

International Centre



for Soil and
Contaminated Sites



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and Contaminated Sites**

MANUAL

FOR

BIOLOGICAL

REMEDIATION

TECHNIQUES



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MANUAL FOR BIOLOGICAL REMEDIATION TECHNIQUES

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1 Introduction

The ability of microorganisms to transform environmental contaminants to final products harmless to man and environment has been a subject of laboratory investigations since the 60ies. Since the early 80-ies biotechnological remediation techniques for treating soil, groundwater and air have been developed and applied on this basis. In Germany the microbiological treatment of contaminated soils is the technique of treatment applied most frequently in remediating contaminated sites.

Proceeding from the high expectations as to the efficiency of microbiological techniques there has to be stated that numerous limiting factors restrict their applicability. This resulted in an extensive research providing, first of all, a scientifically founded basis for the empirical approach practised in the past. In addition, techniques were developed to degrade groups of contaminants so far considered to be persistent. At present we can proceed on the fact that all organic contaminants are, in principle, degradable. However, in nature numerous factors bring about a limitation of the degradation of specific extraneous substances (xenobiotics) so that contaminants put into soil and groundwater by man in the past and at present are, without the intervention of man, frequently not reduced or only reduced in geogenic periods, thus causing a sustained contamination of the respective sites.

Microbiological remediation techniques are applicable on such sites where, on the one hand, in the course of planning remediation the site-specific boundary conditions have been sufficiently clarified and, on the other hand, an appropriate planning is carried out taking sufficiently into account the properties of the biological techniques. Given these conditions, microbiological techniques can show high economic and ecological advantages as compared with other techniques.

The primary target of microbial remediation techniques is to create optimum basic conditions for the degradation of contaminants. To reach this target knowing the various degradation reactions, ways of degradation and the influencing factors is of basic importance.

The present manual is to give a survey of the remediation techniques applied in Germany and their possibilities of application, thus providing for authorities and remediation planners a structured approach to choosing appropriate techniques.

2 Fundamentals of microbial remediation techniques

2.1 Degradation reactions: mineralization, transformation, humification

Contaminants in soil and groundwater are mainly degraded by bacteria and fungi. Though the number of bacteria exceeds by far the number of fungi, fungi are attached ever more importance owing to the results achieved in developing up-to-date techniques.

Microorganisms produce natural catalysts (enzymes) which degrade organic compounds forming CO₂, methane (CH₄), water and mineral salts. Such a complete degradation is called **mineralization**. However, mostly mineralization does not always proceed completely as in

each degradation operation a part of the substrate carbon is used for building up new biomass. Occasionally the (catabolic) enzymes participating in the degradation may transform contaminants also to metabolites which, first of all, accumulate (in particular, given conditions of lack of electron acceptors). Then, we speak of a **transformation**. Metabolites are also mineralizable. This requires possibly the participation of other organisms or other redox conditions. Strongly persistent substances are incorporated into the humic substance matrix mostly by means of radically acting enzyme reactions; this is designated as humification. Such a humification occurs frequently also with metabolites which themselves are reactive compounds. Such metabolites may enter into stable bonds with the natural organic substance of subsoil, the humic substances. In addition, the integration may in spontaneous reactions be also effected purposefully owing to the enzymic activity. Humification proceeds, to a more or less great extent, in all degradation reactions in soil, parallel to the mineralization (Fig. 1), i.e. all microbial transformations result in filling up of the humic substance pool. According to the present state of knowledge we can proceed on the fact that, in particular, aromatics with a few functional groups (such as TNT or PAH degradation metabolites) are integrated into humic substances covalently via stable bonds. Humification in soil is the main way of eliminating TNT.

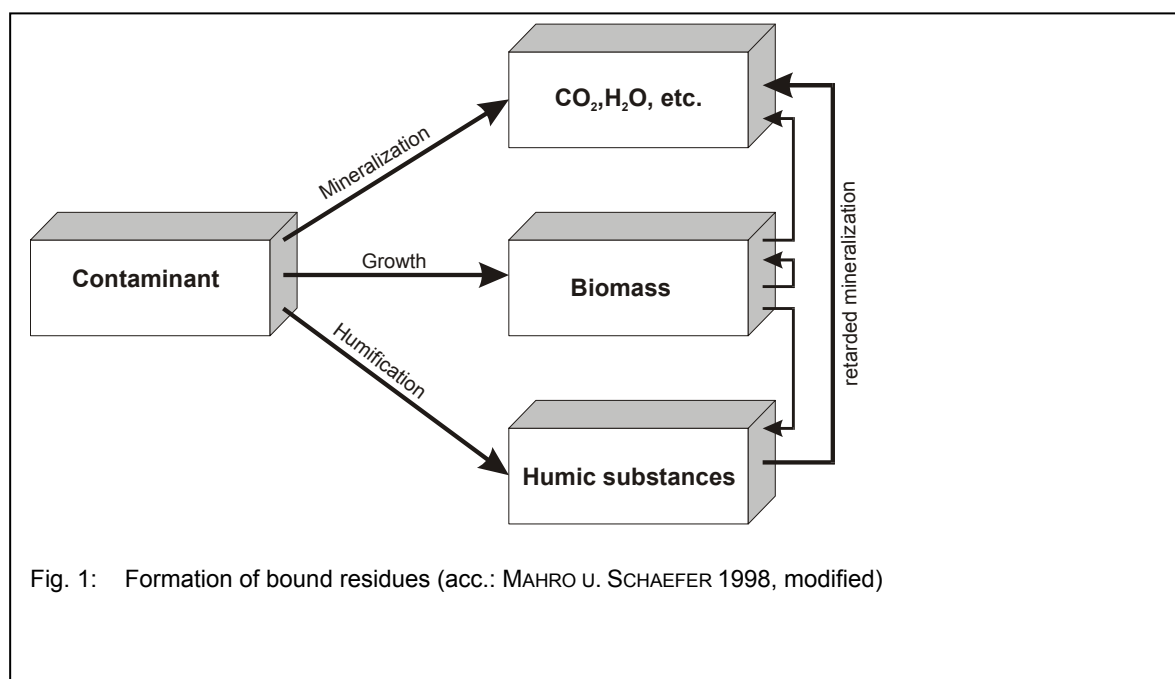


Fig. 1: Formation of bound residues (acc.: MAHRO U. SCHAEFER 1998, modified)

In humification as remediation technique it is decisive that the contaminant molecule is incorporated into the product of humification via stable chemical bonds such as e.g. C-C bonds. Then the contaminant is called "*bound residue*" which lost its chemical identity as well as its toxicological potential in this form.

A „release“ as original contaminant molecules does not seem to take place given natural conditions, the bound residues are rather mineralized in the course of a slow natural transformation of the humic substances. Humic substances may have an age of up to 500 years, caused, among others, by their macromolecular structure preventing specific processes of degradation to proceed, thus resulting in a quasi-stable state.

If, by taking remediation measures, appropriate nutrient situations will be brought about in subsoil the humification process may be even promoted purposefully. Thus, in principle, mineralization and humification may be considered as equal remediation targets. However, an accumulation of metabolites has to be avoided by controlling degradation appropriately. Furthermore, in the case of an appropriate technology being applied also other biochemical processes (e.g. precipitation) causing a reduction of the toxicity of the contaminants may be used.

2.2 Types of degradation

Given suitable environmental conditions, bacteria will grow and reproduce themselves. Microbial metabolization is the basis for growth. Degradative (catabolic) reactions result a. o. in preserving energy, reduction equivalents and elements for building up new cells. Biosynthetic (anabolic) reactions combine the elements to new cells consuming energy and reduction equivalents. To ensure that such a metabolism may proceed the following substances have to be made available in a suitable composition:

- electron donators
- electron acceptors
- carbon sources
- energy sources
- water
- nutrient salts

The so-called chemo-heterotrophic bacteria are the most important group with regard to the degradation of contaminants. They may frequently satisfy many requirements such as demand for carbon, energy source and electron donator by one and the same compound. This compound will be then called primary substrate.

Table 1: Classification of microorganisms according to the use of various electron acceptors in catabolism

respiration			
aerobic respiration	O ₂	®	H ₂ O
denitrification	NO ₃ ⁻ ® NO ₂ ⁻	®	N ₂
reduction of manganese	Mn-IV	®	Mn-II
iron reduction	Fe-III	®	Fe-II
sulphate reduction	SO ₄ ²⁻	®	S ²⁻
methanogenesis	CO ₂	®	CH ₄
organic respiration	organ. compounds (e.g. fumaric ac	®	organ. compounds (e.g. fumaric
fermentation	organic compounds*	®	organic compounds*

*) are not only used as electron acceptor but also as electron donator.

The chemo-heterotrophic microorganisms obtain the required energy by transforming specific substances rich in energy to compounds poor in energy in oxidation-reduction reactions (redox reactions). By oxidation we understand a release of electrons and by reduction an uptake of electrons. The oxidation of a substance is always connected with the reduction of another substance. In degradation processes of microorganisms supplying energy the redox reactions proceed successively in a cascade-like chain: electrons are passed on from one molecule (electron donator) to another one (electron acceptor). At the end there is a terminal electron acceptor taking up electrons, thus being reduced. In the course of this electron transport the energy required for microorganisms is released. Depending on the electron acceptor we distinguish between aerobic and anaerobic respiration and fermentation (Table 1). In fermentation the primary substrate is not only used as C source, energy source and electron donator but also as an electron acceptor. However, the primary substrate is degraded only incompletely. As regards the degradation of contaminants we may distinguish various types:

- **Productive metabolism:** Bacteria may use many contaminants as primary substrate, i.e. as source of energy, carbon and as electron donator. Here, contaminants are transformed by specific enzymes. Thereupon, bacteria grow at the costs of contaminants. The population density grows, thus accelerating the degradation speed (if the biomass represents the degradation limiting factor). Usually, this type of degradation results in a mineralization, i.e. a complete degradation of the organic molecule to the inorganic compounds CO_2 , water and mineral salts.
- **Utilization of secondary substrate:** If the contaminant (as primary substrate) will be available in a concentration smaller than the threshold concentration (S_{\min}) a degradation will no longer take place as its concentration will not be sufficient for a growth of bacteria. That is why another sufficiently concentrated primary substrate (here also designated as auxiliary substrate) will be required which will allow the bacteria to grow. Typical values of S_{\min} of various contaminants in aerobic systems are between 0.1 and 1.0 mg/l, partly also below it. The target contaminant will be mineralized in the course of “utilizing secondary substrate”. Then the residual concentration will be distinctly below the threshold concentration S_{\min} . However, the utilization of the secondary substrate may also result in the fact that principally degradable contaminants will not be mineralized but transformed forming metabolites.
- **Cometabolism:** The transformation of a contaminant will depend on the presence of another substance (cosubstrat). If, in nature, no specific enzymes will be available for degrading contaminants a degradation may, nevertheless, proceed in the course of a cometabolism if the cosubstrate and the contaminant will show “similarities”. In this connection, some catabolic enzymes „accept“ contaminants in the active centres due to their unspecificity transforming them in a kind of “free reaction”. As microorganisms will not obtain energy from this reaction a primary substrate will be required for their growth. Cometabolism will, as a rule, allow only a transformation but not a mineralization. But it can be a part of a mineralization reaction if the transformation products formed cometabolically may serve as suitable primary substrate for other microorganisms.

However, cometabolism does not mean a „selective advantage“. Whereas in specific degradation reactions the substrate will be quickly reduced in the course of the productive metabolism and the degrading germs will reproduce themselves accordingly, in cometabolism the target substrate will be only slowly reduced owing to the substrate competing with the contaminant for the catabolic enzyme and, in particular, a reproduction of the cometabolic bacteria will not take place.

Independent of the type of microbial transformation frequently an initial phase (adaptation phase, *lag* phase) is detected during which, first of all, contaminants are not degraded. In the *lag* phase an adaptation to the transformation of the substances offered takes place by means of multifarious mechanisms such as:

- induction: The formation of the required enzyme equipment (specific or unspecific) has to be first induced.
- growth up to reaching a specific size of the bacterial population to arrive at a significant degradation of contaminants.
- diauxy, a special case of induction: In contaminant mixtures the enzymes degrading a specific contaminant are possibly only induced after other (easily degradable) contaminants have been completely degraded.
- mutation and selection: Mutations or new gene arrangements may result in forming new enzymes which enable bacteria to degrade contaminants. This requires often an adaptation time of a few months.
- horizontal gene transfer: Transmission of genes within a microorganism species, yet also beyond the boundaries of species providing new recombinant or finally complete ways of degradation.

Depending on the predominant mechanism adaptation may require a period of hours up to months. This will play a decisive part in the development of remediation techniques resulting in an essential prolongation of the remediation time. Whereas mineral oil hydrocarbons and BTEX are degraded on a commercial scale without a recognizable *lag* phase e.g. when degrading 1,1,1-trichlorethane given methanogenic conditions an adaptation time of approx. 10 weeks was observed.

3 Factors influencing the microbial degradation of contaminants

3.1 Persistence factors

The degradability of contaminants is affected by a multitude of factors. Many factors ensure that some contaminants will persist in environment over a long period. The most important persistence factors are discussed hereinafter.

Lacking bioavailability: Due to the low solubility of contaminants, their strong sorption to solids, sequestration (physical enclosure) by high-molecular matrices, diffusion in macropores of solids and in sediments or their enclosure in insoluble and lipophilic phases the mass transfer will be reduced. The contaminants will not be bioavailable so that a significant degradation will be prevented and the contaminants will persist.

Concentration of contamination: In the event of their concentrations being too high the contaminants will be able to fully develop their possibly existing toxic effects on bacteria, thus preventing their degradability. In the event of the concentrations of contaminants being too low, however, degradation enzymes will not be induced. Bacteria will, first of all, tend to utilizing the substrate degradable more easily and only then to forming enzymes for degrading more complicated substrates (diauxy). However, in a very heterogeneous soil system degradation will proceed in a way not making frequently these reactions obvious.

Availability of nutrients: If the degradation will be limited owing to nutrients lacking (electron acceptors or donators and nutrient salts) will have to be added to accelerate the degradation processes. As to nutrient salts mostly only nitrogen and possibly phosphorus compounds (macronutrients) will have to be added as other nutrient salts (micronutrients) required for degrading contaminants are frequently available in soil in a sufficient quantity.

3.2 Environmental conditions

The physicochemical environment of microorganisms is designated as environment. The environmental conditions may be determined approximatively by means of measuring the so-called field parameters. Which values will have to be adopted by the individual parameters to ensure that degradation will proceed most quickly will, on the one hand, depend on the contaminants to be degraded and, on the other hand, on the (so-called autochthone) microflora existing on the site. The field parameters involve:

pH: Microbial degradation processes proceed preferably at a pH of approx. 6-8. For fungi a pH of approx. 5 is better suited.

Temperature: Though the microflora on the site is, as a rule, the predominant environment, e.g. adapted to the unvariable groundwater temperature of approx. 10°C in deeper layers a modification of the values within the physiological range (here e.g. increase to 20°C) may, however, result absolutely in an acceleration of the degradation.

Electrical conductivity: It characterizes the ionic strength (i. e. the salt content). In the event of the salt content being too high the degradation speed may be reduced.

Water content: A water content of approx. 40 – 60 % of the maximum water capacity of the soil will be optimal for degradation reactions in the unsaturated soil zone. In drier soils the degradation speed is reduced, in wetter soils water-saturated partial areas (microcompartments) are formed where the supply with oxygen and thus also the degradation will be retarded.

Redox potential and oxygen content characterize oxidizing or reducing conditions. With the aid of these two parameters it was detected whether suitable conditions (depending on the desired degradation reaction oxidizing or reducing) will be reached in the course of remediation.

If a contamination of subsoil will occur the contaminants will be degraded mostly until nutrients will be no longer available or the environment affected also by degradation reactions will

be so unfavourable that a further degradation will stagnate. Yet, as a rule, a lack of electron acceptors will be the dominating persistence factor. Many degradation mechanisms, in particular mechanisms for the degradation of mineral oil hydrocarbons, require aerobic conditions for a sufficiently fast degradation, i.e. oxygen as electron acceptor. However, some hydrocarbon compounds are also degradable under denitrifying conditions. Here, first of all, the available oxygen will be consumed (aerobic respiration), only then nitrate instead of oxygen (denitrification) is utilized as electron acceptor. If the nitrate will be exhausted an iron reduction will take place. The subsequent redox reaction will follow the same pattern: only if the redox process marked by a higher energy level may no longer proceed because the respective electron acceptor has been consumed the next redox process will start. This sequence will be accompanied by a decline of the redox potential (Fig. 2).

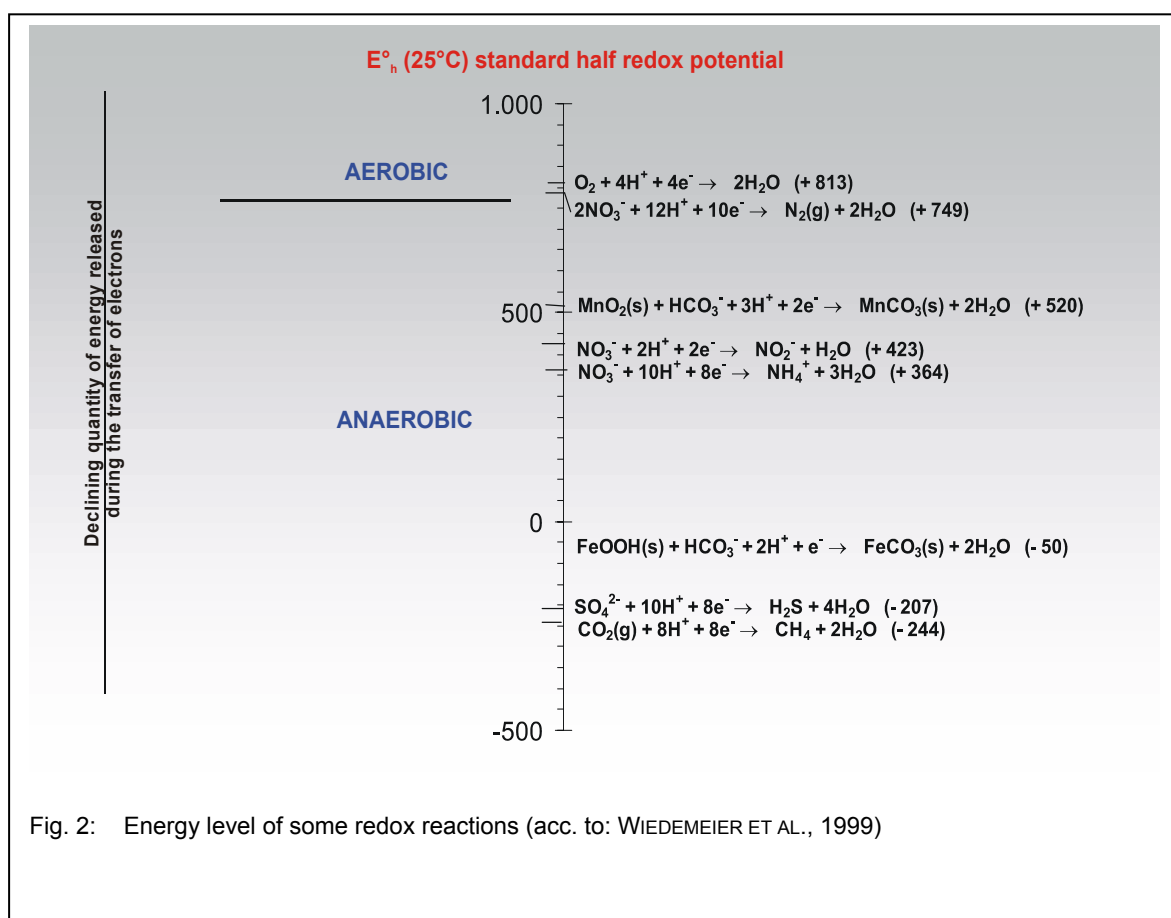


Fig. 2: Energy level of some redox reactions (acc. to: WIEDEMEIER ET AL., 1999)

Represented in a simplified way, with the energy level declining the use for the microorganisms and thus the growth rate and also the degradation speed go down. Thus, it is the aim of active microbial remediation to establish aerobic conditions as far as possible. Yet, some degradation reactions may only proceed given specific redox conditions (e.g. sulphate reduction and/or methanogenesis).

3.3 Adding of electron acceptors and donators

The main tasks of e.g. *in situ* techniques (com. Chapter 7.5) are to transport nutrients (nutrient salts, electron acceptors or donators) to the site of contamination, to remove reaction products and to establish environmental conditions suitable for the degradation of contami-

nants. This may be reached by the extraction of groundwater, treatment and re-infiltration of the water enriched with electron acceptors or donators, injection of air into the aquifer or other techniques.

The most important degradation reactions induced are aerobic processes and denitrification. They are explained in greater detail hereinafter. Biological degradation processes requiring oxygen may be limited by a lack of oxygen. Oxygen may be added by the infiltration of groundwater enriched with O_2 . Oxygen from air or tonnage oxygen dissolve with a maximum concentration of approx. 12 or 50 mg/l. By using hydrogen peroxide (H_2O_2) split up quickly into O_2 and water in the aquifer the supply of O_2 will be improved. However, the concentration of H_2O_2 in the infiltration water shall not exceed 1000 mg/l as otherwise the microflora may be damaged. Furthermore, too high concentrations of H_2O_2 may result in forming of O_2 -gas bubbles and thus in reducing the hydraulic permeability with a subsequent insufficient supply with nutrient salts and oxygen in the areas hydraulically not reached. Apart from that, loss of O_2 may occur by outgassing into the unsaturated soil zone.

In the course of remediation the demand for oxygen declines. Whereas the rate of oxygen consumption is limited by the rate of O_2 supply it will be determined by the mostly lower rate of aftersolution of the contaminants and their diffusion from micropores in a later phase of remediation when all easily available contaminants have been already degraded. Especially in this phase attention should be paid to an adapted supply of O_2 to avoid the formation of gas bubbles.

Owing to the low solubility of oxygen and its faster consumption the range of oxygen supply of subsoil from the infiltration point, is limited. A longer range of supply by electron acceptors is reached by the infiltration of nitrate (s. below).

Also abiotic side reactions may cause a significant consumption of oxygen. The most important reaction might be the oxidation of iron sulphide or soluble Fe(II) to insoluble Fe(III) the result of which might be clogging of the infiltration installations by iron hydroxide (ochering).

In degrading contaminants given denitrifying conditions nitrate as electron acceptor is consumed and nitrogen (N_2) is formed as final product. Also during denitrification gas bubbles may be formed as N_2 shows a low solubility similar to oxygen. As in the case of the nitrate concentrations being too high nitrite also toxic to microorganisms may be accumulated in the course of denitrification and for this reason a not too high nitrate concentration is to be recommended.

Nitrate and oxygen may be also infiltrated jointly. Subsequently, in the vicinity aerobic degradation reactions (with a denitrification being prevented) take their course with a complete consumption of oxygen and a degradation will proceed in the further area under denitrifying conditions.

For electron donators a number of substrates are applied which differ basically by their solubility. Apart from completely soluble substrates (e.g. molasses or lactate) low-soluble substrates (edible oils) or insoluble substrates (e.g. HRC[®], whey) are used. Electron donators are mainly required to support the degradation of halogenated compounds.

3.4 Outgassing loss and residual concentrations

Owing to their volatility the concentrations of some contaminants may be reduced by outgassing. This refers a. o. to (mono)aromatic compounds and low-molecular aliphatic hydrocarbons. In particular, outgassing of aliphatics results in a reduction of the share of inhibitory or toxic components of mineral oil products. The speed of outgassing increases with the temperature rising. For techniques where outgassing loss is unavoidable such as biobed techniques, land farming and techniques applying forced aeration of soil/groundwater the contaminants may be classified with regard to their main elimination processes:

- **High volatility and comparatively slow degradation:** Contaminants are mainly eliminated by outgassing also if the substances are, in principle, biologically degradable.
- **Volatility and biological degradation compete with each other:** Owing to their low volatility bacteria have the chance to degrade contaminants.
- **Low volatility and comparatively fast degradation:** The speed of the reduction of the concentration of contaminations is determined by the speed of their degradation.

Which process will take preferably effect will depend on the chemical composition of the trading item put into subsoil.

During remediation, as a rule, the reduction of the concentration of contaminations slows down until reaching an asymptotic course and the reduction of the concentration per time will be negligible. In this phase the residual concentrations may be scarcely further reduced without taking drastic measures. The level of the residual concentration will depend on the type of the contaminated soil and the type and quantity of the contaminants. The main causes for residual concentrations are:

- The bioavailability may be limited by pore space and diffusion barriers if the contaminants will be enclosed in the soil matrix. The residual phases (pendular residual saturation) fill mostly less than 20 % of the pore space, being scarcely accessible to a biological degradation.
- The bioavailability may be restricted a. o. by bonding to the humic substances contained in soil or formed during remediation.
- The bioavailability of contaminants (e.g. mineral oil hydrocarbons) may be affected by their hardening at the boundary layer oil/water. Diesel oil is liquid though containing solid alkanes ($C_{\geq 17}$) as shorter-chain alkanes serve as solvents for long-chain alkanes. By outgassing and biological degradation at the boundary layer oil/water the share of low-molecular components is reduced there and the remaining oil will become more viscous. Naturally, this effect is most strong at the outer surface of an oil film or oil drop, thus causing the formation of a hardened boundary layer counteracting a further microbial degradation of the residual fraction. The same refers to tar oil in PAHs. This effect is also expressed by the fact that contaminants are faster degradable in a fresh case of damage than in an old one.
- In contaminations with a contaminant mixture (e.g. mineral oil hydrocarbons) a residual fraction of less mineralizable or scarcely attackable substances will remain after degradation. They are detected gaschromatographically as components of the so-called „oil mountain“. They involve shorter-chain and long-chain, highly branched isoalkanes ($> C_{20}$), higher condensed cycloalkanes, long-chain n-alkanes, asphaltenes and strongly alkylated, low-condensed aromatics (apolar residues).

3.5 Soil mechanical and hydrogeological parameters

In remediation of soil and groundwater apart from physicochemical and biological boundary conditions also soil mechanical parameters and the hydrogeological development at the site play a major part. As the transport of substances (feeding of nutrients, removal of metabolic end products) represents one of the dominating processes in remediation a possibly high pneumatic and/or hydraulic permeability is desired. This applies, in particular, to *in situ* remediation. A grain-size distribution analysis gives first indications to the permeability. There are various techniques to determine the permeability:

- pneumatic permeability: radon technique
- hydraulic permeability: pumping tests
- vertical deviations of the hydraulic permeability: flowmeter measurements

Hydraulically suitable conditions are detected in loose sediments with a permeability of $> 10^{-4}$ m/s provided that the remediation is based on a technique with high groundwater pumping rates. The so-called passive techniques may still be applied given a hydraulic permeability distinctly below the value mentioned.

Also knowing the natural direction of the groundwater flow and the direction induced by remediation measures is required in view of a purposeful implementation of measures. However, it may be derived only from detailed data relating to the hydrogeological situation of the site.

3.6 Optimization of the degradation of contaminants

In planning *in situ* remediation there has to be considered that processes of various scales proceed (from nm to km). Whereas the cellular import and export of contaminants and the induction of degradation enzymes take place on a nm scale surface processes are rather assigned to the μ m scale. Diffusion processes (soil micropores) and microinhomogeneities (microcompartments) are in the range from μ m to mm. The m scale comprises small inhomogeneities such as silt lenses in sandy aquifers. Finally the km scale comprises local aquifer systems. In each scale the processes are limited by various factors. Usually the limitations of the biggest scale (e.g. hydraulic permeability of the site) dominate most of the other processes. Furthermore, a multitude of chemical, physical and biological reactions affecting each other proceed in the soil. As these changes in the soil may frequently not be measured the use of quantities measured indirectly, together with an idea of the transformations proceeding in soil, are the only possibility of characterizing the course of remediation. In *in situ* processes only a limited number of „activities specific to remediation“ may be undertaken, e.g.:

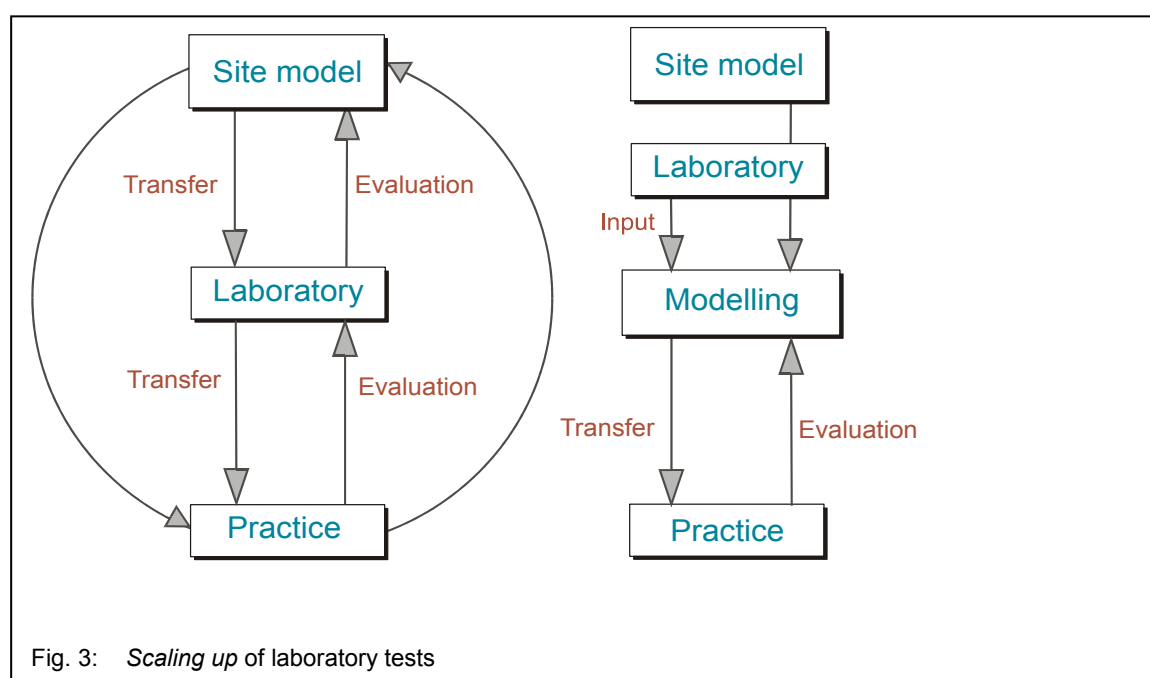
- feeding of electron acceptors/donators and nutrient salts
- change of temperature and water content
- influencing of the transport.

In this connection, it is the aim to affect specific processes proceeding in subsoil (state parameter) so that the microbial degradation of contaminants (target parameter) will proceed with an optimum speed. The interactions between the individual processes are so numerous

that influencing of a parameter may have various effects. The increase of the groundwater temperature may e.g. result in a faster degradation. On the other hand, a fast degradation may result in a massive formation of biomass, thus leading ultimately to blocking of the aquifer and therefore reducing drastically feeding of nutrients on the hydraulic way.

To determine the degradation capacities and kinetics with specific parameters varying, as a rule, laboratory tests have to be carried out (Fig. 3, comp. Chapter 4). The interpretation of the tests requires already a deepened knowledge of the site (conceptual standard model).

To apply the results of laboratory tests to a commercial scale (*scaling up*) the factors influencing *scaling up* have to be known. In addition, the basis should be improved using the respective experience gained to optimize the remediation adapted to the site (evaluation). Sometimes also mathematical modelling may be helpful in *scaling-up*. Finally a reliable data base for planning remediation may be created only by means of the described approach.



4 Preliminary investigations and pilot tests

A general characterization of the site (in particular of the hydraulic permeability) helps already in making a preliminary decision on the applicability of *ex situ* or *in situ* techniques. However, apart from the geological and hydrogeological conditions and the contaminant distribution each site has to be investigated for the degradability of the contaminants in site samples. Some techniques to be applied for this are described hereinafter:

A first, very important indication to the fact that microbial remediation techniques may be applied on a site is the detection of the consumption of electron acceptors along the direction of contaminant migration. In simple cases of damage this may be already sufficient for the further planning of remediation. However, more complex cases of damage require investigations of degradation in the laboratory. The course of such investigations is represented in Fig. 4. The laboratory tests are carried out in two phases. In a first phase (test level) there

will be checked if the contaminants contained in site samples are sufficiently degradable (general degradability). The investigations of numerous sites show that the autochthone microflora contains mostly specific agents degrading contaminants. That is why it is assumed that, as a rule, a degradation potential is available. Soils and groundwater loaded with bacteriotoxic substances so heavily that a significant damage of the microflora occurred are an exception.

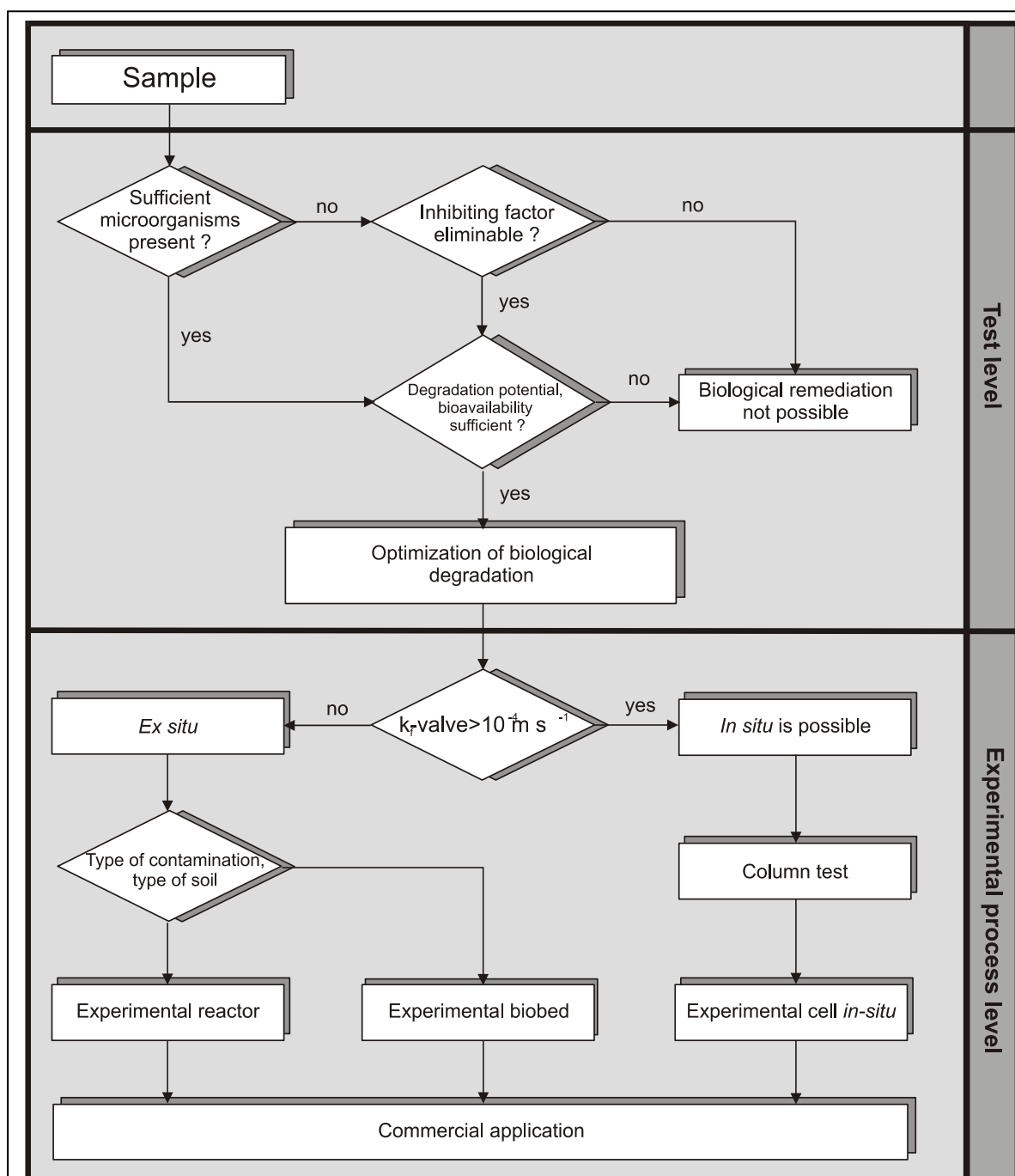


Fig. 4: Sequential diagram of the laboratory tests (acc. to: KLEIN, 1992, LFUG, 1999, modified)

Usually the environmental conditions met are not suited for allowing a fast degradation of the contaminants. That is why in the first phase of investigation, given optimized conditions, rather a maximum than a realistic degradation rate is determined. In the second phase (process test level) basic planning data are determined with the aid of investigation techniques simulating already the chosen remediation technology (bench top scale). Basic planning data involve e.g.:

- demand for nutrients
- possibilities of optimization by varying electron acceptors and nutrient conditions (a. o. cosubstrate),
- duration of remediation,
- final concentrations to be reached.

In simulating *in situ* techniques for treating the water-saturated soil zone (column test) it seems to be appropriate to carry out the tests with real contaminated groundwater. For this purpose a. o. also mobile test units are available.

The results of all site investigations including risk assessment are used for planning remediation. The following points may be answered on the basis of the degradability investigations:

- site specific suitability of preliminarily selected techniques,
- optimization of techniques,
- assessment of the time needed,
- technique-specific basis for interpreting the measures on a commercial scale,
- basis for cost estimates.

For the time being, sufficient experiences are available to transfer, in most of the cases, the results of the tests to the commercial scale. Pilot tests on a semi-commercial scale are only carried out if new technologies are applied without having gained sufficient practical experience or if the site shows unusually complex characteristics or if persistent contaminants are to be treated. Then, the pilot test shall be carried out in such a way that it will reflect the basic conditions of technological remediation. When applying a hydraulic *in situ* technique e.g. the location of infiltration and the infiltration pressure shall be comparable to a commercial scale. Otherwise it will be difficult to transfer the results. Also planning, constructional measures and monitoring connected with the pilot test shall be comparable with a remediation on a commercial scale.

Passive microbial *in situ* remediation of long-chain hydrocarbons are an exception. Here, a pilot test is always required for determining the design parameters for the commercial scale. However, laboratory tests may be renounced if the site investigation will already provide indications to a natural degradation of contaminants.

5 Conceptual site model

The data of a specific site collected will be used for compiling a so-called conceptual site model. The conceptual site model is a descriptive characterization of the site. There the specific hydrogeological conditions, contaminant distribution and the processes proceeding in subsoil (contaminant transport, natural degradation of harmful substances) will be considered. The processes need not be fully detected on the site of investigation but will be derived from the substance distribution analyzed on the basis of expert knowledge.

Based on the site model additional investigations will be derived and finally the remediation measures will be planned on a commercial scale. For *in situ* remediation it might be appropriate to describe the groundwater flow and the contaminant transport and degradation in a mathematical model in addition to the conceptual site model and to include the results of modelling as additional planning elements. The models used consider the specific parameters determined in site and laboratory investigations.

6 Microbial degradation of selected contaminants in soil and groundwater

6.1 Survey

The numerous contaminants contained in subsoil require preferably either aerobic or anaerobic conditions for their degradation. In the past the investigations concentrated on aerobic mechanisms. However, the importance of anaerobic processes should not be underestimated. Substances such as hydrocarbons, aromatic hydrocarbons, ether compounds and others are attacked especially in the presence of oxygen with a comparatively high degradation rate with hydroxyl groups being typically incorporated into the substrate. Thus, it will be easier to degrade them. Here, oxygen will be used as a terminal electron acceptor (respiration). The electrons released in the course of the oxidative degradation of contaminants will be transferred to it. In addition, it will serve to “activate” the compounds to be degraded.

In the past anaerobic ways of degradation of contaminants were detected for most of the contaminant groups. In the absence of oxygen other oxidized compounds such as nitrate, sulphate a. o. take over the role of a terminal electron acceptor. Frequently these anaerobic degradation processes proceed slower than the aerobic processes, however not necessarily less efficiently. In addition, some contaminant groups such as e.g. nitroaromatics and diazo compounds may be aerobically attacked, however their degradation proceeds faster under reductive conditions. Some contaminants (e.g. tetrachloroethylene) are completely mineralizable in some biogeochemical environments in acceptable periods only if a succession (first anaerobic, then aerobic) is ensured. In Tab. 2 the contaminant groups occurring most frequently and the preferred degradation conditions are listed.

The term of a „good“ or „bad“ degradability sums up numerous individual processes, e.g. the degradation speed subjected also to bioavailability besides kinetic factors. In addition the “amount of microbes required” plays a part: compounds requiring the formation of biotensides to be made available for the degradation of which cometabolic conditions are neces-

sary or microorganism groups have to be available demand more microbes than compounds which are easily soluble and effectively degradable only by one type of microorganisms.

Table 2: Compilation of degradability (mineralization, humification) and transformability of some contaminants

contaminant	microbial degradability			preferred conditions
	high	low	no	
mineral oil hydrocarbons				
short-chain mineral oil hydrocarbons	+			aerobic
long-chain/branched mineral oil hydrocarbons		+		aerobic
cycloalkanes		+		aerobic
monoaromatic hydrocarbons				
AHs	+			aerobic
phenols	+			aerobic
cresols		+		aerobic
catechols	+			aerobic
polycyclic aromatic hydrocarbons				
2- to 3-ring-PAHs (e.g. naphthalene)	+			aerobic
4- to 6-membered ring PAHs (benzo(a)pyrene)		+		aerobic
chlorinated aliphatic hydrocarbons				
tetrachloroethylene, trichloroethane	+			anaerobic
trichloroethylene, dichloroethane	+			anaerobic/aerobic
dichloroethylene, vinyl chloride	+			aerobic
chlorinated aromatic hydrocarbons				
chlorophenols (superchlorinated)		+		anaerobic
chlorophenols (low-chlorinated)	+			anaerobic/aerobic
chlorobenzenes (superchlorinated)		+		anaerobic
chlorobenzenes (low-chlorinated)	+			anaerobic/aerobic
chloronaphthalene	+			anaerobic/aerobic
polychlorinated biphenyls (superchlorinated)		+		anaerobic
polychlorinated biphenyls (low-chlorinated)	+			anaerobic/aerobic
nitroaromatic compounds				
mono- and dinitroaromatics	+			aerobic/anaerobic
trinitrotoluene (TNT)	+			aerobic/anaerobic
trinitrophenol (picric acid)		+		aerobic/anaerobic
nitroaliphatic compounds				
glycerol trinitrate	+			aerobic
pesticides				
g-hexachlorocyclohexane (lindane)	+			aerobic/anaerobic
b-hexachlorocyclohexane (lindane)		(+)	+	aerobic/anaerobic
atrazins	+			aerobic
„dioxins“				
PCDD/F (several)		+		anaerobic
2,3,7,8-PCDD/PCDF			+	
xenobiotic polymers	+	+		
inorganic compounds				
free cyanides		+		aerobic
complex cyanides		+		
ammonium	+			aerobic/anaerobic
nitrate	+			anaerobic
sulphate	+			anaerobic
heavy metals*		+		
radioisotopes*		+		

*) Microbially transformable, yet not "degradable"

Apart from considering the degradability from an aspect of microflora it may be also examined from remediation-technological aspects. Then, the completeness of degradability (mineralization and irreversible humification) will play a part.

The classification made in Tab. 2 is not quantifiable but a rough general assessment and may be absolutely modified depending on the conditions given. To choose a suitable remediation technique detailed knowledge of the degradation reactions, of metabolites formed possibly and of the factors influencing degradation will be necessary. As to some contaminants such as e.g. mineral oil hydrocarbons the formation of metabolites need not be attached special importance if their degradation has been initialized by adding sufficient quantities of electron acceptors. In practice, the alcohols, aldehydes, ketones and acids formed intermediately are degraded mostly much faster than the initial substances. In addition, their toxicity is mostly weak.

When degrading e. g. TNT or chlorinated compounds, however, all metabolites formed temporarily which are partly more toxic than the initial substances have to be considered. The environmental conditions have to be adapted in a way that such metabolites will not accumulate or will be further transformed in acceptable periods. For all ways of degradation where considerable metabolites are formed a respective, suitable analyzing technique has to be applied. By means of various biotests (so-called ecotoxicological test batteries) information on the non-toxicity of the remediated soil for selected (groups of) organisms may be obtained (Chapter 9.4).

6.2 Mineral oil hydrocarbons

Mineral oil hydrocarbons are products of processing crude oils, e.g. petrol, diesel, fuel, engine oils or heavy oils. That is why mineral oil hydrocarbons involve a multitude of groups of substances consisting, on their turn, of numerous individual substances. We distinguish aliphatic and aromatic hydrocarbons.

6.2.1 Aliphatic hydrocarbons

Aliphatic hydrocarbons are to be distinguished as to their structure (linear, branched, cyclic) and their „saturation“, i.e. as to the occurrence of single, double and triple bonds between the C-atoms (alkanes, alkenes, alkynes). Linear saturated hydrocarbon chains are called n-alkanes, branched are designated as iso-alkanes.

Aliphatic hydrocarbons are preferably degraded under aerobic conditions. The principle of degradation is uniform for all groups of substances and is described hereinafter by the example of an n-alkane. First of all, a compound is activated by the gradual oxidation of the terminal C-atom: alkane → alcohol → aldehyde → acid. The fatty acid formed is coupled with a carrier molecule. By further oxidation reactions at the 2nd and 3rd C-atoms with a subsequent decomposition an acetate residue is formed at the carrier molecule (which is transformed to CO₂ or cell components in the respiration chain of the microorganism) and a fatty acid reduced by a C₂ unit, also bound to a carrier molecule (incorporated during decomposition). The further degradation proceeds always in the same cycles (“β-oxidation”). By activation, first of all, molecules are formed which are more polar and better soluble and thus better degradable. As only during the first oxidation stages „free“ compounds, i. e. not bound to a carrier molecule, appear an accumulation of free metabolites should not be expected. Yet, in the case of the oxygen concentration being too low this may, nevertheless, occur: O₂ is re-

quired for degrading mineral oil hydrocarbons to activate the molecules, yet also as a terminal electron acceptor. That is why given conditions lacking O₂ partial oxidations may occur. However, if sufficient oxygen will be fed the metabolites accumulated in the meantime will be subject to a fast mineralization owing to their improved degradability as compared with the initial compounds.

Other aliphatic hydrocarbons are prepared for the β -oxidation in various ways. In branched alkanes, first of all, branches are split off, in cycloalkanes the ring has to be opened. The individual reactions vary in difficulty for microorganisms. This results in various degradation rates. In addition, compounds within a group of substances, e.g. n-alkanes, are degraded differently fast. Short-chain alkanes (approx. < C₈) pass easily a cell diaphragm; that is why they may be toxic for microorganisms. This may result in inhibiting the organisms and retarding the degradation of short-chain n-alkanes. With the chain length increasing alkanes show a declining water solubility and thus a declining bioavailability. That is why microorganisms have to make greater efforts, a.o., to form biotensides in order to degrade these substrates. Therefore, the degradation of long-chain n-alkanes takes more time. Altogether, the various degradation speeds cause a fractioning of the residual hydrocarbon fractions. The degradation speed declines approximatively in the order n-alkanes → iso-alkanes → alkenes → cycloalkanes.

The anaerobic degradation of mineral oil hydrocarbons has been comparatively seldom investigated. It is a very slow process. Whereas under denitrifying conditions a degradation is still technologically applied degradation reactions under redox conditions on a lower energy level (e.g. under sulphate reducing conditions) play only a part if techniques of remediation proceeding comparatively slowly (e.g. *natural attenuation*) are applied. In this case degradation attempts are, however, imperative, because among the multitude of the various groups of substances summed up by the term mineral oil hydrocarbons some may be chemically so inert under denitrifying conditions that they are to be regarded as “not degradable” by applying active remediation techniques. In addition to sulphate reduction also other environmental conditions (iron reduction, methanogenesis) allow a degradation of mineral oil hydrocarbons.

A frequently accompanying substance of mineral oil hydrocarbons is methyl tertiary butyl ether (MTBE) added to petrol to increase the octane number. MTBE shows a high solubility, low sorption, insignificant tendency to outgassing and a low degradability, thus resulting in forming large contaminants' plumes in groundwater. Obviously, MTBE may be mineralized under aerobic conditions or degraded cometabolically. The environmental conditions suited for the degradation, however, are not yet sufficiently known. Later investigations have shown that MTBE is degradable also under most of the anaerobic conditions.

6.2.2 (Mono)aromatic hydrocarbons (AHs)

Benzene (C₆H₆) and its alkylated derivatives toluene, ethyl benzene and *ortho*-, *meta*-, and *para*-xylene belong to the classical monoaromatic hydrocarbons (BTEX-aromatics). The group of AHs has been extended by the so-called test petrols (trimethylbenzene, ethylmethylbenzene, isopropylbenzene and cumol (C₃-benzene) and tetramethylbenzene, diethylbenzene (C₄-benzene) etc. In mineral oil products, in particular petrols, numerous fur-

ther various substituted benzene compounds such as e.g. styrol ($\text{CH}_2=\text{CH}-\text{C}_6\text{H}_5$) or linear alkylbenzenes (LAB) which partly may show very long side chains may be contained. Furthermore, alkylbenzenes with branched side chains are available.

Aromatic hydrocarbons are distinctly better water-soluble than aliphatic hydrocarbons. Benzene with 1.76 g/l is best soluble, trimethylbenzene with 20 mg/l is worst soluble. AHs belong to the well degradable contaminants with the degradability declining in a first approximation with the degree of substitution rising. The degradation of all aromatics follows a basic pattern: given aerobic conditions the aromatic ring is activated by the incorporation of an oxygen molecule. In a next step the central metabolite catechol with OH groups in *ortho* position is then formed (Fig. 5).

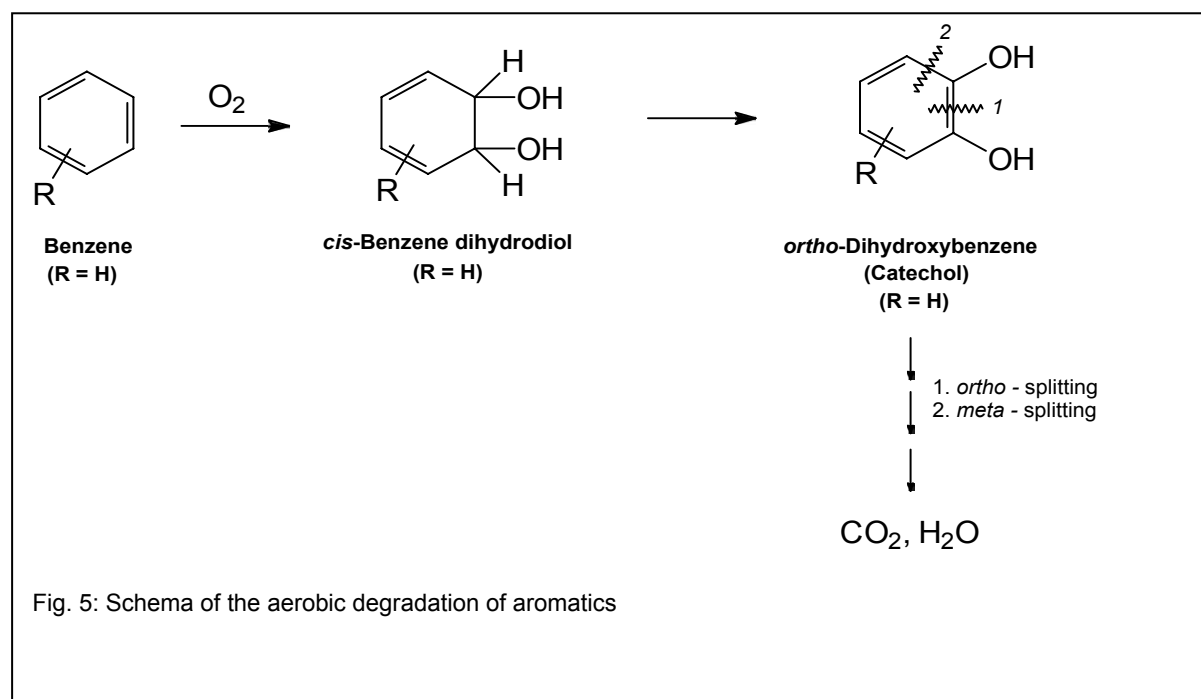
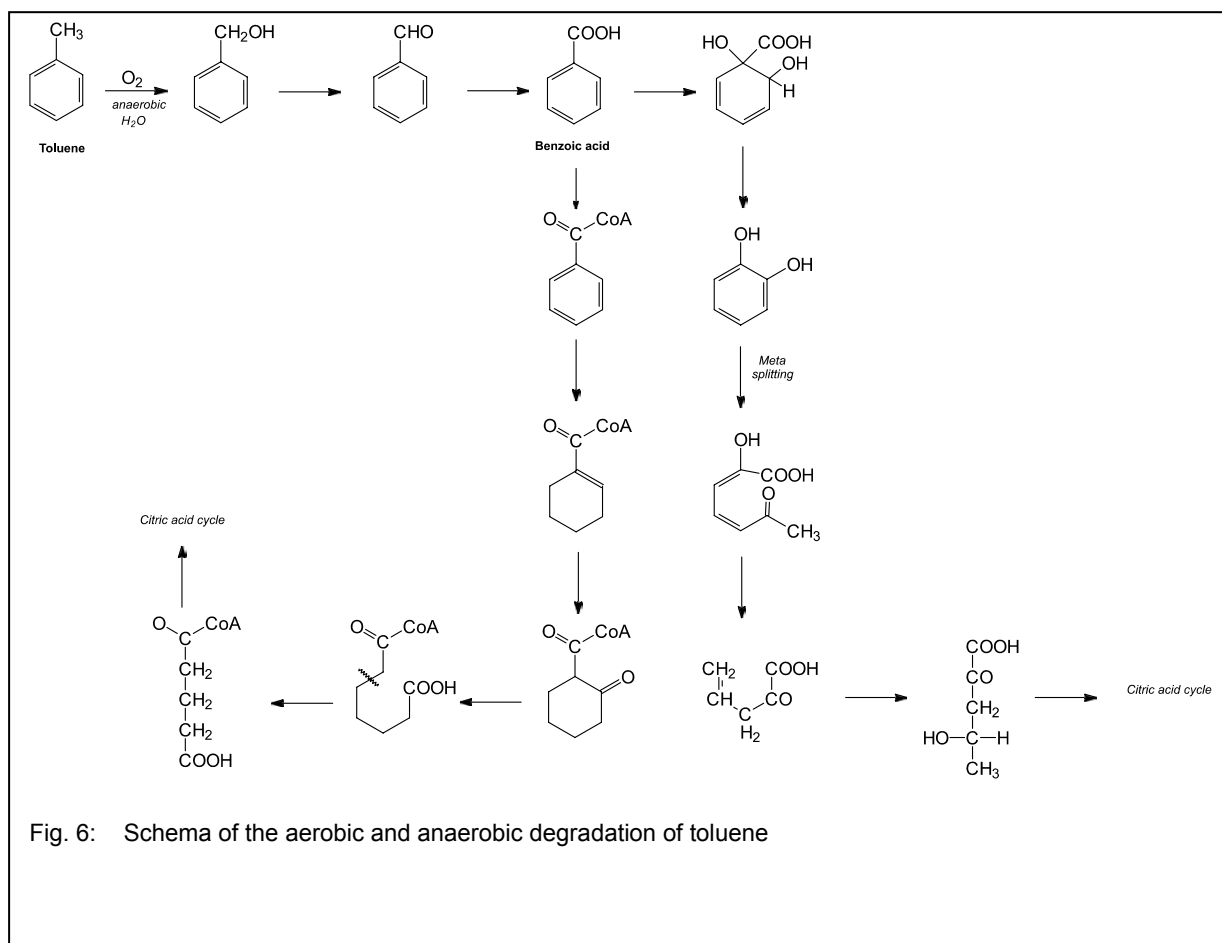


Fig. 5: Schema of the aerobic degradation of aromatics

The substituents of the aromatic ring may either remain at the ring – in this case alkylcatechol will be formed. However, it seems to be more probable that in the course of the formation of catechol the substituent is split off. Thereby, aromatic carboxy acids are formed in the meantime. This is shown by the example of the degradation of toluene (Fig. 6). Longer side chains, in particular if they show still branches, have to be degraded, first of all, comparable to the degradation of mineral oil hydrocarbons. The further degradation is effected by the decomposition of the aromatic ring. The decomposition products are mineralized through the same metabolic pathways used also for degrading aliphatic hydrocarbons.

AHs are degraded under nearly all anaerobic conditions. This is shown by the example of the degradation of toluene (Fig. 6). However, the reachable degradation rates are distinctly lower as compared with the aerobic degradation. Substituents of the benzene ring are split off. First of all, the ring is activated by coupling to the carrier molecule coenzyme A (CoA) with a subsequent degradation to a central metabolite (benzoyl-CoA). The oxygen required for this initial stage comes from the ambient water. The aromatic ring of benzoyl-CoA is then reduced and subsequently decomposed by means of hydrolysis. The degradation products are fed into the cellular energy metabolism.



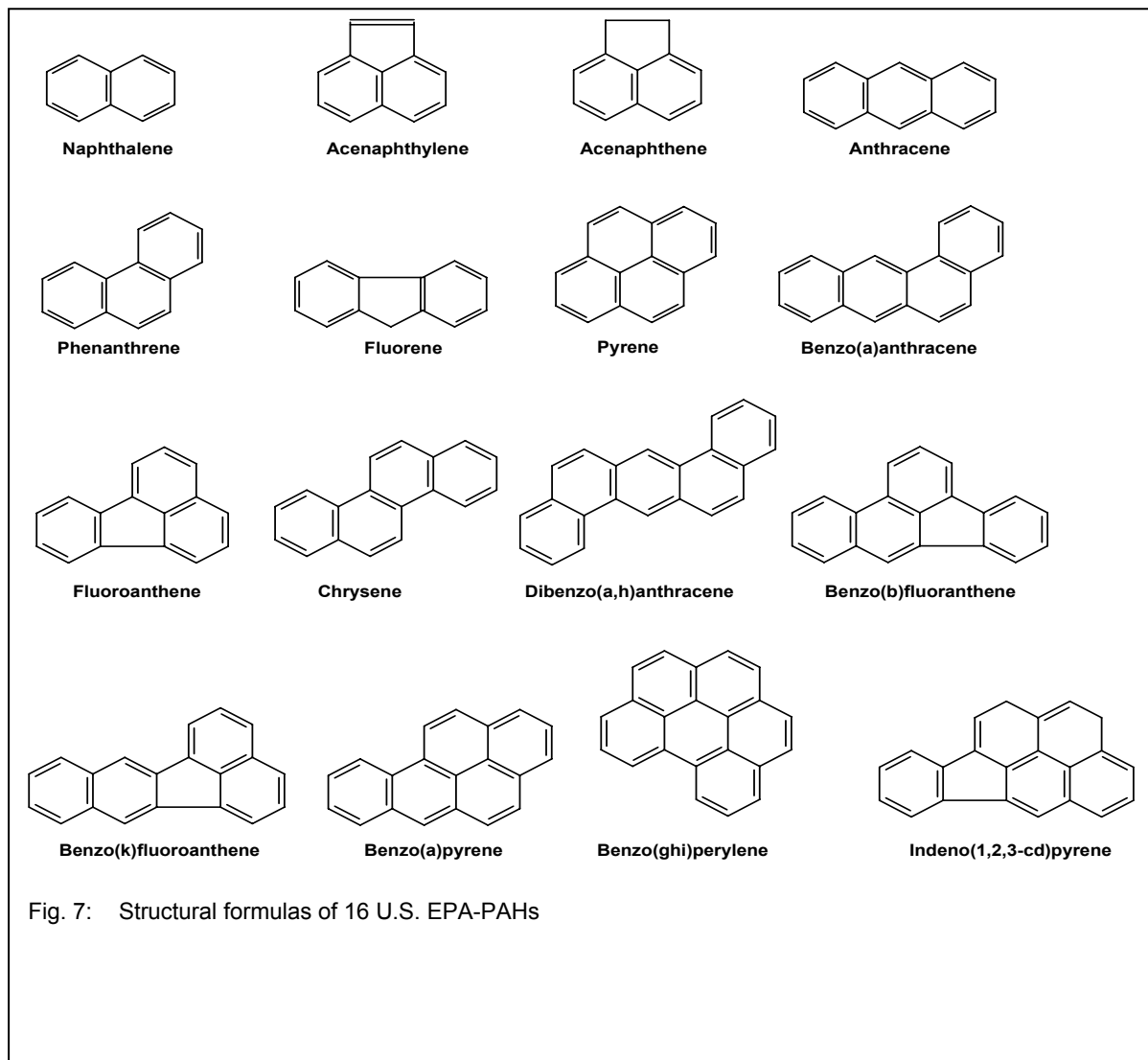
The biochemical details of the anaerobic degradation of aromatics have not yet been clearly detected. Yet, it is of importance that a suitably mixed biozenosis has to be available for the anaerobic degradation, i.e. for eliminating the ring substituents and for the ring fission as individual bacteria are frequently not in a position to bring about a complete mineralization.

6.3 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are a. o. contained in tar, crude and mineral oil products. Pollution of subsoil by PAHs comes frequently from coal utilization plants such as gas works and coking plants. These are mostly mixed contaminations with a high hazard potential for groundwater. PAHs involve numerous compounds with two or more condensed aromatic rings. Due to the number and spatial arrangement of the rings and their substituents, mostly methyl groups, there is a multitude of potential compounds a few hundred of which are known. The Environmental Protection Agency of the USA has selected 16 non-substituted PAHs as key substances for investigating PAH pollution owing to their frequent occurrence in the environment, their comparatively simple detectability and their hazard potential (Fig. 7).

At room temperature PAHs are solid, crystalline compounds. However, they occur mostly in mixtures (as pure PAH mixture or in connection with aliphatic and aromatic hydrocarbons and heterocyclic aromatics with the freezing temperature being so low that the product is liquid at ambient temperature (PAH oils, tar oil). As higher aliphatics PAHs are hydrophobic,

easily soluble in organic solvents, yet slightly soluble in water. Low condensed PAHs (2-3 condensed rings) show still a tendency to outgassing. For naphthalene (two benzene rings) the volatility is an essential factor and phenanthrene (three benzene rings) is designated as semi-volatile.

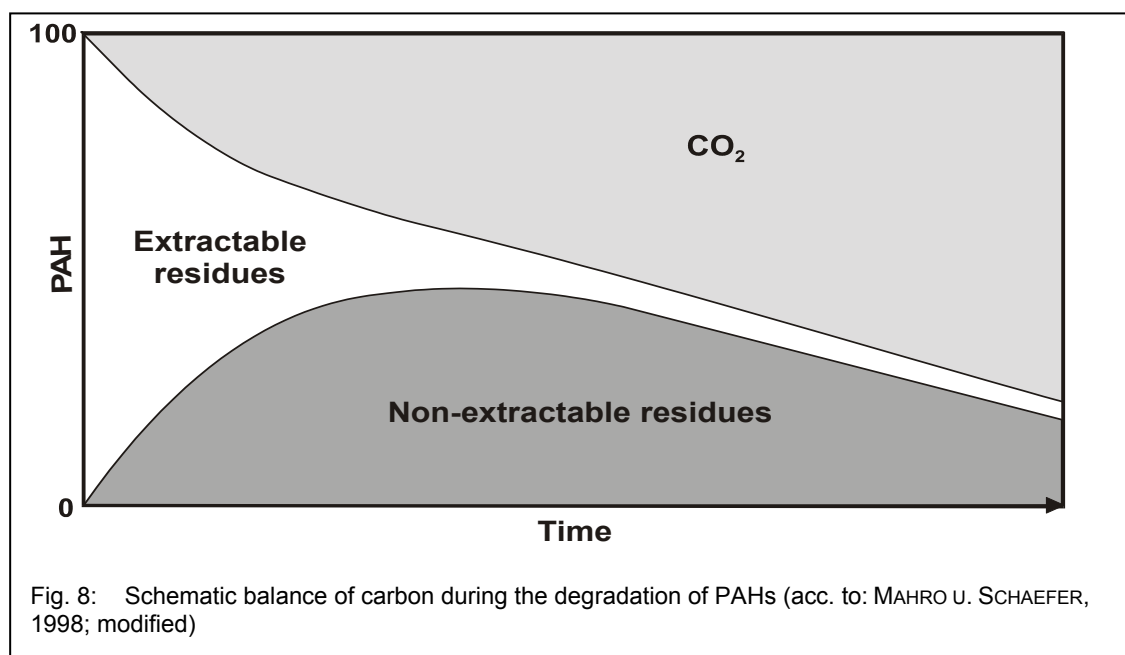


PAHs show altogether a low water solubility. Whereas naphthalene with approx. 30 mg/l is comparatively well soluble benzo(a)pyrene can be dissolved only with maximally 2 µg/l, i. e. with the molar mass increasing the solubility declines quickly. At the same time, the tendency to sorption increases. If PAHs occur together with other contaminations the PAHs are „immobilized“ in tar particles or with tar oil or soil particles glued with tar oil and artificial asphalt. Such aggregates may be only badly supplied with e. g. oxygen when applying remediation techniques. In addition, the substance transfer (solid → dissolved) is strongly obstructed.

In general, PAHs up to 3 rings are considered to be well degradable, thereupon the degradability decreases owing to a decline of the bioavailability. Due to this, a relative enrichment of higher-nuclear PAHs takes place during remediation. Owing to the low solubility the threshold concentration (S_{\min}) for the degradation may not be reached so that higher condensed PAHs may scarcely serve as carbon and energy sources. Here, cometabolic degradation

reactions dominate. That is why after adding e.g. compost an increased degradation may be detected.

The aerobic degradation of PAHs is effected analogously to the degradation of mononuclear aromatics with the individual rings being degraded one after the other. The central metabolite catechol is formed equally which is then subject to a ring fission with a subsequent mineralization. Besides mineralization humification plays a major part in degrading PAHs. Both processes proceed parallel and in competition with each other (Fig. 8). Extractable PAHs remain detectable also over a longer period declining only at a very low rate. This refers to a PAH fraction which only through slow processes such as rediffusion from soil pores or tar particles will have to become only bioavailable before a degradation will lead to its reduction. The amount of the residual PAH fraction measured at a specific time during remediation depends thus mainly on the bioavailability (i.e. indirectly on the quantity of tar particles, organic carbon etc.). Also the quantity of bound residues declines with the time to the benefit of the formation of CO_2 . This shows that the bound residues, together with humic substances, are slowly mineralized in the course of natural transformation. However, a release of the bound residues may not be observed, neither by the action of the great number of influencing factors nor by fungi degrading humus.



Adding of compost promotes mineralization as well as humification. If mineralization is proceeding very slowly as in higher-nuclear PAHs the humification may be the preferred process. Comatabolically PAHs are degraded by white rot fungi. With the aid of enzymes generating radicals of the „lignolytic system“ (= enzymes formed for the degradation of wood) the PAHs are oxidized in an initial step and then transformed further. As these enzymes are extracellular enzymes an uptake of contaminants into the microorganism cell is not required. Thus, also quasi insoluble substances shall be transformable.

To sum up there may be assumed that a PAH load may be treated microbially if the following prerequisites are given:

- low content of organic carbon (C_{org}),
- moderate contamination by PAHs
- composition preferably of low-molecular PAHs,
- low concentration of the accompanying contaminants.

The degradation of some low-molecular PAHs (a.o. naphthalene, acenaphthene) takes place under anaerobic conditions. As we have so far not known much about the course of the anaerobic degradation it has so far scarcely played a part for active biological remediation.

6.4 Phenols

A benzene ring substituted with one OH group is termed phenol. Apart from that, numerous compounds with phenol as basic body and further substituents, e.g. with alkyl residues, are summed up in the “phenol” group. Similarly as benzene derivatives the alkyl side chains may show a great length or branches. Cresols (methyl phenols) and xylenols (dimethyl phenols) are met especially frequently. As phenols are weak acids the solubility of the compounds and thus their bioavailability increase with the pH and the dissociation of the OH group rising. In addition, there is, however, applicable that with the number of substituents rising the solubility goes down. Apart from pure phenol alkyl phenols, halogenated phenols and nitrophenols are of special relevance to contaminated sites. Phenol is obtained as a by-product in coking plants and lignite carbonization power stations.

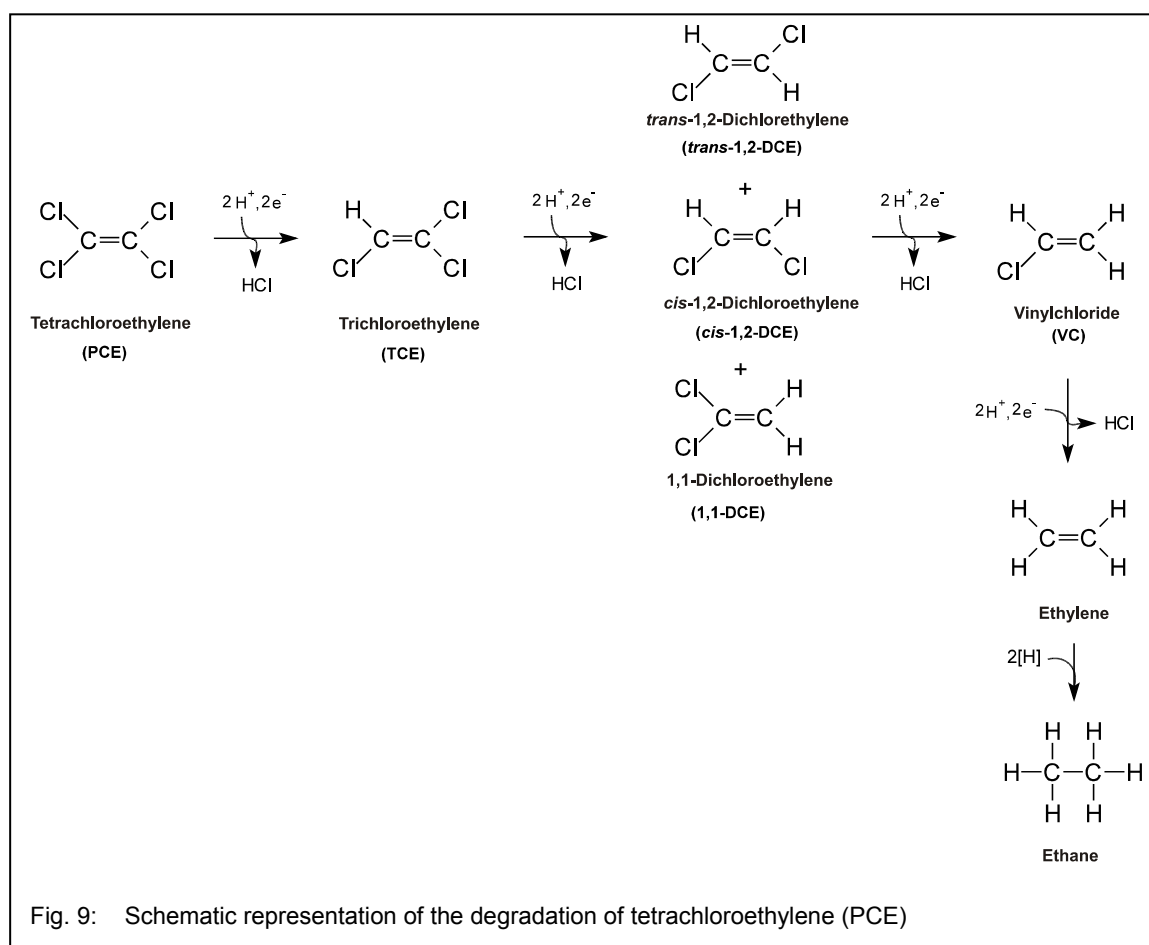
In general, phenols are considered to be easily degradable. Their degradation is effected almost completely analogously to the degradation of the (mono)aromatics with the exception that in the course of initial oxidation not two but only one OH group is incorporated into the molecule. The central metabolite (catechol) formed is the same as in the degradation of AHs. Thus, the subsequent reactions are also identical. However, oxidized phenol metabolites tend strongly to polymerization (black product).

6.5 Degradation of volatile chlorinated organic compounds

The volatile chlorinated organic compounds consist of chlorinated C_2 -compounds where one to all hydrogen atoms are substituted by chlorine. Volatile chlorinated organic compounds have been used as solvents, degreasing and extracting agents in various branches of industry and serve the production of lacquers and varnishes up to edible oils and fats. Being frequently used PCE, TCE, 1,1,1-TCA and DCM are the most important contaminants. Cases of damage with volatile chlorinated organic compounds are sources of contamination for groundwater. Volatile chlorinated organic compounds are, in principle, microbiologically degradable.

Among chloroethylenes tetrachloroethylene (PCE) is not or scarcely attackable under aerobic conditions, i.e. with oxygen. The degradation of chloroethylenes requires preferably anaerobic conditions. As these volatile chlorinated organic compounds may not be used as carbon and energy sources for the growth of bacteria it is necessary to add an appropriate primary anaerobically degradable C source, the so-called auxiliary substrate. This is organically bound carbon (C_{org}). Hydrogen released during the degradation of the auxiliary sub-

strate by an anaerobic biozlenosis ($H_2 \rightarrow 2 H^+ + 2 e^-$) is transferred to the volatile chlorinated organic compounds by chlorine-respiring microorganisms being thus reductively dechlorinated. In this reaction designated as dehalorespiration volatile chlorinated organic compounds serve as electron acceptors. In addition, the volatile chlorinated organic compounds may be also reductively dechlorinated in a cometabolic reaction. However, the cometabolic degradation proceeds slower by some orders of magnitude than dehalorespiration. It is obvious that various organic carbon compounds may serve as an auxiliary substrate during the degradation of volatile chlorinated organic compounds as the hydrogen (H_2) released during an anaerobic degradation of C_{org} is the key substance for reductive dechlorination. Thus follows that the dechlorination efficiency depends primarily on the quantity of the available hydrogen and the type of the auxiliary substrate generating H_2 .



Under anaerobic conditions significant transformations of volatile chlorinated organic compounds take mostly place under methanogenic conditions. Yet, also already under sulphate reducing conditions a transformation may take place, i.e. only if a sufficiently negative redox potential (-200 to -400 mV) and a sufficient quantity of the auxiliary substrate are available a reductive dechlorination up to a mineralization may proceed, i.e. up to a complete dechlorination forming the chlorine-free final products ethylene and ethane which are easily degradable to form CO_2 given aerobic conditions. The degradation sequence of tetrachloroethylene (PCE) is represented in Fig. 9. PCE is anaerobically dechlorinated to form ethylene which is further reduced to ethane. In this degradation sequence, however, not all transformation steps proceed with the same speed. Here, rather the approximation rule applies that at a given redox potential each transformation step proceeds slower than the preceding one.

This results in the fact that given anaerobic conditions, first of all, the low-chlorinated metabolites cDCE and/or vinyl chloride (VC) accumulate and are degraded further only very slowly. Apart from the reductive dechlorination of VC also an oxidative degradation of it is known given anaerobic conditions.

The aerobic degradation may proceed cometabolically or also productively. The low-chlorinated metabolites (cDCE, VC, 1,1-DCA) accumulated under anaerobic conditions are faster degraded under aerobic conditions (by oxidation). The lower the chlorine content of the compound will be the faster will proceed its aerobic degradation, i.e. VC is aerobically better degradable than cDCE and this, on its turn, better than TCE. PCE may not be transformed aerobically.

In specific metabolic pathways enzymes occur which, owing to their unspecificity, are in a position to oxidize also volatile chlorinated organic compounds (cometabolic degradation). As these enzymes are only formed (induced) if their primary substrate will be available the aerobic degradation of volatile chlorinated organic compounds requires the presence of these substrates (i.e. inducers). Inductors for the aerobic degradation of volatile chlorinated organic compounds may be infiltrated or formed quasi-intrinsically. Thus, the mineralization of the auxiliary substrate added under anaerobic conditions results in the formation of methane which given aerobic conditions may then be used as inductor. Furthermore, some further compounds may serve as inducers such as e.g. toluene frequently occurring in accompanying contaminations such as BTEX. Also the compounds phenol, ethylene, propane, cresol, isoprene or ammonium may induce the aerobic degradation of volatile chlorinated organic compounds. Only if one or a few of these compounds and oxygen are available in a sufficient quantity the volatile chlorinated organic compounds may be oxidized cometabolically with a significant rate. TCE and its metabolites are aerobically oxidized to form the respective epoxides decomposing spontaneously to form further products which are either already free from chlorine or may be mineralized by so-called heterotrophic microorganisms under simple conditions. Degradation reactions not forming epoxides are also known. Furthermore, at least VC may be degraded productively under aerobic conditions, i.e. being used as C- or energy-sources.

In **chloroethanes** the initial product 1,1,1-trichloroethane (TCA) is obviously degradable only cometabolically under aerobic conditions and only difficult to be degraded. Anaerobically the degradation is effected by a reductive dechlorination via 1,1-dichloroethane (DCA) to form chloroethane (CA). The metabolite CA is subject to a fast abiotic decomposition to form ethanol. That is why, as a rule, an accumulation of CA does not take place. Apart from that, in the case of TCA a significant abiotic degradation is to be expected forming the metabolites 1,1-DCE and acetate.

The aerobic degradation of the TCA family has been comparatively little investigated. It is, however, quite probable that at the C-atom not substituted an oxidation will take place forming tri- or dichloroacetic acid. Chloroacetic acids and ethanol formed from CA are equally easily mineralizable by heterotrophic microorganisms. During the anaerobic and the aerobic degradation chlorine atoms are released as Cl^- .

As to the **possibility of the biological remediation of volatile chlorinated organic compounds** there may be stated that microbial *in-situ* remediation has been carried out already on a commercial scale. Depending on the initial contamination and the site-specific conditions three various technological variants may be implemented:

- complete anaerobic degradation after infiltration of an auxiliary substrate (PCE, TCA etc.),
- complete aerobic degradation after infiltration of an inductor (cDCE, VC),
- complete degradation by means of a sequential anaerobic-aerobic technique after infiltration of an auxiliary substrate and in the runoff from an inductor (PCE, TCA).

As mostly a mixed contamination with distinct shares of the initial substances occurs in contaminated sites remediation variant (A) is most frequently applied.

6.6 Hexachlorocyclohexanes

The compound hexachlorocyclohexane (HCH) of the group of difficultly volatile non-aromatic chlorinated compounds is considered by way of example. Hexachlorocyclohexanes (HCHs) with the empirical formula $C_6H_6Cl_6$ are chlorinated cyclohexanes occurring in eight stereoisomers. The main source of HCH contamination is the production of lindane. Until the early 1980ies lindane has been one of the insecticides spread most frequently worldwide. It is produced by chlorinating benzene and isolated from the mixtures of isomers formed thereby. That is why it contains mainly γ -HCH and insignificant shares of α - and β -HCH. Therefore the term lindane and γ -HCH are frequently identified. When producing lindane in addition to HCH isomers also chloroaromatics with a partly very high humantoxicological hazard potential are obtained. That is why cases of damage connected with the production of lindane are, as a rule, mixed contaminations. HCHs are strongly sorbed to the soil matrix so that the quantity transported with drainage water into groundwater is only insignificant.

At ambient temperature HCHs are solids and in contrast to volatile chlorinated organic compounds difficultly water-soluble, scarcely volatile and persistent. Volatile chlorinated organic compounds are accumulated in the nutrient chain. By photolysis, i.e. within reach of UV radiation of sunlight, they are abiotically modified or decomposed. The most stable isomer is β -HCH.

The insignificant water solubility of HCHs and the speed of aftersolution or desorption limit connected with it limit the speed of their degradation. The biological degradability of HCHs is affected by the spatial position of the chlorine atoms to the ring. Except β -HCH volatile chlorinated organic compounds may be degraded anaerobically or aerobically with splitting off chlorine. Aerobically γ -HCH is only slowly transformed to γ -pentachlorocyclohexane; α -HCH is transformed analogously to γ -pentachlorocyclohexane. Anaerobically γ -HCH is further dechlorinated reductively via γ -tetrachlorocyclohexane as first metabolite to form chloroaromatics.

Laboratory tests with aged soils contaminated by HCH seem to confirm that β -HCH is not biologically degradable, yet α -HCH and γ -HCH are degradable as has been proved. The presence of bacteria transforming α -HCH and γ -HCH in soil samples was the decisive factor

for a significant degradation of these isomers. The question important for remediation whether these isomers are better anaerobically or aerobically degradable and to which final products they may be transformed may not yet be answered finally.

6.7 Chloroaromatics

Chlorinated aromatics (chlorobenzenes, chlorophenols, polychlorinated biphenyls, dioxins und furans) may occur in a great number. The number of persistent, ecotoxicologically or humantoxicologically alarming chloroaromatics relevant to contaminated sites is comparatively high.

With the number of chlorine atoms rising the number of the potential isomers (= compounds with different structures with the empiric formulas being the same and thus with various physical and chemical properties) increases. With the degree of chlorination rising the biological degradability goes down; in general, higher chlorinated compounds are classified as hardly degradable under the usually prevailing biogeochemical conditions. Similarly as in volatile chlorinated organic compounds highly chlorinated aromatic compounds are degraded preferably anaerobically, lower chlorinated compounds rather aerobically.

Within the individual substance groups volatility, water solubility and mobility decline with the number of chlorine atoms rising. Whereas stability and persistence increase the highly chlorinated aromatics remain in subsurface layers of the soil and may, like highly molecular PAHs, come to deeper soil layers by co-transport, with co-contaminants serving as dissolution mediators. Owing to their lipophily chloroaromatics are stored by organisms accumulating in the nutrient chain. Persistent chloroaromatics are everywhere detectable also if their production has been stopped since long ago or their use (e.g. use of phenol containing wood preservatives and production of lindane) in the OECD countries has been prohibited already for a long time.

6.7.1 Chlorobenzenes and chlorophenols

Chlorobenzenes and chlorophenols are used for producing varnishes, pesticides, wood preservatives and impregnating agents etc. In addition, they may be obtained as intermediate or side products of chemical production, e.g. in connection with the production of lindane. HCB, PCP and 2,4,6-TCP were used as fungicides, 2-, 3- und 4-CP as fungicides and bacteriocides. In chlorobenzenes and in particular in chlorophenols their toxic effect on microorganisms depending on the concentration is an obstacle to biological remediation. The compounds (mono)chlorobenzene (MCB), 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4-DCB), 1,2,4-trichlorobenzene (1,2,4-TCB), hexachlorobenzene (HCB) and 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol and (2,4,6-CP) are relevant to industry. The compounds 1,2,4-TCB, 1,2,4,5-tetrachlorobenzene, 2,4-DCP and 2,4,6-TCP are technologically relevant to contaminated sites.

The lower chlorinated chlorobenzenes are still comparatively well water-soluble with 30 mg/l (1,2,4-TCB) to 460 mg/l (MCB) whereas HCB is nearly insoluble with 8 µg/l. MCB, 1,2-DCB

and 1,3-DCB are liquid and strippable at ambient temperature. For chlorobenzenes with the molecular weight of 1,2,4-TCB and higher the potential loss by outgassing is negligible. Chlorobenzenes may be irreversibly immobilized in soil by sorption. However, chlorophenols are clearly better water-soluble owing to their hydroxyl group. Even the water solubility of PCP, the most highly chlorinated phenol is still in the mg/l range (19 mg/l). The volatility of chlorophenols is insignificant. They may be fixed to particles by sorption and again released by elution. Humic substances and biomass may bind chlorophenols.

Given **anaerobic** conditions chlorobenzenes and chlorophenols are reductively dechlorinated. Hexachlorobenzene (HCB) is only anaerobically attacked (dehalorespiration or cometabolism), whereas the lower chlorinated benzenes are increasingly better degraded aerobically with the degree of chlorination declining and may be used as C- and E-sources. A part of the intermediate products formed during reductive dechlorination of the chlorobenzenes and chlorophenols are, at the same time, products of microbial transformation and chemical industry. During the reductive dechlorination of chlorobenzenes and chlorophenols chlorine is preferably split off at central or adjoining chlorine atoms. (Mono)chlorobenzene is the final product of chlorobenzenes whereas chlorophenols may be mineralized anaerobically.

During the **aerobic** degradation bacteria may use chlorobenzenes and chlorophenols as C- and E-sources or transform them cometabolically. The chlorinated compounds are worse degradable than their basic components benzene and phenol. The speed of mineralizing chlorobenzenes and chlorophenols increases with the number of chlorine atoms declining with the isomers differing in their degradability. The anaerobically persisting 1,3,5-HCB seems to be also aerobically refractory. In contrast to chlorobenzenes the highest chlorinated phenol (PCP) will be aerobically mineralized owing to its hydroxyl group (-OH) and, beyond that, seems to be better degradable aerobically than anaerobically.

A prerequisite to mineralization is the ability of the bacteria to dechlorinate the chloroaromatics and to fission the aromatic ring. Chlorobenzenes and chlorophenols may be mineralized by dechlorination (1) before ring fission (e.g. PCP) and (2) after ring fission (e.g. mono- to tetrachlorobenzenes, chlorophenols). Fungi degrading lignin may transform chlorobenzenes and chlorophenols, including chlorophenols serving as fungicide wood preservatives, only aerobically and cometabolically. Chinones are formed, to a small extent also CO₂, dechlorination products and higher molecular products of conjugation and polymerisation reactions. In addition, they may be transformed to polychlorinated dioxins/furans.

The **consequence for the remediation practice** is that non-toxic concentrations, especially at chlorophenols, are the basic prerequisite to a biological remediation. As cases of damage with chlorobenzenes and chlorophenols are often mixed contaminations containing aromatics with a differing degree of chlorination there should be individually clarified in preliminary investigations whether a biological remediation will promise success. Hexachlorobenzene may be attacked only anaerobically. Also tetrachlorobenzenes are better transformed anaerobically. The positive findings of literature relating to the aerobic mineralization of chlorobenzenes and chlorophenols are not yet a guarantee of success of the practice as the degradation of chloroaromatics is frequently incomplete. In addition the type of soil and cocontaminants may negatively affect the transformation of chloroaromatics. The bioavailability of chlorobenzenes restricted by the water solubility and fixing in contaminated sites is an addi-

tional obstacle affecting the degradability. According to the few experiences available there may not be said to which extent and if cases of damage with chlorobenzenes and chlorophenols are suited for a biological treatment focussed only on these substances.

The biological self-purification potential of chlorobenzenes and chlorophenols seems to be extremely small also if insignificant anaerobic transformations by a reductive dechlorination have been detected. An indicator of a latent self-purification potential is, as in other groups of contaminants, the presence of microorganisms degrading or transforming these contaminants in contaminated soil and groundwater.

6.7.2 Polychlorinated biphenyls (PCB)

PCBs consist of a “dumbbell-shaped” skeleton with two benzene rings being linked with each other through a carbon atom (biphenyl). It may be substituted with one to ten chlorine atoms. Theoretically altogether 209 individual compounds designated as congeners are obtained only about half of which being formed in syntheses.

The production of PCBs was stopped in the USA in 1977 and in Germany in 1983. PCBs served as cooling and isolating liquids in transformers, as hydraulic and heat transfer oils and as softeners for adhesives and lacquers. Industrial mixtures of various highly chlorinated congeners were used. The products arochlor 1221 contains 0-3 substituents for chlorine with simply chlorinated biphenyls totalling more than 50 % of the contained congeners. At the other end there is arochlor 1260 containing preferably hexachlorobiphenyls.

The so-called Ballschmitter congeners are six selected leading substances important from the viewpoint of remediation techniques the content of which is determined in usual PCB analyses to subsequently extrapolate the total PCB content.

Most of the industrially used mixtures are practically water-insoluble, stable and persistent. PCBs are bound by organic substances, accumulating frequently in soils and sediments as e.g. sea mud. Owing to their persistence they form part of the substances relevant to environment. PCBs behave similarly as chlorobenzenes. However, already lower chlorinated biphenyls are worse water-soluble by powers of ten so that sorption is a dominating process. The water solubilities of mono to decachlorobiphenyls are between about 6 mg/l and 0,1 µg/l. The water solubilities of isomers with the same empirical formula differ also in PCBs owing to the spatial position of the chlorine atoms at the benzene rings.

The biological degradation of the PCBs is mostly slowly and incomplete as it will not only be limited by their water (in)solubility. Also the distribution and the number of chlorine atoms at the two benzene rings of biphenyl restrict the aerobic and anaerobic degradation, in general, to transformation or partial degradation processes. When checking the PCBs for their „degradation“ frequently only the reduction of the initial substances is measured (which is understandable from practical aspects) so that it will be further unknown where they remained actually.

Biphenyl, the non-chlorinated parent substance of the PCBs, is mineralizable in a simple way via the “metabolism of aromatics”. However, if the parent substance has been substituted by chlorine atoms the aerobic attack on the molecule will be complicated. Bacteria dechlorinate PCB as chlorobenzenes reductively under anaerobic conditions. And the aerobic transformation of PCB proceeds according to the same basic pattern as the degradation of chlorobenzenes: monochlorobiphenyls are transformed still comparatively smoothly. Among them 4-chlorobiphenyl may serve as growth substrate, i.e. as C- and E-sources, whereas the further isomers seem to be attacked only cometabolically. In PCBs with ≥ 3 chlorine transformation reactions dominate. PCBs with ≥ 5 chlorine atoms represent, as a rule, the upper limit for an aerobic transformation.

Fungi may also cometabolically transform PCBs equally under aerobic conditions as chlorobenzenes. As the attack is unspecific the position of chlorine atoms does not affect the transformation. Nevertheless, the biological attackability of the PCBs declines with the degree of chlorination increasing. Hexachlorobiphenyls are scarcely transformed.

The success of applying biological techniques in soils polluted by PCBs has so far been extremely insecure. A proper control of success is scarcely possible as the criterion „reduction of the reference congeners” does not say where the PCBs are actually left. In addition, products of anaerobic dechlorination may be final stages which may be also scarcely or not further transformable under aerobic conditions. Finally, most of the congeners existing in soils contaminated by PCBs may be transformed only cometabolically under aerobic conditions. Owing to the difficult and incomplete degradability and the predominantly low bioavailability a biological remediation of a site contaminated by PCBs seems to be scarcely appropriate. Whether PCBs are suited for an uncontrolled humification is unclear.

6.7.3 Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF)

Polychlorinated dioxins and furans are aromatic ethers where two benzene rings bearing up to four chlorine atoms per ring are connected with each other through one or two oxygen bridges (Fig. 10). Owing to the big number of the potential positions of the chlorine atoms on both rings there is a multitude of potential congeners the toxicity of which is classified as high. Seven compounds of tetra to octachlorodioxins and ten compounds of tetra to octachlorofurans bearing chlorine substituents in the positions 2,3,7 and 8 are especially toxic.

PCDD/F are chemically stable, predominantly extremely persistent compounds formed as side products in the production of chlorinated chemicals and in various combustion processes. In addition, they may be formed in the biological transformation of chlorophenols by fungi. Their water solubility is still lower than that of PCBs, being in the $\mu\text{g/l}$ - to ng/l range. The water solubility of the „Seveso poison“, 2,3,7,8-TCDD, forming part of the most toxic organic compounds is indicated with 13 ng/l . As the polychlorinated dibenzodioxins and dibenzofurans are practically immobile they remain normally in subsurface soil layers. Their air transport with soil particles by the wind is an important way for their spreading over large areas.

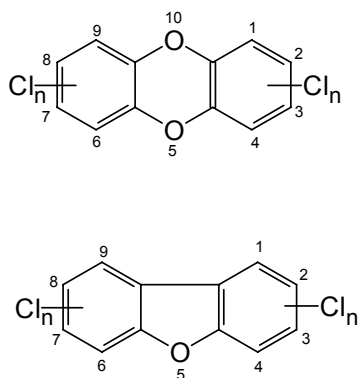


Fig. 10: Structural formula of polychlorinated dibenzo-p-dioxins and dibenzofurans

The non-chlorinated skeletons of PCDD/F (dioxins and furans) may be used as carbon and energy sources by some bacterial strains under aerobic conditions whereas mono and dichlorodioxins and furans and 2,4,8-dichlorodibenzofuran are transformed only cometabolically under aerobic conditions. The reductive dechlorination of PCDD/F was detected anaerobically. A complete dechlorination has not been observed.

Contaminations with PCDD/PCDF are, owing to their toxicity, extremely low bioavailability, low transformation rates and complete detoxification reactions in the sense of mineralization and humification lacking, **unsuited for a biological remediation**.

6.7.4 Trinitrotoluene (2,4,6-TNT)

In the earlier war material production in Germany about half of the explosive production was based on the production of trinitrotoluene (TNT) so that TNT is the main contamination on the abandoned production and processing sites. However, the contaminations found today contain in addition side products of the production and degradation metabolites accumulated in the meantime which are not or only slowly further degradable under the prevailing environmental conditions. In addition to 2,4,6-TNT and its isomers (2,3,4-TNT, 2,4,5-TNT and 2,3,6-TNT) mono and dinitrotoluene, nitrobenzene and nitroxylenes, toluene and benzene and xylene are detected (compounds typical to explosives; CTE). TNT is classified as mutagenic and carcinogenic, 2,6-dinitrotoluene is even more toxic.

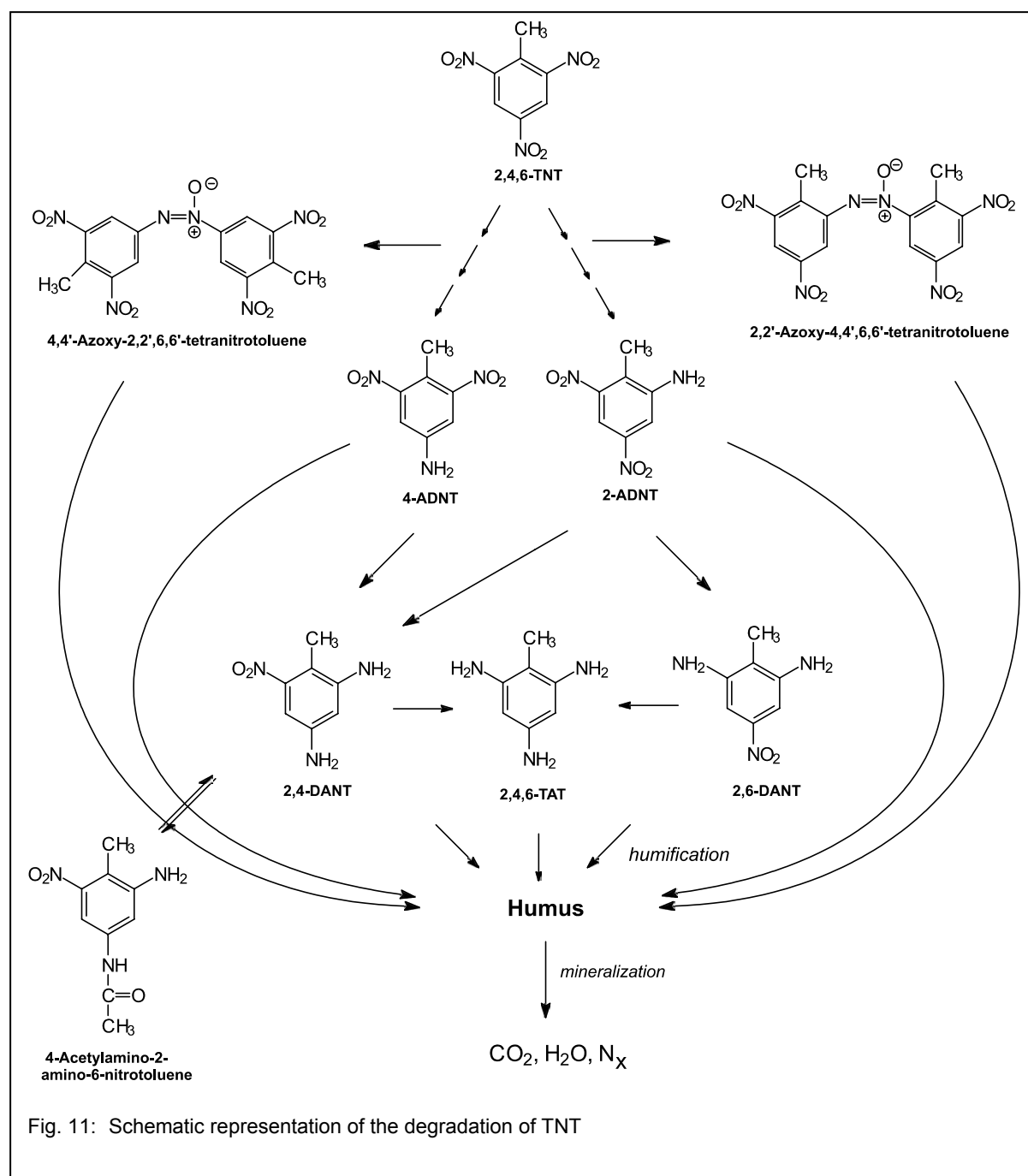
In general, most of the CTE are only cometabolically degradable with numerous metabolites being formed. Under environmental conditions some CTE may be mineralized, for some of them humification is the only efficient elimination mechanism. Owing to the high electron-consuming effect of the symmetrically arranged nitrosubstituents an electron deficit is to be detected at the aromatic ring of TNT so that an oxidative attack of aerobic bacteria is extremely complicated. That is why reductive mechanisms are predominant. As the electron deficit is the more developed the more nitro groups are on the aromatic ring maximally double nitrated dinitrotoluenes may be aerobically mineralized.

The principal way of the bacterial degradation of TNT (Fig. 19) starts with the anaerobic reduction of the nitro group to 4-aminodinitrotoluene (4-ADNT). As a side product also 2-ADNT may be formed. The reduction proceeds via the intermediate formation of hydroxylamino compounds which may dimerize to azoxy compounds. These dimers are subject to a further degradation under anaerobic conditions with nitro groups being reduced and the azoxy compound being again decomposed. ADNT compounds are further reduced forming, first of all, preferably 2,4-dinitroaminotoluene (2,4-DANT). Also aerobic degradation mechanisms may reach this reduction stage. However, the required electrons have to be generated by a cometabolism. Only given very strongly reducing conditions also the last nitro group may be reduced forming 2,4,6-triaminotoluene (TAT). Under aerobic conditions TAT is subject to an autooxidation with a subsequent polymerization.

In an activated anaerobic soil stack, first of all, the reduction steps proceed up to forming DANT. It may not be answered as far as TAT will be formed as this compound will be irreversibly bound to the soil matrix. Owing to the necessarily strong negative redox potential it is hardly probable that TAT will be formed. The amino group of the TNT metabolites may bind covalently with humic substances in a process called humification at the DANT stage already under anaerobic conditions. A subsequent aerobic treatment will increase the extent of binding drastically. In addition, products of fermentation accumulated under anaerobic conditions resulting from the degradation of the auxiliary substrate are mineralized under aerobic conditions. The product of humification does no longer show an ecotoxic effect. Moreover, the bonds seem to be so strong that a release of the metabolites as such is no longer effected under environmental conditions. In the course of the natural degradation of the humic substances such bound residues are very slowly mineralized.

The extent of mineralization may be increased by using white rot fungi. First of all, TNT is transformed in a similar way as described, by the reduction of the nitro groups. The radically working enzymes of the „lignolytic system” of the fungi may aerobically mineralize the metabolites of the TNT (ADNT, DANT) to form CO₂, H₂O and NO₂⁻. Apart from that also humification reactions proceed, based on the reduced nitro aromatics and on the radically activated metabolites. Depending on the environmental conditions and the differing reaction kinetics this will result in a more or less effective mineralization with all residues of contaminants being humified.

There should be taken into consideration that in the course of the degradation of TNT numerous additional polar metabolites may be formed. They involve a.o. 4-acetylamino-2,4-dinitrotoluene (shown by way of example in Fig. 11) and 4-amino-2,6-dinitrobenzoic acid (metabolites of 4-ADNT) or 2,4-diamino-6-nitrobenzylmethyl ether (metabolite of 2,4-DANT). They may be further degraded or humified – which is detected by biotests in remediated soils - but have to be especially monitored owing to their distinctly higher water solubility and thus mobility as compared with the initial compounds.



The co-contaminants DNT and NT are subject to comparable reactions. Yet, NT may be aerobically mineralized already directly or after an anaerobic reduction to 4-aminotoluene (toluidine). The DNT compounds may be aerobically mineralized and humified after the reduction of the nitro groups. Here, the same mechanisms will be of importance as in the degradation of TNT.

The multitude of treatment variants to remediate cases of damage with TNT are based on the principle of humification of the transformation products with the aid of nutrients and aggregates. Microbiologically there are two possibilities of treatment:

1. cometabolic anaerobic transformation of TNT and the accompanying nitroaromatics by bacteria, if necessary, with an aerobic aftertreatment to promote humification.
2. cometabolic aerobic transformation by higher fungi in straw-fungi mixtures and incorporation of the products into humic substances in connection with a potential, insignificant mineralization.

7 Biological techniques of remediation in the soil and groundwater zone

7.1 Fundamentals and classification

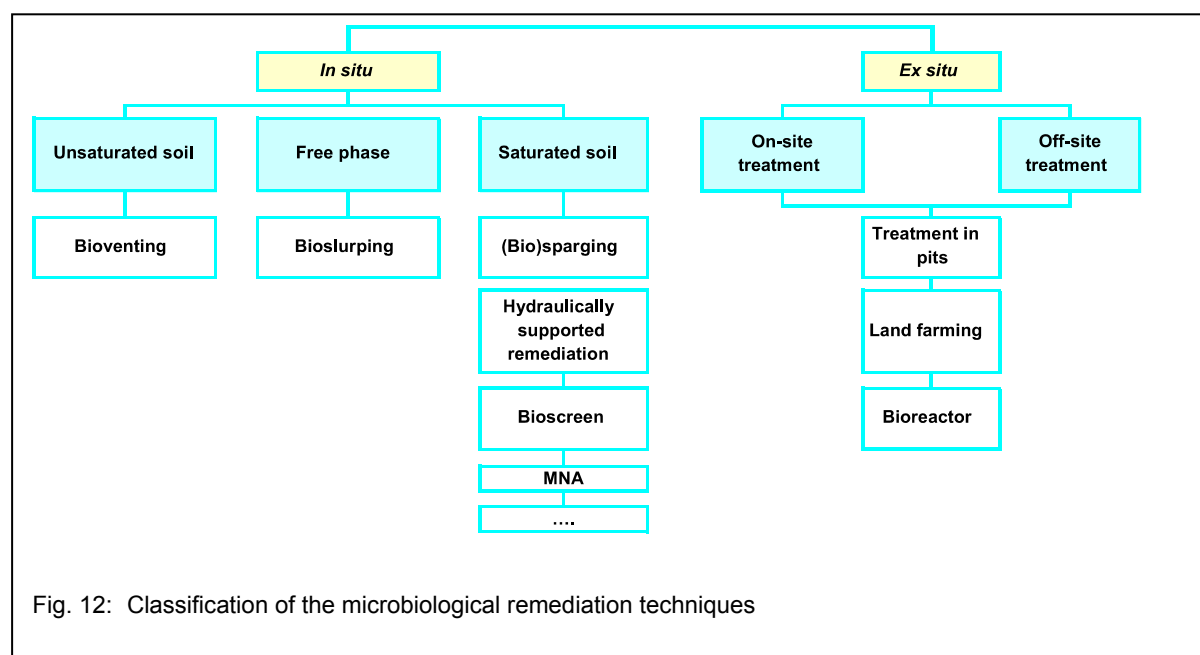
A soil contamination by contaminants is characterized by the fact that the contaminants penetrating into soil migrate mainly vertically downwards. The quantity of contaminants remaining in the unsaturated zone is determined by sorption, diffusion into soil pores and retention by capillary forces. The infiltration area within the unsaturated soil equally as areas where a contaminant phase (s. below) develops shows mostly a high concentration of contaminations. As soon as the contaminants with a low water solubility will reach the groundwater surface by migrating downwards they at a respectively high concentration will form there a separate phase (called LNAPL, light non-aqueous phase liquid). If the density will be higher than 1 kg/l the substances may sink to the base of the aquifer forming there a phase (DNAPL, dense non-aqueous phase liquid). Insignificant shares of contaminants dissolve continuously in groundwater and are transported further with the natural groundwater flow forming a so-called contaminants' plume. The extent of the plume and the concentration of the dissolved contaminants depend on the age of the contamination, the sorption, the characteristic of the transport and on the natural degradation of contaminants. Evaporation reactions proceeding in the unsaturated soil zone result in gaseous contaminations in the case of volatile contaminants. Outgassing from the groundwater surface may, to a limited scale, also contribute to increasing the volume of contaminants existing in the unsaturated zone in a gaseous state.

The heterogeneous distribution of the contaminants ensures that various compartments with always specific (hydro)geological and contaminant-specific characteristics (saturated ↔ unsaturated, highly contaminated ↔ insignificantly contaminated) are to be found in a site. Such various compartments make also various demands on remediation.

The individual remediation techniques are basically aimed at decontaminating soil and/or groundwater, i.e. the contaminants are removed or at least reduced in their quantity and concentration so that permanent hazards, essential disadvantages or remarkable nuisance will no longer exist for the individual or the general public.

In general, the techniques are subdivided into ex-situ- and in-situ remediation techniques. Ex-situ means excavation of soil with a subsequent purification on site or in a different place

(off site). The remediation techniques used on site and off site do not differ basically. Ex-situ techniques are also available for treating groundwater. They involve the extraction of groundwater with a subsequent purification and reinfiltration or drainage. With in-situ techniques the soil remains in its natural place and will be treated there. In-situ techniques are subdivided into techniques suited for treating the unsaturated soil and techniques serving the treatment of saturated soil. A further subdivision is made into active and passive techniques. In-situ techniques comprise mostly also ex-situ components, i.e. plants above ground such as e.g. groundwater and soil air treatment plants. Yet, if the essential elimination of contaminants is brought about by their degradation in subsoil (Fig. 12) we speak always of in-situ remediation.



For the time being, numerous different techniques and technological variants are applied. As a rule, the various techniques were developed also with various targets. Thus, different techniques have to be applied for treating a highly contaminated centre with a possibly still existing contamination phase than for remediating a contaminants' plume in the groundwater discharge area. Only a few techniques are always alternatively available for remediating a specific compartment. Mostly alternative techniques show various limitations so that in practice only one of the techniques is applicable. In Fig. 13 various in-situ techniques and the respective fields of application are shown. Various technological variants consider the various properties of contaminations and the site-specific characteristics, thus representing an adaptation or optimization of the basic techniques.

The techniques discussed individually hereinafter show a various level of development. Most of the bed techniques, in particular if they are applied in soil treatment centres, may be designated as state of the art. Other techniques, in particular *in-situ* techniques and there the latest technologies comprising passive techniques, are not designated as state of the art, even if in some techniques a high measure of successful experiences has been included (as at 2005). This might be explained by the following items:

- *In-situ* techniques are especially adapted to site-specific conditions, thus being unique.
- Owing to the inhomogeneity of subsoil the results of exploration on which the technique is based are available only selectively. This requires that *in-situ* techniques have frequently to be additionally adjusted during their application.

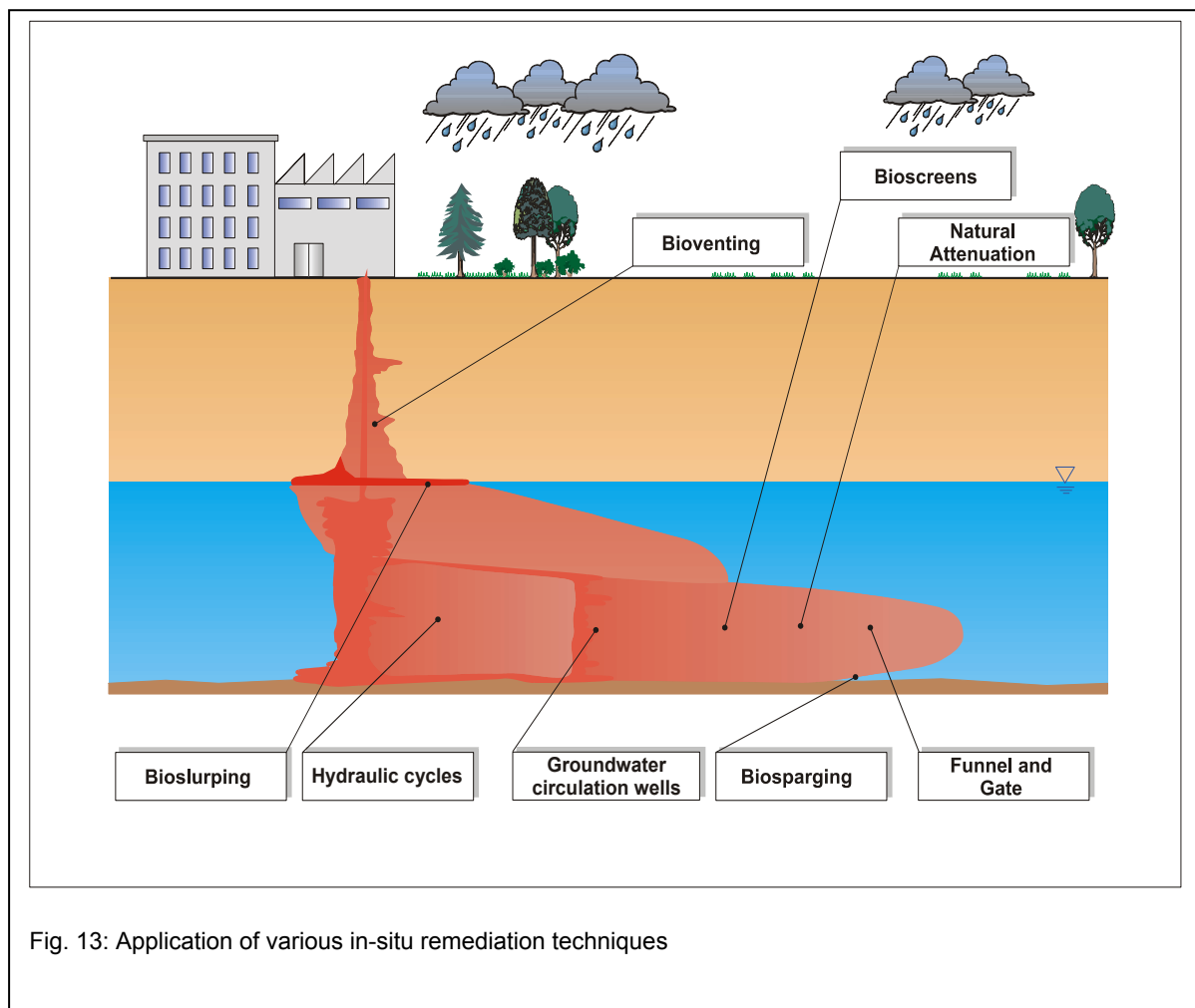


Fig. 13: Application of various in-situ remediation techniques

Nevertheless there is no reason why such techniques should be granted insignificant prospects of success in remediating cases of damage. Which technique will be most suitable may, as a rule, be only decided in the individual case. The application of innovative techniques requires often a comprehensive feasibility study or an intensive monitoring to secure the success of remediation.

7.2 Bioaugmentation

All microbiological remediation techniques may be combined with the so-called bioaugmentation. This concerns the addition of bacteria degrading specific contaminants which were reproduced in a laboratory. These degradation strains may be taken before from site samples, isolated or as collections of microorganisms. However, bioaugmentation is disputed as the mass of bacteria is immobilized in subsoil within some centimeters during an infiltration into subsoil. Also the infiltration of nutrients ensures usually that the microorganisms added are

overgrown by the autochthone microflora and thus the microorganisms added do not bring an advantage as to the speed or quality of degradation of contaminants.

When applying biobed techniques by adding continuously degradation strains a constant presence of the specialists, yet in particular, an always increased biomass concentration, may be reached which resulted in an acceleration of the degradation of contaminants. Also when applying the reactor techniques adding of strains may be possibly appropriate if the environmental conditions may be chosen so selectively that the strains are not replaced by microorganisms present on the site. In the individual case there has to be checked whether the additional costs or the cost advantage predominate owing to the time saved by bioaugmentation treatment.

Only in reductively dechlorinating volatile chlorinated organic compounds bioaugmentation seems to be occasionally required. As, according to the present state of knowledge, only various strains of a unique type of bacteria, *Dehalococcus ethenogenes*, are able to completely dechlorinate the volatile chlorinated organic compounds to get the final product ethylene this remediation technique is also bound to the presence of *D. ethenogenes*. If this bacterium will not be present it may be infiltrated into the aquifer. An appropriate mixed culture is commercially available for this purpose. Yet, for the time being, there is still disputed whether *D. ethenogenes* is not everywhere available or will be needed at all: on some sites a natural complete dechlorination takes place without *D. ethenogenes* having been detected.

7.3 *Ex-situ* techniques

Ex-situ techniques are mostly only applied under specific basic conditions owing to the higher costs resulting, in particular, from constructional measures, as compared with in-situ remediation. They involve frequently also site-specific basic conditions apart from biotechnological limitations. Ex-situ remediation may be required for the following reasons:

- In the course of construction work the contaminated soil will be excavated anyway or a fast subsequent use of the site will not allow time-intensive remediation measures so that only an exchange of soil will come into consideration.
- The mass of contaminated soil may be simply excavated and is only small so that an *in-situ* remediation will be too inefficient requiring, as a rule, already when starting the project significant investments for a remediation plant.
- The soil is so heavily contaminated that *in-situ* remediation seems to be scarcely promising. In this case frequently intensive techniques implementable only in bioreactors or in special biobed techniques are required.
- The contaminated soil shows an insignificant pneumatic or hydraulic permeability. In this case appropriate quantities of structural substances are added so that the soil will be treatable by biobed techniques.

- The type of contaminants or the contamination characteristic (e.g. present in the form of small bits or residual phases) make appear an *in-situ* technique to be little promising.

Ex-situ remediation will start with excavating the contaminated soil. If highly volatile contaminants such as volatile chlorinated organic compounds or AHs are present a significant out-gassing of these contaminants is to be expected. It may be possibly required to excavate the soil housed (with purifying of the exhaust air) (e.g. in the case of a sensible environment or high contaminant loads to be expected) or under a constant suction of the excavation.

Independent of the degradability of the substances it seems to be appropriate to remove the highly volatile components of the total contamination in a separate technological stage (homogenization and intensive stripping). The contaminants stripped may be removed from the gaseous phase by means of various techniques (biofilters, sorption by activated carbon, catalytic afterburning). The soil with the remaining difficultly volatile residual contaminants will be subject to a further treatment. Yet, first of all, the soil will be conditioned. For this purpose, coarse accompanying substances are screened out and, if necessary, separately disposed. Larger components (stones, bricks, concrete) will be screened out, crushed and again added to the soil. Depending on the treatment technique a further preliminary treatment will be required.

7.3.1 Land farming

In land farming the soil is placed in thin layers (max. 40 cm) and cultivated repeatedly with agricultural machinery (plough, harrow, rotary hoe). Thereby nutrients are mixed into the soil, soil aggregates still present are crushed and the oxygen input is supported. The cultivated area is sealed to collect the leachate. Levelled ground bearing for agricultural machinery und sealed by sufficiently dimensioned plastic foils (e.g. HDPE) and sealed clay beds is suited as treatment area. Covering or housing is, as a rule, not envisaged. Sometimes the low bed is covered by oxygen-permeable and water-repellent foils. If a cover is lacking the precipitation water which may be loaded with eluted contaminants has to be collected and subjected to a treatment. An additional aeration of the soil or an irrigation is, as a rule, not carried out. In the course of a regular cultivation in addition to nutrients also structural substances may be mixed in. Mineral granulate, compost, bark mulch etc. may be used as structural substances. By means of structural substances the pneumatic permeability of the conditioned soil mixture may be improved so that the oxygen input will be more efficient. Adding of organic material promotes, on the one hand, cometabolic degradation and, on the other hand, the increase of the biomass concentration promotes degradation, in general. The low bed may be planted as a technological variant. A root penetration of soil will promote adding of oxygen, the supply with nutrients and the environmental conditions for microorganisms.

Fields of application

- For aerobically simply degradable contaminants (e.g. mineral hydrocarbons) if sufficient time and place will be available (is only comparatively rarely applied).

Advantages

- Low demands on the technology and process control
- Comparatively low costs

Disadvantages

- High demand for area owing to the insignificant width of the layer
- Price increase by provision of areas (a. o. rent, base sealing) possible
- Comparatively long period of treatment (months to years)
- In the event of a cover lacking the degradation may be nearly stopped during frost periods. And a water saturation after heavy precipitation may reduce distinctly the oxygen input and thus the degradation speed. Also drying out of the soil may stop the degradation.
- Danger of outgassing of highly volatile contaminants present into atmosphere by crushing the soil aggregates.

7.3.2 Biobed technique

When applying the biobed technique excavated soil is piled up to so-called beds (biobeds) where the microbial degradation of contaminants takes place. When conditioning soil, first of all, coarse parts (building rubbish, metal, wood) are sorted out and disposed separately. The soil is screened and coarse grains (if they contain significant quantities of contaminants) after having been crushed are again added to the soil. Subsequently structural substances are added which – comparable with land farming (Chapter 7.4) - facilitate adding of oxygen and represent an additional source of nutrients and microorganisms. In case of need, further nutrients may be added. The soil/substrate mixture is homogenized and may then be piled up to beds. Highly volatile contaminants may be possibly efficiently expelled by an additional stripping during conditioning and be removed from the exhaust air by means of biofilters or activated carbon adsorbers. The remaining low-volatile contaminants are degraded in the beds. In general, two different bed techniques are distinguished:

Regeneration biobeds are static biobed techniques. Through installed aeration drains at the bed base air is sucked off during operation and thus the oxygen required for degradation is put into the biobed body. The layer width of the beds may be selected nearly voluntarily due to the construction of the aeration plants. However, heights of 2m are normal. The suction affects the bed temperature. In the event of the bed drying out the lacking humidity may be again replaced through irrigation systems.

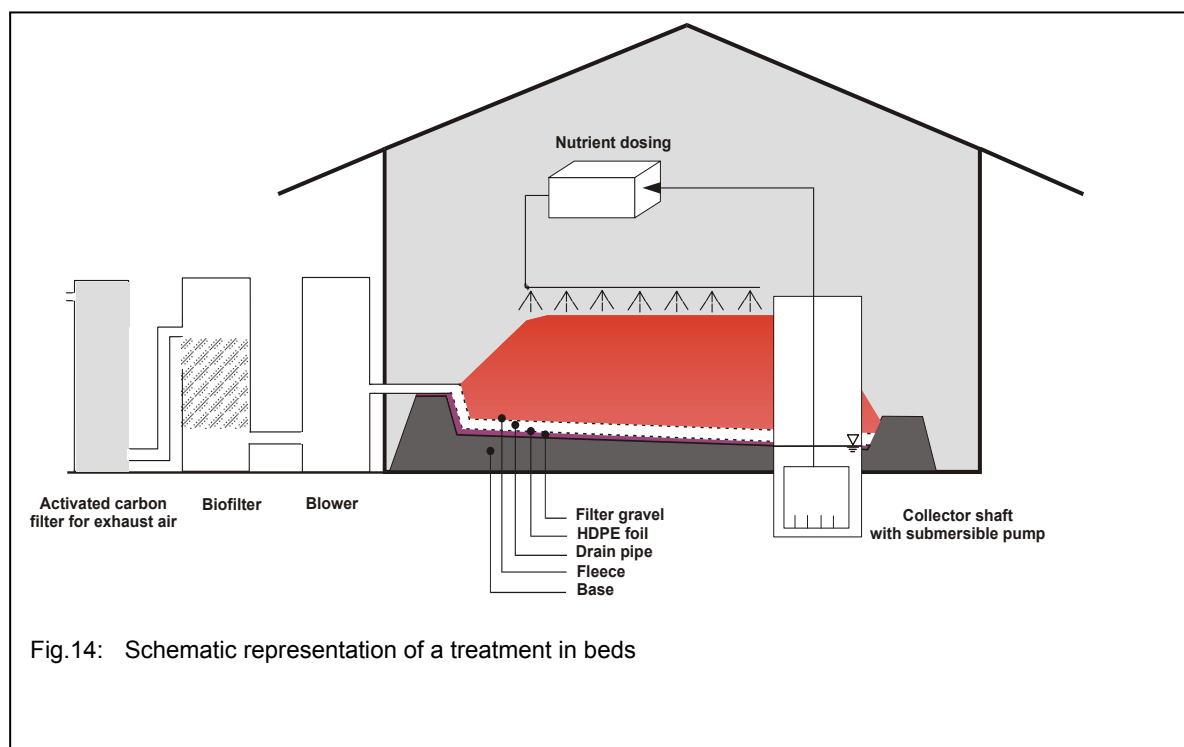
Dynamic biobeds techniques: The soil is aerated by regularly turning the soil by means of special machines. In addition, soil aggregates are increasingly better crushed and supplied with oxygen. During such turning operations nutrients and/or water may be mixed in. The

width of the layers of the beds is about 1.5 m; it is fixed according to the construction-specific features of the turning machines.

Both types of biobeds may be housed or covered by water-repellent, air-permeable foils (e.g. Goretex®). However, when applying the dynamic technique foils are less suited as they have to be removed temporarily for each turning operation. An encapsulation of the beds has the following advantages:

- An increased process temperature due to the greenhouse effect brings about a faster degradation.
- No damming wetness by precipitation → avoidance of anaerobic zones
- Collection of residual emissions and cleaning of exhaust air.

The treatment in beds takes, as a rule, place on specially prepared areas (bed areas) which are prepared in a way to show the required bearing capacity for taking up the soil and the treatment machines and a sufficient density (by means of HDPE foil) to avoid that potentially occurring process water will get into the non-contaminated underlying soil. Fig. 14 shows schematically the structure of a housed mobile bed technique. In stationary soil treatment plants all described components of the process are combined in an ideal way.



Originally the bed technique was designed for the treatment of soil contaminated by mineral oil hydrocarbons. The technology shows that mainly aerobic degradation mechanisms are applied. In the course of developing techniques for remediation of soils contaminated by explosives numerous variants of the bed technique were tested:

Composting: Here, a high share of organic material (up to 40 % by volume) is mixed into the soil. After piling up the bed self-heating with temperatures up to approx. 70°C takes place and thermophilic degradation reactions proceed. The temperature of the bed body may be possibly reduced through aeration. After the thermophilic phase in conformity with a usual composting process further mesophilic transformations will take place, possibly an after-maturation in the form of land farming will be required. Soil composting is basically an aerobic process. Owing to the high content of organic material the soil may have inappropriate properties after treatment for constructional purposes.

Anaerobic bed: Structural substances allowing an improved input of oxygen are not added to an anaerobic bed. Instead of it, strongly oxygen-consuming substances such as e.g. molasses or fresh compost are added. After piling up the bed and compacting it subsequently oxygen-free reductive conditions may be created in the bed body. Following the anaerobic treatment, as a rule, an aerobic aftertreatment will be required for hygienic reasons where accumulated odour-intensive fermentation products are mineralized.

Bioaugmentation bed: Mainly fungi are added to a bioaugmentation bed. Fungi most important with regard to the degradation of contaminants are basidiomyceten (white rot fungi). As they reproduce themselves in soil only badly and prefer wood or straw as natural substrate, first of all, the “production of substrate” should take place. Prepared wood or straw are vaccinated with a fungi culture reproduced in a laboratory. After fungus-mycel has grown through the substrate this substrate/mycel mixture is added to the soil. Subsequently it is homogenized and piled up to a bed. Owing to the sensitivity of the fungi the bed treatment is a static technique.

The bed techniques described may be also combined with each other. Thus, the anaerobic bed was already combined with a subsequent aerobic dynamic bed process for the remediation of soils contaminated by TNT. Also during the anaerobic phase it is possible to turn the soil, however not too frequently. Turning of the soil has the advantage of improving the bioavailability of the contaminants owing to the soil aggregate being crushed. Only if sufficiently high quantities of oxygen-consuming substrates are added to the soil a sufficiently fast anaerobization of the soil will take place after the soil was turned.

Fields of application

Originally applied to soils contaminated by mineral oil after a respective adaptation of the technology the bed technique finds ever new possibilities of application. Thus, soils may be treated which, apart from mineral oil hydrocarbons, contain also further accompanying contaminants to an insignificant extent such as e.g. PAHs. The quantity of accompanying contaminants which is maximally treatable is mostly determined by degradation experiments or by means of diagnostic analyses (e.g. quantity of organic carbon or distribution of congeners in PAHs contamination). Though volatile compounds are expelled during soil conditioning residual quantities of e.g. (mono) aromatics or possibly highly volatile low-chlorinated hydrocarbons may be degraded also by a bed treatment. Bed techniques are finally also applied for the purification of soil contaminated by explosive-typical compounds (ETC). Biobed techniques are frequently applied, for the time being a trend going away from on-site and over to stationary treatment plants is to be stated.

Advantages

- Well controllable microbiological remediation technique

Disadvantages

- Essential use of energy by excavation of the soil, subsequently conditioning and removal or further utilization of the remediated soil
- *On-site* biobeds are technologically expensive so that a transport of the soil to stationary treatment plants may be reasonably priced.

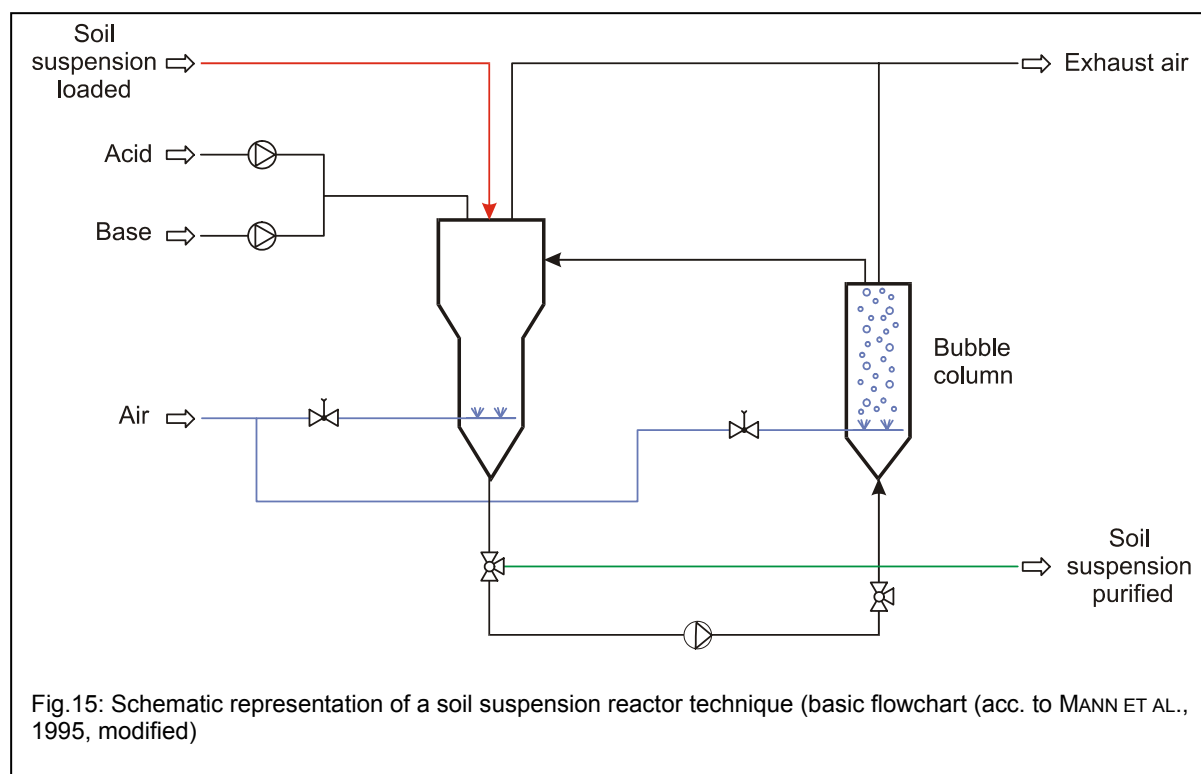
7.3.3 Reactor technique

Similarly as with applying the biobed technique by means of the reactor technique conditioned soil is treated in a closed container, the reactor. By respective measurement and dosing facilities the pH, humidity content, oxygen and nutrient concentration, redox potential and temperature may be optimally adjusted (Fig. 15). This significant improvement of process control results in a shorter time of treatment. In addition, already in conditioning soil the same structural substances (mineral granulate, compost, bark mulch etc.) may be added as with the biobed technique. Emissions do either not occur or are completely collectable and treatable.

Numerous different reactor types may be applied for the treatment of soil. Expressed in simplified terms, each reactor consists of the same components which, however, are different in design and operation.

Depending on the water content dry and suspension reactors are distinguished. Dry reactors work with a water content of 20-50 % of the maximum water retention capacity of soil. Owing to its cloddy structure soil may be mixed. Suspension reactors are used with water-soil sludge being added. The solid share in the suspension is between 30 and 50 % by weight. The soil suspensions are very homogenous mixtures the treatment of which is well controllable. In addition, fine-grained, viscous and hardly permeable soils may be biologically treated. However, the process water has to be separated after treatment and the soil has to be dried.

Furthermore, reactors are distinguished by their mixing facility (rotary drum reactors, stirrer tank reactors, air lift reactors, fluidized bed reactors), according to their structure as horizontal or vertical reactors and according to the way of charging (mostly batch operation). Oxygen is, as a rule, pressed in at the reactor bottom by compressed air, but the reactors may be also operated anaerobically by sweeping them with nitrogen or better helium or the oxygen being consumed by easily degradable substrates.



Fields of application

- Suited for the treatment of fine-grained, cohesive soil or sludge (e.g. the highly loaded residual fraction of soil washing),
- Frequently treatment of soil with significant PAH loads,
- Application of dry reactors, e.g. in treating soils contaminated by TNT, however competitor to biobed or bed composting techniques,
- So far only comparatively little remediation on commercial scale, some designs up to the pilot level.

Advantages

- Treatment of soil materials possible which, owing to their fine granularity and/or level concentration of contamination may not be subjected to a different microbiological treatment.
- Faster degradation by a higher level of soil homogenization
- Frequently higher degree of degradation by a better desorption and solubilization of the mostly hydrophobic contaminants
- Adding of tensides possible as a closed system exists. The tensides are completely degraded upon completion of the treatment or may be separated by the process water and be reused. The advantages of a tenside use should be checked by investigations conducted for remediation because of the high costs of tenside.

- As the processes are controllable adding of degradation strains is possible.

Disadvantages

- High expenditure of energy for the movement of the soil and the separation of the process water and drying of soil possibly required upon termination of the treatment
- High costs

At any rate, reactor techniques have to compete with other techniques suited for the treatment of highly contaminated soil. So far thermal techniques have been more beneficial. In spite of the continuous efforts made to further develop reactor techniques the lacking efficiency in the last few years has sustainably prevented an application of the reactor technique.

7.4 Contaminant phases

7.4.1 Significance of contaminant phases

Basically contaminant phases may not be treated microbiologically. As the microbial degradation takes place only at the phase border (contaminant → water) a complete microbiological degradation of the phase owing to the unfavourable ratio between the active surface and the total volume of the phase requires mostly geogenic periods. That is why contaminant phases have to be treated by means of other techniques. The following techniques which may be also combined with each other are available:

- Hydraulic removal of the phase by groundwater extraction: the phase is collected in the groundwater lowering funnels which develop and may be pumped off by means of special scooping systems. It is disadvantageous that the phase is vertically “spread” and high quantities of the groundwater to be treated are obtained. In addition, e. g. in the case of mineral oil hydrocarbons approx. 30 % of the contaminants remain in phase in the soil which may not be removed by hydraulic measures. The hydraulic removal of a heavy phase at the bottom of the aquifer is accordingly more difficult.
- Removal of the unsaturated soil and skimming of the remaining free phase from the groundwater surface
- Solubilization of the phase with the aid of tensides, extraction of the water/phase/tenside mixture and elimination of the tenside-contaminant complexes in a wastewater treatment plant. This technique may be applied with the aid of hydraulic scavenger cycles and the *push-and-pull* technique
- Removal with the aid of *skimmer* or *scavenger* systems
- Removal of the phase with the aid of the VER (*vacuum enhanced recovery*) technique (sucking off by means of a developed vacuum) which, at the same time, may

be combined with the microbiological *in-situ* remediation technique (bioslurping, comp. Chapter 7.4.2).

- Removal with the aid of groundwater circulation wells (Chapter 7.5.3.3).

Also in Germany practical experience with innovative techniques (tenside washing, VER) is available at present only to a limited extent (as per 2005).

7.4.2 Bioslurping

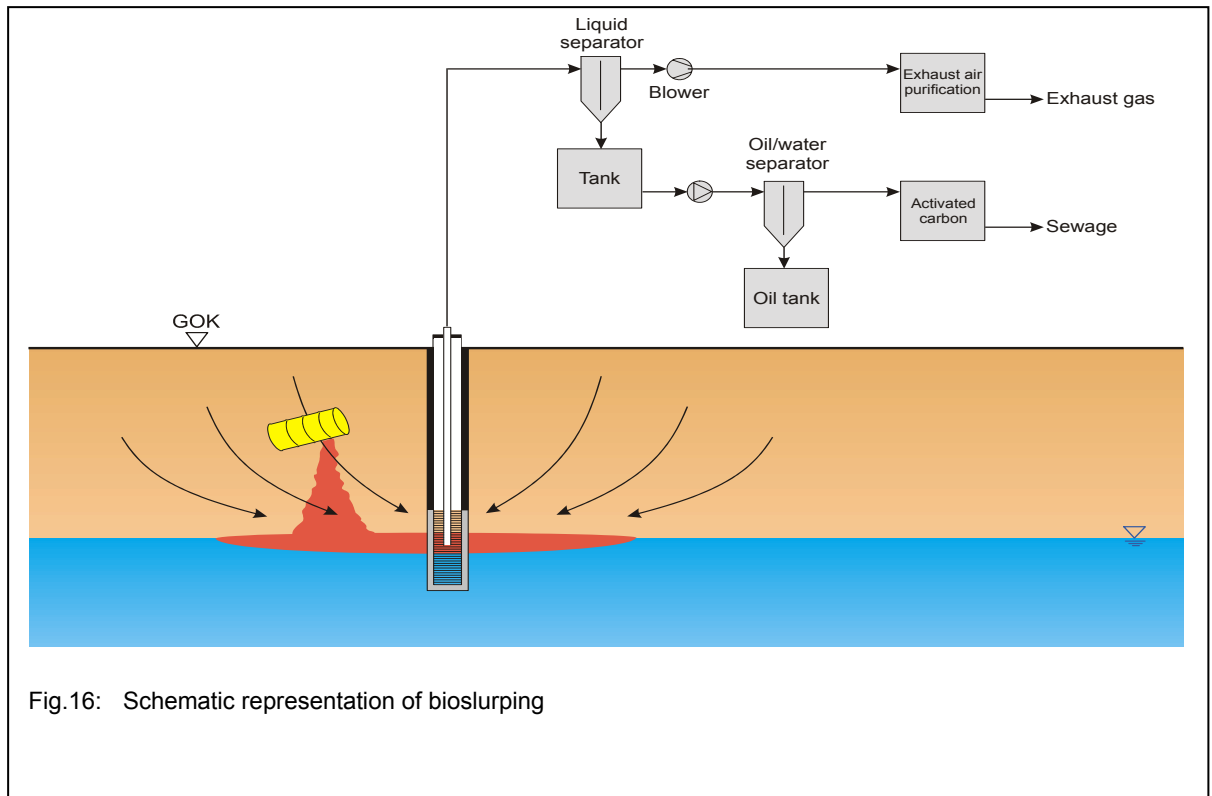
Bioslurping is the only technique serving, on the one hand, the removal of a free contaminant phase (LNAPL) swimming on groundwater and, on the other hand, the support of microbial degradation processes. The phase is removed by sucking it off with the aid of a generated vacuum. Fig. 16 shows the schema of the technique. The bioslurping gauges pressure-tight towards atmosphere are equipped with filters in a zone above the phase up to the groundwater. Within the gauge there is a suction pipe open at the base. The suction pipe is mostly manually adjusted in a way that the suction point will be in the lower zone of the phase. By generating a vacuum in the suction pipe with the aid of a vacuum pump, first of all, the phase and, to a lower extent, also the groundwater are sucked off. If the liquid level will fall below the suction point soil air will be sucked off. This will result in atmospheric air flowing into the unsaturated soil body and thus in a supply with oxygen in conformity with bioventing (Chapter 7.5.2.2). Bioslurping may be combined with the infiltration of nutrient salts into the unsaturated soil zone. The generated vacuum ensures that the phase will flow preferably horizontally to the bioslurping gauge. Thus, phase suction (designated as “vacuum enhanced recovery” VER) and bioventing will alternate. As the groundwater level will remain nearly unaffected only little groundwater will be sucked off during bioslurping.

Above ground, first of all, the contaminant phase and groundwater will be separated in a liquid separator from the soil air sucked off. The contaminated air is mostly treated by biofiltration and/or activated carbon sorption. The liquid mixture will be split up in an oil/water separator. The contaminant phase obtained may be separately disposed. A treatment of water may be effected e.g. by means of sorption of the contaminants to wet activated carbon.

In addition to determining the soil-mechanic parameters, the radius of influence and the *in-situ* degradation rates the investigations mentioned hereinafter are required for designing:

- LNAPL analysis (content of AHs, boiling point distribution of the mineral oil hydrocarbons, viscosity).
- bail down test for the determination of the LNAPL recovery rate

measurement of the vacuum and the groundwater level as a function of the distance from the suction point.



Application

- Especially suited for removing the petroleum product phases.
- Fine sands up to gravel may be treated.

Advantages

- Insignificant volume flow of water and air and therefore comparatively small treatment plants and low treatment costs.
- The main advantage is the horizontally induced flow direction of the phase avoiding a transport of the phase into greater depths.

Disadvantages

- In particular, if the mixture sucked off contains diesel oil the vacuum pumps may cause water oil emulsions to be formed which may no longer be separated in an oil/water separator.
- Contaminant phases showing a high viscosity owing to their weathering are only difficult to be treated.

7.5 *In-situ* techniques

7.5.1 Survey

By biological in-situ remediation techniques are summed up where the polluted soil (unsaturated soil zone) or the contaminated groundwater (saturated soil zone) remain in their natural bedding conditions. Subsoil is so to say to be considered as an „overdimensioned reactor” where the biological remediation process proceeds. When applying in-situ remediation techniques factors limiting the degradation of contaminants in subsoil (lack of nutrients or oxygen) do no longer exist. This is e.g. brought about by the infiltration or injection of suitable substances or aeration techniques. In-situ techniques have the following advantages:

- demolition of structures above ground, excavation of soil, securing of the buildings, soil transport and refilling of the excavation not required;
- may be applied during operation (production, traffic, living etc.);
- preservation of the natural soil structure.

Though soil excavation can be renounced *in-situ* remediation is not more cost-effective in any case. As it requires always an initial investment such as the supply of remediation facilities or construction or connection to the *in-situ* infrastructure the excavation and treatment of the soil in a soil treatment centre may be lower-priced below a minimum quantity of contaminated soil. Above this minimum limit the cost advantages increase with the extent of damage rising by means of *in-situ* measures.

In comprehensively contaminated sites frequently a combination of various techniques is an optimum solution for remediation. The applicability of the individual techniques should be checked in a detailed way in the course of the investigation for remediation (feasibility study).

7.5.2 Technique of treatment for the unsaturated soil zone

7.5.2.1 *In-situ* land farming

In-situ land farming is very similar to ex-situ land farming except for the lacking soil excavation. Deviating from it the soil is treated up to a depth of approx. one meter by means of agricultural machinery. In doing so various aggregates such as nutrient salts, lime for increasing the pH, bark mulch etc. may be added. As the soil is, first of all, loosened by the treatment the input of oxygen is also facilitated. Depending on the technology (e.g. by compaction by means of a roller, adding of oxygen-consuming substrate or covering the soil with a plastic tilt) adding of oxygen may be also again reduced. If organic substrates are added natural, dissolved organic compounds are formed which with the precipitation will be transported into deeper zones of the soil being there available for promoting cometabolic processes. That is why the proper sphere of activity of in-situ land farming will be bigger than the depth of mechanical treatment.

As to the type of soil general restrictions cannot be made. Anaerobic techniques are better suited for more cohesive soils and aerobic techniques for more sandy soils. If structural substances are mixed into cohesive soils to facilitate the supply with oxygen there shall be taken into account that afteruses may be restricted due to the reduced compactability of such soils.

The vegetation of the area may restrict the applicability of the technique; before remediation it may be required to clear the trees.

The technology shall be applied in a way that the mobilized contaminants will be mineralized microbially or immobilized before they with precipitation will be transported to deeper zones. In a respective monitoring with the aid of suction candles or collector drains, however, such effects may be quickly recognized and countermeasures such as e.g. repeated adding of the lacking substrates may be taken.

Application

- Suited in the case of subsurface contaminations. As they are caused predominantly by the input of solids with a low solubility and a high strength of sorption to the soil matrix in-situ land farming is suited, in particular, for treating contamination by TNT and possibly PAHs. In the event of the concentration being too high the contaminants are contained as bigger solid particles difficult to be subjected to a microbial degradation.

Advantages

- Very cost-effective
- Also contaminants with high demands on the conditions of degradation may be treated
- Also an anaerobic degradation environment adjustable
- By suitable aggregates the mass of „raw material“ (e.g. lignin) for humification reactions and the biomass may be increased.

Disadvantages

- Only specific soils are suited for in-situ land farming
- Big contaminant particles shall not be contained
- High demands for a preceding site exploration
- So far only little experience with applying in-situ land farming (as per 2005) was gained.

7.5.2.2 Bioventing

Bioventing is the only microbial in-situ technique available for treating unsaturated soil. The technique is based on a suction of soil air. The pressure differential thus generated causes atmospheric air to enter subsoil, thus resulting in the supply with oxygen for the aerobic degradation of contaminants. Alternatively also atmospheric air may be injected. It may be necessary to add nutrient salts through an irrigation of salt solutions or infiltration through horizontal drains. In some cases adding of nitrogen salts will be required. Nutrients are partly also added as gaseous compounds (e.g. N_2O). Fig. 17 shows a schematic representation of the bioventing technique.

When applying this technique one of the most important tasks is to ensure that an appropriate air flow regime will be reached in subsoil. Especially the geometry of the arrangement of suction wells, the requirement for active or passive aeration gauges and the effects of surface sealing have to be taken into account. A specific preliminary investigation for bioventing is a. o. the determination of the radius of the suction gauges.

High concentration of contaminations may cause a blocking of the soil pores. A supply with oxygen is there drastically reduced. In this case possibly a pulsed soil air suction or purposeful air flow directions by air injection and air extraction may improve the supply with oxygen. However, the injection of air may be only applied if highly volatile contaminants are not present or by the arrangement of the suction gauges there will be ensured that the injection will not cause a transport of contaminated soil air into non-contaminated zones. In addition, there shall be made sure that the injected air will be oil-free which requires special appliances or facilities. If the contaminants to be treated will be volatile the soil air sucked off will have to be treated e.g. by sorption to activated carbon or in a biofilter. A catalytic afterburning will mostly be only useful if a high concentration of contaminations in the gaseous phase exists. However, in this case frequently the biomass will be so damaged that a bioventing will no longer be possible.

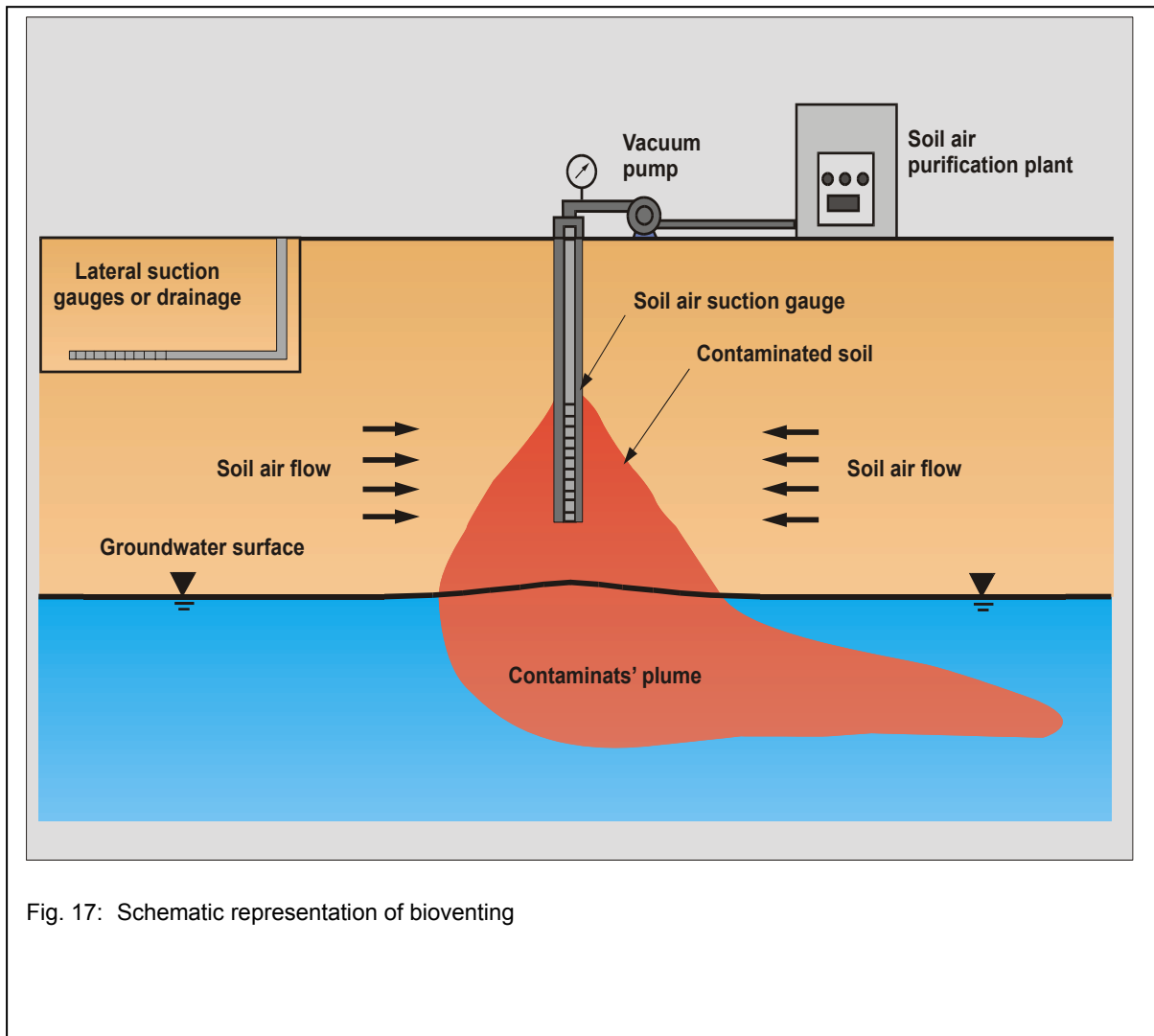


Fig. 17: Schematic representation of bioventing

There is to be considered that soil air extraction will result in a drying-out of soil. By choosing an appropriate rate of nutrient salt infiltration and a nutrient salt concentration based on it drying-out of the soil may be compensated. An optimum rate of degradation requires a water content of 40 to 60 % of the maximum water retention capacity. Higher water contents can result in the formation of water-saturated zones where oxygen cannot be added with air, lower water contents reduce equally the rate of degradation.

It is easy to monitor bioventing (Chapter 9). That is why degradation rates may be indicated here. They total about 0.2 to 20 mg/kg/d e.g. for average permeable soils and a contamination with fuels in the unsaturated soil.

Application

- Suited for remediation of contaminations by mineral oil hydrocarbons (diesel fuel, gasoline), aromatic hydrocarbons or other comparable compounds which are preferably degraded given aerobic conditions.

Advantages

- Owing to the lower viscosity of air as compared with water also soils with a comparatively low k_f -value are remediable. However, the radius of suction has to be determined in the individual case.
- Very cost-effective technique

Disadvantages

- The heterogeneity of soil may reduce the efficiency of remediation. As a rule, areas with a low permeability may be less flown through and the required supply with oxygen is there obstructed.
- Moreover, a high viscosity and a high concentration of the contaminants in hardly permeable soils may result in soils “sticking together” so that a sufficient supply with oxygen and a significant degradation will be ensured only at their boundaries. These aggregates correspond approximatively to pure contaminant phases the degradation of which requires a long time. That is why it is to be recommended to determine reachable degradation rates in a pilot test.

7.5.3 Technique of treatment for the water-saturated soil zone

7.5.3.1 *Hydraulic cycles*

The basic principle of hydraulic cycles consists of pumping off groundwater with a subsequent treatment of it, adding of nutrients to it and clean filtration of the water enriched with nutrients (Fig.18).

The contaminants are degraded in subsoil (in situ) and extracted together with the groundwater (in a dissolved or dispersed form) and removed in a wastewater treatment plant. The distribution between in-situ degradation and hydraulic removal of the pollution depends on their solubility of the contaminants, the in-situ degradation kinetics and the applied technology.

As pumping off and treating of groundwater is cost-intensive the technique has to be optimized with regard to minimizing the groundwater quantities to be pumped off. The whole technique including the in-situ infrastructure and positioning and dimensioning of the infiltration and groundwater extraction wells may a. o. be designed with the aid of a model simulation. The groundwater flow direction can be horizontally or vertically induced by a respective arrangement of the wells. A forced vertical groundwater flow favours the supply with nutrient salts. The groundwater extraction wells have to be arranged in a way that contaminants may not escape. This may be reached by an infiltration being conducted at the border of the area of damage, yet the extraction in the centre. By the arrangement of the wells there should be also ensured that infiltrated nutrients will not get to zones outside the reaction zone. This may be possibly also ensured by protective infiltration or enclosing of the aquifer by means of a bentonite retention wall. To maintain appropriate flow directions it may be necessary to discharge parts of the water extracted into a receiving water.

Usually iron and manganese have to be removed from the groundwater before subjecting it to a clean filtration in a wastewater treatment plant to avoid ochering of the subsequent treatment stages and infiltration facilities. The nutrient salts required for the N/P supply are added to the purified water. This can be done by using urea and phosphoric acid which have the advantage that they do not increase the salt content of groundwater too much. If the groundwater is too sour it is to be recommended to dose polyphosphate salts instead of phosphoric acid. Instead of urea also KNO_3 may be used. This has the advantage that it will not consume electron acceptors but may be used not only as N source but also as electron acceptor. (s. below).

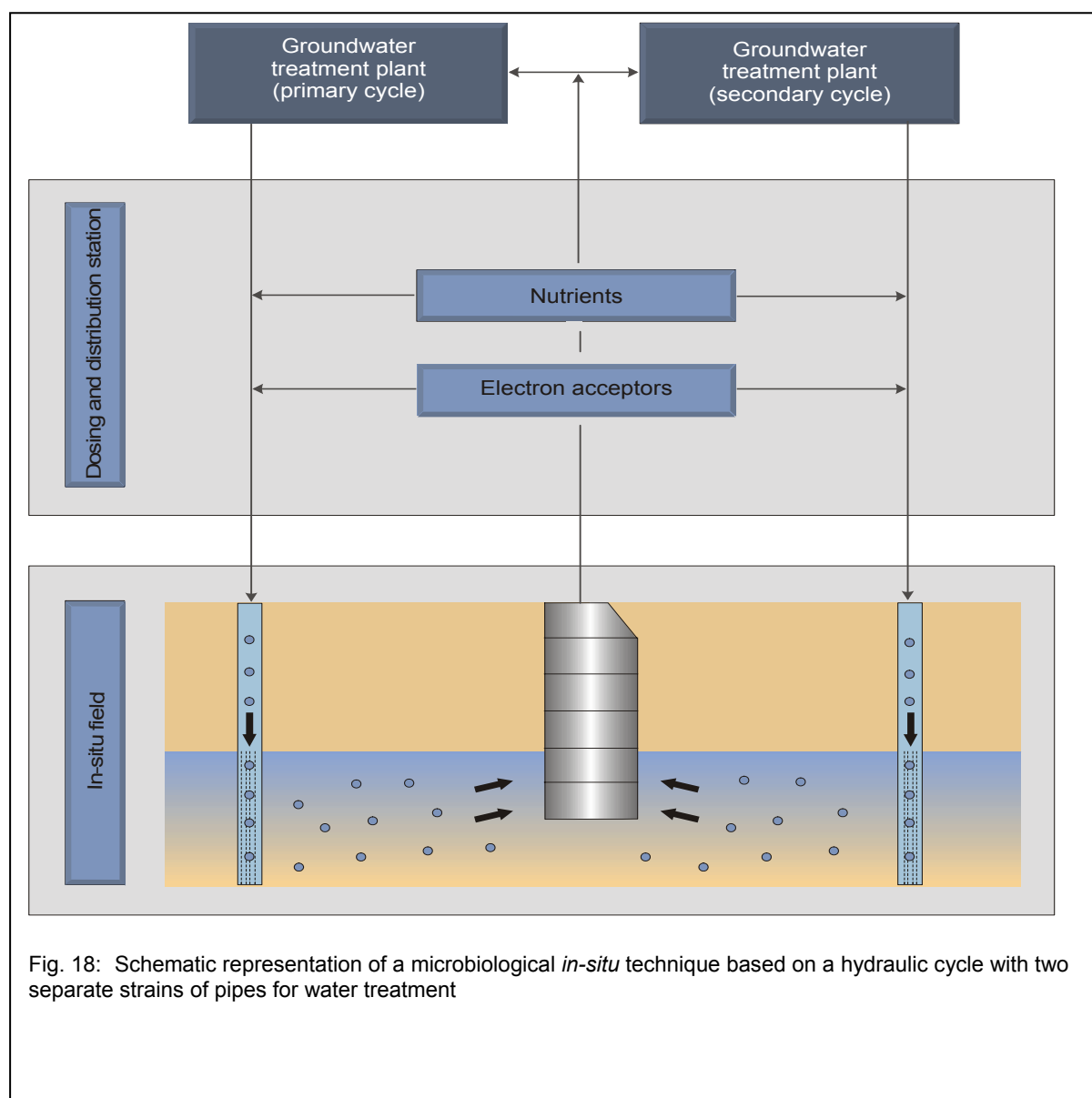


Fig. 18: Schematic representation of a microbiological *in-situ* technique based on a hydraulic cycle with two separate strains of pipes for water treatment

Mostly hydrogen peroxide (H_2O_2) or nitrate (NO_3^-) are applied as electron acceptors. Dosing shall not be too high as otherwise gas bubbles may be formed in the aquifer making it hydraulically nearly impermeable. A re-dissolution of the gas bubbles is connected with remarkable technical problems. It is also possible to infiltrate both electron acceptors in combination. In the close range H_2O_2 supports aerobic degradation reactions whereas the nitrate

will be further transported and denitrifying degradation reactions will be induced in the oxygen-free zone.

If the extracted water will not be clean filtrated but discharged nutrient solutions prepared with fresh water may be used for supplying the aquifer with nutrients.

Application

- Suited for the degradation of all bioavailable contaminants
- Aerobic as well as anaerobic degradation operations are induceable
- The required minimum hydraulic permeability of the aquifer is approximately at a k_f -value of $\geq 10^{-4}$ m/s, in individual cases an application is possible also in the case of low permeabilities.

Advantages

- Also aquifers with a certain homogeneity may be treated by a clever selection of the induced groundwater flow directions
- A high variability and possibility of adaptation to the site conditions are possible by installing also various groundwater flow regimes in small zones, this will allow a fast transport of the necessary nutrients to the place of degradation of contaminants
- Frequently the fastest microbial *in-situ* technique for groundwater treatment

Disadvantages

- Site-dependent minimum time of remediation (months to years) may not be reduced neither at great expenses
- High investment costs (treatment plants) and operating costs (also high demand for energy)
- Intensive monitoring required (to quickly recognize malfunction and take countermeasures)
- High use of process chemicals and big quantities of contaminants
- The infiltration of water into the aquifer is connected with the danger of ochering and other effects leading to a reduction of the receptivity of the infiltration wells (blocking of the subsoil with biomass, shifting of the fine soil grain in the case of too high infiltration speeds), ochering and biomass formation are to be stated notably when the infiltrated water will be enriched with oxygen, hydrogen peroxide or nitrate. That is why at the beginning of remediation the infiltration wells will have to be accordingly dimensioned.

- A regeneration of wells may be required (regeneration with the aid of acids without damaging the biomass in the aquifer is possible)

7.5.3.2

Biosparging (Airsparging)

When applying the biosparging technique (also termed airsparging) oil-free atmospheric air is injected into the aquifer. This induces the formation of a conic area of fine, small branched channels through which air flows into the unsaturated soil area. As the processes proceeding outside these small channels are controlled by diffusion it is desirable that highly branched channels and a cone of influence with a wide opening angle will be formed. To reach this the injection pressure shall be chosen only insignificantly higher than the pressure required for overcoming the water column standing above the sparging point. Also a pulsed air injection may have advantageous effects on the branching of the flow channels. Biosparging can be applied only if the sparging point may be installed distinctly below the contamination. If a significant groundwater flow will be maintained also areas outside the cone of influence will be supplied with oxygen (advective transport).

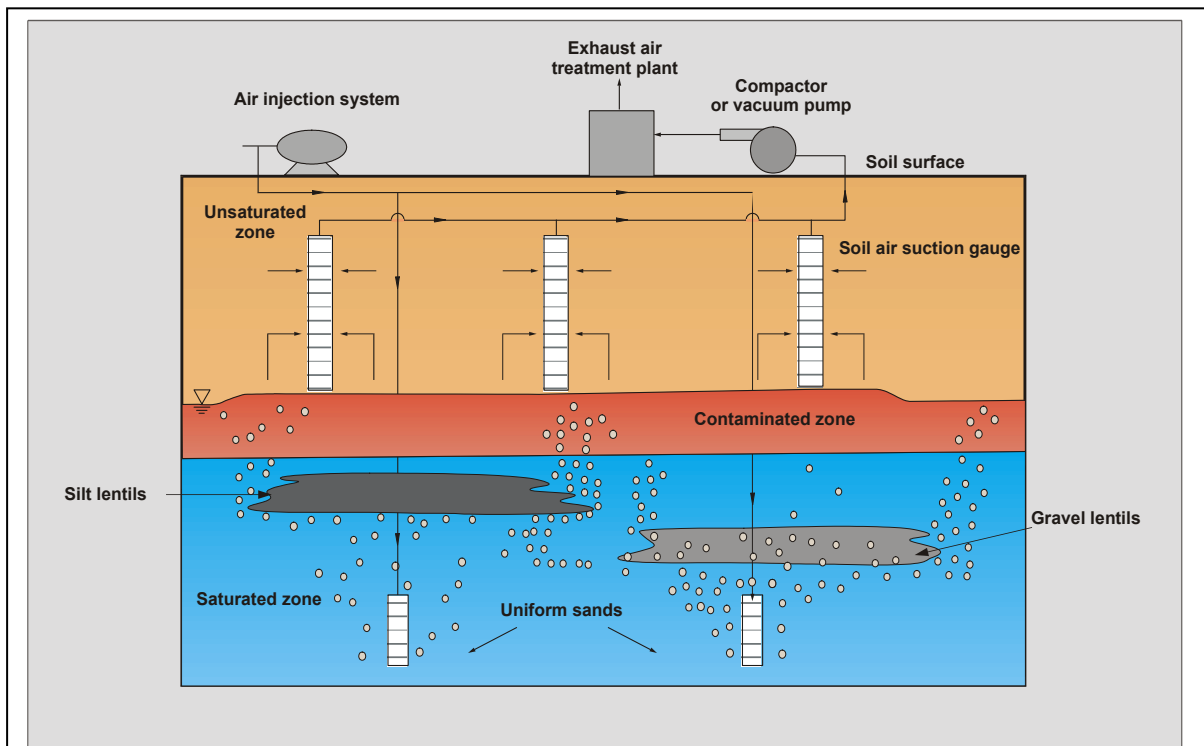


Fig.19: Schematic representation of biosparging

Biosparging supports the *in-situ* stripping of volatile contaminants, the desorption of contaminants and the microbial degradation by enriching groundwater with oxygen.

For highly volatile contaminants stripping plays, obviously, the major part in the elimination of contaminants. The share taken by biological processes is only difficult to quantify. If contaminants are only badly degradable under aerobic conditions (e.g. higher chlorinated chloro-

aliphatics) exclusively an “*in-situ stripping*” is concerned. In the case of difficultly volatile, yet aerobically degradable contaminants (e. g. mineral oil hydrocarbons) stripping plays a subordinate role and sparging serves basically to supply the oxygen required for degradation.

Additional nutrient salts to force the microbial degradation may be put in by the same biosparging gauges or by separate infiltration gauges (Fig. 19). As the contaminants are transported to the unsaturated soil zone biosparging is usually combined with a soil air suction. The volume ratio of injection to suction is mostly in the range between 1:5 and 1:10. As a rule, a pilot test will be required for designing the technique. Mostly an airsparging gauge will be sufficient for this purpose. At regular distances from the airsparging gauge piezometers have to be installed surrounded by filters in the unsaturated as well as in the saturated soil zone. The size of the cone of influence is derived from the measured parameters (change of groundwater level, increase of O₂ concentration in groundwater and concentration of highly volatile contaminants in soil air). After putting airsparging into operation the groundwater level will be raised for a short time, yet will go down to the originally level within a few hours.

Application

- Suited for eliminating highly as well as difficultly volatile contaminants
- Very homogeneously structured aquifers required
- The hydraulic permeability should be in the range of $> 10^{-4}$ m/s, below that the applicability can be distinctly restricted.

Advantages

- Cost-effective method
- By the enrichment of oxygen in the unsaturated soil zone the microbial degradation will be promoted also there (comp. bioventing).

Disadvantages

- Reduced efficiency in the case of inhomogeneities within the aquifer (e.g. redirection of flow channels by insignificantly permeable silt lentils so that the overlying soil area will not be reached).
- In aquifers of a small thickness the cone of influence of an airsparging point may become very small so that a big number of gauges will be required and the technique will become uneconomic.
- A very high pressure to overcome the water column in deep lying contaminations will require an accordingly efficient, yet also comparatively expensive equipment.

- Danger of blocking the aquifer in the case of high concentrations of dissolved iron or also in the case of an efficient microbial degradation of contaminants in subsoil by iron oxide or biomass.

7.5.3.3 *Groundwater circulation wells*

The many special designs of various groundwater wells have two essential joint characteristics:

- They cause a groundwater circulation in the form of a „flow roller“ resulting in an intensive flow rate of water in an ambient aquifer and thus in an efficient supply of nutrients added potentially. The induced flow may be directed from the groundwater level to the aquifer bottom or, with the well being designed differently, in the opposite direction.
- Within the well volatile contaminants are stripped, the polluted exhaust air is purified ex-situ.

In the case of a downward flow the groundwater level is raised and in the case of an upward flow the groundwater level is lowered. If a LNAPL contaminant phase exists it can be collected there in the case of a groundwater level lowering and may be skimmed off. If a DNAPL contaminant phase exists it may come into the well with the downward flow and be stripped there at an accordingly high vapour pressure and thus be removed. Thus, the main task of groundwater circulation wells is stripping of dissolved contaminants and removal of light and heavy contaminant phases. If the wells will be modified accordingly the in-situ degradation potential may be promoted additionally in an efficient way. As an example of techniques such as vacuum evaporator wells (VEW), in-well stripping, coaxial groundwater aeration consisting all of a combined system of groundwater extraction and infiltration within the same well a bioairlift®-well will be explained here (Fig. 20).

At the base and in the area of the groundwater level of the well filtering is effected. Both areas are separated by a full-scale pipe and a bentonite sealing in the ring room. The well consists of an injection pipe, a feed pipe and a jacket pipe. Atmospheric air is pressed into the well with the aid of a lance in the injection pipe below the water table of the well. Thus, an upward flow is produced according to the principle of a mammoth pump. At the same time, volatile contaminants are stripped. The generated vacuum makes groundwater flowing in through the jacket pipe. The water/air two-phase mixture enters through a filter installed above the standing groundwater table into the feed pipe. There, the mixture is separated. The process air loaded with contaminants is stripped and purified. As the water table in the feed pipe is higher than the groundwater table an infiltration will proceed at the base of the well. By the vertical distance between groundwater extraction and infiltration a groundwater circulation will be reached in the surrounding aquifer. This area is flushed thoroughly and thus, first of all, purified hydraulically.

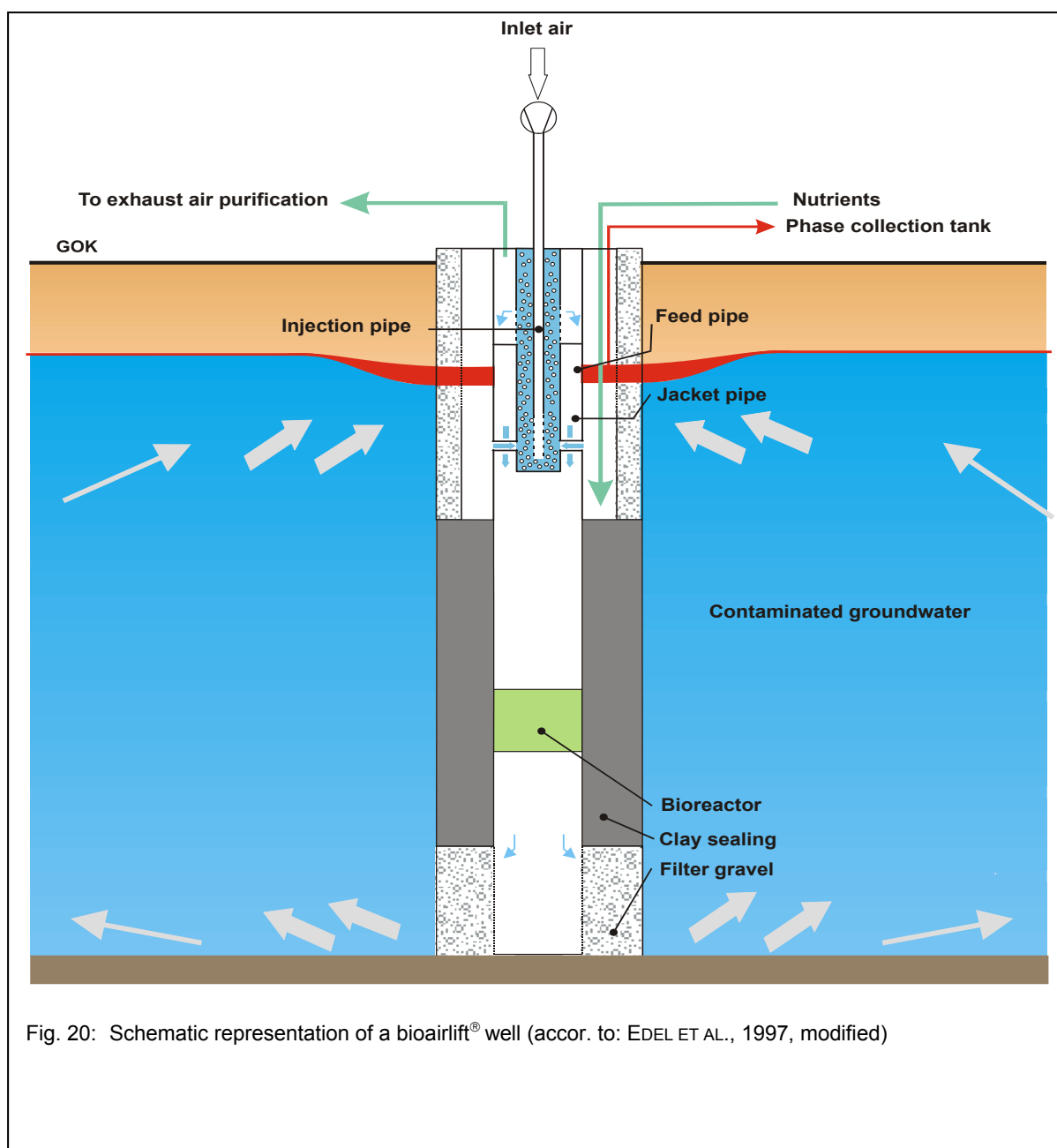


Fig. 20: Schematic representation of a bioairlift® well (accor. to: EDEL ET AL., 1997, modified)

The biological in-situ degradation in the aquifer is promoted in various ways. First of all, water enriched with oxygen is transported to the area surrounding the well by stripping. As the oxygen concentration of approx. 10 mg/l in water will be frequently not sufficient to make available sufficient electron acceptors in an acceptable time of remediation adding of H_2O_2 and/or nitrate may be appropriate. Also further nutrients may be put in through the well.

In addition, a permeable bioreactor containing the immobilized bacteria degrading contaminants may be installed between the points of water extraction and water infiltration. Thus, not only a degradation will be reached within the aquifer but the groundwater passing the well will be purified additionally. As the flow speed is usually too high and thus the retention time in the bioreactor too short to allow a significant degradation these reactors contain additionally activated carbon. Thus, contaminants are, first of all, sorbed to activated carbon and the activated carbon is regenerated by the microbial degradation of the contaminants. Alternatively the reactor can also contain ion exchanger for removing heavy metal ions. If the con-

taminants are not sufficiently degradable under the prevailing conditions they may be also sorbed exclusively to activated carbon. If the unsaturated soil is contaminated, too, the well can be constructed in a way to reach a downward flow in the aquifer. The infiltration point of the well will be shifted to far above the groundwater table. In this case the unsaturated soil may be flushed with water enriched with nutrient. On the other hand, the well may be combined with a soil air suction system. In this case a bioventing of the unsaturated soil will be induced.

Application

- Remediation of contaminations with volatile contaminants such as fuel, aromatic hydrocarbons and chlorinated hydrocarbons
- Owing to the groundwater being enriched with oxygen only aerobic degradation processes will be forced for the remediation of e.g. sites contaminated by volatile chlorinated organic compounds the remediation effect will not be reached by the microbial in-situ degradation but solely by stripping.

Advantages

- Ideal possibilities of combination for various processes (stripping, hydraulic flushing, degradation)
- Completely controlled stripping within the well as contaminated air can no longer escape
- Possibility of treating the subsoil at the same time and removal of lighter and heavier contaminant phases

Disadvantages

- A closed circulation flow will be possibly not reached. In the case of too big distances to infiltration and extraction points or inhomogeneities due to radial displacement the contaminants can be transported to non-contaminated zones
- Dispersive loss at the edge of the flow roller is unavoidable
- By ochering in the case of high iron concentrations in the groundwater a regeneration of the well might be necessary
- To completely collect the contamination a very dense installation of wells may be required → high costs
- The purification targets are often only reachable by a combination with microbial degradation

7.5.3.4 Bioscreen

By the term bioscreen or also passive microbial in-situ techniques a multitude of various techniques is summed up. Nearly no joint features may be detected. Most of the techniques act in situ, i.e. soil air or groundwater are not extracted (exception: injection loops; s. hereinafter). Most of the techniques are applied in defined transects, near the end or at the end of a contaminants' plume, yet partly also in transects directly within the contamination sources. Hereinafter two bioscreen techniques (1. in-situ reactive zone , 2. permeable reactive wall) are described in greater detail.

In-situ reactive zones (IRZ) may consist of a series of closely arranged groundwater wells aligned vertically to the groundwater flow direction within the contaminants' plume (plume transect) or also to the contamination source. Solutions of electron acceptors (e. g. $\text{H}_2\text{O}_2/\text{NO}_3^-$ to force the degradation of non-chlorinated contaminants) or solutions of electron donors (e.g. molasses of lactate to force the degradation of chlorinated contaminants) will then be injected into these wells (pulse injections). Such a system allows to stimulate the autochthone microbial population to adapt to a new redox situation and to develop a suitable contaminant degradation activity.

The so-called "oxygen release compound" (ORC[®]) is used to support aerobic degradation reactions. ORC[®] is a patented magnesium peroxide formulation releasing oxygen when contacted with water. ORC[®] in substance vessels is hanged up in groundwater wells or groundwater measuring points and may release oxygen over a period of up to 300 days. If ORC[®] is exhausted the vessels may be replaced by fresh ones in a simple way. In a typical construction of such dosing facilities PVC may be used as material. The diameter totals approx. 15 cm and the distance between the individual groundwater measuring points is 1.5 m. As the measuring points loaded with ORC[®] have a higher permeability than the surrounding aquifer upstream of the measuring point a convergence and downstream a divergence of the groundwater flow lines will be reached. The dosage points may be constructed also in a staggered form (2 adjacent, staggered series of measuring points erected vertically to the groundwater flow direction).

The electron donors (e.g. hydrogen) mostly required for the anaerobic degradation are added in the form of the so-called „hydrogen release compound" (HRC[®]). This product is mainly used for the in-situ degradation of volatile chlorinated organic compounds. HRC[®], is a highly viscous, insoluble polyacetate which is injected by means of a direct push. In groundwater it hydrolyses to form lactate. Lactate is oxidized to acetate via pyruvate. Hereby, the reduction equivalents required for the reductive degradation of the volatile chlorinated organic compounds are formed. Acetate will be available as growth substrate for autochthone microorganisms.

For extensive contaminations simple injection systems are frequently no longer implementable or economical. In this case, injection loops are installed. These are transects of alternating, directly linked extraction and reinfiltration wells. Concentrated nutrient is added to the closed loops transporting groundwater (Fig. 21).

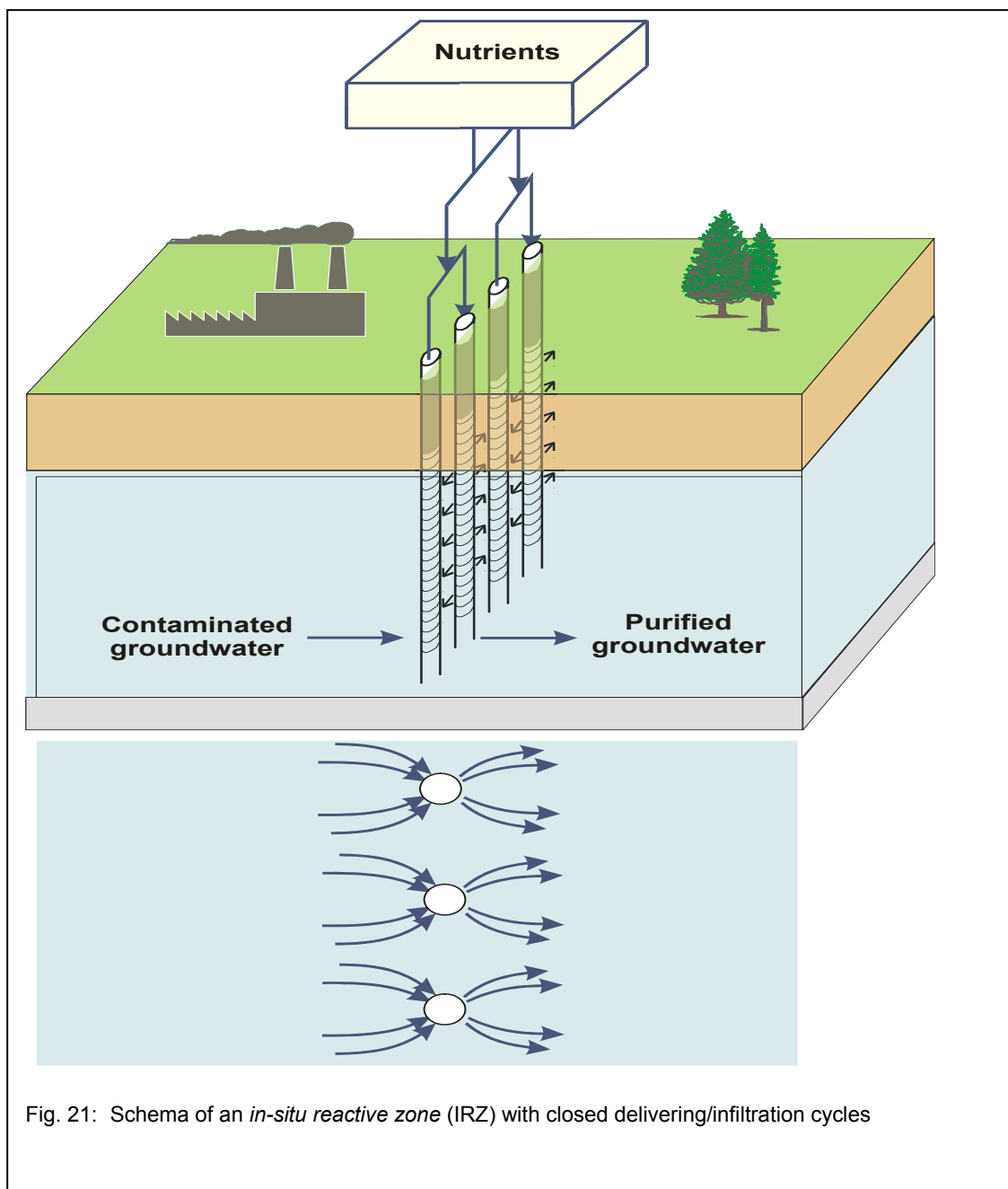
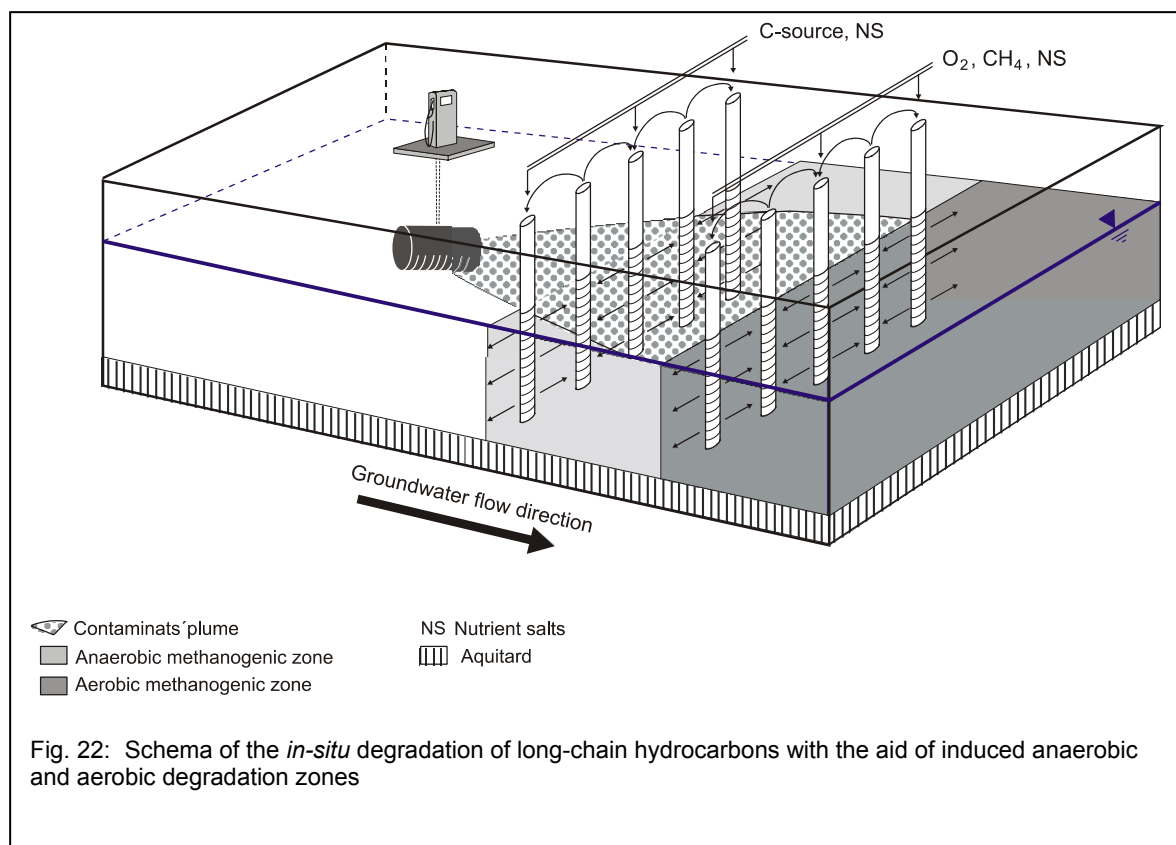


Fig. 21: Schema of an *in-situ* reactive zone (IRZ) with closed delivering/infiltration cycles

By a point by point infiltration in connection with a - with reference to groundwater - induced transverse flow a fast and uniform mixing of the nutrients added is reached in the groundwater, thus creating optimum degradation conditions in every position within the transect.

It is also possible to induce various redox zones one behind the other along the groundwater flow direction where e.g. volatile chlorinated organic compounds can be completely mineralized (Fig. 22).



Application

- Degradation of non-chlorinated contaminants
- Degradation of volatile chlorinated organic compounds (dehalorespiration)

Advantages

- Comprehensive experience, in particular in the USA, relating to the application given various hydrogeological conditions
- Low costs
- Fast success of remediation as compared with conventional techniques
- Low-priced alternatives are available (nitrate, molasses) for the expensive substrates ORC[®] and HRC[®]

Disadvantages

- Extensive contaminations require injection loops
- The formation of methane may be essential given anaerobic conditions.

If contamination sources are not or only insufficiently accessible to a remediation a degradation of contaminants may only take place within a contaminants' plume. The contaminant load to be treated according to the techniques required for that is frequently only small. As the source will not be remediated the techniques serve only securing. The most important technique applied for this is the technique known as permeable reactive barrier. These are permeable, wall-like constructional elements equipped with filling material (reactive media), flown through by groundwater and mostly arranged transversely to the direction of flow. They cause either a retention of the contaminants dissolved in groundwater in the filling material or a reaction of the contaminants with the filling material. Depending on the geometry of the constructional elements flown through a distinction is made between combined constructions of vertical baffles with permeable gates (funnel and gate) and barriers fully flown through.

These systems have mostly a long service life, low or no maintenance is required, nutrients have not to be added during operation. Biological reactive barriers may consist of a mixture of organic waste (compost, wood chips, sewage sludge etc.) in connection with e.g. limestone for the correction of the pH. The organic waste serves as a nutrient source, as structural material, to maintain a high permeability and as a source for bacteria. Also carrier materials (e.g. activated carbon) covered with specific microorganisms degrading contaminants may be used. If the reactive barriers are installed for the whole period of treatment the total quantity of nutrients required has to be calculated based on a mass balance. In this connection there is to be considered that also competing processes for degrading contaminants proceed. Reactive barriers may be also built in a way that the bioreactor materials will be replaceable or regeneratable.

Before installation the materials have to be homogenized to avoid preferred directions of flow within the reactive barriers. Usually the reactive barriers will be designed in a way that the hydraulic permeability will be by about 10 to 100 times higher than that in the surrounding aquifer. As precipitation reactions may result in a decline of the permeability, followed by flowing around the reactive barrier, a regular monitoring of the permeability will be required. The necessary thickness of a reactive barrier depends on the velocity of the groundwater flow within the barrier (i. e. the retention time), the concentration of contamination, the degradation rates and the required degree of degradation. Here, the alterations of these parameters over the time of treatment have to be considered. The groundwater flow through the reactive barrier may be adjusted, if necessary, by pumping the flow minimally downwards. Numerical groundwater models may be helpful for designing the reactive barrier.

Application

- Experience collected on a commercial scale in Germany is still limited (as per 2005).
- Denitrification or „metal barriers“ where a bioprecipitation of heavy metal takes place
- Dehalogenating of volatile chlorinated organic compounds.

Advantages

- Applicable in the event of a homogeneous geological structure of subsoil lacking

- Applicable in the case of a low solubility of hydrophobic organic contaminants and a low velocity of re-diffusion of the contaminants into groundwater
- Well suited for large sites if the application of active techniques is unreasonable
- Low operating costs
- The contaminated area may be used as the remediation plant is at the border of the area.

Disadvantages

- Comparatively little experience with projects on a commercial scale (as per 2005)
- Scarcely knowledge relating to long-term stability (owing to a long time of remediation)
- The long-term behaviour may be only assessed on the basis of the projection of processes followed on the short term
- Respective long monitoring required.

7.5.3.5 Monitored Natural Attenuation (MNA)

In specific cases the monitored natural attenuation (MNA) leads to an elimination of the contaminants' plume (when securing/remediating the contamination source) in an acceptable period. If this will be the case at the site of investigation or not will depend on the site properties. Thereof follows that natural attenuation is a site property and can be only considered as "remediation variant" if the effectivity of natural attenuation has been proved.

Proceeding from the highly contaminated zones (contaminant input locations) within the aquifer (i.e. of the „contamination source“) the dissolved contaminants, are transported away with the natural groundwater flow developing the so-called contaminants' plume. In this plume various processes proceed which may result in a decline of the contaminants. This is termed natural attenuation. Natural attenuation involves all physical, chemical and biological processes causing, without the intervention of man, a reduction of the mass, toxicity, mobility or concentration of the contaminants. These processes may be non-destructive as advection, dispersion, outgassing and solubilization or destructive as biodegradation (mineralization, humification or transformation) and an abiotic degradation (oxidation, reduction or hydrolysis). Only the degradation processes result finally in a removal of the contaminants from environment.

In the genesis of the contaminants' plume, first of all, an expansion along the groundwater flow direction takes place. The length of the plume in the stationary state depends on numerous factors. Expressed in simplified terms, a large plume expansion will occur if the velocity of the groundwater flow will be high and its retarding and degradation will be small. In the event of the velocity of flow being low and/or the retarding or degradation being high the ex-

tension will be smaller. In the case of the source intensity declining (e.g. after removal or separation of the contamination source) the extension of the plume will be also declining with the time. MNA may be only applied if the following prerequisites will be given:

- The microbial degradation of contaminants is the dominating process for their removal.
- A site-specific hazard assessment does not mean an urgent need for action, i.e. sensitive objects of protection (apart from the groundwater itself) are not affected by the contaminants in the plume neither at present nor in future.
- MNA leads to reaching the site-specific remediation targets in an acceptable period.
- The extension of the plume may be prognosticated reliably as to time and space.

MNA as approach consists of the components of microbial in-situ degradation, a scientific proof of the efficiency and quantification and monitoring of the change of concentration of the contaminations. An essential advantage of MNA are the distinctly lower costs as compared with active remediation measures, the long duration of the technology is disadvantageous.

Application

- Dechlorination of non-chlorinated contaminants (aliphatic, mineral oil hydrocarbons)
- Applicable for the degradation of long-chain hydrocarbons on certain conditions

Advantages

- Low costs per year
- No intervention in the subsoil required

Disadvantages

- The contaminated aquifer may not be used over a long period.
- Owing to a long operational life significant overall costs (monitoring) may be incurred.

7.6 Small contaminated sites

Sometime small contaminated sites may occur the elimination of which may not be effected by simple excavation measures as this would demand comparatively high costs (e.g. excavation lining etc.) which are in no proportion to the success. Such cases may e.g. occur when implementing constructional measures. If aerobically degradable substances are concerned it would be appropriate to erect upstream of the area of damage groundwater measuring points without conducting an expensive exploration and planning of remediation and to infiltrate there hydrogen peroxide (H_2O_2) at a low rate. For anaerobically degradable substances the infiltration of molasses or the like as electron donators is to be recommended. Nutrients (nutrient salts and electron acceptors or donators) are transported with the groundwater flow. The dispersion results in a spatial supply with nutrient salts in the contaminated area, pro-

ceeding from a punctiform infiltration point. The extent of dispersion should be estimated before.

7.7 Decision-making matrix for applying microbial techniques

Table 3 contains the techniques described as to their suitability to various basic site conditions.

Table 3: Orientating decision-making matrix for applying microbial techniques

Parameters	Ex-situ methods			In-situ methods								
				unsaturated soil					saturated soil			
	land farming	biobeds	reactors	in-situ land farming	bioventing	bioslurping	hydraulic cycles	biosparging	gw circulation wells	bioscreens	funnel-and-gate	natural attenuation
light non-aqueous phase liquid existing	-	-	-	-	-	+	-	0	+	-	-	-
dense non-aqueous phase liquid existing	-	-	-	-	-	+	-	-	+	-	-	-
mineral oil hydrocarbons	+	+	-	-	+	+	+	+	+	-	-	+
BTEX	-	0	-	-	+	+	+	+	+	+	+	+
PAHs (preferably low-condensated)	-	0	+	0	-	-	0	0	0	0	0	0
phenols	-	+	-	-	+	+	+	+	+	+	+	+
volatile chlorinated organic compounds	-	-	-	-	-	-	+	+	+	+	+	+
chloroaromatics	-	0	+	-	0	-	+	-	-	-	-	-
explosive-typical compounds	-	+	0	+	-	-	-	-	-	-	-	-
unsaturated soil is contaminated (up to 2 m in depth)	+	+	0	+	0	-	-	-	-	-	-	-
unsaturated soil is contaminated (deeper than 2 m)	+	+	-	-	+	0	-	-	-	-	-	-
saturated soil is contaminated	-	-	-	-	-	-	+	+	+	+	+	+
saturated soil is contaminated at great depth	-	-	-	-	-	-	0	0	-	0	-	0
complex mixture of hardly degradable pollutants	-	0	+	-	-	-	0	-	-	0	-	-
soil shows a low permeability	+	+	+	0	-	-	-	-	-	+	+	+
soil shows a high permeability	+	+	-	-	+	+	+	+	+	0	0	0
unsaturated soil is strongly heterogeneous	+	+	+	+	-	-	-	-	-	-	-	-
saturated soil is strongly heterogeneous	-	-	-	-	-	-	-	-	-	+	+	0
small place for remediation plants available	-	-	+	+	+	+	-	+	+	+	+	+
short remediation time required	-	-	+	-	-	-	+	+	-	-	-	-
combination with bioaugmentation possible	0	0	+	+	-	-	0	-	0	-	-	-
small financial funds available	+	-	-	+	+	-	-	-	-	+	+	+

Legende: + suitable or appropriate
 0 suitable with restriction
 - unsuitable or inappropriate

If a contamination phase will exist there shall be taken into consideration that techniques for treating this phase are required. However, as apart from the phase also the unsaturated soil and groundwater are contaminated it will be necessary to apply additional techniques.

8 Additional techniques to accelerate the biological degradation

The most important parameter decisive for the speed of degradation of contaminants is the bioavailability of contaminants. Owing to the insignificant water solubility of the contaminants, their tendency to sorption to the soil matrix and owing to the mostly inhomogeneous or insignificantly permeable subsoil frequently only a small quantity of contaminants is present in a bioavailable form, the additional supply in a bioavailable form is limited because of a slowly proceeding substance mass transfer (desorption, diffusion) the speed of which determines finally the degradation speed. That is why most of the additional measures for accelerating the microbial degradation investigated so far are concentrated on an increase of the bioavailability.

Tensides: Tensides are interface-active substances consisting of a hydrophobic part of a molecule adsorbing to hydrophobic interfaces and a hydrophilic part of a molecule. They act as dissolution mediator and emulsify oil by forming smallest drops (micelles) with a hydrophobic environment developing inside and a hydrophilic environment developing on their surface surrounded by water. Thus, hydrophobic contaminants may be enclosed inside a micelle and brought into „solution“. The formation of micelles is mostly only reached at a tenside concentration in the percentage range. Their addition is only indicated at the earliest if the easily bioavailable contaminant quantity has been already degraded.

By using tensides the bioavailability of the contaminants is to be increased, yet their use is disputed. Because tensides may also obstruct the degradation of contaminants e.g. by sorbing to the soil matrix and thus reducing the bioavailability by co-sorption. There exists also the danger that in in-situ use the solubilized contaminants will be transported with the groundwater flow. The degradability of tensides has to be ensured to prevent them from becoming secondary contaminants, on the other hand, they as the substrate shall not compete with the contaminants. To sum up there can be stated that the efficiency of using tensides in microbial remediation has not yet been proved conclusively. Thus, in every individual case checking of the efficiency and a respective cost-benefit consideration will be required.

Heating: Heating results in an acceleration of all chemical-physical and biological processes. In ex-situ remediation heating is very simple and cost-effective, e.g. implementable by housing, and the degradation speed will be distinctly higher. During in-situ remediation heating of the unsaturated or saturated soil is essentially more difficult and, as a rule, uneconomic. Using the temperature in non-biological remediation techniques is essentially more promising. By means of injecting vapour a significant mobilization of contaminants has been already reached.

Frac-technique: By pneumatic fracturing or hydrofracturing with a high pressure in short pulses in subsoil possibly finely branched cracks are to be produced. Thus the pneumatic or hydraulic permeability of subsoil will be essentially increased. In soils marked by a k^f -value of 10^{-6} to 10^{-4} m/s a good permeability in a radius of 6 to 12 m around the injection point may

be reached with the aid of the frac technique. For this purpose only a borehole has to be made where the PVC pipe will be inserted for injection. The high number of required frac points and the high costs connected with it is of disadvantage to the frac technique – in cases of large sites. Notably in in-situ remediation frequently conducted with the soil surface covered by buildings the frac technique will not be applicable. It might be also essential that the frac technique might presumably cause not only uniformly branched cracks but the development of preferable flow directions.

Result: So far no well suited, cost-effective additional measures are available for promoting the degradation speed. The applicability of individual measures has to be checked in each individual case.

9 Monitoring and efficiency review of remediation

9.1 Targets of monitoring

A prerequisite to achieving success for a remediation measure is the controllability of the biogeochemical, hydrogeological and technological processes. With monitoring accompanying remediation this technology is supervised, on the one hand, and, on the other hand, it is checked or proved if the target of remediation has been reached.

A specific monitoring programme shall be made up for each remediation where the sampling spots, the frequency of sampling and the parameters to be analyzed are fixed. With the monitoring programme an optimum as regards analyzing (as much as necessary) and the costs resulting from it (as low as possible) has to be found.

9.2 Monitoring of *ex-situ* techniques

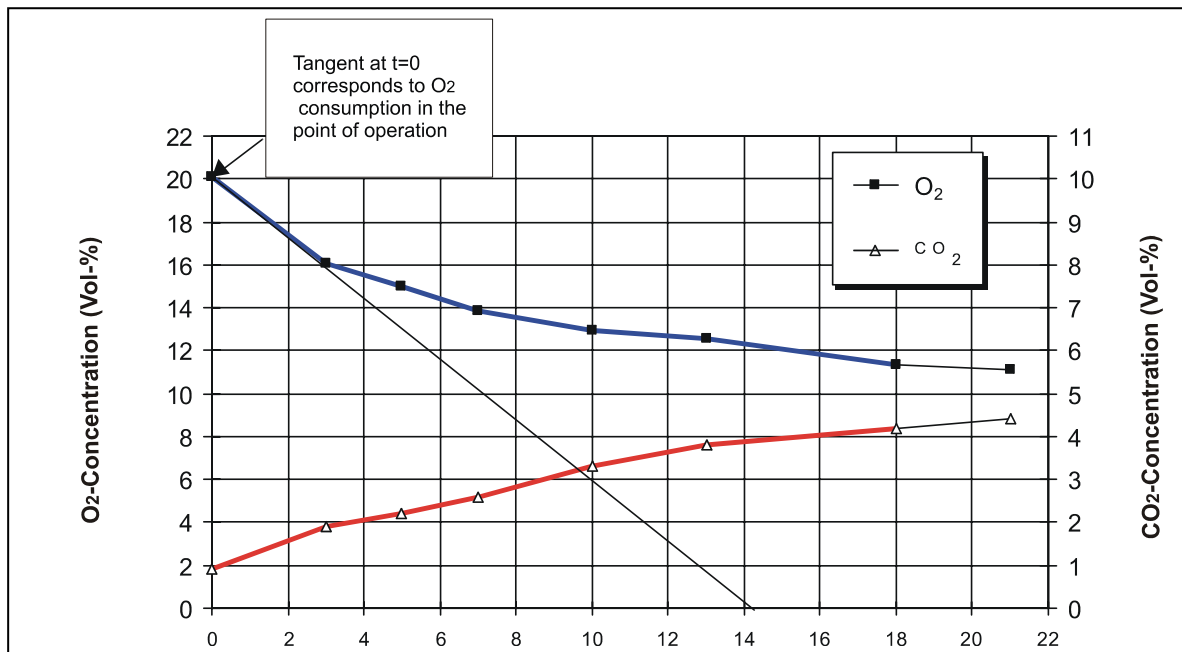
Monitoring of *ex-situ* techniques differs significantly from monitoring of *in-situ* remediation. When applying biobed techniques nearly no other analyses are carried out besides monitoring of the reduction of contaminants. In isolated cases when the remediation does not correspond to the expectations the following parameters may be determined in the soil samples taken:

- humidity
- pH
- nutrient content
- bacterial counts (total bacterial count, count of bacteria degrading contaminants)

By soil air probes the concentrations of O₂ and CO₂ within the bed body can be measured. If an aeration or suction of the biobed do not take place the degradation rate may be assessed via the temporal modification of these parameters (consumption of oxygen and formation of CO₂) (*In-situ* respiration test; ISRT) (Fig. 23).

The determination of the consumption of O₂ is better suited for calculating the actual degradation rate than the formation of CO₂ as CO₂ may be subjected to further reactions. A tempo-

ral modification of the degradation rate allows to assess whether the remediation measures carried out are appropriate. A reduction of the degradation rate in the course of remediation may possibly indicate the lack of water, nutrient salts or degradable contaminants.



When applying reactor techniques the process is completely controlled. By a comprehensive automation the most important parameters (pH, redox potential, oxygen supply, nutrient supply) may be measured on-line and subsequently corrected when deviating. The process control is carried out in the same way as with other biotechnological reactor techniques (e.g. wastewater treatment).

9.3 Monitoring of *in-situ* techniques

In *in-situ* remediation of unsaturated soil (bioventing) the most important monitoring instrument is the *in-situ* respiration test (IRST) to be carried out with the plant shut down. Though the ISRT allows only a selective determination of the degradation rates (at the location of the measuring gauge) the *in-situ* degradation may be followed with it comparatively exactly. Furthermore, with the plant operating the following parameters may be measured in the soil air sucked off and at the monitoring wells:

- concentration of contaminations
- O₂, CO₂
- radon concentration (²²²Rn)
- relative humidity
- volume flow
- vacuum

Thus, the actual reach of suction and the contaminants discharged and soil humidity may be determined. The actual soil humidity may be measured on-line also with the aid of the humid-

ity probes installed in subsoil. Thus, there may be ensured that the soil will not dry up in the course of soil air suction. With the aid of the natural tracer radon the share of atmospheric air mixed with the soil air sucked off may be calculated.

If nutrients are infiltrated and spray irrigated it might be necessary to prove with the aid of groundwater monitoring that neither nutrients are transported into groundwater nor contaminants are significantly eluted and transported into groundwater. The distribution of nutrient salts in subsoil may be determined with the aid of installed suction candles collecting the soil eluate under vacuum. Soil samples for determining the residual contaminant contents should be only taken at comparatively great time intervals.

When remediating the saturated zone the numerous processes proceeding in an aquifer have to be covered possibly in their entirety. Remediation will be only successful if the transport of the nutrient salts and electron acceptors/donators to the location of contamination and the removal of the final metabolic products (e.g. CO_2 , N_2) will be sufficient. If this will not be the case gas bubbles may be formed in subsoil affecting essentially the transport processes. That is why the following parameters (maximum list) have to be measured during monitoring:

- contaminants
- metabolites (determined as dissolved organic carbon; DOC)
- final degradation products (CO_2 , CH_4)
- nutrient salts (z. B. NH_4^+ , PO_4^{3-})
- redox indicators (O_2 , NO_3^- , NO_2^- , $\text{Fe}_{\text{dissolv}}$, $\text{Mn}_{\text{dissolv}}$, SO_4^{2-} , S^{2-}),
- electron donators (as a rule, also analyzed as DOC)
- field parameters (pH, redox potential, electric conductivity, temperature)
- optional: bacterial counts (total count, contaminant degraders, *D. ethenogenes* etc.)

With these data information about the following processes are obtained:

- biogeochemical state of the aquifer,
- success of addition of electron acceptors/donators and nutrient salts,
- functionality of remediation measures,
- reaching of the remediation targets.

As a multitude of various metabolites may be present which are polar and thus better soluble than their initial substances they may be summed up as “dissolved organic carbon” (DOC). In some cases, e.g. if the infiltration facilities will be blocked by biomass, detailed information on the type of DOC is required. Thereby, DOC may be analyzed by means of LC-OCD (liquid chromatography with detection of the organic carbon). Thereby, DOC is classified in subfractions such as humic substances, so-called building blocks, low-molecular acids, amphiphilic substances and polysaccharides.

So far reliable techniques for the determination of the degradation rates in situ are not available for most of the contaminants. In practice this proof is mostly furnished via the reduction of the concentration of contaminations. The degradation of some contaminants (a.o. (mono)aromatics, naphthalene, volatile chlorinated organic compounds and MTBE) may be

quantified by analyzing fractioning of the stable isotopes (mostly $^{12}\text{C}/^{13}\text{C}$). Here, the fact that molecules microbially preferred are degraded with the lighter isotope. Then the remaining residual fraction is enriched in the heavier isotope. This technique will only work for low-molecular compounds and only if various ways of degradation with various isotope fractionating factors are not available. An alternative is carrying out tracer tests. For this purpose, a conservative tracer will be put into the aquifer together with the contaminant marked by heavy stable isotopes. Along the flow direction the degradation may then be calculated in relation to the decline of the concentration of the conservative tracer. However, this technique is questionable from an aspect of approval.

In many projects as regards monitoring mostly a pragmatic solution is chosen, i.e. only a limited number of parameters is analyzed. They involve the determination of nutrient salts, electron acceptors (or donators in the case of anaerobic processes) to ensure a sufficient supply with these auxiliary agents. The field parameters are determined to prove that the measures implemented have brought about environmental conditions favouring degradation.

As a change of the hydraulic permeability of the aquifer (expressed as k_f -value) has far-reaching effects on the functionality of remediation the hydraulic permeability has to be monitored. In the case of need, counter-measures such as e.g. regeneration of wells or change of the nutrient concentrations have to be taken. Monitoring of hydraulic measures (pumping off and infiltration) is carried out by measuring the infiltration rate (Q) and the resistance (N) within the remediation well with the aid of pressure sensors installed at the bottom of the well. Such measurements and the subsequent evaluation may be automated. The hydraulic permeability may be reduced by the formation of gas bubbles (e.g. at an infiltration of too high H_2O_2 concentrations or a denitrification proceeding too quickly at high nitrate concentrations), by the precipitation of iron oxide (if the redox tension is changed), by blocking with the biomass formed or displacement of the fine grain in the case of a fast infiltration.

As, in general, an equilibrium between the gases dissolved in groundwater and their concentration in soil air exists possibly soil air samples will be taken and analyzed for the following parameters:

- volatile contaminants
- final degradation products (CO_2 , CH_4) for balancing the *in-situ* process
- electron acceptors (O_2) to detect a supersaturation or overdosage into groundwater, if necessary
- radon as natural tracer (^{222}Rn) to assess the transport speed of substances from groundwater into soil air and further into atmosphere

Also with passive in-situ techniques a potential change of the hydraulic permeability of the aquifer accompanied by a change of the groundwater flow direction plays a major part. The direction of the groundwater flow as an auxiliary parameter is measured in situ with the aid of photometric techniques.

9.4 Proof of the success of remediation

Soil and/or groundwater samples are, as a rule, taken and chemically analyzed and biologically investigated to furnish proof that the targets of remediation have been reached. By chemical analyses the residues of contaminants left after remediation are determined. As in the microbial degradation, first of all, easily bioavailable contaminants are degraded it might be necessary to determine the mobility of the residual fractions.

The residual contamination consists mainly of less mobile individual substances. This is of importance notably if the contaminants will be recorded only as sum parameters as e.g. in the case of mineral oil hydrocarbons. Furthermore, metabolites may be formed which, however, at a sufficient time of treatment are mostly subject to a mineralization. However, humified contaminants are mineralized only with a high delay but have lost their original chemical identity and their toxic potential.

The ecotoxicological relevance of the residual contaminants in the remediated soil in their entirety are recorded by biotests. In biotests inhibition or toxic effects on various biological systems are measured. They involve a.o.:

- bioluminescence
- growth rates of algae
- mortality of crayfish (daphnias)
- growth rates of plants (e.g. cress)

Depending on the effective path to be considered various biotests are required. These tests may be partly carried out in a miniaturized form. At any rate, however, a laboratory experienced in carrying out these tests should be involved.

10 Development potential of biological remediation techniques

The efforts made in research in the last few years, e.g. as to humification of contaminants or biotransformation of heavy metals, show a lot of remarkable approaches. Techniques such as bioleaching are taken from related disciplines and, in all probability, may be used in a respective adaptation for remediating contaminated soils and groundwater. Some of the innovative methods which are under development have so far been investigated on a laboratory scale, others such as phytoremediation have been already tested on a pilot scale. However, detailed experience is not yet available and a comprehensive commercial spreading is not yet to be stated. Hereinafter, selected biochemical processes will be presented which owing to their outstanding potential are of special interest and/or may be already possibly used in the short run or have been already used to a limited extent.

Phytoremediation: In phytoremediation dissolved contaminants are removed from soil or groundwater by the roots of plants taking them up. The selection of suitable plants depends on the properties of the contaminants, the soil and the three-dimensional spreading of the contaminants. The applicability of this technique is restricted to the root zone of the plants. Though some contaminants are taken up by the plants they are not transported within the plant. They are accumulated in the roots. However, others such as nitroaromatics are subject

to a limited transfer. The transformation of the contaminants within the plant is mostly not sufficiently effective to detoxify them completely and thus of minor importance. Heavy metals are e.g. accumulated without being significantly transformed. That is why the plants have to be harvested and disposed to finally eliminate the contaminants.

Heavy metals: For the time being, contaminations with heavy metals are mainly still treated by means of physicochemical methods. Though these elements are not microbially „degradable“ they are not biochemically inert. A few microbiological transformation reactions are known which cause mainly a change of the physicochemical behaviour of the metals. Examples of their application are:

Bioleaching: Solubilization was originally developed for supporting leaching of metals (bioleaching) in mining. It is based on the ability of specific bacteria such as e.g. *Thiobacillus* ssp. and *Leptospirillum ferrooxidans* to oxidize metal sulphides to soluble metal sulphates. Bioleaching as remediation method requires pumping off groundwater and removing the dissolved metals in a groundwater treatment plant. This may be possibly done by biosorption (sorption of heavy metals to an immobilized biomass). In general, bioleaching may be also designated as pump-and-treat process. The high solubility of the solubilized metals may lead to a faster removal of these contaminants as compared with the classical pump-and-treat process.

Bioprecipitation: An essential problem of mining industry is the acidification of groundwater apart from its pollution by metal ions and sulphate. These problems may be tackled, at the same time, by bioprecipitation. Adding of an organic substrate results in a formation of sulphides and rising of the pH. The metals are anaerobically precipitated as non-toxic metal sulphides in an abiotic reaction. The metal sulphides formed are very stable. A remobilization will only occur if the pH will decline below the value of 3. As in the precipitation reaction frequently big quantities of H_2S will be formed in some cases a second, aerobic treatment is required reoxidizing excessive H_2S to sulphate. By bioprecipitation at least contaminations with Pb, Zn, Cu, Cd, Ni may be treated.

Biovolatilization: Microorganisms may also form or degrade metalorganic compounds. By alkylation and dealkylation some important parameters such a toxicity, volatility and water solubility will be changed. By means of this method e.g. contaminations of the unsaturated soil zone with arsine are treated. With regard to soil and soil microorganisms this process represents a detoxification as the volatile transformation products formed outgas into atmosphere. However, the volatile products are very toxic so that the volatile compounds have to be sucked off (e.g. by suction of soil air) with subsequently purifying the loaded exhaust air (e.g. chemical dealkylation in a gas washer). Yet, till the present day research on environmental conditions allowing a controlled microbial reduction of arsine has not yet been carried out.

11 Bid analysis and costs of biological techniques

11.1 Instructions for a bid analysis

At least by public principals in Germany services in the field of remediation of contaminated sites are usually put out to tender, analogously to construction work, according to the construction contracting procedures (VOB), the contracting procedures for services (VOL) and the contracting procedures for freelance workers (VOF). In the case of simple measures (e.g. ex-situ remediation of soil) a detailed estimate is made. For more complex tasks as it is, as a rule, the case in in-situ remediation invitations to tender are normally made with regard to the purpose. This requires making a detailed analysis of the bids with the respective technical knowledge before a contract will be awarded.

It is essential that, as a rule, only such bidders will be inquired the efficiency of whom will be sufficiently known. The basis for invitations is, as a rule, a feasibility study. There already questions relating to the applicability of various techniques, the reachability of the remediation targets and to the time needed will be answered so that the principal and the contractor will be completely secure as regards the implementation of the work put out to tender. Laboratory tests (LFUG, 1999: preliminary tests in the framework of investigations of remediation and remediation (batch and column tests); materials relating to the remediation of contaminated sites; Landesamt für Umwelt und Geologie (Saxony Land authority for environment and geology) and field tests may serve to assess the time and to specify the costs, to reach a technological optimization and to meet the requirements of approval. A similar approach is also the normal practice adopted in East European countries.

In a German invitation for tenders, in particular, attention is to be drawn to handling „biological agents“ and the special protective measures resulting thereof. They are fixed in the Directive 90/679/EEC (1990) relating to the „Protection of workers from risks related to exposure to biological agents at work“. Similar complexes of rules exist also in other countries.

11.2 Costs of biological techniques

In the last few years a vehement price war has taken place on the remediation market. It resulted in the development of a forced application of low-cost remediation techniques in Germany. They include, among others, land farming and passive microbial in-situ techniques. Also in the past it was scarcely possible to indicate general prices for in-situ remediation as the specific site conditions may result in high price ranges. Only for ex-situ treatment of excavated soil price tables were available. Owing to the constantly high dynamics of the market such tables are today scarcely effective. This refers, in particular, also to East European countries where for reasons of costs remediation is frequently only accompanied by a minimum monitoring program.

Also if in Germany „performance books“ are available for calculating the costs of ex-situ measures they are increasingly less applied. The reasons for that are, on the one hand, that the share of ex-situ measures declines. If they are applied the excavated soil is, as a rule, supplied to stationary soil treatment centres the prices of which have to be always inquired

anew owing to the dynamics of the market. On the other hand, the complexity of in-situ remediation measures has always obstructed the preparation of performance books. Thus, the calculation of costs is, to a special extent, left to the expert/planner and his knowledge of the market – not only in Germany but also in the East European countries.

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13 List of abbreviations

ADNT	aminodinitrotoluene
AH	aromatic hydrocarbons
BTEX	benzene, toluene, ethyl benzene and xylene
CA	chloroethane
CP	chlorophenol
CTE	compounds typical to explosives
DANT	dinitroaminotoluene
DCA	dichloroethane
DCB	dichlorobenzene
cDCE	cis-dichloroethylene
DCM	dichloromethane
DOC	dissolved organic carbon
DNAPL	dense non-aqueous phase liquid
ETC	explosive-typical compounds
HCB	hexachlorobenzene

HCH	hexachlorocyclohexane
HDPE	high-density polyethylene
HRC	hydrogen release compound
ISRT	in-situ respiration test
IRZ	in-situ reactive zones
LAB	linear alkylbenzenes
LNAPL	light non-aqueous phase liquid
MCB	monochlorobenzene
MNA	monitored natural attenuation
MTBE	methyl tertiary butyl ether
NT	nitrotoluidine
ORC	oxygen release compound
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PCE	tetrachloroethylene
PCDD	polychlorinated dibenzo-p-dioxins
PCDF	polychlorinated dibenzofurans
PCP	polychloroprene
PH	polycyclic hydrocarbon
TAT	triaminotoluene
TCA	trichloroethane
TCB	trichlorobenzene
TCE	trichloroethylene
TNT	trinitrotoluene
VER	vacuum enhanced recovery
VEW	vacuum evaporator wells
VOC	volatile organic compounds



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