals radii overlap. As a consequence, the positively charged carbons in the middle of the molecule are completely surrounded by electron dense chlorines which prevents nucleophilic attack.

Reductive dechlorination of hexachloro-1,3-butadiene in the batch incubations with Ti(III) citrate and hydroxocobalamin, yielded pentachloro-1,3-butadiene, trichloro-1-buten-3-yn and 1-buten-3-yn as products. The proposed reductive pathway is depicted in Fig. 4.6. The presence of pentachloro-1,3-butadiene implies that the first step in the reductive dechlorination of hexachloro-1,3-butadiene was a hydrogenolysis, like in the column experiment. Moreover, the retention time in the analytical column differed from the retention time of the intermediate (*E*)-1,2,3,4,4-pentachloro-1,3-butadiene in the methanogenic column. Assuming that dechlorination in this experiment also took place at the 1-position, because of steric protection of the 2-position, we propose (*Z*)-1,1,2,3,4-pentachloro-1,3-butadiene to be the first intermediate of reductive dechlorination of hexachloro-1,3-butadiene by Ti(III) citrate and hydroxocobalamin. Reductions of chlorinated hydrocarbons with cobalamines as catalyst most probably proceed via nucleophilic substitution (Schrauzer and Deutsch 1969, Holliger et al 1992), like the biological and electrochemical reductions. The presence of trichloro-1-buten-3-

yn suggests that pentachloro-1,3-butadiene was dechlorinated via a dihalo-elimination (Fig. 4.6). During reductive dihalo-elimination of an alkane, two vicinal halogens are released from the molecule, giving the respective alkene (Schanke and Wackett 1992). Similarly, reductive dihalo-elimination of an alkene would result in the corresponding alkyn. Finally, trichloro-1-buten-3-yn was dechlorinated by the replacement of two chlorines by two hydrogens (Fig. 4.6). Dihalo-elimination by vitamin B_{12} has also been demonstrated with chlorinated alkanes (Gantzer and Wackett 1991, Holliger et al 1992, Schanke and Wackett 1992). Also, hydrogenolysis of tetrachloro-ethene and chlorinated benzenes (Gantzer and Wackett 1991) by vitamin B_{12} has been demonstrated before.

From our experiments with hydroxocobalamin, one may conclude that methanogenic bacteria and acetogenic bacteria, which contain considerable amounts of this kind of cofactors (50-800 nmol/g dry weight, Dangel et al 1987), should have the potential to reduce hexachloro-1,3-butadiene, by analogy to observations with chlorinated ethanes (Holliger et al 1990, Holliger et al 1992) and tetrachloro-ethene (Fathepure et al 1987, Gantzer and Wackett 1991). However, the column results show that dechlorination under environmental conditions may involve other catalysts, which was also found for the reductive dechlorination of 1,2-dichloroethane by a pure culture of *Methanobacterium thermoautotrophicum* (Holliger et al 1992). While all chlorines can potentially be removed by hydroxocobalamin via a mechanism involving dihalo-elimination and hydrogenolysis, dechlorination in the column followed a different pathway and lead to the formation of (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene and an isomer of trichloro-1,3-butadiene as dead-end products. These products are known as antifungal agents (Malama et al 1984) which means that the dechlorination in the column does not lead to a complete detoxification. However, the dechlorinated products may be susceptible to aerobic degradation, an option that has to be tested in future experimentation.

ACKNOWLEDGEMENTS

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CHAPTER 5

SIMULATION MODEL FOR BIOTRANSFORMATION OF XENOBIOTICS AND CHEMOTAXIS IN SOIL COLUMNS

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ABSTRACT

In this paper a model is presented which can be used to simulate the behaviour of xenobiotic chemicals in soil columns with respect to their physical and chemical properties. Terms describing biological transformation of xenobiotics are also included in the model. It incorporates microbial growth following Monod kinetics and a chemotactic response of the transforming bacteria towards the xenobiotic substrate. The model appeared to yield good simulations of an experiment by van der Meer et al. (1987) who investigated the degradation of 1,2-dichlorobenzene in soil columns inoculated with *Pseudomonas* sp. strain P51. The behaviour and the fate of 1,4-dichlorobenzene as found by Kuhn et al. (1985) can also be simulated using this model, but their results were also adequately simulated using a simple second order model. The results generated by the model correspond to kinetic parameters obtained in other studies. It is concluded that the model is a useful tool for the investigation of the activity of bacteria degrading xenobiotics in soil columns, provided that the microbial parameters can be determined in independent experiments, and that the active microbial mass in the soil can be measured.

INTRODUCTION

In recent years microbiologists have become interested in transformations of xenobiotic chemicals by micro-organisms in the environment. In most studies the transformation by pure cultures was investigated. In some cases degradative pathways (Williams and Worsey 1976, Reineke et al 1982, Schraa et al 1986a) were elucidated. In other cases the main objectives were the kinetics of biotransformation (Simkins and Alexander 1984, Lewis et al 1985, Schmidt et al 1985b). The latter type of studies were recently reviewed by Button (1985).

Examples of the modelling aspects of biotransformation in aquatic systems are studies by Larson (1984), Paris et al. (1981) and Imboden (1986) and in biofilm reactors by McCarty et al. (1984). In general, the fitting of these models to experimental data has been done with non-linear regression techniques (Robinson 1985).

A bottleneck in predicting the behaviour of xenobiotic chemicals in natural systems with a model, is the lack of reliable data about the in situ metabolic activity of micro-organisms involved in transformation reactions of these compounds. By using information from field, laboratory and pure culture studies, we have developed a model which can be used to simulate the behaviour of biodegradable xenobiotic chemicals in laboratory soil columns. The model contains physical, chemical and microbiological terms and it is assumed that bacteria in the soil transform the xenobiotic substrate following Monod kinetics just as pure cultures do. Since van der Meer et al. (1987) and Schraa et al. (1986b) have demonstrated a chemotactic response of bacteria in soil percolation columns to some of these chemicals, a mathematical expression for chemotaxis has been derived and is included in the model.

Chemotaxis is defined as the movement of organisms in response to a chemical stimulus (Berg 1975). It can be compared to phototaxis observed with algae and plants. When bacteria show a positive chemotactic response to a chemical compound, they move against the concentration gradient of this compound. Therefore, it is assumed that the chemotactic response of bacteria is governed by (i) the concentration gradient of the chemical compound and (ii) the number of micro-organisms which is available to move against this gradient (Keller and Segel 1971). The model has been calibrated with results of the experiment by van der Meer et al. (1987) and with data of 1,4-dichlorobenzene obtained from a study by Kuhn et al. (1985).

THEORY

To describe the physical and chemical behaviour of organic chemicals in porous media, a model was developed by Lapidus and Amundson (1952). This model is given in equation (1) where C is the concentration of the compound ($\mu g \cdot L^{-1}$), t is time (h), D is the dispersion coefficient ($cm^2 \cdot h^{-1}$), z is the space co-ordinate (cm), V is the flow rate of the water ($cm \cdot h^{-1}$),

K_p is the partition coefficient ($L.g^{-1}$), ρ is the particle density of the soil ($g \cdot cm^{-3}$), and ε is the porosity of the soil ($L \cdot L^{-1}$).

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - V\frac{\partial C}{\partial z} - K_p\frac{\partial C}{\partial t}\rho(1-\varepsilon)/\varepsilon \qquad (1)$$

Schwarzenbach and Westall (1981) showed that this model is applicable to the transport of various non-polar compounds in soil columns, continuously operated under saturated flow conditions. The above model describes the effects of dispersion, transport due to the flow of water, and adsorption. Transformation of the xenobiotic compounds by micro-organisms which use these compounds as their source of carbon and energy is not included.

Assuming Monod kinetics (Monod 1949) the uptake rate of a xenobiotic chemical is described by equation (2) where μ_{max} is the apparent maximum specific growth rate (h^{-1}) which will be discussed later, X is the bacterial density ($\mu g \cdot L^{-1}$), Y is the yield coefficient ($\mu g \cdot \mu g^{-1}$) and K_s is the half saturation constant ($\mu g \cdot L^{-1}$).

$$\frac{dC}{dt} = -\frac{\mu_{max} CX}{Y(K_s + C)} \qquad (2)$$

Equation (2) assumes that degradation only occurs in the liquid phase of the column. It is possible that this assumption is not valid and that degradation also takes place on the solid phase. However, the site of biotransformation is irrelevant in the model because equation (1) assumes local sorption equilibrium for the substrate.

Combining and rearranging of equations (1) and (2) yields equations (3) and (4) where R_d is the retardation factor (dimensionless).

$$R_d\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - V\frac{\partial C}{\partial z} - \frac{\mu_{max} CX}{Y(K_s + C)} \qquad (3)$$

$$R_d = 1 + K_p\rho(1-\varepsilon)/\varepsilon \qquad (4)$$

The value of the retardation factor (R_d) for a known compound can be predicted from its octanol/water partition coefficient (Schwarzenbach and Westall 1981, Schwarzenbach 1986), or estimated from a breakthrough curve by dividing its retention time by that of water.

When a substrate is degraded according to the kinetics of equation (2), microbial growth is described by equation (5), where b is the decay coefficient (h^{-1}). It is assumed that b is proportional to the bacterial density (Sinclair and Topiwala 1970).

$$\frac{dX}{dt} = \frac{\mu_{max} CX}{K_s + C} - bX \qquad (5)$$

During the calibration of the model with the data of Kuhn et al. (1985), it appeared that μ_{max} and b were highly correlated: an increase of μ_{max} could be completely compensated for by an increase of b. In addition, we have not been able yet to determine values of b in independent

pure culture experiments. Therefore, the second term of equation (5) was eliminated from the model. This implies that μ_{max} represents only the apparent maximum specific growth rate since it is the resultant of both the growth and decay of bacteria.

Results presented by van der Meer et al. (1987) and Schraa et al. (1986b) give evidence that chemotaxis may play a role in the biotransformation of xenobiotic chemicals in soil percolation columns. The effect of chemotaxis is therefore incorporated in the model. As mentioned in the introduction, the chemotactic response of bacteria is proportional to the microbial density and the concentration gradient of the substrate. This is expressed in equation (6) where K_c is the chemotactic coefficient ($cm^2 \cdot L \cdot \mu g^{-1} \cdot h^{-1}$).

$$\frac{\partial X}{\partial t} = -K_c \frac{\partial}{\partial z}\left(X \frac{\partial C}{\partial z}\right) \qquad (6)$$

When growth and movement due to chemotaxis are considered simultaneously, and eliminating the second term of equation (5), the change of bacterial density in an infinitesimal segment of a soil column is obtained from equation (7) where μ_{max} is the apparent maximum specific growth rate (h^{-1}).

$$\frac{\partial X}{\partial t} = \frac{\mu_{max} C X}{K_s + C} - K_c \frac{\partial}{\partial z}\left(X \frac{\partial C}{\partial z}\right) \qquad (7)$$

The simulation model for chemotaxis and biotransformation of xenobiotics is defined by equations (3) and (7). Its parameters are listed in Table 5.1. The simulations were carried out using CSMP III (IBM 1975). Both equations are partial differential equations because they contain more than one independent variable (namely z and t). The equations were converted to two sets of ordinary differential equations by eliminating the space co-ordinate with the method of lines. These two sets of differential equations were implemented in CSMP III - a simulation language that is designed to solve ordinary differential equations numerically. Several integration methods are available in CSMP III of which the Runge-Kutta-Simpson method (IBM 1975) was used.

The values of the unknown parameters were calculated by non-linear regression analysis with the PODS-module developed by Birta (1977). The module is especially fitted to perform non-linear regression with models written in computer languages like CSMP III. The least squares function J (equation 8) in which n is the number of data, C_{mi} is the measured concentration at data point i and C_{ci} is the calculated concentration at the same data point, was minimized during the regression analysis.

$$J = \sum_{i=1}^{n} (C_{mi} - C_{ci})^2 \qquad (8)$$

Initial guesses of the parameters were obtained from data which are available from pure culture experiments (Table 5.4).

Table 5.1: Names and dimensions of the symbols used in the model

Symbol	Definition	Dimension
C	Concentration of substrate	$mg \cdot L^{-1}$
X	Bacterial density	$mg \cdot L^{-1}$
t	Time	h
z	Space co-ordinate	cm
D	Dispersion coefficient	$cm^2 \cdot h^{-1}$
V	Flow rate of water	$cm \cdot h^{-1}$
μ'_{max}	Apparent maximum specific growth rate	h^{-1}
K_s	Half saturation constant	$mg \cdot L^{-1}$
Y	Yield coefficient	$mg \cdot mg^{-1}$
R_d	Retardation factor	-
K_c	Chemotactic coefficient	$cm^2 \cdot L \cdot mg^{-1} \cdot h^{-1}$

MODEL PERFORMANCE

The performance of the model was tested with data of column experiments by Kuhn et al. (1985) and van der Meer et al. (1987). These investigators used small columns (length ca. 20 cm) which were wet-packed with sand from a river/ground water infiltration site. The columns were percolated with a mineral salts medium containing a mixture of xenobiotics. Kuhn et al. (1985) showed that their results were qualitatively comparable with field data (Schwarzenbach et al 1983).

The chemotaxis term of the model was calibrated with the experiment by van der Meer et al. (Van der Meer et al 1987). In this study, a soil column showing no transformation of 1,2-dichlorobenzene was inoculated with *Pseudomonas* sp. strain P51 which was able to use 1,2-, 1,3-, and 1,4-dichlorobenzene and 1,2,4-trichlorobenzene as sole carbon and energy source (Van der Meer et al 1987). The inoculum was added 10 cm beyond the influent port of the column and for the simulation of this experiment, it has been assumed that the cells had subsequently colonized the rest of the column beyond this point homogeneously. Initially, 1,2-dichlorobenzene was only transformed at and beyond the point of inoculation. In time, the transformation also occurred before this point. After 42 days, 1,2-dichlorobenzene transformation took place already at the beginning of the column (Fig. 5.1). The model was calibrated with this experiment using K_c as fitting parameter. The values of the other parameters were determined independently. The dispersivity of the column material was measured with a pulse of tritiated water, R_d was determined by dividing the residence time of 1,2-dichlorobenzene in the column by that of the tritiated water and the Monod parameters of *Pseudomonas* sp. strain P51 were determined with pure culture experiments. The parameter values are listed in Table 5.2. The concentration profiles yielding the best fit with the data of van der Meer et al. (1987) are shown in Fig. 5.1. The resulting value of K_c was $4 \cdot 10^{-4} \pm 0.4 \cdot 10^{-4}$ $cm^2 \cdot L \cdot \mu g^{-1} \cdot h^{-1}$.

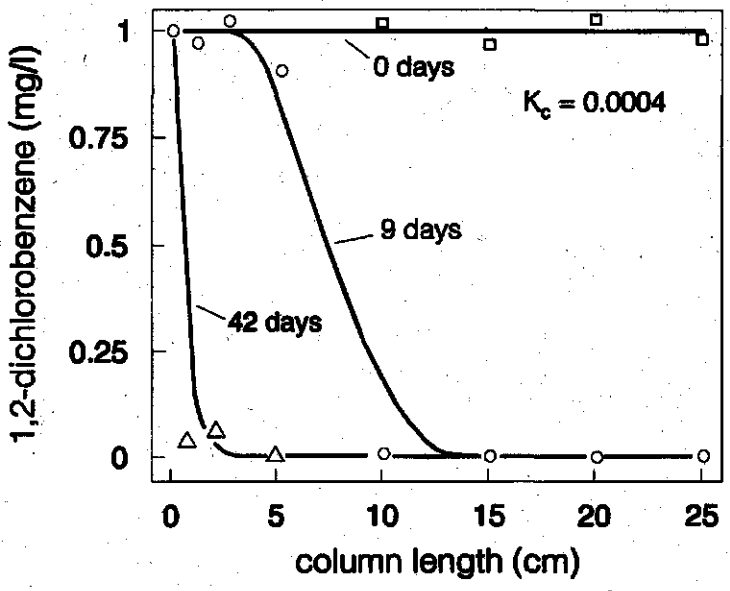

Figure 5.1: Calculated concentration profiles of 1,2-dichlorobenzene at 0,9, and 42 days after inoculation of a soil column with Pseudomonas sp. strain P51. *the experimental data are indicated with squares (0 days), circles (9 days) and triangles (42 days).*

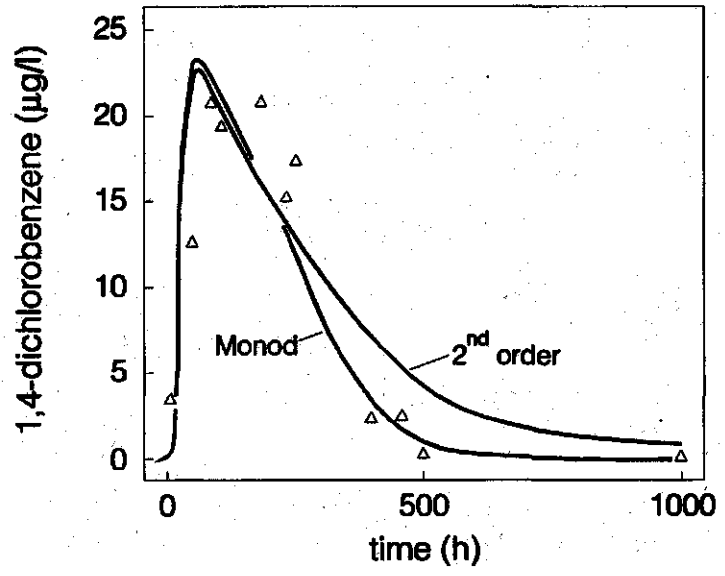

Figure 5.2: Calculated breakthrough curves of 1,4-dichlorobenzene assuming Monod kinetics and second order kinetics. The experimental data of 1,4-dichlorobenzene are indicated with triangles.

The model was also calibrated with the data of 1,4-dichlorobenzene by Kuhn et al. (1985). In this case, independent measurements of the Monod parameters were not available. Additionally, the initial bacterial density (X_o) was not known. Therefore, the model was calibrated with μ'_{max}, Y, K_s, K_c, and X_o as fitting parameters. The values of D and R_d were available from Kuhn (1986). The parameter values which yielded the best fit with the experimental data are listed in Table 5.2. The resulting breakthrough curves and concentration profiles are shown in Fig. 5.2 and 5.3.

***Table 5.2:** Model parameters for the experiments with 1,2-dichlorobenzene by van der Meer et al. (1987) and 1,4-dichlorobenzene by Kuhn et al. (1985).*

Parameter	1,2-dichlorobenzene	1,4-dichlorobenzene
Influent concentration (mg/l)	1000[a]	32.1[c]
Initial bacterial density (mg/l)	1500[a]	25.0 (2.0)[f]
D ($cm^2 \cdot h^{-1}$)	0.4[b]	1.0[d]
V ($cm \cdot h^{-1}$)	4.0[b]	4.0[c]
R_d (-)	3.6[b]	6.7[e]
Y ($mg \cdot mg^{-1}$)	0.16[b]	0.10 (0.02)[f]
μ_{max} (h^{-1})	0.01[b]	0.009 (0.0007)[f]
K_s ($mg \cdot L^{-1}$)	32.0[b]	18.0 (3.0)[f]
K_c ($cm^2 \cdot L \cdot \mu g^{-1} \cdot h^{-1}$)	4.0×10^{-4} (0.4×10^{-4})[f]	0.045 (0.019)[f]

[a] Van der Meer et al (1987).

[b] Determined independently.

[c] Kuhn et al. (1985).

[d] Kuhn (1986).

[e] Calculated with the method of Schwarzenbach and Westall (1981).

[f] Obtained by fitting (standard errors for the fitted parameters are given in brackets)

Since the experimental data of Kuhn et al. (1985) had to be calibrated with five fitting parameters, it is possible that these data can be simulated using a simpler model without a statistically significant decrease of the quality of simulation. Therefore, a sensitivity analysis was performed by omitting or changing some of the assumptions of the model. The statistical significance of the difference between the quality of fit of two models was tested using an F-test (Robinson 1985). The results of the sensitivity analysis are summarized in Table 5.3. The first step was to omit the assumption of chemotaxis and to replace Monod kinetics by simple second order kinetics (Paris et al 1981). This model is given in equations (9) and (10), where k_2 is the second order growth constant ($L \cdot h^{-1} \cdot \mu g^{-1}$).

$$R_d \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - V \frac{\partial C}{\partial z} - k_2 C X / Y \qquad (9)$$

$$\frac{\partial X}{\partial t} = k_2 C X \qquad (10)$$

The best fit of this model with the experimental data by Kuhn et al. (1985) did not significantly differ from the best fit obtained with the Monod with chemotaxis model (Table 5.3). The value of the second order growth constant obtained after calibration was $2.4 \cdot 10^{-4} \pm 0.2 \cdot 10^{-4}$ $L \cdot h^{-1} \cdot \mu g^{-1}$. This value is approximately two orders of magnitude larger than values found by Paris et al. (1981) for the butoxy-ethylester of 2,4-dichloro phenoxy acetic acid (2,4-DBE) in aquatic systems. Nor the combination Monod without chemotaxis, nor second order with chemotaxis, yielded a fit of the experimental data that differed significantly from the simple second order model (Table 5.3). However, it is interesting to note that the difference between the Monod models with and without chemotaxis are significant (Table 5.3). This difference is illustrated in Fig. 5.3. Despite this fact it can be concluded from the sensitivity analysis that the data provided by the experiment from Kuhn et al. (1985) can adequately be simulated with both the Monod with chemotaxis or a simple second order model.

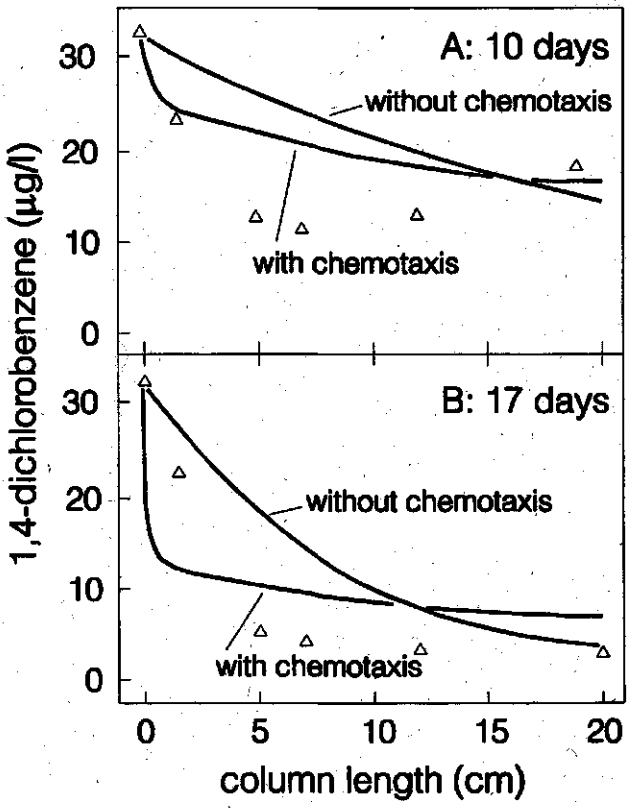

Figure 5.3: Calculated concentration profiles of 1,4-dichlorobenzene assuming growth with chemotaxis and growth without chemotaxis after 10 days (A) and after 17 days (B). The experimental data are indicated with triangles.

Table 5.3: The statistical significance (P=0.1) of the difference between the simulations of the data by Kuhn et al. (1985) according to an F-test (Robinson 1985).

	Monod		Second order
	+ chemotaxis	- chemotaxis	+ chemotaxis
Monod			
+ chemotaxis	S[a]	-	
Second order			
+ chemotaxis	NS[b]	NS	-
- chemotaxis	NS	NS	NS

[a] S = significant
[b] NS = not significant

DISCUSSION

From the results of the simulation of the experiment by van der Meer et al. (1987), it can be concluded that the model combining Monod with chemotaxis provides a good description of 1,2-dichlorobenzene degradation by *Pseudomonas* sp. strain P51 inoculated in a soil column. All parameter values for this experiment were determined independently, except the value of K_c. The sensitivity analysis reveals that the degradation of 1,4-dichlorobenzene as found by Kuhn et al. (1985), is equally well simulated with the simple second order model (equations 9 and 10), as with the Monod model with chemotaxis. However, there are several reasons to use the Monod model:

(i) The Monod equation provides a description of substrate uptake and growth of bacteria which is generally accepted among microbiologists;

(ii) The Monod model converts to the simple second order form when the substrate concentration is low in comparison to K_s. During the column experiment of Kuhn et al. (1985) 1,4-dichlorobenzene concentrations were almost always below the fitted K_s value of 18 $\mu g \cdot L^{-1}$. Thus, it might be that the soil column experiments do not have the proper design to discriminate between the two models;

(iii) The values of the Monod parameters obtained after fitting the model with the 1,4-dichlorobenzene data from Kuhn et al. (1985) show a good agreement with the pure culture data for 1,2-dichlorobenzene obtained with *Pseudomonas* sp. strain P51 (Table 5.2). As 1,2- and 1,4-dichlorobenzene are very similar substrates, it is reasonable to compare these values;

(iv) The fitted k_2 value of $2.4 \cdot 10^{-4}$ $L \cdot h^{-1} \cdot \mu g^{-1}$ obtained with the simple second order model, is approximately two orders of magnitude larger than values found by Paris et al. (1981) for 2,4-DBE, obtained with the same model.

If for the reasons mentioned above, the Monod model is thought to be more realistic than the simple second order model, the significance of the difference between the Monod models with and without chemotaxis (Table 5.3, Fig. 5.3) is important. The results from the experiment by van der Meer et al. (1987) indicate that chemotaxis may be an important process in the soil

columns. However, the difference between the values of K_c determined from the two column experiments is very large. With the results which are available now, this cannot be explained. Literature data to which the K_c values can be compared, were not found.

From the literature, it is known that chemotaxis may have a great influence on the growth of bacteria in heterogeneous environments. Pilgram and Williams (1976) showed that a chemotactic strain of *Proteus mirabilis* outgrew a non-motile mutant in a semi-solid (heterogeneous) medium while the two strains grew equally well in a homogeneous medium. Kennedy and Lawless (1985) observed that a chemotactic strain of *Pseudomonas fluorescens* survived significantly better in soil than a non-motile strain of the same species. In an elegant experimental set-up, Walsh and Mitchell (1978) showed that bacteria are able to move perpendicular to flow rates of up to 2 cm·min^{-1} in a cylinder with a length of 6 cm. From these results it may be concluded that chemotactic bacteria have advantages in environments where concentration gradients of substrates occur. With the simulation model, a large bacterial density was predicted in a sharp band at the inlet of the column. This is consistent with experimental data of Dahlquist et al. (1972) and Holz and Sow-Hsin Chen (1979), who observed bands of chemotactic bacteria as a response to a steep increase in substrate concentration.

Van der Meer et al. (1987) have found that in their experiments 1,2-dichlorobenzene was not transformed below a threshold concentration of ca. 5 µg·L^{-1} in inoculated and ca. 0.1 µg·L^{-1} in "naturally adapted" soil columns. Such a threshold or S_{min} value has also been demonstrated by Bouwer and McCarty (1984) and Rittmann and McCarty (1980). They have shown that its value in biofilm columns can be predicted with equation (11), where b is the loss rate of bacteria (h^{-1}). The existence of b can be caused by (i) decay, (ii) maintenance requirements, and/or (iii) sheer loss of bacteria.

$$S_{min} = K_s \frac{b}{\mu_{max} - b} \qquad (11)$$

However, as mentioned in the "Theory" section, we did not succeed in incorporating the loss rate of bacteria in the model. Predictions of threshold concentrations can only be made using our model, if b is included. It is obvious that the absence of a decay term in the model is unrealistic because it results in an indefinite growth of bacteria in the simulations. Therefore, it is needed that experiments be designed in the future where growth of bacteria in relation to degradation of xenobiotics in the soil is followed quantitatively. Such research is in progress in our laboratory.

***Table 5.4**: Values of the maximum specific transformation rate ($\mu'_{max}Y^{-1}$), the half saturation constant (K_s) and the yield coefficient (Y) obtained from different studies.*

Substrate	Organism	Culture conditions	$\mu'_{max}Y^{-1}$ (h^{-1})	K_s ($mg \cdot L^{-1}$)	Y	Reference
1,2-dcb[1]	*Pseudomonas* P51	pure culture	0.06	32.0	0.16	-
1,4-dcb[2]	mixed culture	soil column		18.0	0.1	this study
	mixed culture	field study		0.4	ND	Schwarzenbach et al (1983)
	Pseudomonas P51	pure culture		ND	0.16	unpublished data
	Alcaligenes A175	pure culture		ND	0.18	Schraa et al (1986a)
1,2,4-tcb[3]	*Pseudomonas* P51	pure culture	0.3	ND	0.1	unpublished data
1,2-dmb[4]	*Arthrobacter* sp. 125	pure culture	0.17	ND	0.56	unpublished data
	Pseudomonas sp.	pure culture, induced cells	ND	43.2	ND	Button (1985)
	Pseudomonas sp.	pure culture, uninduced cells	ND	33.1	0.01	Button (1985)
benzoate	*Pseudomonas* sp.	pure culture	0.43	450	ND	Simkins and Alexander (1984)
4-cp[5]	*Pseudomonas* sp.	pure culture	ND	1295	ND	Paris et al (1981)

1 1,2-dichlorobenzene.
2 1,4-dichlorobenzene.
3 1,2,4-trichlorobenzene.
4 1,2-dimethylbenzene.
5 4-chlorophenol.
ND = not determined.

Some of the parameter values for 1,4-dichlorobenzene which were calculated with our model, may be compared to values obtained in batch culture studies with chemically and structurally related compounds (Table 5.4). The maximum specific growth rate is converted to a maximum specific transformation rate (μ'_{max}/Y) because in some studies only the transformation rates were measured. The values of the maximum transformation rate (ca. 0.1 $\mu g\,L^{-1}$) and the yield coefficient (0.1 $\mu g \cdot \mu g^{-1}$) are of the same order of magnitude as those obtained in other studies. However, the value of the half saturation constant of 18 $\mu g \cdot L^{-1}$ is lower than values measured by others (Paris et al 1981, Simkins and Alexander 1984, Button 1985). As the soil columns used by Kuhn et al. (1985) were operated with low input concentrations, this low value of K_s may be interpreted as an adaptation of the transforming bacterial population to a low concentration of their carbon and energy source (Button 1985). In this light, it is interesting to compare the results of this study with the field observations made by Schwarzenbach et al. (1983). These authors measured a concentration profile of 1,4-dichlorobenzene at a river water infiltration site in Switzerland. From this published concentration profile, the K_s for 1,4-dichlorobenzene can be estimated with a Lineweaver-Burk plot. In order to accomplish this, it is assumed that the 1,4-dichlorobenzene concentration in the river was the initial concentration for the transformation until the first sampling point. The concentration at this point serves as initial concentration for the next point, and so on. The resulting estimate for the K_s in the field was 0.4 $\mu g \cdot L^{-1}$. The low

1,4-dichlorobenzene concentration in the river (ca. 0.2 $\mu g \cdot L^{-1}$) seems to induce a lower K_s in the field. It should be remembered here that the soil material used by Kuhn et al. (1985) was obtained from the same site where Schwarzenbach et al. (1983) made their observations. Therefore, it is reasonable to compare the field data with the laboratory column data.

It must be stressed that from the results obtained by simulation as presented in this paper, no definite conclusions can be drawn about the details of the processes occurring in the soil percolation columns. The main problem is that one of the variables of the model, the amount of microbial biomass, which is responsible for the transformation of the xenobiotic chemicals, was not measured by Kuhn et al. (1985) and is indeed very difficult to measure. This complicates the development of models describing biological transformations in the soil because they have little predicting value, when they contain variables which cannot be measured (Imboden 1986). A first solution to this problem has been provided by the inoculation experiment by van der Meer et al. (1987). It has been possible to follow the activity of *Pseudomonas* sp. strain P51 in the column quantitatively. Additionally, Monod parameters were determined independently in pure culture experiments, while the amount of inoculum was known. Similar experiments to determine the microbiological parameters in the soil independently are needed and are in progress in our laboratory. In addition efforts are made to develop methods for the determination of active microbial mass in the soil.

ACKNOWLEDGEMENTS

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CHAPTER 6

TRANSPORT OF INTRODUCED BACTERIA AND BIOTRANSFORMATION OF ORGANIC COMPOUNDS IN SATURATED SOIL COLUMNS

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ABSTRACT

The biotransformation of organic compounds by introduced bacteria was studied in saturated soil columns percolated with aqueous media of varying ionic strength. *Pseudomonas* sp. strain B13 and *Rhodococcus* sp. strain C125 growing on 3-chlorobenzoate and benzoate respectively, were used as model organisms. *Rhodococcus* sp. strain C125 attached better to the column material than did *Pseudomonas* sp. strain B13. Attachment of both strains improved with increasing ionic strength. Biotransformation of benzoate by introduced *Rhodococcus* cells at low ionic strength was best described by a model that allowed growing cells to detach from the solid to the aqueous phase. In contrast, biotransformation at high ionic strength was best described assuming no detachment. The ionic strength of the water in which bacteria are introduced into soil controls the transport and attachment of bacteria during bioremediation. The occurrence of a uniform residual concentration at all levels of the ionic strength, could not be explained by the model approach. A heterogeneous distribution of the bacteria in the soil columns most probably causes the observed residual concentration.

INTRODUCTION

The objective of *in situ* bioremediation is to stimulate growth of micro-organisms in contaminated aquifers and soils, and thus increase biotransformation rates of organic contaminants. Sometimes, specific micro-organisms are introduced, especially when compounds are present which are not readily degraded by the autochthonous microflora. The success of the addition of micro-organisms depends on their ability to reach the contamination, to survive, and to carry out the desired reaction. A better understanding and control of the transport of bacteria in soil and ground water will help to optimize techniques for bioremediation employing introduced bacteria. The transport of bacteria under saturated flow conditions is controlled by surface characteristics of bacteria and soil particles and by the ionic strength of the flowing water (Elimelech and O'Melia 1990, Martin et al 1992, Rijnaarts et al 1993a). The adhesion of bacteria to solids can be understood with concepts derived from the DLVO-theory which predicts a positive correlation of adhesion of negatively charged cells and negatively charged solids with their hydrophobicities and with the ionic strength of the water (Van Loosdrecht et al 1989, Rijnaarts et al 1993a, Rijnaarts et al 1993b).

The objective of the present study was to investigate the transport and biotransformation of organic compounds and the transport of bacteria in saturated soil columns simultaneously. *Pseudomonas* sp. strain B13 and *Rhodococcus* sp. strain C125 which have different surface properties and are able to grow on 3-chlorobenzoate and benzoate respectively, were selected as model systems. The adhesion of the strains to the column material was controlled by using aqueous media of varying ionic strength. Experimental results were used to validate a mathematical model incorporating approaches originating from colloid filtration theory and classical microbial growth kinetics with parameters obtained from independent sources as input.

MATERIALS AND METHODS

Cultivation and preparation of bacteria

Pseudomonas sp. strain B13 (Dorn et al 1974) was a kind gift of Prof. Reineke (University of Wuppertal, Germany). Cells are hydrophilic and have a moderate negative charge at pH=7, as demonstrated by contact angle (θ_w=32°) and electrophoretic mobility (u=-2.91·10^{-8} m^2·V^{-1}·s^{-1}) measurements (Rijnaarts et al 1993a). *Rhodococcus* sp. strain C125 was originally described as *Corynebacterium* sp. strain C125 (Schraa et al 1987) and later renamed to *Rhodococcus* (Bendinger et al). In contrast to *Pseudomonas* strain B13, cells are hydrophobic (θ_w=70°) and have a high negative charge (u=-3.34·10^{-8} m^2·V^{-1}·s^{-1}).

Both strains were grown in a mineral medium (Schraa et al 1986) amended with 5 mM 3-chlorobenzoate for *Pseudomonas* sp. strain B13 and 5 mM benzoate for *Rhodococcus* sp.

strain C125. Cells from two 250 ml cultures were harvested in the late exponential phase and washed by repeated centrifuging (3x10 min., 20,000 g) and resuspension of the pellet in mineral medium. Finally, cells were resuspended in 10 ml mineral medium and were stored on ice until experimentation began (within an hour). In the case of column experiments, cells were washed and resuspended in a tenfold dilution of the mineral medium.

Rifampicin resistant cells of *Pseudomonas* sp. strain B13 were obtained by striking 100 µl of a suspension containing at least 10^9 cells on nutrient broth (Difco, 8 g/l) agar plates amended with 50 mg/l of the antibiotic. Plates were incubated in the dark at 30°C. After 3 days of incubation, colonies were picked and inoculated in mineral medium amended with 3-chlorobenzoate (5 mM) and rifampicin (50 mg/l). The stability of the obtained rifampicin resistance was checked by subculturing the obtained resistant cells in medium without the antibiotic 3 times. No loss of resistance was observed. Contact angles and electrophoretic mobilities of resistant and wild-type cells were the same.

Column experiments with Pseudomonas *sp. strain* B13

Columns constructed of hard PVC (10 cm length, 2.5 cm i.d.) were wet packed with sand (organic matter content < 0.1%, grain size 0.25-0.50 mm) from the Rhine River near Wageningen, The Netherlands. Columns were operated in an up-flow mode at a flow rate of 4 ml/h with media of varying ionic strengths (adjusted by addition of NaCl). Columns were percolated with the appropriate media overnight, before suspensions of rifampicin resistant *Pseudomonas* sp. strain B13 (about. 10^6 CFU/ml), were applied to study the transport of the cells. Cell numbers in in- and effluent were determined by means of tenfold counts on agar plates prepared with mineral medium containing 10 mM 3-chlorobenzoate and amended with 50 mg/l rifampicin, and expressed as Colony Forming Units per ml (CFU/ml). The detection limit of the method was 100 CFU/ml. Plates were always counted within 48 h of incubation. Samples of the sand used for the experiments were checked for the presence of indigenous, 3-chlorobenzoate degrading and rifampicin resistant bacteria by blending 1 g with 10 ml of a phosphate buffer (10 mM) during 1 min. and applying 3x100 µl on nutrient broth plates containing the same amount of rifampicin as used for counting. No colonies appeared on the plates during 48 h of incubation.

Biotransformation of 3-chlorobenzoate by *Pseudomonas* sp. strain B13 was studied with the same set-up. Columns were inoculated either by injection of 1.5 ml of a cell suspension (10^7 CFU/ml) just above the influent port, or by wet packing the columns with mineral medium of appropriate ionic strength containing rifampicin resistant cells (10^4 or 10^9 CFU/ml). Columns were left overnight without pumping to establish equilibrium between suspended and sorbed cells, before medium containing 3-chlorobenzoate (3.2 µM) was applied. The residence time in the columns was determined using chloride (100 mM) as conservative tracer.

Batch degradation and uptake experiments with Rhodococcus sp. strain C125

The net yield (mg dry weight/mmol) of *Rhodococcus* strain C125 during growth on benzoate was determined by growing the organism on 1, 2, 3, 4, and 5 mM benzoate. The maximum biomass obtained was measured spectrophotometrically as optical density at 660 nm (OD_{660}). The relation between OD_{660} and dry weight was established with a serial dilution of a culture of known dry weight, which was measured by putting 3x2.5 ml of culture suspended in mineral medium at 105°C overnight. Sterile mineral medium served as control.

The maximum specific growth rate was determined by following substrate consumption in 20 ml cultures with a known initial dry weight, amended with a series of initial benzoate concentrations in the range of 5 µM to 1 mM. Cultures were incubated on a rotary shaker at 20°C at 400 rpm.

Benzoate uptake kinetics were quantified in short term experiments by measuring benzoate depletion in a series of 1 ml Eppendorf centrifuge tubes containing culture with a known dry weight, amended with initial benzoate concentrations of 5, 10, 20, 40, 80, 100, 200, 300, 400, and 500 µM (seven tubes for each concentration). The reaction was carried out at 20°C. It was stopped at 5 minute intervals by the addition of 20 µl of concentrated H_2SO_4 to 1 tube of each concentration. The depletion rate for each concentration was calculated by only taking into account the initial slope of the curve. For concentrations above 40 µM this always comprised at least 3 data points. Lower concentrations were depleted within 10 minutes, and therefore, only the first 2 data points could be used to calculate the slope of the curve.

The half saturation constant for the uptake of benzoate was also determined by measuring the oxygen uptake rate at different benzoate concentrations polarographically with an oxygen electrode at 20°C. Uptake rates were corrected for endogenous respiration.

Column experiments with Rhodococcus *sp. strain* C125

Autoclaved glass columns with an internal diameter of 1 cm and a length of 10 cm were wet packed with autoclaved sand (organic matter content < 0.1%, grain size 0.09-0.25 mm) obtained from the Rhine River near Wageningen, The Netherlands. Columns were operated in an down-flow mode at a flow rate of 10 ml/h. The in- and outlets were equipped with glass filters to obtain a homogeneous flow through the column. Bacterial suspensions of different ionic strength were obtained by diluting samples of the concentrated suspension to a final optical density at 280 nm (OD_{280}) of 0.6 (Rijnaarts et al 1993a) corresponding to 25 mg dry weight/l. This value remained constant throughout the experimental time. OD_{280} was related to cell dry weight the same way as described for the OD_{660}. The ionic strength of media was adjusted by the addition of different amounts of NaCl, such that the negative logarithm of the ionic strength (pI) varied between 1 and 2.5. Columns were equilibrated by percolating

medium overnight. Then, cell suspensions were applied to the columns for 60 minutes followed by cell-free solutions of the same pI for an additional hour. The OD_{280} of the effluent was monitored in effluent samples throughout the experimental time of 2 hours. Finally, the influent solutions were replaced by cell free solutions of the same pI amended with 50 μM benzoate. The effluent of two columns (one low and one high pI) was sampled for 9 hours by means of fraction collectors. Samples were collected in glass tubes containing 30 μl H_2SO_4, to prevent benzoate transformation after sampling. The effluent of all columns was sampled after 16 and 40 hours of continuous operation to determine steady state residual benzoate concentrations. The residence time in the columns was determined using chloride (100 mM) as conservative tracer.

Analyses

Benzoate and 3-chlorobenzoate were analyzed by HPLC (Schraa et al 1986). Chloride was measured with a chlor-o-counter (Marius, Nieuwegein, The Netherlands).

THEORY

Transport of bacteria

The transport of bacteria under saturated flow conditions can be understood with the concepts and models from colloid filtration theory (Iwasaki 1937, McDowell-Boyer et al 1986, Harvey and Garabedian 1991, Martin et al 1992). Cells are transported with the flowing water. Sorption may retard their movement while irreversible attachment, sedimentation and interception leads to a removal of cells from the flowing water. These processes were originally combined in an equation that stated that the concentration of suspended cells declines exponentially with column length (Iwasaki 1937):

$$\frac{dB_{aq}}{dz} = -\lambda B_{aq} \qquad (1)$$

where B_{aq} is the aqueous bacterial density, z the axial co-ordinate, and λ the filtration coefficient. A complete list of symbols, their meaning and dimensions is given in the Notation list. Microbial transport in a saturated column can be described in an equation that combines terms for dispersion, advection, sorption and filtration respectively (Matthess et al 1988, Harvey and Garabedian 1991, Matthess et al 1991):

$$\frac{\partial B_{aq}}{\partial t} = D\frac{\partial^2 B_{aq}}{\partial z^2} - V\frac{\partial B_{aq}}{\partial z} - K_p\frac{\rho}{\theta}\frac{\partial B_{aq}}{\partial t} - \varphi B_{aq} \qquad (2)$$

where D is the dispersion coefficient, V the interstitial pore water velocity, K_p the microbial partition coefficient, φ a first order filtration rate constant, ρ the bulk soil density, θ soil

porosity, and t is time. The use of a first order sink term to account for filtration is equivalent to the approach in equation (1) since the travel distance represents a travel time through the sand filter (Lindqvist and Bentsson 1991). Equation (2) can be rewritten as:

$$R_m \frac{\partial B_{aq}}{\partial t} = D\frac{\partial^2 B_{aq}}{\partial z^2} - V\frac{\partial B_{aq}}{\partial z} - \varphi B_{aq} \qquad (3a)$$

with

$$R_m = 1 + \frac{\rho}{\theta} K_p \qquad (3b)$$

The accumulation of cells at the solid phase as a result of filtration can be described as:

$$\frac{\rho}{\theta}\frac{dB_{att}}{dt} = \varphi B_{aq} \qquad (4)$$

where B_{att} is the density of attached bacteria. The accumulation of sorbed bacteria (B_{sorbed}) is given by the relationship:

$$\frac{dB_{sorbed}}{dt} = -K_p \frac{dB_{aq}}{dt} \qquad (5)$$

Equations (3), (4) and (5) are subject to the following initial and boundary conditions:

$$B_{aq}(t=0,z) = R_m^{-1} B_i \qquad (6a)$$

$$B_{att}(t=0,z) = 0 \qquad (6b)$$

$$VB_{aq}(t,z=0) - D\frac{\partial B_{aq}(t,z=0)}{\partial z} = VB_0(t) \qquad (6c)$$

$$\frac{\partial B_{aq}(t,z=L)}{\partial z} = 0 \qquad (6d)$$

where B_i is the initial aqueous bacterial density, B_0 the bacterial density in the column influent and L column length. The first initial condition (6a) defines the equilibrium between sorbed and aqueous bacteria after inoculation via the aqueous phase. The second (6b) assures absence of attached, irreversibly bound bacteria at the start of the experiment. The first boundary condition (6c) equals the influx of bacteria to the sum of the dispersive and advective flux at the boundary, while the second (6d) imposes a constant density of aqueous bacteria over the effluent boundary. The latter condition is necessary to make a numerical solution possible.

Transport and biotransformation of a growth limiting organic compound

Transport of organic compounds is described using an advective dispersive transport equation, similar to the transport equation for bacteria:

$$R_d \frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - V\frac{\partial C}{\partial z} - P \qquad (7)$$

where C is the concentration of the organic compound, R_d its retardation factor, and P a sink term describing biotransformation. The initial and boundary conditions for equation (7) are:

$$C(t=0,z) = C_i \qquad (8a)$$

$$VC(t=0,z) - D\frac{\partial C(t=0,z)}{\partial z} = VC_0(t) \qquad (8b)$$

$$\frac{\partial C(t,z=L)}{\partial z} = 0 \qquad (8c)$$

where C_i is the initial and C_0 the influent concentration of the organic compound. The initial condition in equation (8a) defines a constant initial concentration of the organic compound in the columns. The first boundary condition (8b) equals the influx of substrate to the sum of the dispersive and advective flux at the boundary, while boundary condition (8c) defines a constant contaminant concentration over the effluent boundary.

Bacteria may be reversibly sorbed or irreversibly attached to the solid phase or suspended in the aqueous phase. Suspended and bound cells both have to be considered in the description of microbial growth and the biotransformation of organic compounds. Biotransformation by the total biomass is given by:

$$P = \mu B_{tot} Y^{-1} \qquad (9)$$

where μ is the specific growth rate, Y the yield coefficient and B_{tot} the total bacterial density which is calculated as the sum of the aqueous and solid phase densities. Solid phase densities are corrected using ρ and θ for dimensional consistency:

$$B_{tot} = B_{aq} + \frac{\rho}{\theta}(B_{sorbed} + B_{att}) \qquad (10)$$

Combining equations (7) and (9) leads to:

$$R_d \frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - V\frac{\partial C}{\partial z} - \mu B_{tot} Y^{-1}. \qquad (11)$$

Using classical microbiological approaches and assuming that no detachment of attached cells takes place after cell division, growth rates of attached (B_{att}) and aqueous cells (B_{aq}) are given by:

$$\frac{dB_{att}}{dt} = (\mu - b)B_{att} \qquad (12a)$$

and

$$\frac{dB_{aq}}{dt} = (\mu - b)B_{aq} \qquad (12b)$$

respectively, where b is the specific maintenance rate of the bacteria. The growth rate of sorbed cells is automatically obtained via the relationship in equation (5). The specific growth rate is given by the Monod equation:

$$\mu = \mu_{max} \frac{C}{K_s + C} \tag{13}$$

where μ_{max} is the maximum specific growth rate and K_s the half saturation constant.

Equations (1)-(13) provide a full description of the transport of introduced bacteria in saturated soil columns and the simultaneous transport and biotransformation of an organic compound. A basic assumption in the model derivation is that attachment of cells is irreversible and that the daughter cells resulting from cell division remain attached to the solid phase. It is also conceivable however, that the daughter cells are released into the aqueous phase instead of remaining bound to the solid phase. As a consequence, growth will only lead to accumulation of cells in the aqueous phase and equation (12a,b) gets replaced by:

$$\frac{dB_{aq}}{dt} = \mu\left(B_{aq} + \frac{\rho}{\theta} B_{att}\right) - bB_{aq}. \tag{14a}$$

and

$$\frac{dB_{att}}{dt} = -bB_{att} \tag{14b}$$

The daughter cells in the aqueous phase will be transported with the water flow and re-attachment may take place as described in equations (3) and (4). As mentioned above, the growth rate of sorbed cells is automatically obtained via the relationship in equation (5).

Steady state

The model will reach a steady state when the net accumulation rates of attached and aqueous bacteria are equal to zero, i.e. when the contributions of filtration, growth and decay do not result in accumulation of cells anymore. Hence, the staedy states can be calculated from

$$\frac{\rho}{\theta}\frac{dB_{att}}{dt} = (\mu - b)\frac{\rho}{\theta} B_{att} = 0 \tag{15}$$

when no detachment of attached cells occurs, from

$$\frac{\rho}{\theta}\frac{dB_{att}}{dt} = \varphi B_{aq} - b\frac{\rho}{\theta} B_{att} = 0 \tag{16a}$$

and from

$$\frac{dB_{aq}}{dt} = \mu\left(B_{aq} + \frac{\rho}{\theta} B_{att}\right) - bB_{aq} - \varphi B_{aq} = 0 \tag{16b}$$

when detachment of daughter cells from dividing attached cells is assumed. Solving these equations together with equation (13) yields:

$$C_{res} = K_s \frac{b}{\mu_{max} + b} \qquad (17)$$

where C_{res} is the steady state residual concentration which is equal to the threshold concentration for growth from the extended Monod equation (Bouwer and McCarty 1984, Middeldorp et al in prep., Chapter 5). Equation (16b) does not take into account the advective transport of bacteria. Hence, equation (17) yields a minimum value for C_{res} if cell division results in detachment of daughter cells.

Simulation strategy

Estimates of V and D were obtained by fitting measured breakthrough curves of chloride to equation (7) with the computer program CXTFIT (Parker and Van Genuchten 1984), keeping values of R_d and P fixed at values of 1 and 0 respectively. Estimates of R_m and φ were obtained by fitting the results of the breakthrough and displacement experiments with *Pseudomonas* sp. strain B13 and *Rhodococcus* sp. strain C125 respectively, to equation (3a), fixing V and D at the values obtained from the chloride breakthrough curves.

Model equations were solved numerically, using a finite difference method to discretize the axial grid (Chapter 7). The resulting set of non-linear differential equations was implemented in FORTRAN-77 and solved with the stiff stable Gear method (Gear 1971) implemented in IMSL 9.2 (IMSL 1987). The computer program did not make use of ρ, θ, and K_p. Therefore, $(\rho/\theta)B_{sorbed}$ and $(\rho/\theta)B_{att}$ were treated as variables instead of B_{sorbed} and B_{att} and equation (5) was implemented as:

$$\frac{\rho}{\theta}\frac{dB_{sorbed}}{dt} = -(R_m - 1)\frac{dB_{aq}}{dt} \qquad (18)$$

The cell density during column packing for the high ionic strength experiment with *Pseudomonas* sp. strain B13 was used as value of the initial aqueous bacterial density B_i. The initial 3-chlorobenzoate concentration (C_i) was estimated from the measured effluent concentration in the first hours of the experiment in Fig. 6.2. The cells injected just above the influent port in the low ionic strength experiment were assumed to be present in the first cm of the column initially and the value of B_i was chosen accordingly. It was set at zero in the rest of the column. All parameters defining the kinetics of growth of *Pseudomonas* sp. strain B13 on 3-chlorobenzoate were taken from chemostat and recycling fermentor studies (M.E. Tros, personal communication). Calculations used equation (14a,b) instead of (12a,b) because it was evident from the data that cells were always detectable in the effluent.

In the first stage of simulation of the experiments with *Rhodococcus* sp. strain C125, the full displacement experiments presented in Fig. 6.4, were simulated using equations (3) and (4) with the parameter values obtained by fitting as described above. The value of B_i was taken at zero while B_0 was set at the measured bacterial density in the influent. The value of C_0

was set at zero during these calculations. Thus, a distribution of attached bacteria was obtained that served as the initial condition for the simulation of the transport and biodegradation of benzoate in these columns. The aqueous bacterial density at the end of the simulations of the displacement experiments was always close to zero. In the second stage of simulation, the value of B_0 was reset to zero while the value of C_0 (the benzoate concentration in the influent) was set at 50 μM. Simulations were done both with equation (12a,b) and equation (14a,b) to describe microbial growth. The microbial parameters describing the kinetics of growth of *Rhodococcus* on benzoate, were obtained from independent batch experiments as described in the "Materials and Methods" section. The value of b was estimated to be 5% of μ_{max}.

RESULTS

Experiments with Pseudomonas *sp. strain* B13

Biotransformation of 3-chlorobenzoate by rifampicin resistant *Pseudomonas* sp. strain B13 introduced to non-sterile Rhine River sediment columns was initially chosen as model system to study the transport of bacteria and the transport and biotransformation of organic compounds simultaneously. Experiments were carried out with high (pI=1.3) and low (pI=2.3) ionic strength media to vary the extent of adhesion. Numbers of *Pseudomonas* sp. strain B13 were detected in the column effluent by colony counts on agar plates amended with 3-chlorobenzoate and rifampicin.

The transport of *Pseudomonas* sp. strain B13 was studied separately without 3-chlorobenzoate to determine its adhesion behavior independently. The bacterial density in the influent was about 10^6 CFU/ml. Cells adhered stronger to the sand in the columns at high ionic strength than at low ionic strength (Fig. 6.1).

Biotransformation was initially studied with a high ionic strength medium (pI=1.3). Columns were wet packed using mineral medium containing *Pseudomonas* sp. strain B13 at a density of 10^9 CFU/ml. They were equilibrated overnight without pumping to establish equilibrium between suspended and sorbed cells. Then, percolation with mineral medium amended with 3-chlorobenzoate (3.2 μM) was started. A low concentration (about 1 μM) of 3-chlorobenzoate was still present in the cell suspension used for column packing. This was detected in the column effluent immediately after the start of the experiment (Fig. 6.2). Then, a short and incomplete breakthrough of 3-chlorobenzoate (C/C_0=0.6) was observed. After breakthrough, the effluent concentration decreased until a stable residual effluent concentration of 0.6 μM (C/C_0=0.2) had established after 1 day (Fig. 6.2). This concentration remained stable during 10 days of column operation (not shown).

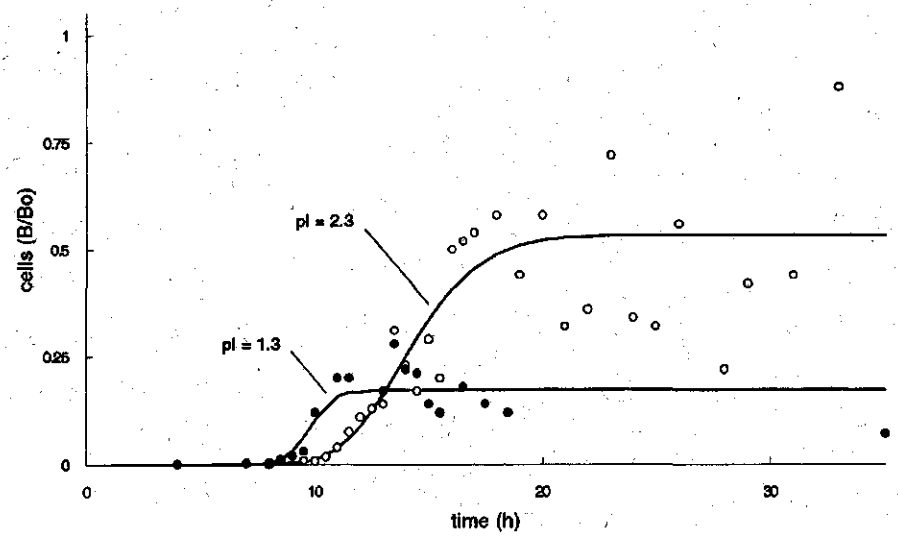

Figure 6.1: *Breakthrough of Pseudomonas sp. strain* B13 *at pI = 1.3* (●) *and pI = 1.3* (O). *Cell numbers are plotted as the ratio of effluent and influent numbers* (B/B_0).

Cell numbers in the effluent were determined during the first 2.5 days of column operation. Large quantities of cells (about 10^9 CFU/ml) were initially detected in the effluent (Fig. 6.2), followed by a gradual decrease to a steady state level of about 10^7 CFU/ml. Model calculations were performed using the parameter values from a fit of equation (2) to the breakthrough curve of *Pseudomonas* sp. strain B13 at pI=1.3 and from kinetic parameters for growth of the strain on 3-chlorobenzoate from chemostat and recycling fermentor experiments (M.E. Tros, personal communication, Table 6.1). Values of V and D were estimated using chloride as conservative tracer (Table 6.1). Equation (14a,b) instead of equation (12a,b) was used to calculate the growth rate of the bacteria because it was evident from the data that cells were present in the aqueous phase throughout the experiment. The initial 3-chlorobenzoate concentration in the column was taken at 1 μM and the initial aqueous bacterial density at 10^9 CFU/ml. The model correctly predicts a rapid wash-out of cells in the first day of the experiment. However, predicted values are close to zero afterwards which is a considerable deviation from the measured level of about 10^7 CFU/ml. The model reproduced the 3-chlorobenzoate data qualitatively, although the actual level of 3-chlorobenzoate breakthrough is higher than the level calculated by the model. On the other hand, the model accurately predicts the 3-chlorobenzoate concentration of 0.6 μM reached after day 1. However, the residual concentration was expected to be about 5 μM based on data from chemostat and recycling fermentor experiments (M.E. Tros, personal communication). Model simulations over 100 days predicted a complete disappearance of all biomass from the column and a

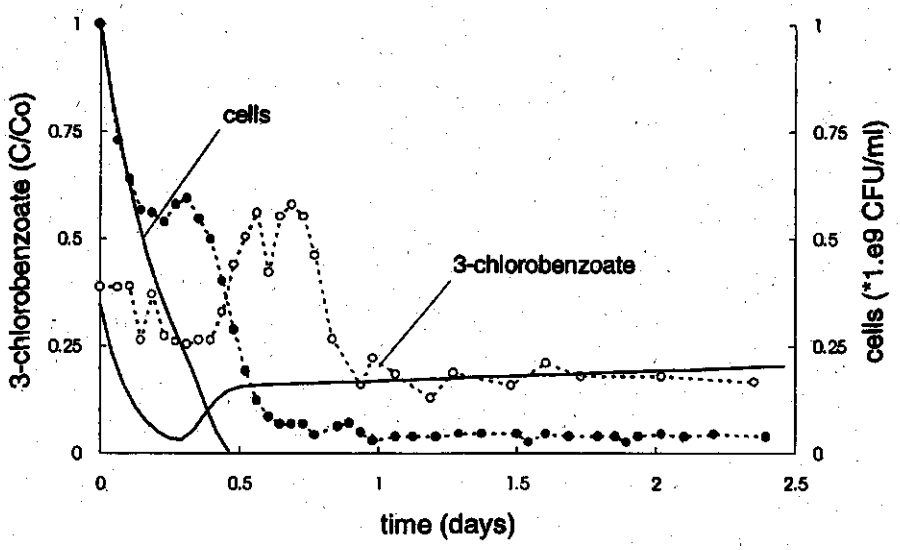

Figure 6.2: Behavior of 3-chlorobenzoate (O) *and* Pseudomonas *sp. strain* B13 (●) *in a column operated at a pI of 1.3. Solid lines represent model simulations using parameter values given in table 6.1. The 3-chlorobenzoate concentration is plotted as the ratio of effluent and influent concentration (C/C_o).*

complete breakthrough of 3-chlorobenzoate because the influent concentration was below the residual steady state concentration of 8.7 μM calculated from equation (17).

Another experiment was done with a low ionic strength solution (pI=2.3) to see if the breakthrough pattern of 3-chlorobenzoate and the wash-out of cells would be different due to less adhesion. Columns were wet packed using mineral medium with *Pseudomonas* sp. strain B13 at a density of 10^4 CFU/ml and were equilibrated overnight without pumping to allow equilibrium between suspended and sorbed cells to establish. No transformation of 3-chlorobenzoate was observed during 3 weeks of operation with the low ionic strength medium amended with 3.2 μM 3-chlorobenzoate (not shown). Then, after washing the column with the same influent solution without 3-chlorobenzoate, 1.5 ml cell suspension (10^7 CFU/ml) was injected in the column at 2 cm from the inlet and the column was left without pumping overnight. An initial breakthrough of 3-chlorobenzoate followed by a short period of transformation and breakthrough thereafter, was observed after resumption of percolation with 3-chlorobenzoate (Fig. 6.3). Final breakthrough was almost complete (C/C_o=0.9) and was preceded by a wash-out of high numbers of inoculated cells from the column. Only small numbers of cells (< 200 CFU/ml) were detectable in the effluent at the end of the experiment. The results confirm that *Pseudomonas* sp. strain B13 performed worse under conditions of low ionic strength. The simulation model predicted an initial wash-out of cells at a level that was 50 times higher than observed in the experiment and it predicted a breakthrough of 3-chlorobenzoate until >99% of the influent concentration without the temporal removal

observed in the experiment (not shown). The deviations between the experimental data and model simulations may have resulted partly because it was not possible to obtain a reliable value for the filtration rate constant φ (a value of 0.05 h^{-1} was used) from the curve at pI=2.3 (Fig. 6.1). Furthermore, the initial conditions were badly defined due to the second addition of cells. Finally, it became clear from parallel experiments with columns packed with glass and teflon beads that attachment of strain B13 is reversible under the experimental conditions. This contradicts predictions based on the DLVO-theory (Rijnaarts et al 1993a).

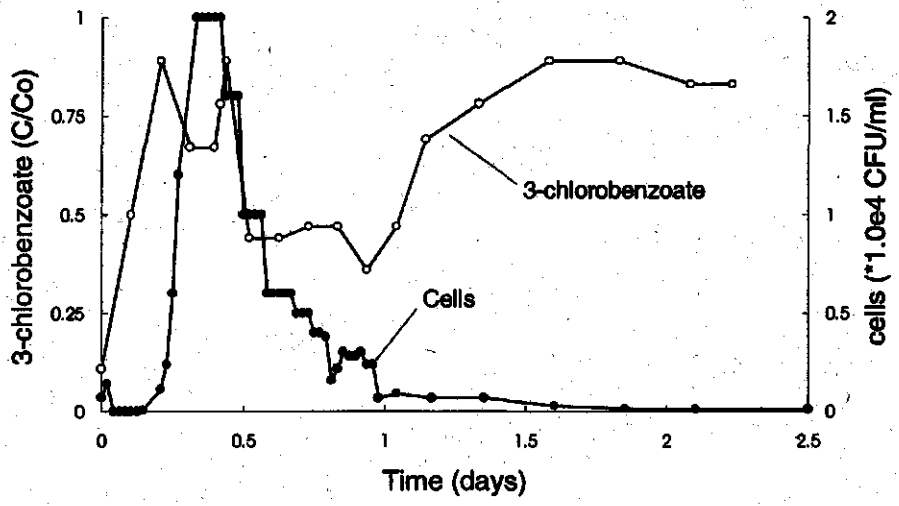

***Figure 6.3:** Behavior of 3-chlorobenzoate and* Pseudomonas *sp. strain* B13 *after re-inoculation of a column operated at a pI of 2.3. The 3-chlorobenzoate concentration is plotted as the ratio of the effluent and influent concentrations (C/C_0).*

Experiments with Rhodococcus *sp. strain* C125

Another set of experiments was done with *Rhodococcus* sp. strain C125 as model system. This strain is highly hydrophobic and has a high negative charge (Rijnaarts et al 1993a, Rijnaarts et al 1993b) which allowed accurate control of adhesion. Its biomass was quantified by OD_{280} measurements to obtain more accurate data. Inoculation of the columns for the biotransformation experiments was done by means of displacement experiments to obtain an accurately defined initial biomass distribution. Cells suspended in mineral medium with pI-values varying from 1 to 2.5 were applied to small columns packed with sand from the Rhine River. After 1 hour the suspensions were replaced by cell free mineral medium of the same pI to eliminate suspended cells. The lower the pI, the more cells were retained in the columns, as expected (Fig. 6.4).

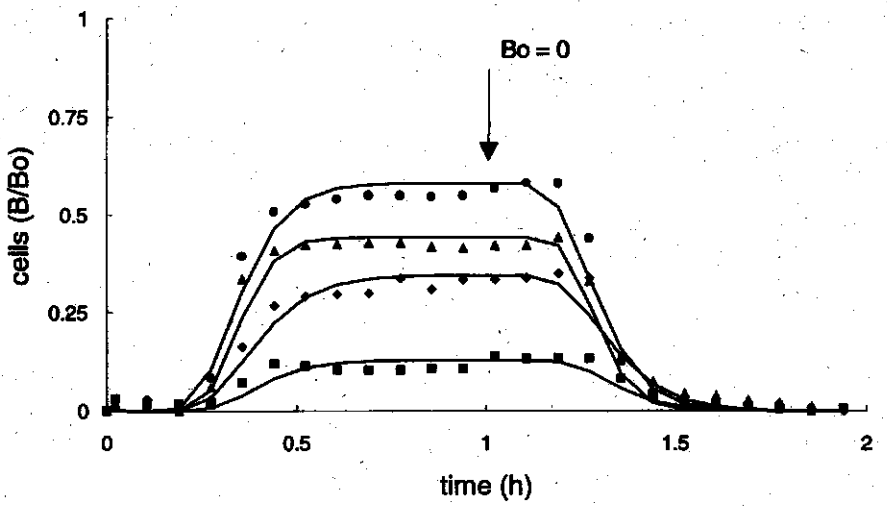

Figure 6.4: Displacement of Rhodococcus C125 *in columns packed with sand from the Rhine at pI-values of 1 (■), 1.5 (◆), 2 (▲), and 2.5 (●). Solid lines represent fits of the data to equation* (2). *Cell densities are plotted as the ratio of effluent and influent cell densities (B/B_0).*

The transport and biotransformation of benzoate (50 µM) was measured in the same columns immediately after completion of the displacement experiments. Thus, the initial distribution of cells in the columns was exactly defined. Benzoate breakthrough curves were measured in 2 low (pI = 2.5) and 2 high (pI = 1.5) ionic strength columns, while steady state effluent concentrations of benzoate were determined in all columns after 24 and 48 hours of operation. Both at high and low ionic strength, a partial breakthrough of benzoate was observed, followed by decreasing effluent concentrations, indicative of increasing biotransformation rates, most probably due to growth of *Rhodococcus* (Fig. 6.5). The initial removal was highest in the columns operated at high ionic strength, as expected. Complete breakthrough without degradation was observed in control columns without added cells and operated under similar conditions (Fig. 6.5).

Model calculations were performed using the parameter values from a fit of equation (2) to the data from the displacement experiments at pI=1.5 and pI=2.5, respectively (Table 6.1). Values of V and D were estimated from tracer experiments. The microbial parameters μ_{max}, K_m and Y were obtained from the batch experiments (Table 6.1). The value of b was not determined experimentally and estimated to be 5% of μ_{max} (Table 6.1). Equations (12a,b) and (14a,b) were both used in the model calculations to see how the assumptions regarding detachment of daughter cells resulting from cell division would affect the results. The model predicts a partial breakthrough followed by a rapid decline of the benzoate concentration in the effluent if it is assumed that daughter cells do not detach from the solid phase (Fig. 6.5). A

similar initial breakthrough followed by a much slower decline of the effluent concentration is predicted if detachment of daughter cells is being assumed (Fig. 6.5). The calculations assuming detachment of daughter cells give the closest simulation of the data at pI=2.5, while the calculations assuming no detachment yield the closest simulation at pI=1.5. However, neither of the model calculations predicted the data exactly. Especially the slow rise of the benzoate concentration after initial breakthrough (Fig. 6.5) is not represented in the model calculations. The reason for this rise is unclear. Either the activity of *Rhodococcus* sp. strain C125 decreased or there has been an unexpectedly high wash-out of cells during the first hours of the experiments. The possibility of a high wash-out of cells could not be checked by means of the OD_{280} measurements because benzoate also absorbs light at a wavelength of 280 nm. The predicted steady state effluent concentration of benzoate is 0.95 µM. This value is well below the concentrations of 5 to 10 µM which were observed after 24 and 48 hours of column operation. The measured steady state effluent concentrations had no correlation with the ionic strength of the aqueous medium.

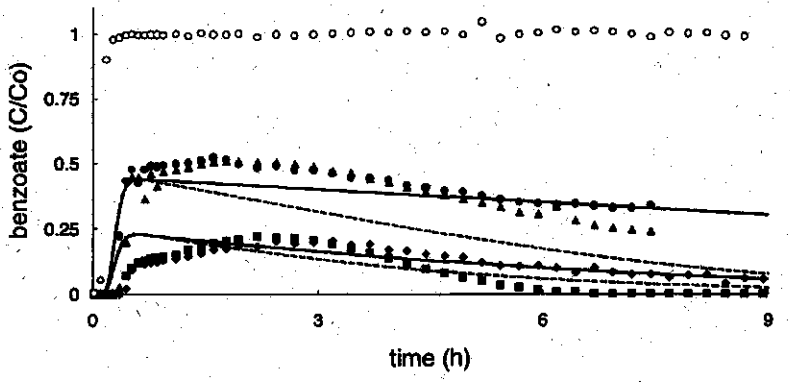

***Figure 6.5:** Breakthrough of 50 µM benzoate in duplicate columns inoculated with* Rhodococcus C125 *and operated at pI-values of 1.5 (■,◆) and 2.5 (▲,●). No biotransformation of benzoate took place in non-inoculated controls (O). Dashed lines represent model simulations assuming no detachment, solid lines simulations assuming detachment of newly formed cells. Concentrations are plotted as the ratio of the effluent and influent concentrations (C/C_0).*

DISCUSSION

The adhesion of bacteria to solid surfaces in aqueous environments depends on the surface characteristics of the bacteria and solids and on the chemical composition of the water. If all surfaces are negatively charged, the DLVO-theory predicts a positive correlation of adhesion with the hydrophobicity of cells and solids and with the ionic strength of the water (Van Loosdrecht et al 1989, Rijnaarts et al 1993a, Rijnaarts et al 1993b). Though the attachment of

Table 6.1: Column parameters used in model calculations

	Pseudomonas B13		*Rhodococcus* C125		
Parameter	pI = 1.3	Source	pI = 1.5	pI = 2.5	Source
D	0.047 $cm^2\ h^{-1}$	Tracer experiment	14.6 $cm^2\ h^{-1}$	12.5 $cm^2\ h^{-1}$	Tracer experiment
V	1.18 cm h^{-1}	Tracer experiment	29.8 cm h^{-1}	31.4 cm h^{-1}	Tracer experiment
L	10.0 cm	-	10 cm	10 cm	-
R_d	1.0	Control experiment	1.0	1.0	Control column
R_m	1.17	Breakthrough experiment	1.33	1.20	Displacement experiment
μ_{max}	0.13 h^{-1}	M.E. Tros (pers. comm.)	0.2 h^{-1}	0.2 h^{-1}	Benzoate depletion[a]
K_s	150 µM	M.E. Tros (pers. comm.)	20.0 µM	20.0 µM	Benzoate uptake[a]
Y	63 mg mM^{-1}	M.E. Tros (pers. comm.)	44.4 mg·mM^{-1}	44.4 mg·mM^{-1}	Batch experiment[a]
b	0.008 h^{-1}	M.E. Tros (pers. comm.)	0.01 h^{-1}	0.01 h^{-1}	estimated as 5 % of μ_{max}
φ	0.21 h^{-1}	Breakthrough experiment	3.33 h^{-1}	1.74 h^{-1}	Displacement experiment
C_0	3.2 µM	-	0[b]	0[b]	-
			50 µM[c]	50 µM[c]	
B_0	0	-	24 mg/l[d]	24 mg/l[d]	-
			0[e]	0[e]	

[a] As described in Materials and Methods
[b] $-2\ h < t < 0\ h$
[c] $t > 0\ h$
[d] $t < -1\ h$
[e] $t > -1\ h$

bacteria to negatively charged hydrophilic surfaces like glass is predicted to be irreversible with the DLVO-theory, it becomes reversible at lower ionic strengths in the medium due to steric hindrance (Rijnaarts et al 1993a). Adhesion of both strains was indeed positively correlated with the ionic strength of the aqueous phase (Fig. 6.1, 6.4). Moreover, the value of the filtration rate constant obtained for *Pseudomonas* sp. strain B13 at pI=1.3 was an order of magnitude lower than the value obtained for the much more hydrophobic *Rhodococcus* sp. strain C125 at pI=1.5 (Table 6.1).

Only a limited reversibility of attachment was allowed in the model (via equation 14a,b) and this may explain why the model failed to yield correct simulations of the biotransformation experiments with *Pseudomonas* sp. strain B13. Attachment of this strain to glass beads was reversible at pI-values down to 1 (Rijnaarts et al 1993a). A similar behavior may be expected in the experiments presented here since the organic carbon content of the sand used in the columns was less than 0.1%. However, the data from the breakthrough experiments (Fig. 6.1) do not allow the estimation of possible detachment rates and therefore no attempts were made to include reversible behavior other than detachment of daughter cells resulting from growth, in the model.

The different levels of benzoate breakthrough observed at low and high ionic strength in the experiments with *Rhodococcus* sp. strain C125 (Fig. 6.5) reflect the different numbers of bacteria that adhered to the sand in the columns during the displacement experiments. The decline of the benzoate concentrations after initial breakthrough which was observed in all

experiments suggest that *Rhodococcus* grew on benzoate as source of carbon and energy. At low ionic strength, the closest fit of the model to the data was obtained when it was assumed that daughter cells resulting from cell division detach to the aqueous phase (Fig. 6.5). In contrast, the assumption that no detachment occurs at all yielded the closest fit at high ionic strength, which could be explained by a decreasing detachment rate with increasing ionic strength (Rijnaarts et al 1993a).

Steady state effluent concentrations after 24 and 48 hours of column operation varied between 5 and 10 μM in all column experiments. These had no correlation with the ionic strength of the aqueous medium. Based on the values of μ_{max}, K_s and b, a steady state effluent concentration of 0.95 μM can be calculated from equation (17). If equation (17) is used inversely to calculate b from the observed steady state effluent concentration, the result is a value between 0.07 and 0.2 h^{-1} or 30 to 100% of μ_{max}. Such values are unrealistic since values of b are normally less than 10% of μ_{max}. Apparently, other factors then microbial decay and maintenance requirements alone, contribute to the establishment of the steady state residual concentrations. The much higher steady state concentrations in the column experiments point to a mechanism that has nothing to do with the adhesion behavior of the introduced cells. A heterogeneous distribution of cells in the column material can account for the high steady state effluent concentrations. Such a heterogeneous distribution may result from the fact that micro-organisms never occupy space as a continuum like in a biofilm, but are present as microcolonies varying in size from a few to hundreds of cells (Hirsch and Rades-Rohkohl 1983, Harvey et al 1984). As a result, the column material consists of a number of "hot spots" with microbial activity, separated by desert areas with no activity at all. A certain fraction of the organic compounds fed to the column will then travel through areas without microbial activity. Some bacteria will only "see" substrate after diffusion of these molecules through the desert areas. Thus, diffusion through the soil matrix may limit the biotransformation rate. Biotransformation will stop as soon as the supply rate of substrate cannot meet the maintenance requirements anymore (Schmidt et al 1985a). The resulting residual concentration can be calculated from the effective diffusion coefficient of the organic compound in the soil under consideration and from the maintenance coefficient, dry weight density and radius of the micro-organisms (Chapter 8). The present model formulation does not account for such a distribution of cells in the column and hence, the model is not able to exactly reproduce the experimental data.

CONCLUSIONS

The biotransformation of organic compounds by introduced bacteria in saturated soil can be understood by taking into account the transport of degrading bacteria and the transport of organic compounds simultaneously. The ionic strength of the water in which bacteria are

introduced can be used to control the transport and attachment of bacteria. If a low ionic strength is used, bacteria may travel long distances which can be advantageous when the contamination is located far below the soil surface. On the other hand, a high ionic strength will generally stimulate the attachment of bacteria to the solid phase and hence, the effectiveness of their application.

Steady state effluent concentrations as observed in the column experiments can only partly be explained by the presented model approach. A full explanation most probably requires the incorporation of a heterogeneous distribution of bacteria in the column material together with the kinetics governing diffusion of the organic compounds to spots of microbial activity. Efforts to reduce residual amounts of contaminants after bioremediation should include a maximization of diffusion rates of the contaminants.

NOTATION

Subscripts *b* and *c* are used to distinguish between bacteria and organic compound.

b	specific maintenance rate of bacteria (T^{-1})
B_0	Influent bacterial density ($M_b \cdot L^{-3}$)
B_{an}	Attached bacterial density ($M_b \cdot M_s^{-1}$)
B_{aq}	Aqueous bacterial density ($M_b \cdot L^{-3}$)
B_i	Initial bacterial density ($M_b \cdot L^{-3}$)
B_{sorbed}	Sorbed bacterial density ($M_b \cdot M_{ss}^{-1}$)
B_{tot}	Total bacterial density ($M_b \cdot L^{-3}$)
C	Concentration of the organic compound ($M_c \cdot L^{-3}$)
C_0	Influent concentration of organic compound ($M_c \cdot L^{-3}$)
C_i	Initial concentration of organic compound ($M_c \cdot L^{-3}$)
C_{res}	Residual steady state concentration ($M_c \cdot L^{-3}$)
D	Dispersion coefficient ($L^2 \cdot T^{-1}$)
K_p	Microbial partition coefficient ($L^3\ M_s^{-1}$)
K_s	Half saturation constant ($M_c \cdot L^{-3}$)
L	Column length (L)
λ	Filtration coefficient (L^{-1})
μ_{max}	Maximum specific growth rate (T^{-1})
P	Sink term describing biotransformation ($M_c \cdot L^{-3} \cdot T^{-1}$)
R_d	Retardation factor of organic compound (-)
R_m	Microbial retardation factor (-)
ρ	Bulk soil density ($M \cdot L^{-3}$)
t	time (T)
V	Interstitial pore water velocity ($L \cdot T^{-1}$)

Y Yield coefficient ($M_b \cdot M_c$)

z Axial co-ordinate (L)

θ Soil porosity ($L^{-3} \cdot L^{-3}$)

φ First order filtration rate constant (T^{-1})

CHAPTER 7

MODELING TRANSPORT AND RADIAL DIFFUSION LIMITED BIODEGRADATION OF ORGANIC CONTAMINANTS IN SATURATED COLUMNS

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to be submitted to *Water Resources & Research*

ABSTRACT

A numerical model to describe the transport of organic contaminants in columns operated under saturated flow conditions was developed. Retarded dispersed flow, external mass transfer, intra-aggregate diffusion, and biotransformation in aggregates were represented in the model. Column breakthrough was characterized by 8 independent parameters in the model equations: the Peclet number, the retardation factor, a non-equilibrium index, an external mass transfer modulus, a maximum specific growth rate modulus, the half saturation coefficient, and the yield coefficient. The basic model run resulted in an initial breakthrough of contaminant followed by an almost linear decrease of the effluent concentration due to radial diffusion limited biotransformation and microbial growth. Breakthrough dynamics were most sensitive to the non-equilibrium index and the specific growth rate modulus. In contrast, model steady state is exclusively determined by the maximum growth rate modulus, the half saturation constant, and the decay rate modulus. Leaching dynamics from a contaminated column only depend on the non-equilibrium index, and not on any of the microbial parameters. Therefore, pumping water amended with nutrients in a bioremediation scheme will merely result in a hydrodynamic wash-out of contaminants instead of stimulating biotransformation.

INTRODUCTION

The behavior and fate of organic contaminants in ground water depends on a complex interaction of physical, chemical and biological processes. Infiltration of contaminated water and leaching of soil bound contaminants are responsible for migration of contaminants through aquifers. Dispersion and diffusion tend to spread contaminants, while sorption onto soil retards their movement. Chemical reactivity and biologically mediated decomposition determine contaminant persistence in soil and ground water. Microbial transformation is often required for their complete mineralization.

Rates of sorption and desorption during transport of contaminants are largely influenced by pore and surface diffusion (Wu and Gschwend 1986). This explains non-equilibrium phenomena such as equilibration times in the order of months in batch experiments (Ball and Roberts 1991), and both strong tailing of breakthrough curves of sorbing species in column experiments (Schwarzenbach and Westall 1981) and a flow rate dependency of the observed retardation factor in column experiments (Valocchi 1985, Brusseau 1992). The existence of an immobile fraction of the pore water in soil enhances such non-equilibrium phenomena in column experiments (Van Genuchten and Wierenga 1976, Van Genuchten and Cleary 1979, Schwarzenbach and Westall 1981, Valocchi 1985, Brusseau 1992).

The majority of indigenous bacteria was hypothesized to colonize surfaces in the interior of aggregates of soil particles (Stotzky 1972, Hattori and Hattori 1976), rather than being freely suspended in mobile pore water. Indigenous bacteria in the subsurface are indeed mainly associated with the solid phase (Hirsch and Rades-Rohkohl 1983, Harvey et al 1984). Soil aggregates may serve as micro-habitats for micro-organisms, where they are protected from adverse environmental influences such as drying out and predation by protozoa and nematodes (Stotzky 1972).

At old waste sites, biotransformation of previously sorbed contaminants must take place for remediation. Biodegradable contaminants in soil are often metabolized more slowly than in aqueous culture. Sorbed substrates are less available for biotransformation (Ogram et al 1985) and biotransformation rates are limited by diffusion of the chemical from soil aggregates to the bulk liquid (Rijnaarts et al 1990). Thus the overall reaction rate in aggregated soil is - at least partly - controlled by sorption rates (Bouwer and Zehnder 1993).

Mathematical modeling provides an effective means to integrate physical, chemical and biological processes and to determine the relative contributions of each process to the overall behavior of organic chemicals under saturated flow conditions. This paper focuses on the interaction between biotransformation and retarded intra-aggregate diffusion and their effects on the transport of organic chemicals that are the sole source of carbon and energy for soil micro-organisms. A mathematical model for one-dimensional flow was developed that considers advection, dispersion, external mass transfer, sorption retarded intra-aggregate diffusion, and biotransformation of an organic contaminant. A sensitivity analysis was

performed to determine the relative contribution of diffusion, sorption and biotransformation in describing the fate and behavior of organic contaminants under saturated flow conditions. Two scenario's were evaluated. The first scenario considered the transport of contaminated water through an initially uncontaminated column with an initial homogeneous distribution of bacteria in soil aggregates representing the migration of contaminated ground water in an aquifer. The second involved the percolation of an initially contaminated column with microbial activity at the aggregate surface only, with clean water to simulate a ground water remediation scheme.

MODEL FORMULATION

Several models have been proposed to describe the transport of biodegradable organic substances under aerobic saturated flow conditions. The biological reaction rate is generally modeled using the Monod relationship (Monod 1949). Assuming constant biomass levels, the Monod equation becomes zero order at high contaminant concentration and first order at low concentration. These simplified equations are often used for modeling biodegradation kinetics in a well-defined concentration range. A dual Monod expression that contains a term for the substrate (contaminant) and a nutrient is often used to describe the combined consumption of nutrients and contaminants because they both have the potential to limit microbial activity (Borden et al 1986, Molz et al 1986, Kindred and Celia 1989). Microbial migration with the advective flow (Borden et al 1986) or even chemotactic movement against nutrient concentration gradients are also included sometimes (Corapcioglu and Haridas 1984, Chapter 5). Other more complex kinetic relationships that account for toxic inhibition and competitive and non-competitive inhibition have been employed to model biodegradation process (Bailey and Ollis 1986). Recent approaches successfully include kinetically controlled sorption in the description of transport and biotransformation in columns with introduced bacteria (Brusseau et al 1992, Chen et al 1992).

A micro-colony approach to simulate biodegradation coupled to advective-dispersive transport demonstrated that biodegradation would be expected to have a major effect on contaminant transport when proper conditions for growth exist (Molz et al 1986). Micro-colonies were represented as disks of uniform radius and thickness attached to pore surfaces. A boundary layer of given thickness was associated with each colony across which substrate and oxygen were transported to the colonies by diffusion.

A model considering advective flow in macropores, external mass transfer from macro- to micropores, and combined pore and surface diffusion in the micropores was shown to give appropriate simulations of contaminant transport in aggregated soil (Crittenden et al 1986). We combined this approach with biotransformation inside soil aggregates to simulate the transport of biodegradable organic substances under saturated flow conditions (Fig. 7.1).

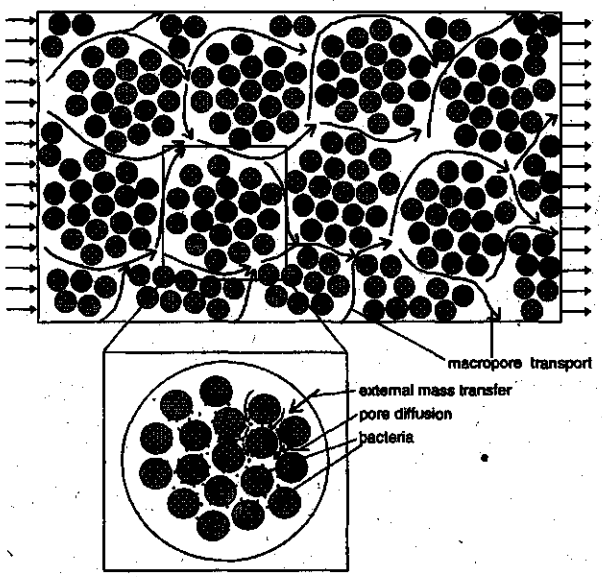

Figure 7.1: Mass transport mechanisms in the mobile phase (macropores) and the stationary phase (micropores). Modified after Crittenden et al (1986).

Because the majority of indigenous ground water bacteria is associated with the solid phase, it is assumed that they are only present in the aggregates and hence, that biotransformation can only take place in aggregates (Fig. 7.1). For the same reason, chemotaxis and migration with advective flow were not included to describe the fate and behavior of micro-organisms. Finally, we assumed that the organics are the only limiting nutrients. With these assumptions the mobile phase mass balance becomes (Crittenden et al 1986):

$$R_d \frac{\partial C(t,z)}{\partial t} = D_c \frac{\partial^2 C(t,z)}{\partial z^2} - V \frac{\partial C(t,z)}{\partial z} - 3 \frac{1-\theta_c}{\theta_c} R^{-1} k_m [C(t,z) - c(t,r=R,z)] \qquad (1)$$

where R_d is the axial retardation coefficient, D_c the axial dispersion coefficient, V the axial flow rate, t the temporal, z the axial, and r the radial co-ordinate, C the macropore, and c the micropore contaminant concentration, θ_c the macropore porosity, k_m the external mass transfer coefficient and R the aggregate radius. A listing of all symbols and their dimensions is given in the Notation section. The term on the left hand side in equation (1) describes the retarded concentration change of contaminant in the mobile phase with time. The terms on the right hand side represent the contaminants' axial dispersion and diffusion, advective flow, and liquid phase mass transfer from the mobile to the stationary phase, respectively. It is subject to the following initial and boundary conditions:

$$C(t=0,z) = f(z) \qquad (2a)$$

$$VC(t,z=0)-D_c\frac{\partial C(t,z=0)}{\partial z}=VC_0(t) \qquad (2b)$$

$$\frac{\partial C(t,z=L)}{\partial z}=0 \qquad (2c)$$

where L is the column length and $C_0(t)$ the influent concentration. The initial contaminant distribution in the macropores is given in equation (2a). In the first scenario, simulating the infiltration of contaminated water in a clean environment, the initial concentration was set at zero throughout the column. The second scenario involving bioremediation of polluted soil with a pump and treat method, was simulated with an initial homogeneous distribution of contaminant throughout the column. The influx of contaminant via the inlet is defined in equation (2b). The influent concentration C_0 does not have to be constant in time. It has the shape of a pulse in a typical displacement experiment, where the breakthrough of a substance is studied followed by displacement by clean water. However, we used a constant positive non-zero value in the first scenario and kept it at zero in the second scenario. The outlet boundary condition in (2c) establishes that no reaction and no dispersive flow takes place beyond the column.

The equation for radial transport of a contaminant in the micropores of a spherical aggregate of radius R is presented as:

$$\frac{\partial c(t,r,z)}{\partial t}=D_i r^{-2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c(t,r,z)}{\partial r}\right)-\frac{\mu(t,r,z)}{Y}B(t,r,z) \qquad (3)$$

where D_i is the effective intra-aggregate diffusion coefficient, B the microbial density, μ the specific growth rate of micro-organisms, and Y the yield coefficient. The left hand term in equation (3) describes the concentration change of contaminant in the stationary phase with time. The right hand side terms represent radial diffusion in the aggregate and contaminant removal by biotransformation, respectively. The following initial and boundary conditions apply:

$$c(t=0,r,z)=g(r) \qquad (4a)$$

$$\frac{\partial c(t,r=0,z)}{\partial r}=0 \qquad (4b)$$

$$D_i\frac{\partial c(t,r=R,z)}{\partial r}=k_m[C(t,z)-c(t,r=R,z)] \qquad (4c)$$

Equation (4a) gives the initial contaminant distribution in the aggregates. The boundary condition in (4b) ensures a symmetric solution around the center of the aggregate, which is an imperative because of the spherical symmetry. Equation (4c) is the boundary condition for the aggregate surface, stating that the mass flux at the aggregate surface is proportional to the concentration difference between macro- and micropores. The external mass transfer

coefficient k_m expresses the mass transfer resistance between the mobile and stationary phase. If k_m approaches infinity, condition (4c) is equivalent to imposing the Dirichlet boundary condition $c(t,r=R,z)=C(t,z)$, which treats the two phases as a continuum without mass transfer resistance.

Microbial growth and biotransformation of organic contaminants are described by Monod kinetics in which the uptake rate saturates to a maximum value as contaminant concentration increases (Monod, 1949). Hence the specific growth rate is given by:

$$\mu(t,r,z) = \mu_{max} \frac{c(t,r,z)}{K_s + c(t,r,z)} \tag{5}$$

where μ_{max} is the maximum specific growth rate and K_s the half saturation constant. Microbial growth is described as:

$$\frac{\partial B(t,r,z)}{\partial t} = [\mu(t,r,z) - b]B(t,r,z) \tag{6}$$

where b is a first order coefficient describing decay of bacteria.

Model scaling

The model was scaled to perform a sensitivity analysis with dimensionless parameters. The advantage of scaling is that it helps to compare results obtained at different scales, by means of the resulting dimensionless parameters. Time and the axial and radial co-ordinates were scaled using the following definitions:

$$t = \bar{t}\frac{L}{V} \tag{7a}$$

$$z = \bar{z}L \tag{7b}$$

$$r = \bar{r}R \tag{7c}$$

where $\bar{t}$ is dimensionless time, $\bar{z}$ the dimensionless axial, and $\bar{r}$ the dimensionless radial co-ordinate. The following dimensionless parameters result from scaling:

$$Pe = \frac{VL}{D_c} \tag{8a}$$

$$\kappa = \frac{k_m R}{D_i} \tag{8b}$$

$$m_f = \mu_{max}\frac{R^2}{D_i} \tag{8c}$$

$$b_f = b\frac{R^2}{D_i} \tag{8d}$$

$$T_f = \frac{L/V}{R^2/D_l} \tag{8e}$$

The Peclet number Pe is a dimensionless measure for axial dispersion, whereas κ is an external mass transfer modulus. The parameters m_f and b_f are the maximum growth rate modulus and decay rate modulus, respectively. T_f is the ratio of the time scales of axial and radial transport of a conservative tracer (R_d=1, m_f =0) and may be viewed as a non-equilibrium index (Valocchi 1985), values >5 indicating equilibrium behavior (Rao and Jessup 1983). Given the above definitions, the scaled model equations become:

$$\frac{\partial C(\bar{t},\bar{z})}{\partial \bar{t}} = Pe^{-1}\frac{\partial^2 C(\bar{t},\bar{z})}{\partial \bar{z}^2} - \frac{\partial C(\bar{t},\bar{z})}{\partial \bar{z}} - 3\frac{1-\theta_c}{\theta_c}T_f\kappa[C(\bar{t},\bar{z}) - c(\bar{t},\bar{r}=1,\bar{z})] \tag{9}$$

$$\frac{\partial c(\bar{t},\bar{r},\bar{z})}{\partial \bar{t}} = T_f\bar{r}^{-2}\frac{\partial}{\partial \bar{r}}(\bar{r}^2\frac{\partial c(\bar{t},\bar{r},\bar{z})}{\partial \bar{r}}) - \frac{\bar{\mu}(\bar{t},\bar{r},\bar{z})}{Y}B(\bar{t},\bar{r},\bar{z}) \tag{10}$$

$$\bar{\mu}(\bar{t},\bar{r},\bar{z}) = m_f\frac{c(\bar{t},\bar{r},\bar{z})}{K_s + c(\bar{t},\bar{r},\bar{z})} \tag{11}$$

$$\frac{\partial B(\bar{t},\bar{r},\bar{z})}{\partial \bar{t}} = [\bar{\mu}(\bar{t},\bar{r},\bar{z}) - b_f]B(\bar{t},\bar{r},\bar{z}) \tag{12}$$

subject to initial and boundary conditions:

$$C(\bar{t}=0,\bar{z}) = f(\bar{z}) \tag{13a}$$

$$C(\bar{t},\bar{z}=0) - Pe^{-1}\frac{\partial C(\bar{t},\bar{z}=0)}{\partial \bar{z}} = C_o(t) \tag{13b}$$

$$\frac{\partial C(\bar{t},\bar{z}=1)}{\partial \bar{z}} = 0 \tag{13c}$$

$$c(\bar{t}=0,\bar{r},\bar{z}) = g(\bar{r}) \tag{14a}$$

$$\frac{\partial c(\bar{t},\bar{r}=0,\bar{z})}{\partial \bar{r}} = 0 \tag{14b}$$

$$\frac{\partial c(\bar{t},\bar{r}=1,\bar{z})}{\partial \bar{r}} = \kappa[C(\bar{t},\bar{z}) - c(\bar{t},\bar{r}=1,\bar{z})] \tag{14c}$$

The interpretation of the initial and boundary conditions for the scaled and non-scaled model formulation are analogous. The scaled model was solved numerically as described in the Appendix.

PARAMETER ESTIMATION

Parameters of the non-reactive transport of n-octanol in small columns (H.H.M. Rijnaarts, personal communication) were used to perform a sensitivity analysis. The value of D_c was estimated independently from the breakthrough response of chloride that was used as conservative tracer (H.H.M. Rijnaarts, personal communication). The mass transfer coefficient k_m was estimated using the correlation (Wilson and Geankoplis 1966):

$$k_m = 1.09 \frac{V}{\theta_c} \left(\frac{VR}{D_{aq}} \right)^{-2/3} \qquad (15)$$

The aqueous diffusion coefficient of octanol was taken from the literature (Oelkers 1991). All other transport parameters were measured independently (H.H.M. Rijnaarts, personal communication). The measured breakthrough of n-octanol was successfully described with the model using D_l and R_d as fitting parameters (Fig. 7.2).

Microbial parameters were taken from a previous model effort that simulated 1,4-dichlorobenzene biotransformation in columns packed with river sediment (Chapter 5). The initial biomass was also included as parameter because it is difficult to measure accurately in soil. All parameter values used in the sensitivity analysis are listed in Table 7.1.

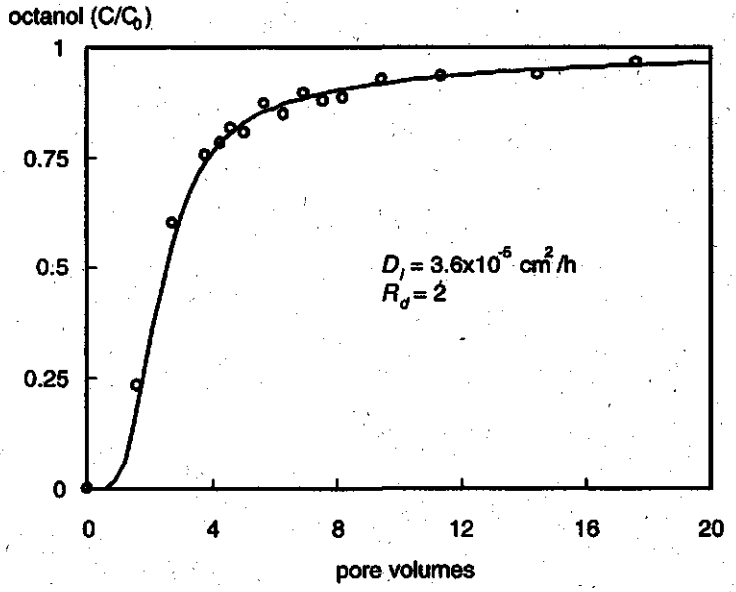

Figure 7.2: Breakthrough of n-octanol in small columns. Solid line represents model simulation. Data were taken from the literature (H.H.M. Rijnaarts, personal communication).

SENSITIVITY ANALYSIS

A sensitivity analysis was performed to determine the relative contribution of diffusion, sorption and biotransformation in describing the fate and behavior of organic contaminants in saturated columns. Two scenario's were evaluated. The first scenario considered the transport of contaminated water through an initially uncontaminated column with an initial homogeneous distribution of bacteria in soil aggregates representing the migration of contaminated ground water in a clean aquifer. The second involved the percolation of an initially contaminated column with clean water to simulate a ground water remediation scheme.

Calculations were made for an organic compound with a hydrophobicity similar to n-octanol, which may be representative for contaminants like chlorinated benzenes. The breakthrough curve calculated in the standard run appears in Fig. 7.3, together with the criteria that served as a basis for the sensitivity analysis. We did the sensitivity analysis by systematically halving and doubling each of the parameters. It is ensured that model sensitivity

Table 7.1: Column parameters used to examine model sensitivity

Parameter	Value	Source
Column Length L	10 cm	H.H.M. Rijnaarts (pers. comm.)
Flow rate V	158 cm/h	H.H.M. Rijnaarts (pers. comm.)
Axial dispersion coefficient D_e	63 cm^2/h	H.H.M. Rijnaarts (pers. comm.)
Retardation factor R_d	2	H.H.M. Rijnaarts (pers. comm.)
Intra-aggregate diffusion coefficient D_i	$3.6 \cdot 10^{-5}$ cm^2/h	H.H.M. Rijnaarts (pers. comm.)
Macropore porosity θ_c	0.296	H.H.M. Rijnaarts (pers. comm.)
Aggregate radius R	0.019 cm	H.H.M. Rijnaarts (pers. comm.)
Aqueous diffusion coefficient (D_{aq})	$0.77 \cdot 10^{-5}$ cm^2/s	Oelkers (1991)
Mass transfer coefficient k_m	25.6 cm/h	Wilson and Geankoplis (1966)
maximum specific growth rate μ_{max}	0.01 h^{-1}	Chapter 5
half saturation constant K_s	20 mg/l	Chapter 5
Yield coefficient Y	0.1	Chapter 5
Decay coefficient b	0.001 h^{-1}	Chapter 5
Influent concentration C_o	50 mg/l	set for sensitivity analysis
Initial biomass density B_o	100 mg/l	set for sensitivity analysis
Dimensionless parameters		
Peclet number Pe	25	-
scaled mass transfer coefficient κ	6500	-
Non-equilibrium index T_f	0.063	-
maximum specific growth rate modulus m_f	0.1	-
Specific decay rate modulus b_f	0.01	-

to each parameter is compared on an equal basis with this approach. The results are useful to rank the parameters according to their impact on chosen model properties. Based on such a ranking, the level of accuracy needed for each parameter value can be assessed. Results of the sensitivity analysis were compared based on (i) the maximal concentration breaking through, and (ii) the observed decline in the effluent after breakthrough (Fig. 7.3). Both criteria are important characteristics of the dynamics occurring during saturated flow.

Table 7.2 reports the impact of each parameter on each of the chosen criteria. In general, the decline after breakthrough appears to be a more sensitive criterion than the maximum concentration reached after breakthrough. An increase of the highest concentration correlates with a decrease of the decline after breakthrough, as expected. Parameters can be grouped into three classes of sensitivity. The most sensitive parameters (with respect to model dynamics) are the non-equilibrium constant T_f and the maximum growth rate modulus m_f, which have similar effects on the breakthrough of contaminant (Fig. 7.4, 7.5).

The microbial parameters Y, K_s, and B_0, have similar impacts on breakthrough, although less pronounced, as m_f has. The model is insensitive to changes in the rate modulus b_f, the retardation factor R_d, the Peclet number Pe, and the mass transfer modulus κ. So, simulation of the breakthrough of a biodegradable contaminant requires an accurate determination of T_f and m_f, while order of magnitude estimations of b_f, Pe, and κ are sufficient. The only effect of R_d is that it moves the breakthrough front in time. It does not have an observable effect on the highest concentration breaking through or the decline afterwards (Table 7.2).

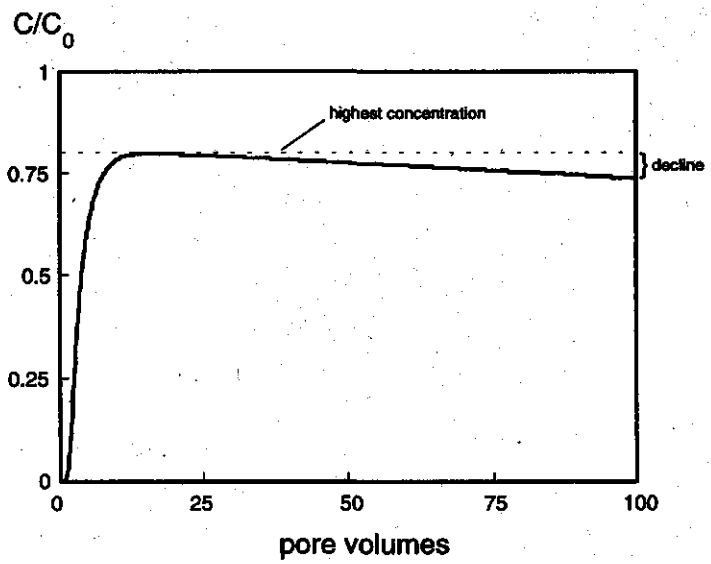

Figure 7.3: *Basic model run. The highest concentration reached and the decline after breakthrough were used as criteria in the sensitivity analysis*

***Table 7.2:** Relative changes of the highest concentration breaking through, and the decline after breakthrough, as a result of halving and doubling parameter values*[a]

Parameter	highest concentration[a]		decline[a]	
	halving	doubling	halving	doubling
Non-equilibrium constant T_f	1.12	0.77	0.22	3.46
Scaled specific growth rate m_f	1.12	0.78	0.21	3.13
Initial biomass B_0	1.12	0.80	0.11	1.38
Yield coefficient Y	0.80	1.12	1.38	0.11
Half saturation constant K_s	0.95	1.06	1.52	0.52
Scaled decay coefficient b_f	1.00	1.00	1.10	0.79
Retardation factor R_d	1.00	1.00	1.02	0.96
Peclet number Pe	1.00	1.00	0.98	1.01
Scaled mass transfer coefficient κ	1.00	1.00	1.00	1.01

[a] normalized with respect to the highest concentration breaking through and the concentration decline after breakthrough in the standard run. Values greater than 1 represent an increase, values below 1 a decrease

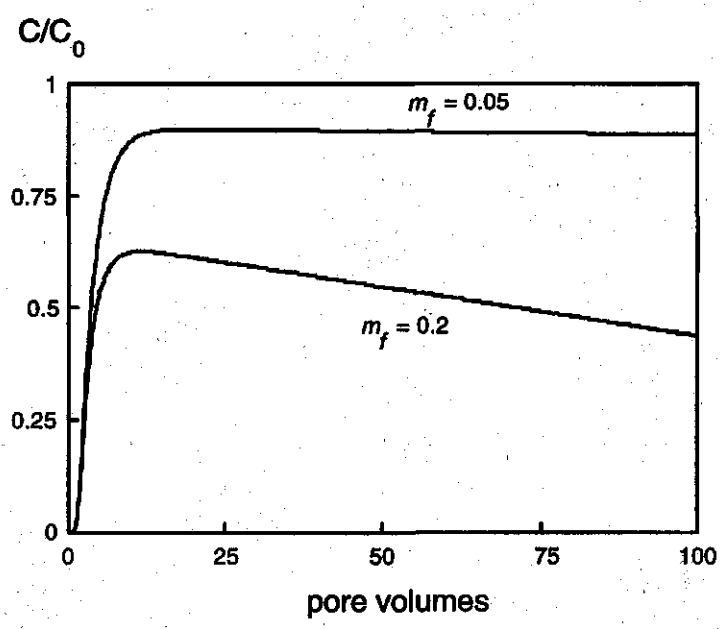

***Figure 7.4:** Model sensitivity to the scaled maximum specific growth rate m_f*

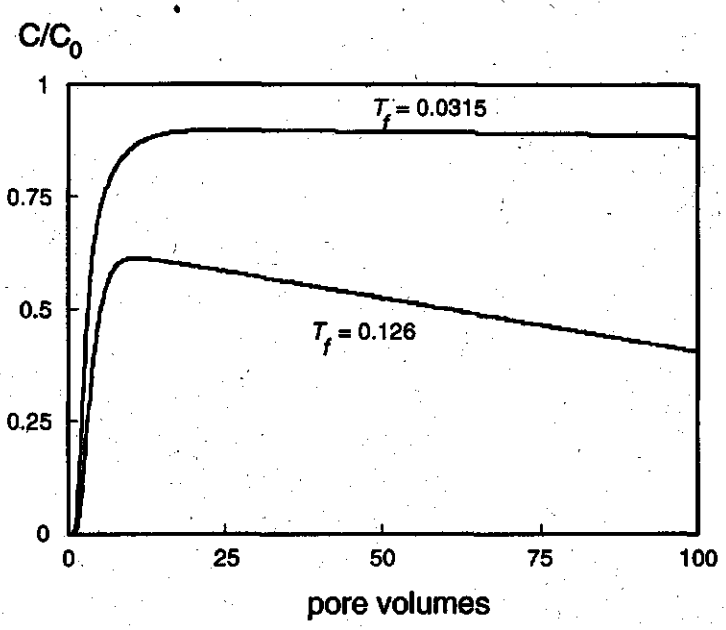

Figure 7.5: Model sensitivity to the non-equilibrium constant T_f

The second scenario involved leaching of contaminants entrapped in soil aggregates, to simulate pump and treat systems that are used to stimulate biotransformation. The initial conditions were a homogeneous distribution of contaminant at a concentration of 50 µg/l throughout the column and biomass present only at the boundary of the aggregates at a density of 100 µg/l. The influent concentration was set at a value of zero. A model run with the basic parameter set showed that the percolation of 20-25 pore volumes of clean water was sufficient to decrease the effluent concentration to near zero (Fig. 7.6). The contaminant was also completely removed from the aggregates in this period of time. Leaching dynamics were not sensitive to variation of any of the microbial parameters, in contrast to the observation with contaminant breakthrough. T_f appeared to be the only sensitive parameter in this scenario. A similar result was found with a model describing leaching of non-aqueous phase pollutants from a soil column, where dynamics were most sensitive to pore water velocity and dissolution kinetics and not affected by microbial kinetics (Seagren et al 1993). Decreasing T_f, which means shifting towards non-equilibrium, causes more tailing in the leaching curve (Fig. 7.7). The mass flow in the macropores is sufficient to maintain a steep diffusion gradient between micro- and macropores and almost no biotransformation of leaching contaminant takes place.

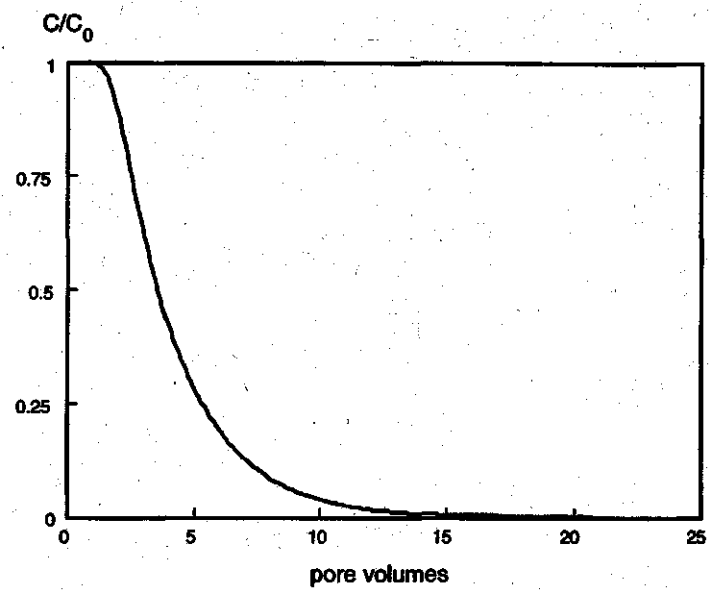

***Figure 7.6:** Leaching of contaminant during flushing of a column with clean water. The shape of the curve did not change in response to halving and doubling any of the microbial parameters.*

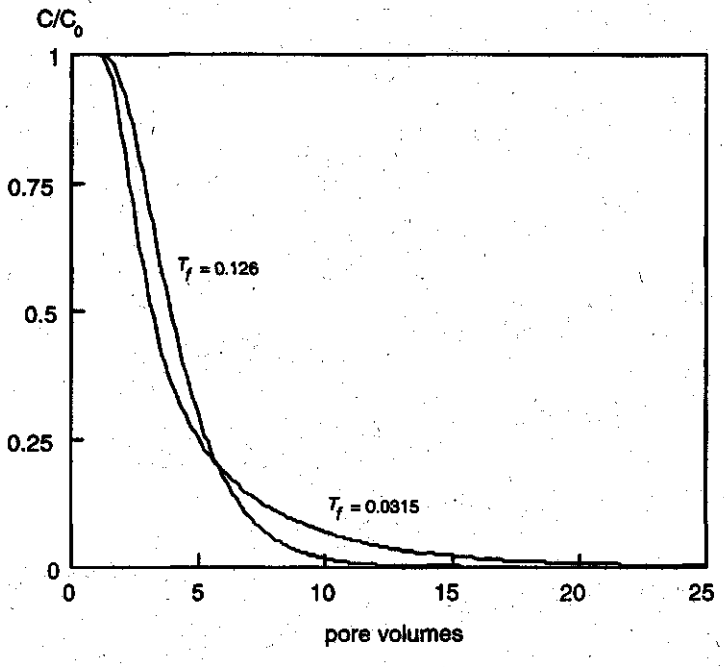

***Figure 7.7:** Sensitivity of contaminant leaching to the non-equilibrium index T_f*

DISCUSSION

According to our calculations the behavior and fate of biodegradable organic contaminants in the saturated zone will mainly be controlled by the values of the dimensionless variables T_f and m_f, while the effectiveness of flushing of a contaminated aquifer with clean water will be exclusively determined by the value of T_f. Values of T_f <5 indicate non-equilibrium in the system under consideration, while values >5 point at equilibrium behavior. The actual value of this parameter is determined by the dimensions and geometry of the system, as expressed in the pore water velocity (V), the travel path (which is equivalent to the column length L), the aggregate radius (R), and the intra-aggregate diffusion coefficient (D_i). As can be inferred from equation (8e), relatively high values of L and D_i, and small values of V and R, lead to high values of T_f and hence, equilibrium behavior. Of these parameters, the effective intra-aggregate diffusion coefficient may vary by several orders of magnitude (Chapter 8), from ca. 10^{-5} cm^2/s (aqueous diffusion, Weber et al 1991) to 10^{-10}-10^{-11} cm^2/s for highly chlorinated benzenes in organic rich sediments (Wu and Gschwend 1986, Ball and Roberts 1991). Values as low as 10^{-13} and 10^{-17} cm^2/s, have been reported for soils that have been contaminated for decades with hexachlorocyclohexane (Rijnaarts et al 1990) and 1,2-dibromoethane (Steinberg et al 1987). Hence values of T_f are also expected to vary by many orders of magnitude in natural soils and sediments.

Under certain restrictions, the non-equilibrium index T_f may be used to interpret results at the larger field scale. Assuming that the size of aggregates and intra-aggregate diffusion rates are similar in columns and in the field, transport under field conditions would tend more to equilibrium behavior than transport in a laboratory column, because the travel path will in general be longer and the pore water velocity smaller. However, other factors like the presence of preferential streamlines in wide pores, will have similar and maybe even more noticeable effects on contaminant transport in comparison to the smaller scale aggregation in laboratory columns (Rappoldt 1992). The counterbalancing effects during up-scaling suggest that transport at the field scale may be described with a model similar to the one presented here. The aggregate radius used in the model then represents an average diffusion length in the field, which may be determined e.g. by measuring the average distance between wide pores (Rappoldt 1992).

Varying the microbial parameters Y, K_s, and B_0, caused similar features in the breakthrough behavior as changing m_f but the deviations were smaller. The model was insensitive to variation in b_f. So, it seems plausible to apply second order growth kinetics and to neglect microbial decay, if one is only interested in the breakthrough dynamics of a contaminant during infiltration. The steady state that will arise during the percolation of contaminated water through a column was not addressed in the sensitivity analysis. A steady state solution will develop when the growth rate of bacteria, and hence the biotransformation rate, equals zero, as given by equation (16):

$$\frac{\partial B}{\partial t} = (\bar{\mu} - b_f)B = \left(m_f \frac{c}{K_s + c} - b_f \right) B = 0 \qquad (16)$$

It can be easily inferred that a stable residual micropore concentration c_{res} exists, below which no degradation will occur:

$$c_{res} = K_s \frac{b_f}{m_f - b_f} = K_s \frac{b}{\mu_{max} - b} \qquad (17)$$

This relation is the same as found for continuous cultures (Middeldorp et al in prep.) and biofilm models (Bouwer and McCarty 1984). The macropore concentration will approach c_{res} also because the net flux over the aggregate boundary will equal zero at steady state, which is only the case if the micro- and macropore concentrations are equal (equation 12c). So, within the framework used in this paper, the steady state residual concentration of a chemical percolated through a column will not be affected by physical and chemical parameters, but only by microbial kinetics. The reason for this is the assumption that bacteria are homogeneously distributed in soil aggregates. As a consequence, all biotransformation will take place at the surface of the aggregates, as demonstrated by calculations with an analytical solution to equations describing microbial growth in aggregates (Priesack 1991). The effect of intra-aggregate diffusion rates on residual concentrations can only be accounted for by explicitly including diffusion kinetics in the terms describing microbial uptake of contaminants (Chapter 8), and by assuming discrete zones of microbial activity in the aggregates. Residual concentrations in aggregated soil are probably inversely proportional to the effective diffusion coefficient D_i as demonstrated by calculations for a single cell surrounded by a matrix with diffusion resistance (Chapter 8).

In conclusion, the physical and chemical processes of diffusion and sorption appear to be of primary importance in determining the breakthrough of biodegradable contaminants in aggregated soil. Microbial kinetics appear to be of secondary importance. A dependency on diffusion kinetics of the residual concentration reached in steady state can only exist if discrete zones of microbial activity are present in soil aggregates. Pumping clean water amended with nutrients will probably not be effective to enhance biodegradation rates during *in situ* bioremediation of aggregated soil, because its efficacy completely depends on the ratio between pore water velocity and radial diffusion kinetics and not on microbial kinetics. Instead, efforts should be focused on decreasing diffusion distances between microbes and bulk contaminants by cracking and pulverizing the soil, and on minimizing transport by mass flow, by applying only slow flushing rates when water amended with nutrients has to be added to the soil.

ACKNOWLEDGEMENT

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NOTATION

α_{-1} $\dfrac{2r_i - r_{i+1}}{r_i - r_{i-1}} \dfrac{2}{r_i(r_{i+1} - r_{i-1})}$

α_1 $\dfrac{2r_i - r_{i-1}}{r_{i+1} - r_i} \dfrac{2}{r_i(r_{i+1} - r_{i-1})}$

b Bacterial decay coefficient (T^{-1})

B Microbial density ($M_m \cdot L^{-3}$)

b_f Bacterial decay rate modulus (dimensionless)

β_{-1} $\dfrac{r_i - r_{i+1}}{(r_i - r_{i-1})(r_{i+1} - r_{i-1})}$ $(i=0, i=I)$

β_1 $\dfrac{r_i - r_{i-1}}{(r_{i+1} - r_i)(r_{i+1} - r_{i-1})}$ $(i=0, i=I)$

C Macropore contaminant concentration ($M_c \cdot L^{-3}$)

C_0 Influent concentration ($M_c \cdot L^{-3}$)

c Micropore contaminant concentration ($M_c \cdot L^{-3}$)

c_r First derivative of c with respect to r

c_{rr} Second derivative of c with respect to r

c_{res} Residual micropore contaminant concentration under steady state conditions ($M_c \cdot L^{-1}$)

γ_{-1} $\dfrac{2}{(r_I - r_{I-1})^2}$

D_c Axial dispersion coefficient ($L^2 \cdot T^{-1}$)

D_i Effective intra-aggregate diffusion coefficient ($L^2 \cdot T^{-1}$)

δ_0 $\dfrac{4r_I - 2r_{I-1}}{r_I(r_I - r_{I-1})}$

θ_c Macropore porosity

I Number of radial nodes

i Radial node index (0,...,I)

j Axial node index (0,...,number of axial nodes)

k_m External mass transfer coefficient ($L \cdot T^{-1}$)
K_s Half saturation constant ($M_c \cdot L^{-3}$)
κ External mass transfer modulus (dimensionless)
L Column length (L)
m_f Maximum specific growth rate modulus (dimensionless)
μ Specific growth rate (T^{-1})
$\overline{\mu}$ Scaled specific growth rate (dimensionless)
μ_{max} Maximum specific growth rate (T^{-1})
Pe Peclet number (dimensionless)
R Aggregate radius (L)
r Radial co-ordinate (L)
$\overline{r}$ Dimensionless radial co-ordinate
R_d Axial retardation coefficient (dimensionless)
t Temporal co-ordinate (T)
$\overline{t}$ Dimensionless time
T_f Non-equilibrium index (dimensionless)
V Linear pore water velocity ($L \cdot T^{-1}$)
Y Yield coefficient ($M_m \cdot M_c^{-1}$)
z Axial co-ordinate (L)
$\overline{z}$ Dimensionless axial co-ordinate

APPENDIX: NUMERICAL APPROXIMATION

Because of the non-linear Monod kinetics, a numerical approach is necessary to solve the transport equations formulated in the previous section. The numerical approximation consists of two stages. The first stage involves a discretization of the spatial co-ordinates (i.e., the axial co-ordinate z and the radial co-ordinate r). The differential equations are approximated by finite difference equations and a large system of ordinary differential equations is obtained. The second stage involves an approximate solution of the system of ordinary differential equations by means of a stiff-stable integration method.

Both axial and radial co-ordinates are discretized by finite difference methods. For the radial co-ordinate we set $r=r_i$, $i=0,...,I$, with $r_0=0$, $r_I=1$. Writing $c_i=c(t,r_i,z_j)$ (where z_j denotes the axial co-ordinate) we have:

$$r^{-2}\frac{d}{dr}\left(r^2\frac{d}{dr}c(t,r,z_j)\right)_{r=r_i} \cong \alpha_{-1}c_{i-1}-(\alpha_{-1}+\alpha_1)c_i+\alpha_1 c_{i+1} \qquad (18a)$$

with

$$\alpha_{-1} = \frac{2r_i - r_{i+1}}{r_i - r_{i-1}} \frac{2}{r_i(r_{i+1} - r_{i-1})} \tag{18b}$$

$$\alpha_1 = \frac{2r_i - r_{i-1}}{r_{i+1} - r_i} \frac{2}{r_i(r_{i+1} - r_{i-1})} \tag{18c}$$

For arbitrary sequences $\{r_i\}$, the truncation error of this approximation is first order in $(r_i - r_{i-1})$ and in $(r_{i+1} - r_i)$. If, however, the sequence $\{r_i\}$ is equidistant with stepsize $h = r_i - r_{i-1}$, the truncation error is second order in h.

For $i = 0$ and $i = I$, the above approximation involves the virtual points r_{i-1} and r_{i+1}, and the virtual values c_{i-1} and c_{i+1}, respectively. Virtual points and virtual values are eliminated using the boundary conditions. These involve the derivative $d/dr\, c(r)$, which is approximated by

$$c_r(r_i) = \beta_{-1} c_{i-1} - (\beta_{-1} + \beta_1) c_i + \beta_1 c_{i+1} \tag{19a}$$

where $c_r(r_i)$ denotes the first derivative of c_i with respect to r, and

$$\beta_{-1} = \frac{r_i - r_{i+1}}{(r_i - r_{i-1})(r_{i+1} - r_{i-1})} \tag{19b}$$

$$\beta_1 = \frac{r_i - r_{i-1}}{(r_{i+1} - r_i)(r_{i+1} - r_{i-1})} \tag{19c}$$

This relation may be used to eliminate the virtual point and virtual value for $i = I+1$ from equations *(15a-c)*. We find:

$$r^{-2} \frac{d}{dr}\left(r^2 \frac{d}{dr} c(t, r, z_j) \right)_{r=r_I} \cong \gamma_{-1} c_{I-1} - \gamma_{-1} c_I + \delta_0 c_r(r_I) \tag{20a}$$

with

$$\gamma_{-1} = \frac{2}{(r_I - r_{I-1})^2} \tag{20b}$$

$$\delta_0 = \frac{4r_I - 2r_{I-1}}{r_I(r_I - r_{I-1})} \tag{20c}$$

The virtual point and virtual value for $i = -1$ are eliminated by direct use of the symmetry at $r=0$:

$$
\begin{aligned}
r^{-2}\frac{d}{dr}\left(r^2\frac{d}{dr}c(t,r,z_j)\right)_{r=0} &= \lim_{r\to 0}\left\{r^{-2}\frac{d}{dr}\left(r^2\frac{d}{dr}c(t,r,z_j)\right)\right\} \\
&= \lim_{r\to 0}\left(c_{rr}(t,r,z_j)+\frac{2}{r}c_r(t,r,z_j)\right) \\
&= \lim_{r\to 0}\left\{c_{rr}(t,r,z_j)\right\}+2\lim_{r\to 0}\left\{\frac{c_r(t,r,z_j)-c_r(t,0,z_j)}{r}\right\} \\
&= 3c_{rr}(t,0,z_j)
\end{aligned}
\qquad (21)
$$

where c_{rr} denotes the second derivative of c with respect to r. Hence

$$
r^{-2}\frac{d}{dr}\left(r^2\frac{d}{dr}c(t,r,z_j)\right)_{r=0} \cong 3\frac{c_{-1}-2c_0+c_1}{(r_1-r_0)^2}=\frac{6}{(r_1-r_0)^2}(c_1-c_0) \qquad (22)
$$

The discretization of the axial co-ordinate z was done similarly. The resulting set of non-linear differential equations was implemented in FORTRAN-77 and solved with the stiff-stable Gear method (Gear 1971) implemented in IMSL-9.2 (IMSL 1987).

CHAPTER 8

BIOAVAILABILITY: A LIMITING FACTOR IN THE BIOREMEDIATION OF POLLUTED SOIL.

FACTORS AFFECTING THE MOBILITY AND UPTAKE OF POLLUTANTS

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ABSTRACT

Organic pollutants in soil can be removed by means of biological treatment. A major problem in the application of these treatments is the efficiency of biodegradation in soil. The bulk of the pollution can often be removed but certain residual amounts remain unaltered and biodegradation rates are often much slower than expected on the basis of laboratory trials. Sorption retarded diffusion kinetics are shown to have a big impact on biotransformation rates in soil and on residual concentrations remaining after biotransformation. Residual concentrations are predicted to be inversely proportional to effective diffusion coefficients in soil and proportional to the metabolic efficiency of the degrading micro-organisms. Bioremediation techniques should try to increase diffusion rates in soil to overcome the limitation. Dissolution kinetics mainly slow down biotransformation rates but do not affect residual concentrations. Biotransformation rates may be increased by adding solvents that enhance the solubility of pollutants. Furthermore, a dispersion of the chemical would also be effective. After long exposure times, pollutants may become covalently bound to soil organic matter via polymerization reactions with natural organic compounds. Thus, a chemically and biologically inactive bound residue is generated that remains unaltered for decades or even centuries. The occurrence of such reactions in soil could be stimulated to inactivate pollutants. However, smaller intermediates which are formed initially, may form highly mobile colloids which are easily transported over long distances, together with bound pollutants. Moreover, similar reactions may also lead to formation of dioxins. Therefore, it is not recommended to stimulate the formation of bound residues as a way to detoxify soil.

INTRODUCTION

Organic pollutants in soil can be removed by means of biological treatment. A major problem in the application of these treatments is the efficiency of biodegradation in soil. The bulk of the pollution can often be removed but certain residual amounts remain unaltered (Valo and M.S. 1986, Wang and Bartha 1990). In addition, biodegradation rates are often much slower than expected on the basis of laboratory trials. Prerequisites for biotransformation are that the overall reaction is thermodynamically favorable and that micro-organisms posses biodegradative capacities, possibly after acquisition via gene transfer (Van der Meer et al 1992, Middeldorp et al in prep.). The kinetics of microbial growth are not always sufficient to explain the slow biological removal rates in soil and the occurrence of residual amounts after bioremediation (Middeldorp et al in prep.). This chapter describes how biotransformation rates and residual concentrations may be controlled by dissolution, sorption and diffusion kinetics and by coupling to soil organic matter via chemical bonds (bound residue formation).

SORPTION AND DIFFUSION

Sorption can be defined as the partitioning of a compound between a solid (e.g. soil particles) and a liquid phase. The adsorption isotherm of hydrophobic organic pollutants in soil is linear at low concentrations, which means that sorption is linearly correlated with the concentration in the liquid phase (Karickhoff et al 1979, Karickhoff 1981):

$$S = K_p C \qquad (1)$$

where S is the amount of compound adsorbed on the solid phase, K_p the partition coefficient and C the concentration of the compound in the liquid phase. A listing of all symbols and their dimensions is given in the Notation section. Sorption of hydrophobic compounds in soil can be viewed as a partitioning process between the aqueous and organic phases (Chiou et al 1979, Karickhoff et al 1979, Schwarzenbach and Westall 1981). Equilibrium partition coefficients for sorption of hydrophobic organics to soil organic matter (K_{oc}) can be estimated from 1-octanol/water partition coefficients or water solubilities, using linear free energy relationships (Karickhoff et al 1979, Schwarzenbach and Westall 1981, Chiou et al 1983, Hasset et al 1983, Curtis 1986). K_{oc} can also be estimated using a molecular topology model, that uses the molecular structure to calculate the first-order molecular connectivity index of a compound (Sabljic' 1987). A linear relationship was demonstrated between the first-order molecular connectivity index and empirically determined $\log(K_{oc})$-values of hydrophobic compounds. Linear free energy relationships also hold for simultaneous sorption of dilute non-polar compounds (Schwarzenbach and Westall 1981) and in aquifer materials with an organic carbon content (f_{oc}) as low as 0.1% (Barber et al 1992). However, in aquifer materials with lower organic carbon contents, the presence of several non-polar compounds together can have a synergistic effect resulting in stronger sorption than predicted from linear free energy

relationships (Brusseau 1991). Furthermore, the contribution of mineral surfaces as sorption sites may become significant (Schwarzenbach and Westall 1981). K_{oc}-values can be converted to K_p-values when the f_{oc} of a soil is known, by using the relationship:

$$K_p = f_{oc} K_{oc} \tag{2}$$

The partitioning of hydrophobic organic contaminants between organic matter and water is a physical process that reaches site equilibrium within milliseconds or, at most, seconds (Weber et al 1991). Sorption rates of organic pollutants found in soil and sediment are generally slower, reaching apparent equilibrium within a day, as a result of the existence of a diffusion layer between the bulk fluid and the sorption site (Weber et al 1991). However, several researchers have observed that, after contact times in the order magnitude of years, aggressive extraction methods are needed to remove organic chemicals sorbed to soil (Karickhoff 1980, Steinberg et al 1987). A two-site model can be used to account for this apparent irreversible sorption (Karickhoff 1980). One site accounts for a rapid equilibration and the second, defined by a small rate constant, accounts for the apparent strong binding after long contact times. Later, a sorption-retarded radial diffusion model was successfully applied to explain very slow sorption of hydrophobic chemicals (Wu and Gschwend 1986, Ball and Roberts 1991):

$$\frac{\partial C}{\partial t} = D_{eff} r^{-2} \frac{\partial}{\partial r}\left(r^2 \frac{\partial C}{\partial r} \right) \tag{3}$$

with

$$D_{eff} = \frac{D_{aq} \theta_a}{K_p (1-\theta_a) \rho_s} f(\theta_a, \tau) \tag{4}$$

where r is the radial co-ordinate, D_{eff} the effective intra-particle diffusivity, D_{aq} the pore fluid diffusivity of the sorbate, θ_a the porosity of the sorbent, t the tortuosity factor, and ρ_s the solid phase density. The function $f(\theta_a, \tau)$, yielding the correction factor for pore geometry, is given by (Ullman and Aller 1982):

$$f(\theta_a, \tau) = \theta_a^{\,i} \tag{5}$$

where the exponent i has a value between 1 and 2.

Effect on biotransformation rates

The effect of sorption kinetics on biotransformation kinetics was evaluated by assuming that bacteria are present inside soil aggregates containing micropores with immobile water (Hattori and Hattori 1976, Fig. 8.1). Uptake and biotransformation result in a depletion of organic pollutants close to the bacterium, which leads to a diffusion gradient between the bulk soil and the region surrounding the bacterium. Resupply of pollutants takes place via diffusion through the micropores.

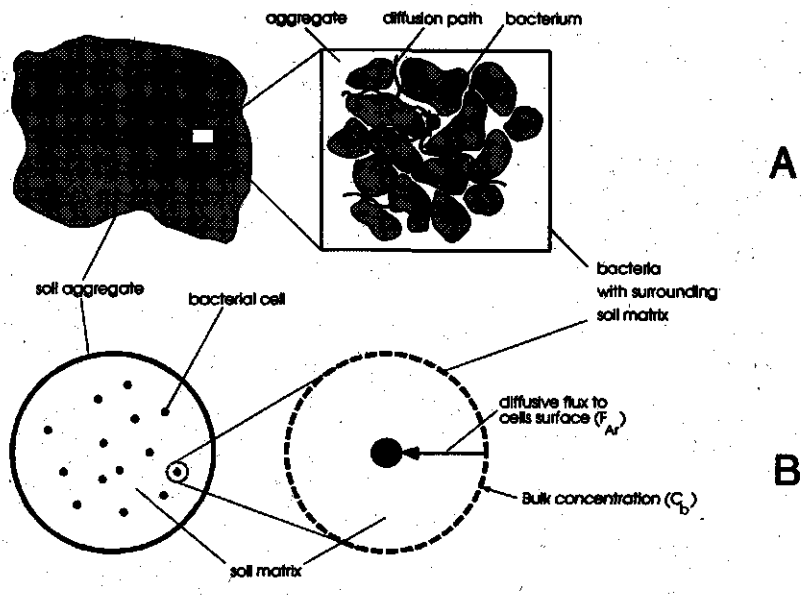

***Figure 8.1:** View of and of a bacterium in a soil particle illustrating the diffusion paths of substrate to the cell (A) and a schematic representation (B) illustrating the concept used in the derivations.*

Bacteria which are faced with low supply rates of nutrients synthesize a lot of transporter enzymes relative to the amount of metabolic enzymes. This strategy allows the cell to operate rate-limiting metabolic enzymes at maximal rates (Button 1991). So, below some critical rate of substrate diffusion to the cell surface, each molecule approaching the cell surface will be taken up and the uptake rate will equal the diffusive flux at the cell-water interface. Then, the growth rate is fully controlled by the diffusive flux. The steady-state flux of a chemical to a spherical cell, is obtained when equation (3) equals zero or when:

$$\frac{d}{dr}\left(r^2 \frac{dC}{dr}\right) = 0 \qquad (6)$$

(Crank 1975). The following boundary conditions apply to equation (6):

$$C(r = R) = 0 \qquad (7a)$$

$$C(r = \infty) = C_b \qquad (7b)$$

where R is the radius of the cell and C_b is the bulk pollutant concentration (see also Fig. 8.1B). Equation 7a is the mathematical equivalent of the assumption that each molecule diffusing to the cell surface is taken up immediately. Solving equation (6) with these boundary conditions yields:

$$C = -C_b \frac{R}{r} + C_b \qquad (8)$$

(Von Smoluchowski 1918, Crank 1975). Thus, the flux at the cell surface becomes

$$F_{r=R} = -D_{eff}\left(\frac{dC}{dr}\right)_{r=R} = D_{eff}\frac{C_b}{R} \qquad (9)$$

where $F_{r=R}$ is the flux per unit area at the cell surface. Multiplication of $F_{r=R}$ with the surface area of the cell ($4pR^2$) yields the total flux of substrate at the cell surface:

$$(F_{tot})_{r=R} = D_{eff}C_b(4\pi R) \qquad (10)$$

The growth rate of a cell is given by (Pirt 1982)

$$\frac{dB}{dt} = Yq - mYB \qquad (11)$$

where Y is the observed yield, q the substrate uptake rate, m the maintenance coefficient, and B the dry cell weight. When all substrate diffusing to a cell is taken up, the uptake rate equals the total diffusive flux at the cell surface:

$$q = D_{eff}C_b(4\pi R) \qquad (12)$$

and the growth rate becomes:

$$\frac{dB}{dt} = YD_{eff}C_b(4\pi R) - mYB \qquad (13)$$

Hence, uptake and growth rates are first order in the bulk concentration of contaminant in case of diffusion limitation and negligible maintenance requirements. This explains why first order degradation terms are applicable so often to account for biodegradation of chemicals in soil. $D_{eff}C_b$ should be kept as high as possible to be able to maintain high transformation rates in a bioremediation process. In other words, one must try to create a significant flux of pollutants to the degrading micro-organisms by increasing the diffusion rate. Pump-and-treat methods which are often used in bioremediation schemes, are not suitable for this purpose, because they do not affect the soil structure that is causing the diffusion limitation. Similarly, the addition of solvents that increase the water solubility of pollutants does not remove limitations caused by diffusion. Pulverization has been shown to be an effective way to enhance rates of desorption (Steinberg et al 1987) and mineralization of organic compounds (Rijnaarts et al 1990).

Effect on residual concentration

The biotransformation rate in soil depends on the rate of pollutant diffusion to the surface of a microbial cell, as shown above. When the diffusive flux, i.e. the value of $D_{eff}C_b$ in equation (13), decreases, maintenance requirements significantly reduce growth rates until the amount of substrate taken up is just enough to meet the maintenance requirements. Cells will either die or enter a dormant state when the supply rate of substrate cannot meet the maintenance requirements anymore (Stevenson 1978). Residual concentrations are believed to result from

this situation. To obtain an expression for the residual concentration that does not depend on the cell mass, B in equation (13) can be replaced according to

$$B = \rho_b(\tfrac{4}{3}\pi R^3) \qquad (14)$$

where ρ_b is the dry weight density and $\frac{4}{3}\pi R^3$ the volume of a cell. Consequently, the growth rate becomes:

$$\frac{dB}{dt} = YD_{eff}C_b(4\pi R) - Ym\rho_b(\tfrac{4}{3}\pi R^3) \qquad (15)$$

The term $D_{eff}C_b(4\pi R)$ at the right hand side of equation (15) represents the total flux of substrate at the cell surface while $m\rho_b(\frac{4}{3}\pi R^3)$ is the minimal total flux at the cell surface required to satisfy the maintenance requirements. Equating the growth rate to zero and replacing C_b by the residual concentration C_r yields:

$$\frac{D_{eff}C_r}{R} = \tfrac{1}{3}m\rho_b R \qquad (16)$$

Both the left and right hand sides of equation (16) represent the minimal flux per unit area required to satisfy the maintenance requirements of a bacterial cell. It can easily be solved for the residual concentration:

$$C_r = \frac{\frac{1}{3}m\rho_b R^2}{D_{eff}} \qquad (17)$$

where the denominator is the product of the cell radius R, and the flux per unit area $\frac{1}{3}m\rho_b R$ required to satisfy the maintenance requirements of a bacterial cell. It can be viewed as a parameter which expresses the efficiency of cellular metabolism. High values reflect a low efficiency typical of eutrophic bacteria, whereas low values reflect a high efficiency typical of oligotrophic bacteria. Since ρ_b is not expected to vary strongly among bacteria, mainly m and R will determine the range of variation of this parameter among different bacterial species. It can easily be inferred that it is advantageous for oligotrophic bacteria to be small, since the efficiency, i.e. $\frac{1}{3}m\rho_b R^2$, is proportional to R^2.

Equation 17 shows that the residual or threshold concentration of a biodegradable organic compound in soil is inversely proportional to D_{eff} and proportional to $\frac{1}{3}m\rho_b R^2$. Fig. 8.2 graphs the calculated aqueous threshold concentration as a function of the effective diffusion coefficient in the range from 10^{-5} (aqueous diffusion, Weber et al 1991) to 10^{-12} $cm^2 \cdot s^{-1}$ (diffusion in soil, Wu and Gschwend 1986, Myrand et al 1992). The upper line represents large copiotrophic bacteria whereas the lower line represents small oligotrophic bacteria. The difference between the upper and lower line stands for the variation of threshold concentrations that are the result of differences in metabolic efficiency between bacterial species. Values of the efficiency parameter $\frac{1}{3}m\rho_b R^2$ were based on published values of m

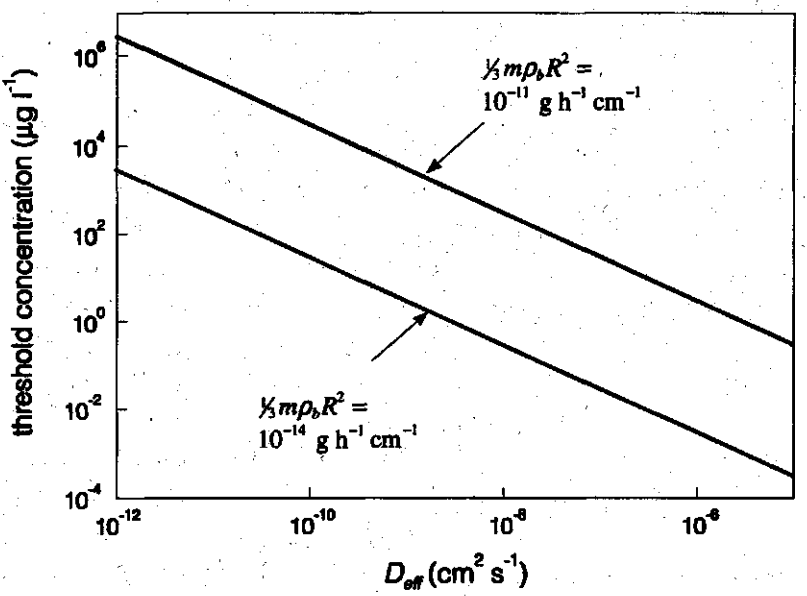

***Figure 8.2:** Threshold for growth as a function of the effective diffusivity in soil. The upper line represents large copiotrophic bacteria, whereas the lower line represents small oligotrophic bacteria.*

(Chesbro et al 1979, Bouillot et al 1990), ρ_b (Schmidt et al 1985a), and R (Ishida and Kadota 1981, Carlucci et al 1986). With the chosen range for $\frac{1}{3}m\rho_b R^2$, the resulting variation in residual concentration is three orders of magnitude. The effect of D_{eff} is even more drastic. A variation of seven orders of magnitude of the residual concentration may be expected based on its published values (Wu and Gschwend 1986, Myrand et al 1992). The overall variation in the residual concentrations estimated from our approach, is as large as 10 orders of magnitude, with a lower limit of about $5 \cdot 10^{-4}$ µg·l^{-1} and an upper limit of 5 g·l^{-1} (Fig. 8.2).

The literature does not provide data to check the validity of the presented approach. Maintenance requirements of bacteria are well studied for pure cultures (Chesbro et al 1979, Pirt 1982, Bouillot et al 1990) but not for indigenous soil bacteria. The role of intra-particle diffusion in sorption of hydrophobic chemicals in soils and aquifers is also well studied (Wu and Gschwend 1986, Ball and Roberts 1991) but reports relating effective diffusion of organic substrates to biotransformation are scarce (Rijnaarts et al 1990). No data are available that relate effective diffusion of organic substrates to residual concentrations that remain after biotransformation. However, some indirect evidence for the validity of the derived expression for residual or threshold concentrations is provided by the observation that biotransformation of chlorobenzenes in columns packed with sediment from a dune infiltration area, resulted in residual concentrations that were positively correlated with compound hydrophobicity (Chapter 2). Residual concentrations varied from 0.03 mg·l^{-1} for chlorobenzene to 2.6 mg·l^{-1} for 1,2,4-trichlorobenzene. In a related study, a stable residual effluent concentration of

0.1 $mg \cdot l^{-1}$ was found after biotransformation of 1,2-dichlorobenzene by indigenous bacteria in columns packed with Rhine river sediment (Van der Meer et al 1987). Reported residual concentrations did not depend on the influent concentration in a range from 1-100 µg/l. In a column with sediment from the same source and inoculated with the polychlorobenzene mineralizing *Pseudomonas* sp. strain P51, the residual effluent concentration of 1,2-dichlorobenzene was 6 $\mu g \cdot l^{-1}$. So, the residual concentration of 1,2-dichlorobenzene obtained with indigenous bacteria differed from that obtained with a pure culture by a factor of about 50. The observed difference may reflect the differences in efficiencies of 1,2-dichlorobenzene metabolism between indigenous cells and P51. The residual concentration after 1,2,4-trichlorobenzene transformation by inoculated *Pseudomonas* sp. strain P51 in the same column was 20 $\mu g \cdot l^{-1}$ (Van der Meer et al 1987). Assuming that the strain has a similar metabolic efficiency during growth on both 1,2-dichloro- and 1,2,4-trichlorobenzene, the residual concentration values of 6 and 20 $\mu g \cdot l^{-1}$ would reflect a lower value of D_{eff} for 1,2,4-trichlorobenzene compared to 1,2-dichlorobenzene, which is expected because of the greater hydrophobicity, i.e. the greater value of K_p of the trichlorobenzene.

The calculated values of the residual concentration are valid only in the region immediately surrounding each individual cell. High values occur in soil particles where low values of D_{eff} are prevalent, lower values in soil particles with higher effective diffusivities. So,

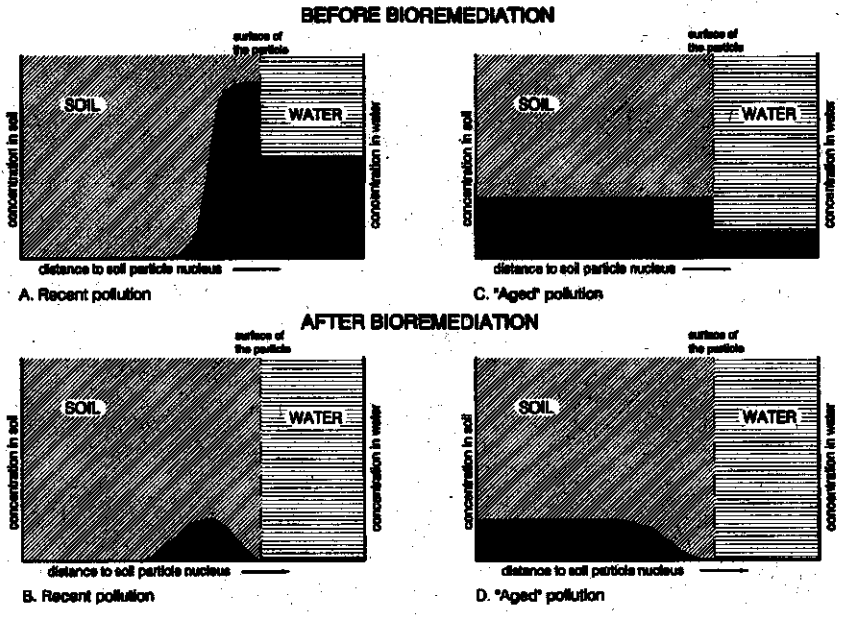

Figure 8.3: Schematic visualisation of the distribution of a pollutant in a soil particle and surrounding liquid (Douben and Harmsen 1991). The vertical scales are different in a way that the soil concentration >> water concentration.

each micro-environment surrounding a bacterial cell, has its own unique residual concentration. Since both soil and microbial properties vary spatially, a pattern of varying threshold concentrations result for a soil or aquifer as a whole. This implies that, in the course of bioremediation, a decreasing fraction of the total bacterial population will contribute to biodegradation. As a consequence, more and more regions in a particular soil or aquifer do not contribute anymore to the process of biodegradation as soon as the local residual concentration has been reached, which means that the diffusion distances between pollutants and still active micro-organisms also increase in the course of time. This may account for the very slow biotransformation rates that are often found in later stages in soil bioremediation.

The occurrence of inaccessible residual concentrations after bioremediation, particularly in the case of "aged" pollutions was reported by several research groups (Salkinoja-Salonen et al 1989, Weissenfels et al 1992, Beurskens et al 1993). Different degradation patterns were found in two soils contaminated with chlorophenols (Salkinoja-Salonen et al 1989). Pollution in one soil occurred only recently, while the other soil was already polluted for over 40 years. Although sufficient amounts of chlorophenol degrading micro-organisms were detected in both soils, degradation only occurred in the recently polluted soil. A freshly added amount of pentachlorophenol in the old polluted soil however, was mineralized instantaneously. When the residual chlorophenols were extracted from the old polluted soil and then added again followed by inoculation of the soil with a polychlorophenol degrading pure culture, part of the chlorophenols were degraded (Middeldorp and Salkinoja-Salonen, unpublished data). Similar results were reported for two soils contaminated with polycyclic aromatic hydrocarbons (Weissenfels et al 1992), the reductive dechlorination of hexachlorobenzene in contaminated sediment (Beurskens et al 1993).

The difference in the process of bioremediation in freshly and "aged" contaminated soil is illustrated in Fig. 8.3. In freshly contaminated soil, contamination only reaches the macropores and the outer, relatively easy accessible, regions of aggregates (Fig. 8.3A). After bioremediation, the pollutant is completely depleted in the macropores, while a small residue remains in the outer regions of the aggregates (Fig. 8.3B). Opposed to this, "aged" contaminations have penetrated the aggregates completely, including regions with extremely narrow and tortuous micropores (Fig. 8.3C). After bioremediation, macropores and the outer regions of the aggregates are clean, but a considerable residual amount of contaminant remains in the internal part of the aggregates (Fig. 8.3D). As a result of the bioremediation process, a steep diffusion gradient exists between the inner part of the aggregates and the macropores, which may result in a very slow diffusion to the macropores again. This explains why sites which are supposed to be cleaned by *in-situ* bioremediation, sometimes appear to be polluted again after a few years. It is easy to infer from equation (17) that residual concentrations can be decreased by increasing the value of D_{eff}. So, as mentioned before, the most appropriate way to remove limitations caused by sorption and diffusion, is to pulverize soil particles with

micropores having high tortuosity. In an *in-situ* design, the development of long diffusion distances may be prevented by ploughing the soil regularly. It may be difficult to pulverize soil particles *in-situ*.

DISSOLUTION OF SOLID AND LIQUID PHASE POLLUTANTS

Since hydrophobic pollutants are generally not equally dispersed in soil, they tend to stick together at high concentrations, forming particles or droplets. Normally, it is assumed that only biodegradation of dissolved pollutants is possible. So, there is a possibility that dissolution kinetics limit biotransformation. The dissolution from a solid or non-polar liquid to the aqueous phase can be described as (Perry et al 1963):

$$J = KA(C_{max} - C) \tag{18}$$

where J is the dissolution rate, K the mass transfer coefficient governing dissolution, A the solid/water contact surface, and C_{max} the maximum water solubility. When the uptake of pollutant by the degrading micro-organisms is limited by the dissolution rate, C will approach zero and the dissolution rate approaches its maximum value:

$$J_{max} = KAC_{max} \tag{19}$$

Under these conditions, q in equation (11) may be substituted by J_{max}. Thus, an expression for growth limited by dissolution of pollutant is obtained:

$$\frac{dB}{dt} = YKAC_{max} - YmB \tag{20}$$

Equation (20) shows that biotransformation rates are positively correlated with the surface area of the pollutant droplets or particles (Thomas et al 1986). Furthermore, as long as the surface area A does not change significantly, the biomass will increase linearly with a rate that is controlled by Y and C_{max} (Volkering et al 1992). So, in case of a low water solubility of a pollutant or a small contact surface A, bioremediation of soil may be limited by the dissolution rate. This limitation may be overcome by the addition of solvents that enhance the solubility of pollutants. Furthermore, a dispersion of the chemical resulting in a higher contact area, would also increase biotransformation rates. However, in the course of the biotransformation process, the size of droplets or particles will decrease until they completely dissolve in the aqueous phase. Then, biotransformation can be described using Monod kinetics. Thus, residual concentrations after bioremediation will not be affected by the presence of droplets or particles purely consisting of pollutant.

BOUND RESIDUE FORMATION

Pollutants like chlorinated phenols, benzoic acids, and anilines, are very similar to natural organic compounds and can be incorporated in humus-like structures via oxidative coupling in a similar manner as their natural analogues (Bollag and Loll 1983). This reaction is catalyzed by a series of inorganic materials, such as sesquioxides, clay, oxides and oxyhydroxides of iron, silica, and allophane (Scheffer et al 1959, Ziechmann 1959, Kyuma and Kamaguchi 1964, Wang et al 1986), and by peroxidases and phenol mono-oxygenases (Martin and Haider 1971). Oxidative coupling proceeds through a radical mechanism which ultimately yields high molecular weight polymers of considerable stability (Sjoblad and Bollag 1981, Stevenson 1982). Both phenolic lignin derivatives, such as vanillic acid, vanillin, ferulic and syringic acid, and a number of man-made organics such as chlorinated phenols, naphtholic compounds and halogenated anilines can be cross-coupled with natural phenols in soil (Bollag et al 1980, Berry and Boyd 1985, Bollag and Liu 1985, Fig. 8.4). The susceptibility of substituted anilines and phenols to enzymatic coupling is enhanced by electron donating substituents on the aromatic ring, which increase electron density at the reaction centers, i.e. the $-NH_2$ and -OH groups, and stabilize the positively charged transition state (Bordeleau and Bartha 1972, Berry and Boyd 1984). Chemicals which are thus incorporated in organic matter, loose their original chemical and biological activity, and hence, are less available, less toxic and less mobile than the "free" compounds (Bollag 1991). However, macromolecular components of dissolved organic matter form colloids in soil which have the potential to serve as carriers facilitating the transport of contaminants which bind to organic matter (McCarthy and Zachara 1989, Dunnivant et al 1992). Thus, the incorporation of chlorinated compounds in macromolecular precursors of organic matter may initially increase instead of decrease their mobility. The stability of soil colloids is determined by a complex interaction between physical and chemical conditions (McCarthy and Zachara 1989). Colloids moving through soil become exposed to varying environmental conditions which may lead to their deposition, decomposition and the release of bound chemicals. Moreover, oxidative coupling reactions can also yield by far more hazardous products than the substrates, like polychlorodibenzodioxins and -dibenzofurans (Svenson et al 1989, Öberg et al 1990, Fig. 8.5).

Little is known of the biodegradability of pollutants which are chemically bound to soil organic matter. It has been postulated that, once pollutants have been incorporated in organic matter, they may be considered harmless and will biodegrade in a similar fashion as soil organic matter, with a half-live of more than 500 years (Bollag 1991). An initial step in the biotransformation of organic matter is the release of basic components like phenols, anilines, and benzoic acids as a result of fungal activity. Then, these molecules are degraded rapidly by other micro-organisms (Calderbank 1989). There is indeed some evidence that soil bound pollutants are mineralized via such a pathway (Haider and Martin 1988). Degradation rates may be increased by stimulating microbial activity in soil via the addition of easily degradable

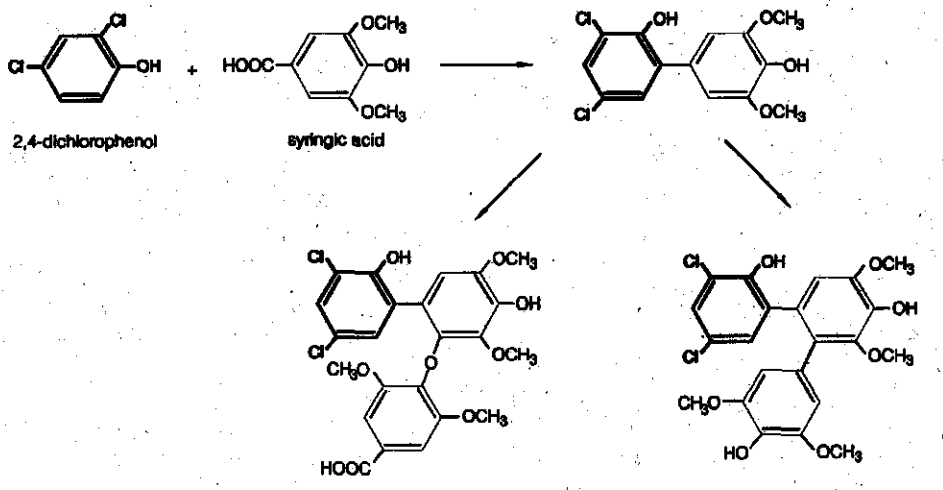

Figure 8.4: Suggested hybrid products resulting from the combined incubation of syringic acid and 2,4-dichlorophenol with a fungal laccase (Bollag et al 1980).

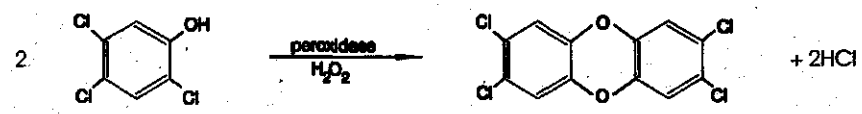

Figure 8.5: Formation of 2,3,7,8-dibenzo-p-dioxin from 2,4,5-trichlorophenol after hydrogen peroxide/peroxidase treatment (Svenson et al 1989, Öberg et al 1990).

organic carbon (Mac Rae 1986). Light may stimulate a sequential photochemical and microbial degradation of organic molecules bound to humic acid (Amador et al 1989). In the top soil, such a mechanism could lead to the breakdown of small organic molecules which are initially formed via oxidative coupling.

It has been proposed to treat contaminated soil with oxidative enzymes to incorporate man-made anilines and phenolic compounds in organic matter, thereby reducing their bioavailability and toxicity (Klibanov et al 1980, Klibanov et al 1983, Berry and Boyd 1985, Bollag et al 1988, Shannon and Bartha 1988, Bollag 1991, Bollag and Myers 1992). However, based on present knowledge, it is not possible to predict the effectiveness of oxidative coupling with regard to the decontamination of soil.

FUTURE PERSPECTIVE

A limited availability of organic pollutants for biodegradation may be caused by physiological or thermodynamic factors or from the presence of undissolved pollutants, slow desorption rates, or oxidative coupling to soil organic matter. In combination, all these factors result in

reduced biotransformation rates. In addition, slow desorption and coupling to organic matter result in high residual concentrations after bioremediation.

Dissolution rates may be enhanced by dispersion of the pure component through the soil, and by the addition of surfactants that increase its maximum solubility. No effect of surfactants on residual concentrations are to be expected however, because these are mainly the result of slow desorption and coupling to organic matter. Removal of desorption limitation can be achieved by pulverization of the soil, which leads to a decrease of diffusion distances, and the disappearance of highly tortuous micropores. However, such an approach seems not to be feasible in an *in-situ* bioremediation scheme.

The role of coupling of pollutants to soil organic matter in bioremediation, is still unclear. On one hand, it can be argued that thus immobilized pollutants are not hazardous anymore, because they have lost their chemical and biological activity. On the other hand, macromolecules of dissolved organic matter may form colloids which are highly mobile and may thus serve as carriers for pollutants which are coupled to them. The application of enzymatic oxidative coupling as a bioremediation technique, requires that more is known of the potential remobilization of pollutants coupled to soil organic matter.

So, we have to face the fact that high residual concentrations remain after bioremediation of "aged" pollutions, due to strong sorption and coupling to organic matter, unless special measures are being taken to mobilize the pollutants. In *ex-situ* remediation, the soil may be pulverized to increase biotransformation rates and decrease potential residual concentrations. It is imaginable to remove the mobile fraction of pollutant in a relatively short time via an intensive biological *in-situ* treatment, leaving the immobilized fraction where it is, trapped in the inside of soil aggregates, where it is biologically and chemically inactive. Then, in a second stage, an extensive treatment should suffice to monitor and control pollutants that are slowly desorbing from the soil aggregates. One approach could be to monitor concentration levels in the macropores continuously and to stimulate biotransformation by the addition of nutrients, as soon as some critical level is reached. Another could be to apply a slow pump-and-treat method continuously. Treatment in the second stage can be stopped as soon as total pollution concentrations in the soil are below acceptable limits.

Since long exposure times have a negative effect on the expected result of bioremediation, new soil pollutions should be treated biologically as soon as possible to achieve optimal results. This implies that biological soil treatment should be included in activities which almost inherently lead to soil pollution with organic chemicals.

NOTATION

Subscripts c and b are used to distinguish contaminant and bacteria.

A solid/water contact surface area (L^2)

B cell dry weight (M_b)

C liquid concentration ($M_c \cdot L^{-3}$)

C_b bulk contaminant concentration ($M_c \cdot L^{-3}$)

C_{max} maximum water solubility ($M_c \cdot L^{-3}$)

D_{aq} aqueous diffusion coefficient ($L^2 \cdot T^{-1}$)

D_{eff} effective intra-particle diffusion coefficient ($L^2 \cdot T^{-1}$)

f_{oc} fraction organic carbon

$F_{r=R}$ flux per unit area at the cell surface ($M_c \cdot T^{-1} \cdot L^{-2}$)

F_{tot} total flux at the cell surface ($M_c \cdot T^{-1}$)

J dissolution rate (

J_{max} maximum dissolution rate (

K mass transfer coefficient governing dissolution ($L \cdot T^{-1}$)

K_{oc} partition coefficient for sorption of hydrophobic organics to soil organic matter ($L^3 \cdot M_c^{-1}$)

K_p partition coefficient for sorption of hydrophobic organics to soil ($L^3 \cdot M_c^{-1}$)

m maintenance coefficient ($M_c \cdot M_b^{-1} \cdot T^{-1}$)

q substrate uptake rate ($M_c \cdot T^{-1}$)

θ_a porosity of the sorbent

R cell radius

r radial co-ordinate (L)

ρ_b cell dry weight density ($M_b \cdot L^{-3}$)

ρ_s solid phase density ($M_s \cdot L^{-3}$)

S sorbed concentration ($M_c \cdot M^{-1}$)

t tortuosity factor

Y observed yield ($M_b \cdot M_c^{-1}$)

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SYNOPSIS

Hydrophobic organic contaminants like DDT, Polychlorobiphenyls (PCB's) and polyaromatic hydrocarbons (PAH's), have been detected all over the world. They tend to accumulate in the atmosphere and in the soil as a result of their physical and chemical properties. Breakdown mainly proceeds by (photo)chemical reactions in the atmosphere and via microbial transformation in the soil. Microbial transformation can be viewed as part of the ecological process of decomposition, that is, the remineralization of organic material by biota. This Chapter discusses the ecological significance of biotransformation and the dependence of biotransformation rates on environmental conditions, and suggests ways to improve the effectiveness of biological soil remediation techniques.

Contaminant cycling in ecosystems

Chemicals are released into the environment by human activities. Normally, they enter the abiotic part of the ecosystem which may be viewed as a contaminant pool (Fig. S.1). Biota take up contaminants directly from the abiotic environment e.g. via leaves or the skin, or ingest them by feeding on a lower trophic level. Organisms have systems at their disposal to excrete or detoxify contaminants. Excretion brings contaminants back to the contaminant pool, while detoxification results in a decontamination as indicated in Fig. S.1.

Plants and animals are not always able to detoxify or excrete contaminants after uptake. The inability of organisms to handle xenobiotic compounds may have several causes. One example is the absence of appropriate enzymes to transform the compounds, another the accumulation in (animal) fat tissue before excretion or enzymatic transformation has taken place. Contaminants accumulate in the food chain when organisms are not able to detoxify or excrete them. Accumulation is indicated by the use of different grey shades in Fig. S.1.

The population of "decomposers" (Fig. S.1) is specialized in the uptake and conversion of all kinds of dead organic material, like for instance dead animals and plant debris. Decomposers are crucial for the functioning of ecosystems because they recycle nutrients to the nutrient pool. Contaminants which are accumulated in the tissue of organisms are recycled to the contaminant pool simultaneously. Some bacteria and fungi are able to detoxify and mineralize man-made organic compounds like chlorinated benzenes and polyaromatic hydrocarbons. Thus, they prevent their accumulation in the environment. These micro-organisms may therefore be viewed as the "decontaminators" of ecosystems (Fig. S.1). Many micro-organisms live in soil and ground water where hazardous compounds may accumulate. Microbial transformation is the only mechanism leading to the effective detoxification of such compounds. Therefore, it is of interest to know under which environmental conditions biotransformation is inhibited or stimulated. The potential of micro-organisms to transform contaminants under various environmental conditions is discussed in the following together with the factors governing exposure of micro-organisms to contaminants in soil and ground water.

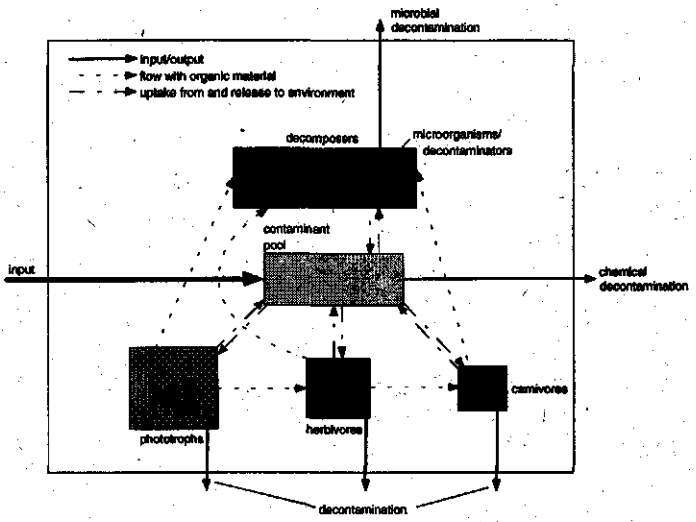


Figure S.1: *Cycling of hydrophobic organic contaminants in ecosystems. Input to the system occurs as a result of human activities. Output occurs via chemical or biological pathways resulting in the formation of harmless products or in complete mineralization. Micro-organisms that are able to transform and mineralize hazardous organic compounds may be viewed as "decontaminators" and belong to the ecological group of the decomposers.*

Potential of micro-organisms to transform organic contaminants

The capacity of micro-organisms to detoxify anthropogenic chemicals under similar environmental conditions is variable among various habitats. This may be related to previous exposure of the micro-organisms to the compound under consideration. An adapted microflora capable of converting and mineralizing new compounds may evolve after a long exposure time. The microflora in a not pre-exposed environment may not be able to detoxify the same compound. Dichloropropene and 2,4-D (dichlorophenoxy acetic acid) are examples of pesticides that micro-organisms "learned" to transform. Degradation of these compounds in the field can be so rapid nowadays that their effectiveness as pesticide is strongly reduced. As a result, farmers have to apply considerably larger amounts of these pesticides than was necessary in the early times of their use.

Many non-chlorinated organic compounds can be mineralized by aerobic bacteria. Well documented examples are simple aromatic compounds like benzene, toluene, and xylenes. More complicated aromatic structures like PAH's are also susceptible to aerobic degradation. Heavily chlorinated compounds are not readily degraded under aerobic conditions. However, anaerobic bacteria have a great potential to dehalogenate all kinds of such chemicals. Dehalogenation changes the environmental impact of the parent compounds considerably. Partly dechlorinated compounds are often more toxic and more mobile than the original compounds. The carcinogenic compound vinylchloride for example, may arise from the anaerobic dechlorination of tetra- and trichloroethene (PER and TRI). The anoxic

transformation products are often biodegradable under aerobic conditions. The increased mobility of the more toxic products allows them to travel to aerobic environments where they can be mineralized. Thus, the anaerobic process of dehalogenation may be an important mechanism to initialize the complete mineralization of heavily chlorinated contaminants in the subsurface environment.

Most of the information regarding the potential of micro-organisms to degrade organic contaminants is obtained from laboratory studies at 20°C. Studies carried out at temperatures down to 4°C, reveal only a slight temperature dependency of aerobic biotransformation rates. Anaerobic dehalogenation rates are reduced and intermediary dehalogenation products accumulate at lower temperatures. It seems that activities of aerobic micro-organisms involved in these processes are less dependent on temperature than those of anaerobes. Therefore, the aerobic removal rates of non- or partly halogenated compounds may be similar in summer and in winter in natural systems, while heavily halogenated compounds will tend to persist more in winter because of the reduced activity of anaerobes.

A microscopic view of soil pollution and micro-organisms

A picture of a versatile microbial community that is able to transform and mineralize a variety of hazardous organic compounds arises from the previous section. Nevertheless, biodegradable organic contaminants can persist in soil for decades. The microbial transformation rate of an organic compound is strongly affected by the potential uptake rate which is influenced by the transport rate to individual micro-organisms. The very slow *in situ* biotransformation is probably caused by the properties of the soil matrix surrounding the micro-organisms which reduces the transport rate. The microscopic spatial distribution of contaminants and micro-organisms will affect biotransformation rates in soil. This paragraph discusses how a spatial separation between micro-organisms and contaminants may develop in case of pollutions from point and non-point sources.

Soil is polluted from point sources like for instance accidental spills and landfills (local pollution), or from non-point sources like atmospheric deposition and application of pesticides (diffuse pollution). The general characteristic of a local pollution is the presence of high contaminant concentrations in a small volume of soil (Fig. S.2A, upper part). Diffuse pollution is characterized by low contaminant concentrations over a wide area (Fig. S.2B, upper part). The lower part of Fig. S.2 schematically shows the local distribution of micro-organisms and contaminants in both situations. Bacteria are normally present inside soil aggregates. Low concentrations of contaminants flow around these aggregates in the case of diffuse pollution (Fig. S.2B). Local pollution initially contaminates pores around soil aggregates. The easily accessible part in wide pores may be biotransformed rapidly until nutrients become exhausted. This leads to a rapid growth of bacteria in the wide pores. Pollutants which are not biotransformed initially will diffuse into the aggregates. Thus, a situation arises with relatively

high numbers of bacteria surrounding contaminated aggregates (Fig. S.2A). Degradation activity is drastically reduced as a result of spatial separation. A similar situation may arise when spots containing pure contaminant exist, where no biological activity is possible anymore. Hence, micro-organisms and contaminants are spatially separated both in the case of local and diffuse pollution. Biotransformation can only take place after diffusion of contaminant through the soil matrix to the micro-organisms.

Computer calculations based on the concept presented in Fig. S.2 show that intra-aggregate processes of sorption and diffusion are of primary importance in determining the kinetics of biotransformation in soil. Effective diffusion rates in soil aggregates can be up to 1-10 orders of magnitude smaller than in water, depending on the characteristics of the soil matrix. As a consequence, biotransformation rates in different soils are subject to the same variation. When the diffusivity in soil aggregates is small, a steep concentration gradient is needed to maintain a flux of nutrients and contaminants that is sufficient to sustain microbial activity. As soon as the contaminant concentration drops below the value that is needed to maintain the gradient, biotransformation will stop. This threshold concentration is inversely proportional to the effective diffusion rate of contaminant. So, residual concentrations after biotransformation are expected to differ by many orders of magnitude, just like the biotransformation rates do.

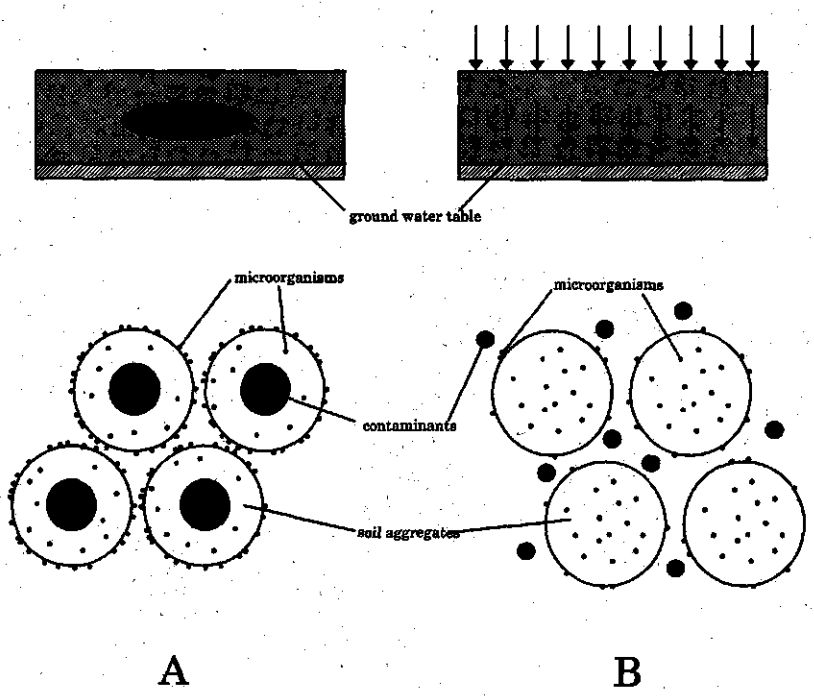


Figure S.2: Schematic drawing of "aged" local (A) and diffuse (B) pollution at a macro- (upper part) and microscopic (lower part) level.

Optimization of bioremediation techniques to relieve limitation of biotransformation

Limitation of biotransformation is not only the result of slow diffusion rates in soil, but may also be due to physiological or thermodynamic factors, to the presence of undissolved pollutants, or to the coupling of pollutant to soil organic matter via covalent bonds (bound residue formation). All these factors may result in reduced biotransformation rates. A strong association between the pollutants and the soil matrix especially develops at sites which have been polluted for years or decades already. Bound residue formation and extremely slow diffusion into small, highly tortuous pores have both been proposed as causes for this strong association. As a result, bioremediation is particularly difficult for these so-called "aged" pollutants (Fig. S.2A).

Dissolution rates of undissolved pollutants may be enhanced by dispersing the pure component through the soil, and by the addition of surfactants that increase maximum dissolution rates. These methods have been shown to increase biotransformation rates in practice. However, surfactants do not dissolve bound residues which are covalently bound to organic matter. In addition, they do not increase diffusion rates in small, highly tortuous pores. The existence of bound residues and the extremely slow diffusion rates are causes for high residual concentrations that remain after bioremediation. Therefore, surfactants are not expected to decrease these residual concentrations.

The possible application of procedures that enhance bound residue formation as means of bioremediation is disputed. It can be argued that pollutants which are present as bound residues are not hazardous anymore, having lost their specific chemical characteristics. Thus, they have also lost their biological activity. However, pollutants not only bind to the humus fraction of the soil, but also to dissolved or colloidal fractions of soil organic matter. This may lead to a mobilization of pollutants instead of the intended immobilization. In addition, dioxin-like products are formed when pollutants with phenolic or carboxylic groups bind to each other. The use of applications involving enhancement of bound residue formation requires that the possible hazards are better understood and that ways are provided to prevent them.

Considerable residual concentrations will always remain after bioremediation of "aged" pollutions, due to strong sorption and incorporation in organic matter, unless special measures are taken to mobilize the pollutants. In an *ex situ* scheme, the soil may be pulverized to increase biotransformation rates and decrease residual concentrations. It is imaginable to remove the mobile fraction of pollutant in a relatively short time via a biological treatment during *in situ* remediation. The residual immobilized fraction which is trapped inside soil aggregates is biologically and chemically inactive. It should be sufficient to monitor and control pollutants that are slowly desorbing from the soil aggregates in an "after-care" phase. An approach may be to monitor the concentration level in the macropores continuously and to stimulate biotransformation by the addition of nutrients as soon as some critical level is reached. An alternative would be to apply a slow pump-and-treat method continuously. The

after-care phase can be stopped as soon as total pollution concentrations in the soil are below acceptable limits.

New pollutions have to be treated biologically as soon as possible to achieve optimal results because long contact times between pollutants and soil have a negative effect on the expected result of bioremediation. A possible strategy is to include biological soil treatment in human activities which almost inherently lead to soil pollution with organic chemicals. Thus, the establishment of a strong association between contaminants and soil can be prevented. This strategy has shown to be effective at tank stations where leaking of benzine or diesel is unavoidable.

Introduction of specialized bacteria is used as a strategy to enhance the biotransformation of compounds that are not degraded by the indigenous microflora. The success of the addition of micro-organisms depends on their ability to reach the contamination, to survive, and to carry out the desired reaction. A better understanding and control of the transport of bacteria in soil and ground water will help to optimize techniques for bioremediation which employ introduced bacteria. Surface characteristics of bacteria and soil particles together with the ionic strength of the flowing water control the transport of bacteria under saturated flow conditions. The adhesion of bacteria to soil particles is positively correlated with the hydrophobicity of bacteria and the ionic strength of the flowing water. Hence, the ionic strength of the water in which bacteria are introduced can be used to control microbial transport and attachment. If a low ionic strength is used, bacteria may travel long distances and disperse around the point where they are introduced. On the other hand, a high ionic strength will generally stimulate the attachment of bacteria to the solid phase and may prevent bacteria from moving away from the polluted site.

Concluding remarks

Microbial transformation is required to achieve detoxification of hydrophobic organic contaminants that accumulate in soil. Micro-organisms can therefore be viewed as a sub-population of the decomposers with a special function, namely detoxification of the environment. The effectiveness of microbial transformation can severely be reduced by the relative immobility of organic compounds in the soil matrix where micro-organisms live. Limitations resulting from slow diffusion can only be removed effectively during *ex situ* remediation, e.g. by pulverizing the contaminated soil. During a biological *in situ* treatment the bulk of contamination can be removed rapidly. The treatment should be followed by an after-care period in which the possible leaking of the residual amount is monitored to be able to take measures if necessary.

From an ecological stand-point, it can be argued that production and release rates of toxicants have to be smaller than *in situ* biotransformation rates to keep environmental pollution within acceptable limits. Treatment as close to the source as possible during the

manufacturing and use of chemicals will be an important strategy to reach such a goal. The use of pesticides should be regulated such that the amount applied in a growth season is completely transformed *in situ* in the same season.

ACKNOWLEDGEMENT

Discussions with Clay L. Montague were very helpful in the conception of the Synopsis.

MIKROBIOLOGISCHE AFBRAAK: EEN NATUURLIJK ONTGIFTINGSPROCES

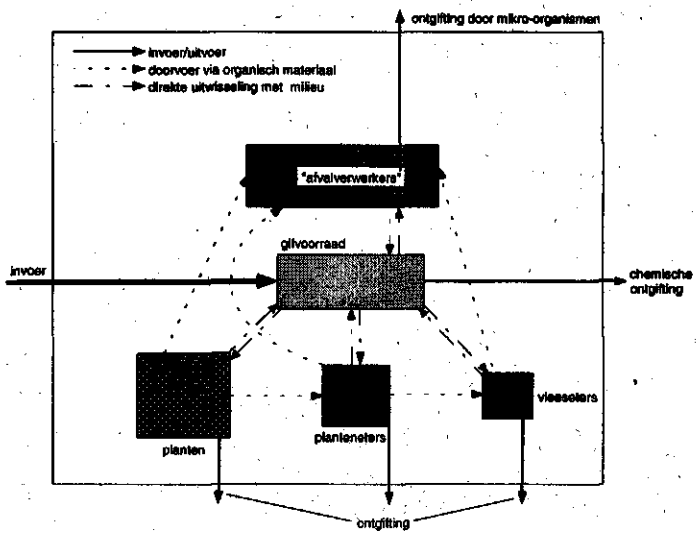
De kringloop van schadelijke stoffen in de natuur

Als gevolg van menselijke aktiviteiten belanden vele schadelijke stoffen in het milieu. Afhankelijk van de wijze van gebruik komen ze terecht in de bodem, het water of in de lucht. Een aantal persistente schadelijke organische verbindingen is inmiddels wereldwijd verspreid. Klassieke voorbeelden zijn dichloor-diphenyl-trichloorethaan en polychloorbiphenylen - beter bekend als DDT en PCB's - die zijn aangetroffen op de Noord- en Zuidpool en in vissen die kilometers diep in de oceanen leven. Het probleem van deze chemikaliën is niet alleen dat zij zich zo sterk verspreiden, maar ook dat zij de neiging hebben zich op te hopen in dieren die aan de top van voedselketens staan (bio-akkumulatie, fig. S.1). Zo heeft massale sterfte onder zeeroofvogels geleid tot een verbod van het gebruik van DDT in de westerse wereld en massale sterfte onder zeehonden tot een herbezinning op de toepassing van PCB's. Bio-akkumulatie treedt op als planten en dieren niet in staat zijn om schadelijke stoffen uit hun lichaam te verwijderen of af te breken. Hieraan kunnen verschillende oorzaken ten grondslag liggen, zoals het ontbreken van enzymen die de giftige stoffen kunnen afbreken (ontgiften) of opslag in dierlijk vet voordat uitscheiding of ontgifting heeft kunnen plaatsvinden. Dit heeft tot gevolg dat de konsentratie in plant en dier alleen maar kan toenemen in de tijd dat ze leven. Planteneters en roofdieren hebben hier in versterkte mate last van omdat zij met hun voedsel tegelijkertijd ook de schadelijke stoffen opeten (fig. S.1).

Een speciale groep organismen heeft tot taak het afval op te ruimen. Zij vormen samen de biologische afvalverwerkers van de natuur (fig. S.1). Dit zijn onder andere dieren, zoals mieren die in al hun bedrijvigheid veel plantaardig en dierlijk afval opruimen. Bij deze opruiming komen persistente stoffen die in het weefsel van de dode organismen waren opgeslagen, weer terug in het milieu. Gelukkig behoren ook bakteriën en schimmels (samen ook wel aangeduid als mikro-organismen) die bekend staan om hun vermogen tal van organische stoffen te kunnen opnemen en "verbranden", tot de afvalverwerkers. Sommige mikro-organismen blijken zich te hebben gespecialiseerd in het onschadelijk maken van door de mens gemaakte giftige stoffen (ontgifting door mikro-organismen). Een groot aantal daarvan leeft in de ondergrond, waar bepaalde schadelijke organische stoffen zich ophopen en onder andere een bedreiging vormen voor de kwaliteit van het grondwater.

Een mikroskopische kijk op bodemverontreiniging en mikro-organismen

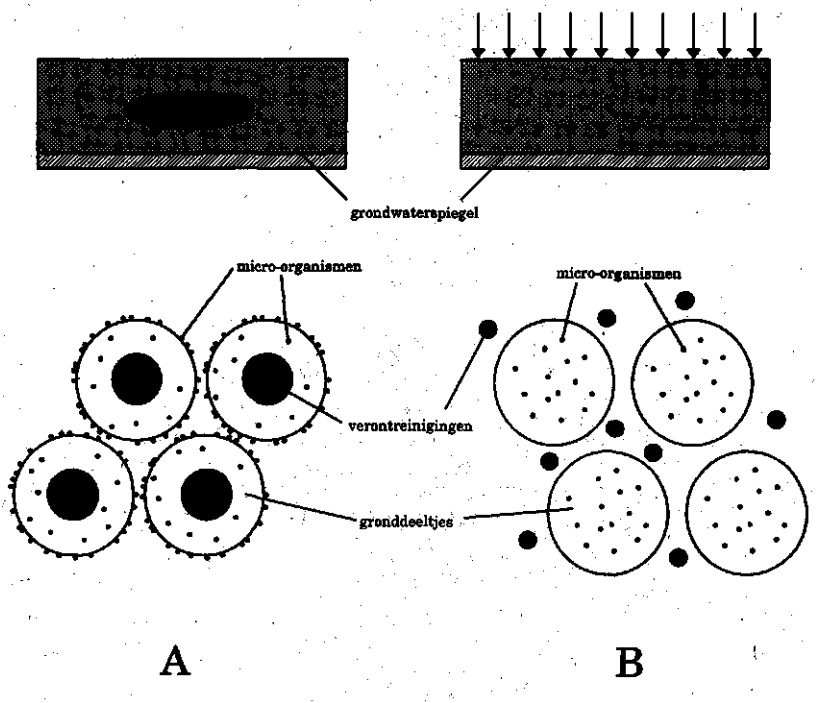
Naast DDT en PCB's worden door de mens nog tal van andere stoffen gebruikt met soortgelijke eigenschappen. Dit zijn onder andere bestrijdingsmiddelen voor de landbouw en organische oplos- en schoonmaakmiddelen (bv. tetra- en trichlooretheen, beter bekend als PER en TRI). Veel van deze stoffen kunnen door mikro-organismen onschadelijk worden gemaakt, maar desondanks kunnen ze tientallen jaren persisteren in de bodem en in het grondwater. Waarom doen de mikro-organismen in de ondergrond nu niet gewoon hun werk en breken deze stoffen af, zoals ze dat in het laboratorium zo netjes doen? Het antwoord op deze vraag moet worden gezocht in de omgeving waarin mikro-organismen in de ondergrond



Figuur S.1: *Kringloop van persistente schadelijke stoffen in de natuur. De mate van ophoping in elk kompartiment is aangegeven door middel van verschillende grijstinten.*

moeten leven en de moeilijkheden die zij als gevolg daarvan ondervinden om te kunnen overleven.

Laten we allereerst eens kijken hoe bodemverontreiniging zich manifesteert. De aanwezigheid van grote hoeveelheden schadelijke stoffen op bepaalde lokaties, bijvoorbeeld onder vroegere gasfabrieken en in stortplaatsen, is de meest in het oog springende vorm. Daarnaast komt ook een veel meer gespreide bodemverontreiniging voor, bijvoorbeeld als gevolg van het besproeien van gewassen in de landbouw of bij tuinonderhoud, de uitstoot van schadelijke stoffen die neerslaan in de nabije en verre omgeving van fabrieken of vuilverbrandingsinstallaties, en onbedoeld weglekken tijdens transport. Het verschil tussen beide wordt schematisch weergegeven in fig. S.2. In het eerste geval is sprake van een sterk gekonsentreerde vervuiling in een (relatief) schone omgeving (fig. S.2A, bovenste deel). In het tweede geval wordt de bodem van boven af voortdurend gevoed met kleine hoeveelheden verontreiniging die met het bodemwater meegevoerd worden naar het grondwater (fig. S.2B, bovenste deel). Wat heeft dit nu voor konsekwenties voor de mikro-organismen ter plaatse waarvan wij zo graag zouden zien dat ze onze rommel weer opruimen? Om dit te begrijpen moeten we ons verplaatsen in de leefwereld van een mikro-organisme. Hun blik reikt niet verder dan een fraktie van een millimeter en daarom is het onderste deel van fig. S.2 een uitvergroting van een detail van de twee bovenste tekeningen, waarin de verspreiding van mikro-organismen én van de verontreiniging schematisch is getekend.



Figuur S.2: *Oude, lokale verontreinigingen lijken vaak te bestaan uit een kontinue massa van schadelijke stoffen in de grond (A, bovenste tekening). Op mikroskopische schaal blijkt dat de verontreiniging aan de binnenkant van bodemaggregaten aanwezig is terwijl de mikro-organismen op de buitenkant zitten (tekening onder). Bij het transport van relatief kleine hoeveelheden van verontreinigingen naar grondwater (B, bovenste tekening) doet zich de omgekeerde situatie voor. Hier bevinden de bakteriën zich juist in de aggregaten terwijl de verontreinigingen met de waterstroom langs de buitenzijde worden gevoerd. In beide gevallen bemoeilijkt de ruimtelijke scheiding tussen mikro-organismen en verontreiniging de biologische afbraakprocessen.*

Het eerste wat opvalt op deze kleine schaal, is dat het niets overheerst. Er zijn veel meer "schone" dan verontreinigde plekken en ook mikro-organismen komen slechts sporadisch voor. In werkelijkheid is dit nog extremer dan in de tekeningen. Daarnaast is het verschil tussen de lokale, sterk gekonsentreerde verontreiniging en de met het bodemwater meegevoerde verontreiniging ook op deze schaal zichtbaar. In sterk verontreinigde lokaties wordt de verontreiniging die zich tussen aggregaten bevindt relatief snel afgebroken. Als gevolg daarvan bevindt de verontreiniging zich na verloop van tijd alleen nog binnenin aggregaten van bodemdeeltjes, terwijl de overlevende mikro-organismen zich alleen nog in het buitenste deel ophouden, in de schonere zones (fig. S.2A). Daartegenover staat dat mikro-organismen zich juist aan de binnenkant van aggregaten bevinden in de situatie waar verontreinigingen met het water door de bodem worden getransporteerd (fig. S.2B). De beide situaties komen overeen wat betreft het bestaan van een ruimtelijke scheiding tussen verontreiniging en mikro-organismen. Dit heeft konsekwenties voor de snelheid waarmee de verontreiniging door de mikro-organismen afgebroken kan worden. De enige wijze waarop het

transport van de schadelijke stoffen naar de mikro-organismen kan plaatsvinden is diffusie, het mechanisme dat ervoor zorgt dat een druppel inkt die voorzichtig in een kopje water wordt gelegd zich tergend langzaam door het water verspreidt totdat er sprake is van volledige menging. Het behoeft geen betoog dat diffusie in grond nog veel langzamer gaat, vaak meer dan een miljoen keer langzamer dan in water. Dit is de oorzaak dat de afbraak van schadelijke stoffen in de ondergrond zoveel langzamer gaat dan wij zouden verwachten op grond van onze kennis over de kapaciteiten van de mikro-organismen. Een ander gevolg is dat mikro-organismen niet alle verontreiniging verwijderen, maar er na verloop van tijd de brui aan geven omdat de aanvoer zo langzaam is dat ze niet meer het vermogen hebben de verontreiniging nog aan te pakken.

Biologische reiniging van bodem en grondwater

Wat is nu de betekenis van dit alles voor de rol die mikro-organismen kunnen vervullen bij het opruimen van schadelijke stoffen? In een bodem waar de mens niet ingrijpt en schadelijke stoffen diffuus voorkomen zoals geschetst in Fig. S.2B, wordt het natuurlijk afbraakproces sterk geremd door het langzame transport van de verontreinigingen naar de mikro-organismen. Als gevolg hiervan kan een aanzienlijk groter gedeelte dan wordt voorspeld op grond van de uitkomsten van laboratoriumtoetsen, naar het grondwater uitspoelen. Het lijkt daarom van belang om in modellen waarmee uitspoeling naar grondwater wordt voorspeld, rekening te houden met de ruimtelijke scheiding tussen mikro-organismen enerzijds en de schadelijke stoffen anderzijds.

Biologische reiniging van grond zou zich sterk moeten richten op het verkleinen van de van nature aanwezige afstand tussen mikro-organismen en verontreinig. Dit zou bereikt kunnen worden door de grond weg te nemen en te vermalen voor of tijdens het reinigingsproces. Het perspektief voor volledige reiniging op de korte termijn is slecht indien men de grond liever op zijn plaats laat liggen dan af te voeren. In dat geval is het wel mogelijk om snel de ergste verontreiniging biologisch te verwijderen, maar men blijft zitten met een rest die slechts zeer langzaam, d.w.z. in een tijdspanne van vele jaren, verdwijnt. Hoe erg dat is, hangt af van de kontekst waarbinnen de reiniging geschiedt. Men moet zich realiseren dat een verontreiniging weinig schade aanricht zolang hij netjes is verpakt in een bodemaggregaat. Men zou dus kunnen overwegen in korte tijd de ergste verontreiniging te verwijderen en vervolgens het vrijkomen van verontreinigingen uit de lokatie te bewaken en in te grijpen indien bepaalde grenzen worden overschreden, totdat de grond geheel schoon is. Het nadeel van een dergelijke aanpak is dat men lange tijd aktief moet blijven, zij het met een geringe inspanning. Een voordeel is dat men de grond op zijn plaats kan laten en weer in gebruik kan nemen na de eerste fase van intensieve reiniging. Misschien nog belangrijker is dat de struktuur van de grond bij een dergelijke aanpak slechts weinig wordt aangetast, waardoor het zijn natuurlijke funktie kan blijven vervullen. Er zijn geen niet-biologische reinigingstechnieken

voorhanden die deze voorwaarde vervullen. Het maakt de mikrobiologische aanpak bij uitstek geschikt voor reiniging van grond in natuurgebieden waar in het algemeen geen sprake is van een direkt gevaar voor de volksgezondheid en waar de biologische funktie van de grond juist zwaar telt.

Konklusies

Bakteriën in de ondergrond spelen een belangrijke rol bij het opruimen van schadelijke stoffen in de natuur. Eigenlijk zou aan alle giftige stoffen die wij mensen in het milieu verspreiden, als eis moeten worden gesteld dat het natuurlijke afbraakproces (de ontgifting) zo snel verloopt dat schadelijke effekten in de natuur achterwege blijven. In het geval van DDT en PCB's is dit overduidelijk niet het geval en dit is dan ook de reden voor het verbieden van respektievelijk de herbezinning op het gebruik van deze stoffen. Er zijn echter nog tal van stoffen waarvan we eigenlijk niet weten of aan deze voorwaarde wordt voldaan. Zijn we nu in staat te voorspellen hoe groot de afbraaksnelheid onder natuurlijke omstandigheden zal zijn en hoe groot de rest zal zijn die niet meer afgebroken kan worden? Helaas is het antwoord voorlopig nog: "nee". Het is echter wel duidelijk dat de afbraak van veel stoffen tijdens het transport naar grondwater van nature zeer veel langzamer verloopt dan wij zouden willen. Een getalsmatige invulling vraagt onderzoek dat zich specifiek richt op de vraag hoe de hier geschetste mikroskopische konsepten kunnen worden toegepast in een natuurlijk, makroskopisch systeem.

Het perspektief voor biologische reinigingsmethoden van sterk verontreinigde grond lijkt beter te zijn. Het is duidelijk dat het afbraakproces verbeterd kan worden door de verontreinigingen geforceerd uit de grond te verwijderen, bijvoorbeeld door vermalen van de grond. Indien een dergelijke aanpak niet mogelijk of gewenst is, kan worden overwogen het snel afbreekbare gedeelte van de verontreiniging biologisch te verwijderen en het restant te laten zitten. Het is dan wel noodzakelijk om een bewakingssysteem op te zetten om het langzaam vrijkomen van het restant te volgen en in te grijpen als dat nodig blijkt te zijn.

CURRICULUM VITAE

Op 8 november 1960 werd ik geboren onder de naam Tom Nicolaas Pieter Bosma, roepnaam Tommetje; 313 dagen te laat om op eenvoudige wijze de militaire dienst te ontlopen. De geboorte vond net niet in de taxi plaats. Het was één van de weinige momenten in mijn leven dat ik sneller was dan de meeste andere mensen. Bijzaken hebben gedurende mijn hele leven de hoofdzaken gedomineerd. Dit waren achtereenvolgens: spelen (lagere en middelbare school), schaatsen (middelbare school), zigeunermuziek en attraktieve baantjes als student-assistent (studie biologie aan de Landbouwhogeschool), en tennis en vervangende dienst (promotie-onderzoek). Het zal geen verwondering wekken dat zowel de studie als het gereedkomen van het proefschrift meer tijd vergden dan gewoonlijk. Alleen het diploma VWO behaalde ik in de tijd die ervoor staat. Momenteel woont ons mini-gezinnetje in Zwitserland, waar pa en ma wat bijklussen bij het Onderzoekscentrum voor Limnologie van het Zwitsers "Eedgenootschappelijk Instituut voor Drinkwaterbereiding, Zuivering van Afvalwater, en Bescherming van Oppervlaktewater", ook wel bekend als de "Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz", afgekort EAWAG.