7 Use of microorganisms in soil sanitation

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Summary

The persistence of xenobiotic compounds at numerous locations in nature has raised questions about the potential of microorganisms to degrade these pollutants.

In laboratory experiments has been demonstrated that many of these compounds can be degraded by microorganisms. Specific conditions and special microorganisms are often required and the degradation may vary from mineralization to only a limited change in the structure of the molecule. The long persistence in nature of in principle biodegradable compounds suggests that other factors than the interactions between a microorganism and a xenobiotic molecule play a role. In fact, nature, degree and rate of biodegradation are determined by several factors.

One has to do with the xenobiotic compound. Both intrinsic properties like complexity of structure, presence of halogen substituents and physical characteristics determine whether the compound can be degraded.

A second factor are 'environmental' conditions. Redox conditions determine the prevailing microbial species and thus the type of biodegradation reactions. Temperature, concentration and availability are influencing the rate with which a compound is transformed.

7.1 Introduction

The presence of organic compounds is essential for the survival of many microorganisms. Via *metabolic* reactions they are capable to convert an organic molecule into inorganic constituents like CO_2, NO_3^-, SO_4^{2-} and PO_4^{3-} (mineralization). Growth and proliferation of the microorganisms is then a result of the use of the released energy and of the incorporation of a portion of the carbon into biomass. In addition to metabolic reactions, *co-metabolic* reactions may take place. In these reactions, in which only a small alteration of the molecular structure occurs (transformation), neither the energy nor the carbon of the molecule are used by the microorganisms. Co-metabolism of an organic molecule only takes place if in addition other molecules are used for biosynthetic purposes.

In the twentieth century many organic compounds, which have been produced by chemical synthesis for industrial or agricultural purposes (sometimes called *xenobiotics*) have entered the environment. Some of these organic compounds are similar to natural ones, but many are quite different and may never have existed in natural environments. As a consequence, microorganisms may have difficulties to degrade the latter ones. All xenobiotic compounds, of which many are toxic, will eventually show up in the soil. Here they are exposed to enzymatic and non-enzymatic reactions.

Non-enzymatic reactions in the soil can cause only minor changes in the chemical

structure of a compound, while major changes (leading to degradation) have to occur through biological reactions. This paper will deal with a number of aspects which are of influence on the nature and rate with which these xenobiotic compounds can be transformed by microorganisms in soil systems.

7.2 Biodegradation

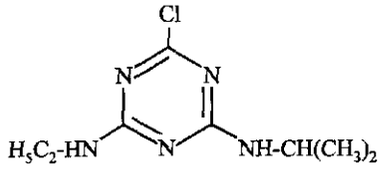
For the ultimate disappearance of xenobiotic compounds in soil and groundwater, they have to undergo biodegradation and not only biotransformation. Although for many xenobiotic compounds has been found that they can indeed be degraded by microorganisms to harmless inorganic end products, many have also been found just to undergo transformations.

Several bacteria have been isolated which can use di- and trichlorobenzenes as only carbon and energy source under aerobic conditions (Schraa et al. 1986, van der Meer et al. 1987, Haigler et al. 1988), with only inorganic compounds as end products. In the absence of molecular oxygen, at low redox conditions, these chlorinated benzenes have been found to undergo reductive dechlorination (Bosma et al. 1988). Monochlorobenzene was formed as a stable end product and additional transformations under these conditions have not yet been witnessed. In general, we can say that highly chlorinated compounds need anaerobic conditions for the transformation to lower chlorinated compounds, before under aerobic conditions mineralization will occur.

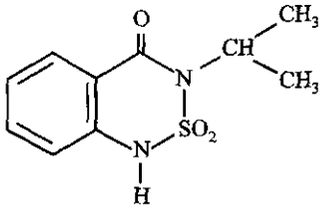
Co-metabolism has also been observed with xenobiotic compounds. Trichloroethylene (TCE) is transformed by methanotrophic bacteria in one reaction step into an epoxide. The enzyme methane monooxygenase is, because of its low specificity, responsible for the reaction. The bacteria do not seem to have any benefit from this one reaction.

If a compound is stable to degradation it is often referred to as being persistent or recalcitrant. This persistence can be due to the structure of the molecule or to the prevailing environmental conditions. Pesticides are in general produced via non-enzymatic reactions and their structure may be just too complex for degradation by the enzymes of microorganisms. Atrazine and bentazon are examples of pesticides, which are notorious soil and groundwater pollutants and which have a 'complex' structure (Figure 7.1).

Environmental conditions include physical conditions (*e.g.* temperature and availability of the compound), chemical conditions (pH, redox potential, concentration of the compound, type of electronacceptor, essential growth factors, etc.) and biological conditions (presence of the desired microorganisms). An initial persistent compound may



2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine)



bentazon

Figure 7.1. Pesticides which seem to be persistent because of their structure.

undergo biodegradation by varying a specific environmental parameter. Tetrachloroethene (PCE), a widespread groundwater contaminant, is persistent in the presence of molecular oxygen, but under anaerobic conditions a complete dechlorination to ethene may take place. In Table 7.1 examples are given of the biodegradation of a number of problematic compounds under aerobic and anaerobic conditions.

In the following paragraphs several factors that determine nature, degree and rate of biodegradation of xenobiotic compounds in soil and groundwater will be discussed. These factors are: structure of the compound, availability in the presence of soil particles, aerobic/anaerobic conditions, concentration of the compound, temperature and water content.

Table 7.1. Examples of what is known about biodegradation under aerobic and anaerobic conditions.

Classes of compounds	Biodegradation*	
	aerobically	anaerobically
Chlorobenzenes		
pentachlorobenzene	-	+ (L)
hexachlorobenzene	-	+ (L)
Chlorophenols		
3,4-dichlorophenol	+ (L)	+ (L)
2,3,5-trichlorophenol	+ (L)	+ (L)
2,3,4,5-tetrachlorophenol	+ (L)	+ (L)
pentachlorophenol	+ (L,F)	+ (L,F)
Chloroanilines		
2-chloroaniline	+ (L)	-
3,4-dichloroaniline	-	-
2,4,5-trichloroaniline	-	-
Polychlorinated biphenyls (PCBs)		
PCB 28	+ (L)	-
PCB 52	+ (L)	?
PCB 138	-	?
Polycyclic aromatic hydrocarbons		
fluoranthene	+ (L)	-
benzo(a)pyrene	+/- (L,V)	-
benzo(ghi)perylene	?	-
Dioxins and furans		
as TCDD equivalents	+ (L)	?

* Biodegradation observed in laboratory- (L) or fieldstudies (F).

7.3 Structure of the xenobiotic compound

Degradation of a xenobiotic compound via existing metabolic routes in microorganisms may take place when the structure of the molecule is identical or shows a strong resemblance to molecules which are normally used for cell synthesis. An inhibition can often be witnessed when the molecule has specific substituents (*e.g.* halogen atoms and methyl groups). The rate of degradation is then mainly determined by the kind and number of substituents and the location in the molecule (Table 7.2).

Table 7.2. Relative conversion rates of different chlorinated benzoates by Alcaligenes eutrophus B9. From Knackmuss 1975.

Substrate	Product (dihydro-dihydroxybenzoate, DHB)	Rate of oxidation of substituted benzoates
benzoate	DHB	1000
2-chlorobenzoate	----	0
2-methylbenzoate	----	0
3-chlorobenzoate	3-chloro-DHB	50
	5-chloro-DHB	100
3-methylbenzoate	3-methyl-DHB	31
	5-methyl-DHB	215
3,5-dichlorobenzoate	3,5-dichloro-DHB	2
3,5-dimethylbenzoate	3,5-dimethyl-DHB	2
4-chlorobenzoate	4-chloro-DHB	1
3,4-dichlorobenzoate	4,5-dichloro-DHB	1
4-methylbenzoate	4-methyl-DHB	4

For compounds with a 'new' structure, enzymes may not be present. A bacterium, capable of using propionate as carbon and energy source, did not possess the enzyme(s) to degrade 2,2-dichloropropionate. Exposure during 120 days to a mixture of propionate and 2,2-dichloropropionate, led to spontaneous genetic changes which resulted in a metabolic degradation of 2,2-dichloropropionate (Senior et al. 1976).

Molecule size is another aspect of the importance of the structure of a compound which is determining the rate of degradation. The influence of the molecule size on the

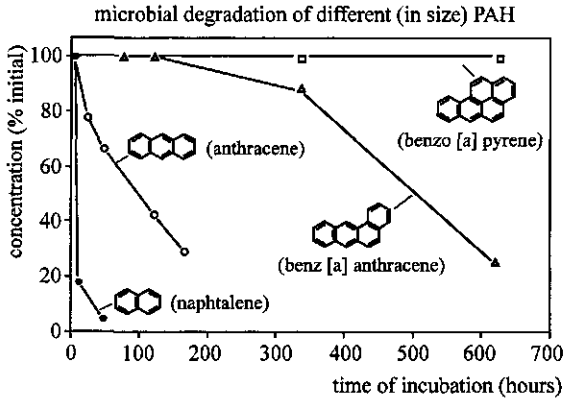


Figure 7.2. Microbial degradation of several polycyclic aromatic compounds.

rate of biodegradation of a number of polycyclic aromatic compounds is shown in Figure 7.2.

An increase in number of aromatic rings leads to a lower degradation rate. Although the simultaneous decrease in solubility has an effect, uptake by the microbial cell and positioning in the active centre of the oxygenase enzyme will be hindered by the increase in molecule size.

7.4 Availability

The distribution of pollutants in the soil is often very irregular. Many compounds are hydrophobic and tend to adsorb to soil aggregates (*e.g.* clay minerals and organic matter). It also seems that in time organic pollutants become an integral part of the organic matrix. Of chloroanilines is known that they can form covalent bonds with humic acids ('bound residues'). In addition, soil pollution with large, irregular particles can take place. Examples are tar globules during oil spills and the formation of calcareous aggregates in a soil in which hexachlorocyclohexane (HCH) wastes were disposed of in the presence of large quantities of lime.

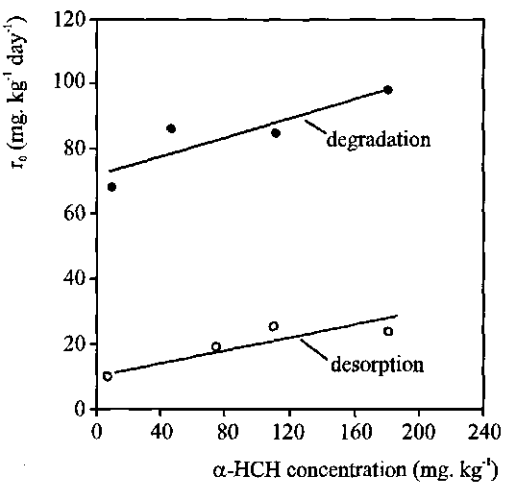


Figure 7.3. Comparison of maximum desorption and biodegradation rates of α-HCH.

It is generally accepted that the uptake of compounds by bacteria proceeds via the waterphase. This was for instance demonstrated by Ogram et al. (1985) for the uptake of 2,4-dichlorophenoxyacetic acid from soil. In a study by Rijnaarts et al. (1990) it was found that within certain limits the degradation rate of α-HCH under aerobic conditions was limited by mass transport in and desorption from soil aggregates and not by the activity of the degrading microorganisms. These results are visualized in Figure 7.3, in which a comparison has been made between the maximum desorption and biodegradation rates of α-HCH at different HCH concentrations in a soil slurry.

7.5 Environmental factors

In addition to the above mentioned availability, other important environmental factors which determine whether biodegradation takes place and what the rate of degradation is are: redox condition, concentration of the xenobiotic compound, presence of nutrients, moisture content, pH and temperature. Degradation only takes place if, and that is partly dependent on the xenobiotic compound, for each factor certain conditions are met.

7.5.1 Redox condition

The presence of a specific electronacceptor determines both the sort of microbial population as the possibility to degradation. The mineralization of a hypothetical chlorinated compound with four different electron acceptors is given in simple reactions in Figure 7.4.

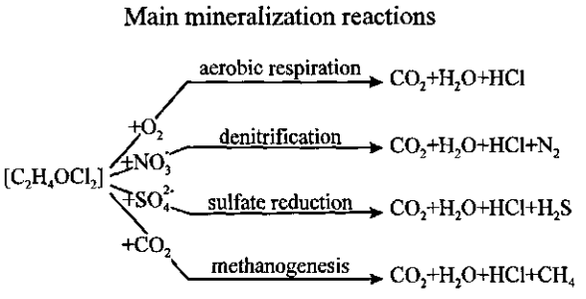


Figure 7.4. Mineralization of a chlorinated compound with four different electronacceptors.

Molecular oxygen is somewhat different from the other electron acceptors, because it does not only function as an acceptor but also as a reactant in oxidation reactions of *e.g.* aromatic compounds (oxygenases).

Some compounds require aerobic conditions for biodegradation (monochlorobenzene), while others are degraded under strict anaerobic conditions (tetrachloroethylene, hexachlorobenzene). In a study by Bachmann et al. (1988a) it was shown that α-HCH could be degraded under both aerobic and methanogenic conditions, while it was persistent in the presence of nitrate and sulfate as electron acceptor (Figure 7.5). There were two big differences between the aerobic and the methanogenic bio-degradation. Under aerobic conditions the degradation was much faster, while also complete mineralization occurred. Under methanogenic conditions several toxic intermediates accumulated (*e.g.* monochlorobenzene).

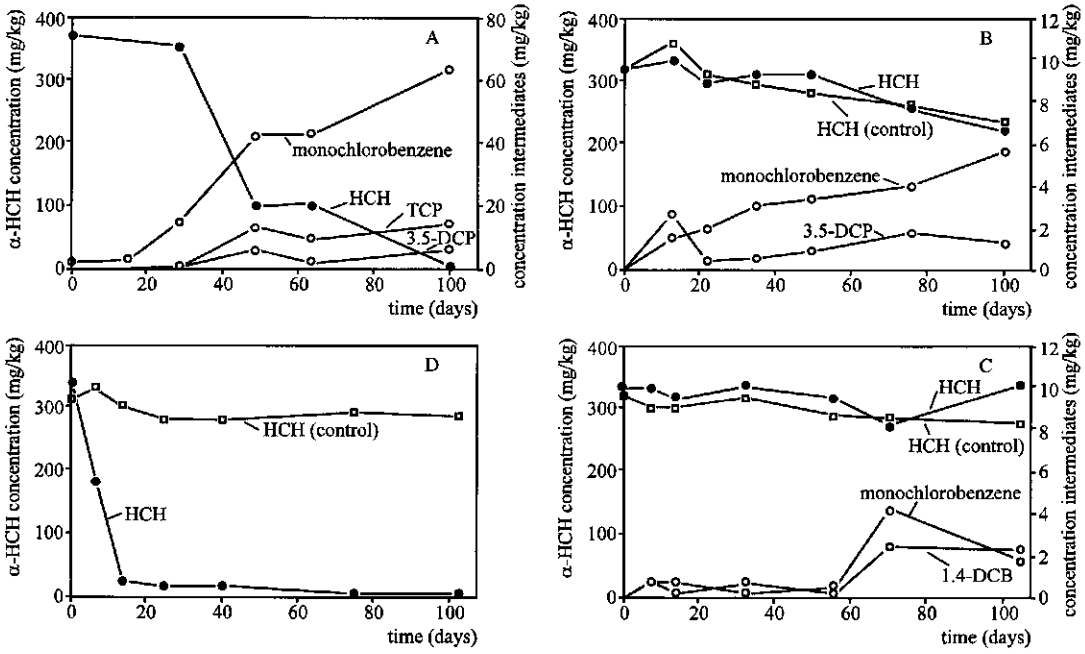


Figure 7.5. Biodegradation of α-HCH in soil slurries under different redox conditions (methanogenic [A], sulfate reducing [B], denitrifying [C] and aerobic [D]).

7.5.2 Concentration

The rate of biodegradation of a xenobiotic compound can in general be described by first order kinetics. This is valid as long as a) the microorganisms use the compound as a carbon and energy source, and b) the availability does not become a limiting factor. Biodegradation may be hampered at high and low substrate concentrations.

At high concentrations the compound can become toxic for the microorganisms (Figure 7.6). A *Corynebacterium* sp., isolated from river Rhine sediment and capable of mineralizing *o*-xylene at a concentration of 80 mg/l, was unable to degrade it at double the concentration (Schraa et al. 1987). At low concentrations, often in the µg/l - ng/l range, there may not be enough energy and carbon available for growth or maintenance. Rest concentrations have been observed for several xenobiotic compounds in soil and groundwater. Initially it was assumed that microorganisms were not able to degrade these compounds at these low concentrations ('threshold concentration'). Recent experiments do suggest that this inability is not a characteristic of the microorganisms, but is caused by not yet fully understood interactions between the microorganisms, the xenobiotic compound and the soil structure.

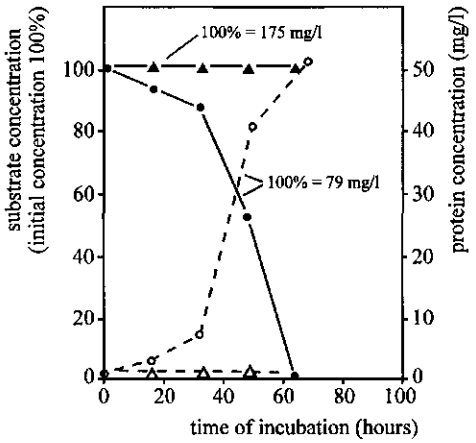


Figure 7.6. Degradation of *o*-xylene by *Corynebacterium* sp. at two different concentrations.

7.5.3 Temperature

This factor influences just as the presence of nutrients (especially nitrogen and phosphorus), moisture content and pH, the general metabolic activities of microorganisms. Within a certain temperature range the optimum activity of soil microorganisms is more or less constant, but outside this range drastic decreases occur. This effect can be seen in Figure 7.7, in which the biodegradation rate of α-HCH as a function of the temperature is given (Bachmann et al. 1988b).

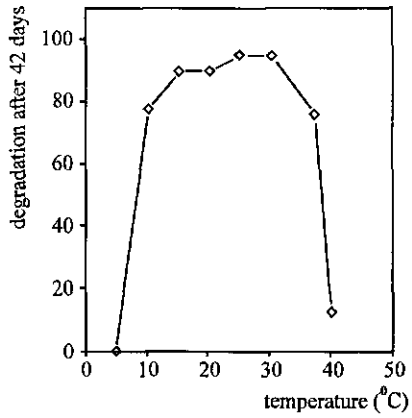


Figure 7.7. Effect of temperature on the aerobic biodegradation of α-HCH in soil.

7.5.4 Water content

Water content is one of the most important factors affecting the growth of microorganisms in natural environments. On one hand there is the water availability for microorganisms, which is determined by the presence of solid substances and surfaces and by the solutes in the water. On the other hand water serves in soil as a transport medium to make nutrients and xenobiotic compounds more accessible to the microorganisms.

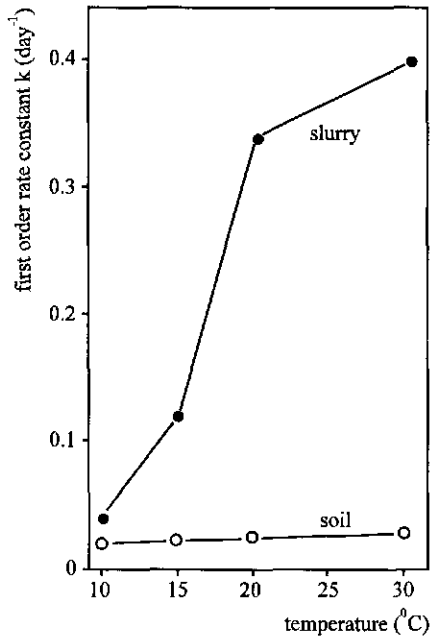


Figure 7.8. Effect of water content at different temperatures on the first-order rate of α-HCH mineralization in soil (slurry: 100 g/l, dry weight; soil: 20% moisture, w/w).

The water content may therefore influence the availability of the compound and thus the rate of biodegradation. This is demonstrated in Figure 7.8, in which the effect of water content on the biodegradation rate of α-HCH in soil at different temperatures is given (Bachmann 1987).

7.6 Concluding remarks

Laboratory experiments have demonstrated that many organic compounds that are responsible for soil and groundwater pollution, like (halogenated) solvents, polycyclic aromatic compounds and several pesticides, can be degraded by microorganisms. The fact that these organisms have often been isolated from polluted sites, also demonstrates the potential of *in situ* degradation. Several factors, among which availability and environmental conditions, determine the rate and degree of degradation.

In addition, for some compounds the structure may be so complex that microorganisms capable of degrading them do not naturally occur.

7.7 References

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