Use of surfactants to improve the biological degradability of petroleum hydrocarbons

Marion Martienssen*, Ole Reichel and Mario Schirmer

*PD Dr. rer. nat. habil. M. Martienssen
PD Dr. habil. M. Schirmer

UFZ Centre for Environmental Research Leipzig-Halle, Department of Hydrogeology,
Theodor-Lieser-Strasse 4, D-06120 Halle/Saale, Germany;

Dipl.-Ing. O. Reichel,

Martin Luther University Halle-Wittenberg, Institute for Biotechnology,
Kurt-Mothes-Strasse 3, D-06099 Halle/Saale, Germany.

Abstract:

Mineral oil products are produced and utilized to a large extent. As a result, mineral oil contamination is one of the main problems in soil remediation.

The predominant portion of the different oil components is easily degradable by microorganisms. But, its biodegradation in natural environments is often limited by the availability of the substrate and the concentrations of nutrients and oxygen.

The optimization of the environmental conditions can significantly enhance the efficiency of degradation in both soil and ground water (Enhanced Natural Attenuation). In recent experiments at a real contaminated site, more than 50 g/kg soil could be degraded within 15 months.

Because of its limited solubility, the bioavailability of mineral oil is low. So it is often necessary to enhance the solubility of the contaminants. This can be realized by the use of surfactants. But, for the purpose of bioremediation only those surfactants may be used, that are completely biodegradable. Moreover, they have to be compatible to the surfaces of the bacteria. These requirements are met by biosurfactants and by substances that contain structures comparable to natural occurring microbial surfactants.

1   Introduction

Petroleum Hydrocarbons are among the most frequent contaminants in soil and ground water because of their widespread use. Although most of the components of mineral oil products can be considered to be readily biodegradable, their natural degradation rates are frequently reduced considerably as a result of the limited natural resources, especially of nutrients and oxygen. Optimizing the environmental conditions within the framework of Enhanced Natural Attenuation (ENA) can significantly increase the performance of pollutant degradation in soil and ground water. As an example, up to 50 g petroleum hydrocarbons per kg soil was able to be degraded at optimal environmental conditions within a pilot scale project.

Pollutant mobilization has a key role as part of the ENA process. In should be noted in this context that only those surfactants which are themselves completely biodegradable and biocompatible with the existing bacteria should be used. Surfactants of natural origin or those having their structure largely related to natural biosurfactants are especially suitable.

2   Formulation of the problem

Mineral oil products are now among the most widely produced and handled chemical products throughout the world. Contamination of soil and ground water caused by leakages during storage and filling and also as a result of numerous salvage operations are correspondingly frequent.

The mineral oils currently available on the market are predominantly obtained from crude oil. Some products are also of synthetic origin. However, the recovery of hydrocarbons by the hydrogenation of coal has largely ceased.

The oils and oil products currently used have a wide range of compositions. However, the components of the crude oil are largely reflected in all mineral oil products.

An overview of the compositions of crude oil and various crude oil products can be found, among others, in [1].

The main components of all mineral oil products are saturated and unsaturated chain-like hydrocarbons, alkanes or alkenes. The chain lengths differ very substantially according to the intended usage, and vary between C1 (methane) and >C25 in waxes and asphalts. With regard to biological degradability, a distinction should be made between straight-chain alkanes, the n-alkanes, and those with branched chains. The n-alkanes, especially those of medium chain length (C12 to C20) are in principle degraded very well by micro-organisms. The degradation takes place mainly by terminal or subterminal oxidation at the carbon chain to give fatty acids which discharge into the basal metabolism of the cells [1]. Branched alkanes having an isoprene structure and bearing a methyl group at every fourth C atom are also highly degradable because their structure is the same as that of a widely occurring group of biological active substances, the isoprenoids. Other singly-branched alkanes with longer side chains are mineralized significantly more slowly because the chain branching impedes the degradation sequence at the alkyl chain. A small fraction of alkanes with tertiary chain branching is problematical for biological degradation. Only a few specialists have succeeded in cleaving this structure. Consequently, these substances are difficult to degrade by natural occurring bacteria.

The n-alkanes and singly branched isoalkanes are normally degraded at high rates by autochthonous organisms present at the contaminated site so that there is no need for additional bacteria. The use of specialists may only be indicated for higher branched-chain alkanes [3].

With a fraction of up to 30%, monoaromatic compounds, especially the so-called BTEX group (benzene, toluene, ethyl benzene, xylenes), are an important component of many mineral oil products. These compounds are also efficiently degraded by micro-organisms. The capacity to degrade aromatic compounds is widespread among the micro-organisms because substituted aromatic compounds occur in numerous forms from natural substances e.g. from the degradation of lignin and other

plant ingredients. Aromatic compounds are degraded to give CO_2 and water like the alkanes [1].

Another important component of all mineral oils are the cycloalkanes or cycloparaffins. Most of the cycloalkanes are difficult to degrade. As the number of carbon atoms in the molecule increases however, the degradation rate becomes equal to that of the aliphatic compounds. An important requirement for the degradation of many cycloalkanes is the simultaneous presence of other sources of carbon because they are only metabolized co metabolically by many types of bacteria.

With reference to biological biodegradability, attention should also be paid to N and S heterocyclic compounds contained in small fractions in mineral oils. These substances require special metabolic performance for their degradation. Polycyclic aromatic compounds, the PAH, play scarcely any role in commercially available flow able oils. They are mainly found in tar and asphalt.

In addition to the substances originally contained in mineral oils, specifically added compounds, additives, play an important role. The degradability of many additives used in the past (e.g. lead tetraethyl or PCB) was often problematical. This resulted in considerable environmental contamination and health hazards. Even after their substitution (e.g. lead tetraethyl by MTBE), their natural degradation is in some cases still continuing today. However, additives currently permitted in fuels such as MTBE are in principle biologically degradable although in some cases their degradation takes place considerably more slowly than that of hydrocarbons [4, 5].

Although the vast majority of mineral oils and their concomitant components are biologically highly degradable, they are frequently mineralized only slowly in the environment and can accumulate in the soil particularly at high pollutant concentrations. This can be essentially attributed to two factors. Firstly, the predominantly hydrophobic mineral oil components are frequently only inadequately bio-available. Secondly, the natural resources of nutrients and suitable electron acceptors (especially oxygen) are frequently not sufficient to ensure rapid degradation of pollutants. The existing

environmental conditions thus determine the actual natural self-cleaning potential of a site.

An important problem in the degradation of hydrocarbons is the bio-availability of the substrates to be degraded. Hydrophobic substances such as mineral oils are almost immiscible with water but separate as an independent phase on contact with water. Contact with the micro-organisms in the aqueous phase then only takes place at the phase boundary of oil and water. Only the short-chain hydrocarbons (hexane approximately 60 mg/L) and the simple aromatic compounds (benzene 1.7 g/L) and some additives such as MTBE (50 g/L) are water-soluble to any significant extent and are transferred into the water phase at the phase boundary. On the other hand, the solubility of long-chain hydrocarbons and multi-ring systems is, for example, significantly lower than 1 µg/L. As a result of the lower bio-availability, the rate of degradation of the various compounds decreases dramatically with increasing insolubility in water under natural conditions. This non-uniform mineralization results in enrichment of various mineral oil components and in the so called oil ageing. During the oil ageing process primarily the more easily degradable n-alkanes and low-boiling hydrocarbons are initially degraded while higher-molecular compounds and other less easily degradable substances remain at the site. The subsequent remediation of these aged oil contaminations thus became ineffective even if the environmental conditions are later optimized [3]. The necessary concomitant components for co metabolic degradation are lacking. The degradation of aged oils is made additionally difficult by chemical processes which result in polymerization and huminification and by the accumulation of microbial metabolic products, especially steroids [3].

A reduced bio-availability also arises as a result of adsorptive binding of the hydrophobic hydrocarbons to soil particles and by the incorporation of oil droplets in the capillary space of the unsaturated soil zone. As with free phases, in these cases also there is only a small contact surface between oil and bacteria. Such NAPLs (Non Available Pollutant Liquids) are also barely bio-available and are mineralized very slowly.

3   Increase in the natural self-cleaning potential

As already explained, organisms for the degradation of petroleum hydrocarbons are mostly present in sufficient numbers in the soil. Provided that the entire ecosystem has not already been severely damaged by other poisons, there is thus normally no need for additional organisms. This was demonstrated among others using the examples of diesel fuel and spindle oil in Research Project FE-50003 of the UFZ Centre for Environmental Research Leipzig-Halle (xenobiotics and micro-organisms: limits for metabolisability or loading capacity) for various loaded and unloaded soils [6]. This is why adapted micro-organisms are only rarely added during the remediation of petroleum hydrocarbon contaminated sites.

Among the nutrients, it is mainly the micro-nutrients, nitrogen and phosphate, which are used directly for building the microbial cell structure which can limit the biological degradation. Approximately 5 g N and approximately 1 g P is required for every 100 g C. If no adequate nutrient supply is available, this must be supplied from outside or this is only released again when biomass dies off. An insufficient supply of nutrient thus reduces the degradation rate.

How high is the available natural potential however? In the soil nitrogen particularly has a limiting effect. Various studies, among others at the UFZ have shown that the nitrogen loading at our latitudes can be given as around 40-60 kg N/ha per year [7]. This is around 10-20 mg/m^2 per day or 40-60 mg N/kg soil per year. Generally only one to two mg N/kg of soil is available in the soil for microbial degradation. This gives a capacity for the degradation of environmental pollutants of around 200 mg/m^2 per day. Thus, a maximum of 6 g hydrocarbons per m^2 can be degraded in four weeks, that is around 30 mg/kg soil. At higher concentrations the ensuing nutrient deficiency must be compensated by adding additional fertilizers. When remediating old loadings where several g oil/kg soil are generally present, it is thus absolutely essential to add nutrients to ensure pollutant degradation in justifiable time intervals.

The nitrogen content in the ground water can be subject to large fluctuations and must be determined for each individual case.

In addition to a limited nutrient supply, the available oxygen is another important factor for efficient mineralization of environmental pollutants. Natural, barely loaded soils are usually aerobic in the upper layers of soil. In cases where there is a limited metabolism as a result of the N limitation to 1 to 2 mg hydrocarbons/kg soil per day, no oxygen deficiency in the upper soil layers is to be predicted even after a contamination. As a result of the degradation of organic substances in contaminated soils however, an oxygen deficiency may arise in deeper soil layers which must be compensated by specific oxygen dosing. The same applies to the ground water. If the metabolism is additionally accelerated by adding nutrients as part of remediation measures, a sufficient supply of oxygen must also be provided to the same degree in order to counteract the rapid consumption of the available oxygen reserves. Alternatively, other electron acceptors for the natural degradation of pollutants can also be considered. It can be concluded both from laboratory experiments and from balance calculations in natural soil systems that petroleum hydrocarbons can be mineralized at considerable rates under anoxic or anaerobic conditions [1, 8]. However, more detailed studies are required for accurate calculations of the actual anaerobic hydrocarbon degradation potential.

In environmental technology the bio-availability is improved in several different ways. The simplest way is to mechanically suspend an oil phase floating on the water. For this purpose suitable dispersing tools are used in bioreactors to break the oil down into minute droplets which are suspended in the water. In soils the bio-availability is improved by mechanical methods, for example, by off-site remediation by intensive mixing. Both methods are unsuitable for use as part of Natural Attenuation. The use of surfactants (dispersants or surfactants) can be considered as an alternative to the mechanical methods. However, as a result of the insufficient experience and especially because of the environmentally harmful properties of numerous commonly used industrial surfactants, this is controversial as before.

4   Use of surfactants - possibilities and risks

Since, as a result of its limited bio-availability under natural conditions, the degradation of petroleum hydrocarbons frequently takes place more slowly than would be possible using the natural degradation potentials, many attempts had already been made early on to improve the water solubility of mineral oils and thus their bio-availability by physical-chemical methods. In this case it was obvious to use surfactants as used in various chemical compositions in washing and cleaning agents. A summary of the structure, mode of action and history of surfactants can be found, for example in Römmp [9]. An important factor for all surfactants is their bipolar structure. They consist of a polar head group which faces the water phase on contact with water and a hydrophobic section (e.g. a long-chain alkyl group) which establishes a contact with hydrophobic substrates (liquids or surfaces). Figure 1 gives an overview of the most common industrial surfactants.

Figure 1.
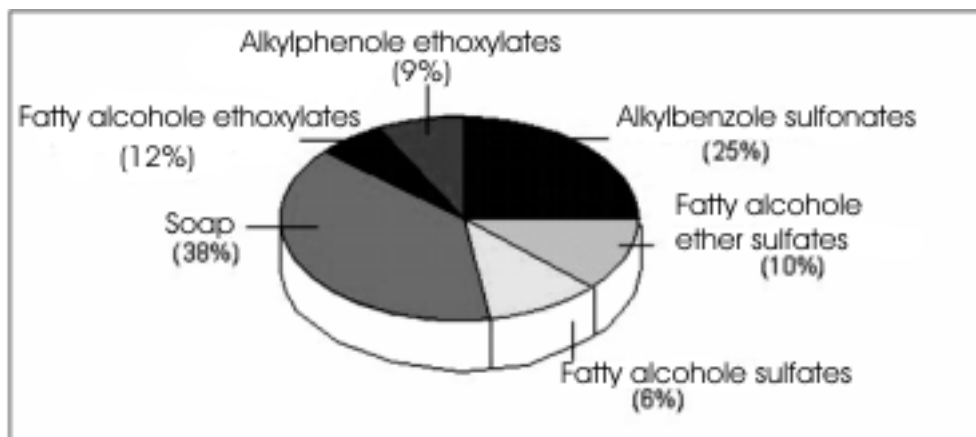
The mode of action of surfactants is based on their capability of mediating as a thin layer as it were between two immiscible phases. In this case, the surface tension between the hydrophobic and the hydrophilic components is reduced to such an extent that the two phases become miscible with one another. At low surfactant concentrations the bipolar molecules initially accumulate at the interface between oil and water. Above a so-called critical micelle concentration the oil phase is dissolved. Individual

colloidal particles completely enclosed by surfactant molecules (micelles) are formed and become dispersed in the aqueous phase.

Over the last twenty years a complete remediation strategy, known as surfactant technology, has developed on the basis of using surfactants. In this case two different objectives are being pursued. Surfactants can on the one hand disperse the oil in the aqueous phase (solubilisation) and thus make it available for biological degradation, or they can merely make the oil flow by lowering the surface tension (mobilization). A comprehensive overview of the use of surfactants in soil remediation is given by Danzer and Herbert [10]. Surfactant technology is playing an increasing role in in-situ remediation where its suitability for removing hydrocarbons (fuels, cooling lubricants etc.) has been demonstrated in numerous applications [11]. The separation of floating oil by mobilization can be regarded as state of the art in some countries (e.g. the USA). The chemical-physical properties of the industrial surfactants used are known to such an extent that a suitably optimized solution is available for almost every oil.

On the other hand, there is little experience with solubilisation for improving bio-availability. The technology is still at the stage between development and industrial application. There have been many pilot projects but only a few complete remediations have been carried out. The results achieved in the past were very variable and in some cases, extremely unsatisfactory. In addition to efficient acceleration of degradation, there were some cases with no effect, some cases where degradability was reduced considerably and even some cases where the existing micro-organisms were significantly damaged. A summary of the results of various remediation experiments is given by Volkering et al. [11].

The reason for these non-uniform effects lies in the structure of the industrial surfactants used. These have been designed to transfer as much oil as possible stably to an aqueous phase using as little surfactant as possible. The focus of the development of industrial dispersants was the solubility not the biological degradability of the dispersed oils. It is thus no wonder that as the level of knowledge increased, some

especially poorly degradable products and some having properties especially harmful to bacteria have been removed from the market (e.g. sulphonic acids with highly branched alkyl groups).

A major problem with most of the industrial surfactants available today is their inadequate biocompatibility. Developed with the aim of converting hydrophobic substances into substances having the highest possible water solubility, most surfactants are very strongly polar or even of an ionic nature. They form electro statically charged micelles in water or micelles having a very high polarity. These are confronted by hydrocarbon-degrading bacteria which are specialized firstly by staying in the water without any major expenditure of energy (being hydrophilic) and secondly by establishing contact with their hydrophobic substrates. In order to meet these requirements, many hydrocarbon-degrading bacteria have hydrophobic domains on a previously hydrophilic surface for taking up the hydrocarbons. As can be seen from Table 1 for selected isolates of a pilot remediation, the loading density on the surface of hydrocarbon-degrading bacteria is astonishingly high. Some species only acquired their hydrophobic properties by the self-assembly of differently structured species (see Chol1 and Chol2 in Table 1). If the hydrophobic hydrocarbons are incorporated in polar or even charged micelles, contact with the hydrophobic domains of the cell surface and therefore the uptake of the hydrocarbons in the cell is made difficult. In addition, the charges on the surface of many conventional surfactant micelles result in an additional repulsion of like-charged bacteria. The hydrocarbons to be degraded are now miscible with water but they no longer come in contact with the bacteria.

Another problem with many conventional, primarily older surfactants, is their high bacteriotoxic potential. As a result of their structure, many surfactants are capable of becoming incorporated uncontrollably into the membranes of the bacteria which results in loss of functionality and thus causes severe damage or even death to the bacteria. Typical representatives of such bacteriotoxic detergents are the alkylsulphonates. Thus, the substance SDS (sodium dodecyl sulphonate) is specifically used in biotechnology to permeabilise bacteria and specifically eliminate or separate cell constituents. Such surfactants are completely unsuitable for use in bioremediation.

Table 1.

Surface structure of selected strains of the bacteria population of a pilot remediation. The selected isolates are capable of degrading differently structured hydrocarbons.

Isolate	Substrate utilised	Contact angle [°]	Adsorption on model carrier (% total cell mass)			
			hydrophilic	hydrophobic	anionic	cationic
Amyl1/2	n-alkane and isoalkane	17	53.8	10.5	44.4	51.3
Amyl1/3		17	37.5	18.0	21.4	37.5
Amyl2/1		18	40.4	29.8	33.6	67.2
Amyl2/2		19	61.6	53.4	54.8	53.4
TBU1	Tertiary-chain branched alkanes	13	0	0	15.6	69.0
TBU2		20.5	11.9	19.3	8.8	9.5
Chol1		20	50.0	56.3	44.3	56.0
Chol2	Steroids	14.7	46.0	22.4	43.6	0
Chol1 and Chol2 together		25	22.1	76.5	-	-

5   Natural or nature-related surfactants as alternatives for environmental use

Nature herself offers a functional alternative to the problematical use of conventional industrial surfactants. In order to solve the problem of the limited bio-availability of

hydrocarbons, many hydrocarbon-degrading bacteria themselves produce biosurfac-tants. These so-called biosurfactants can disperse the hydrocarbons in the same way as industrial surfactants. The micelles formed in this case then accumulate on the bacteria surface and the hydrocarbons contained therein are either directly oxidized by exoenzymes or the total content of the micelle is taken up by the bacteria cell and subsequently utilized. The mode of action of biosurfactants during the utilization of hydrocarbons is shown schematically in Fig. 2.

Figure 2.
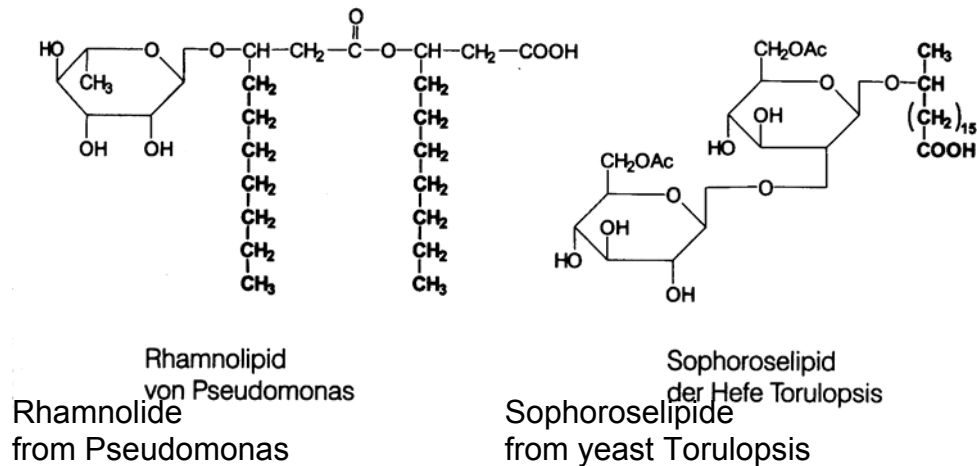Mode of action of biosurfactants during the mobilization of mineral oils
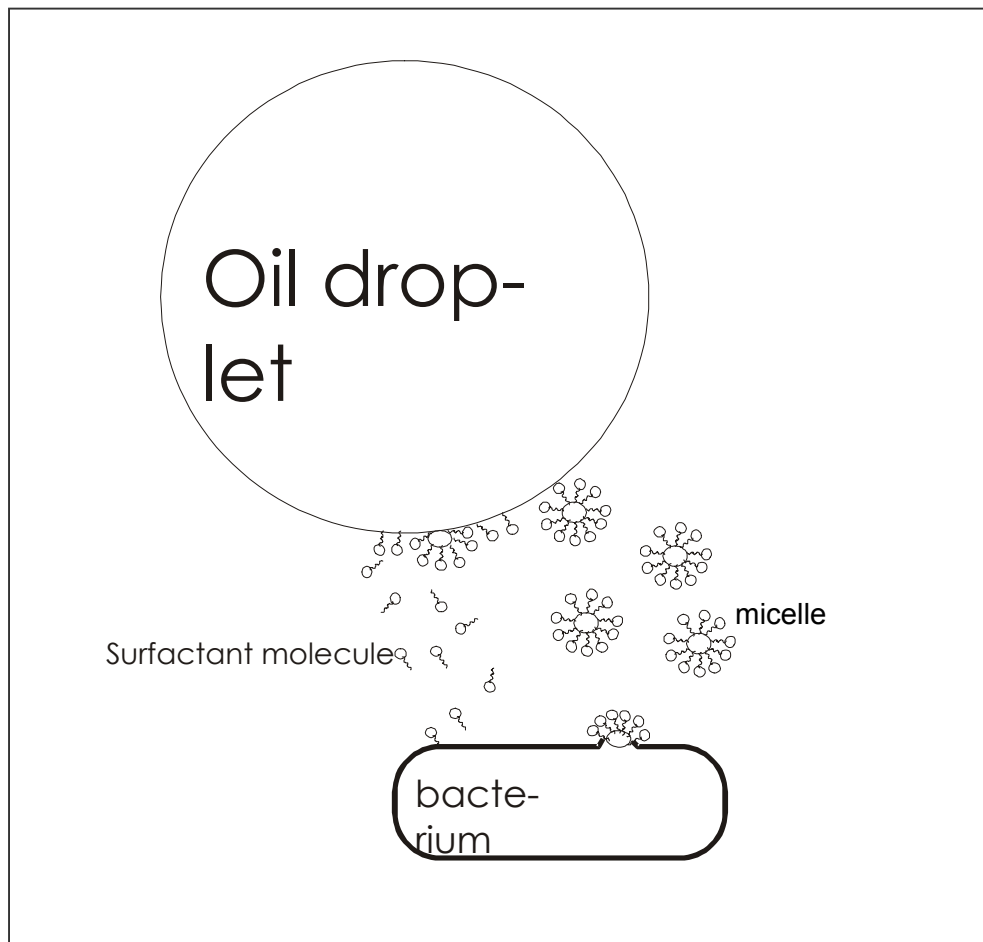
Figure 3.

Structure of selected biosurfactants (from Fritzsche [2])

Rhamnolipid
von Pseudomonas
Rhamnolide
from Pseudomonas

Sophoroselipid
der Hefe Torulopsis
Sophoroselipide
from yeast Torulopsis

Unlike conventional industrial surfactants, biosurfactants are higher-molecular and polyfunctional (they especially combine several different structures and functional groups in the hydrophilic region) and as a result of their structure related to the membrane lipids, they can mediate between the hydrophobic hydrocarbons and the bacteria without damaging the bacteria cell. Biosurfactants are synthesized in large quantities in the environment and are degraded again naturally. Figure 3 shows the structure of two selected examples.

Surfactants specially designed for environmental use closely base the structure of their surface-active components on the structure of natural biosurfactants. The basic building blocks can be vegetable fatty acids without chain branching which are specifically esterified with hydrophilic components such as sugar structures or hydroxycarbonic acids. Nature-related surface-active substances do not have some of the important disadvantages of conventional surfactants in terms of biocompatibility. In particular, the close-to-nature structure prevents any damage to the bacteria membrane during the surfactant contact. As a result of this chemical feature, the toxicity of BioVersal active substances is generally around 100 times lower than that of most conventional surfactants (see Table 2). Bacteriotoxic effects were only determined for luminescent bacteria (*Vibrio fischeri*) and only at extreme concentrations (>25 g/L).

The degradation of petroleum hydrocarbons is also not impeded by dispersion with natural or nature-related biocompatible surfactants.

Table 2.

Toxicities (LC 50 in mg/L) of nature-related surface-active substances compared with conventional industrial surfactants (compiled by Dallinger [12]).

Group of organisms	Anionic detergents	Non-ionic detergents	BioVersal products
Fresh water algae	1-300	-	65*-750**
Marine invertebrates	1-800	0.1-50	-
Fresh water invertebrates	1-270	1-350	100*-1400**
Fish	0.05-15	1-70	100*-300**

* QG product; ** HC product

Figure 4.

Accumulation of bacteria on a fluorescence-labeled model activator. The model lectin labeled with Texas Red binds specifically to the glycoside structures of the bacteria and enriches these at the model surface.
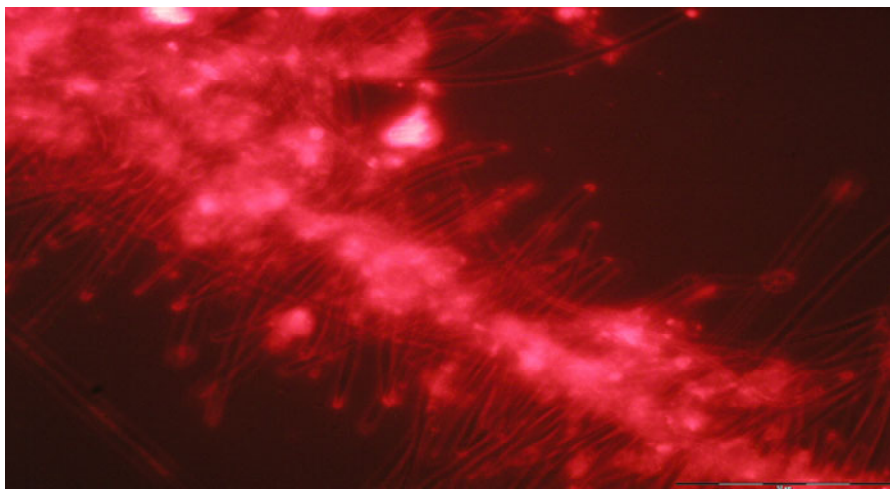
Compared with microbial biosurfactants which are produced by the bacteria cell in the concentration used, industrial products must be available in the highest possible concentration for economic reasons in order to minimize expenditure on transport. This is why the number of anionic charge carriers in the molecule was increased e.g. in BioVersal active substances in order to improve the water solubility. However, this modification increases the kinetic repulsion with the bacteria surface. In order to overcome this thermodynamic disadvantage, additional components, for example, based on plant lectins can be used. The lectins produced by various plants are specific substances which accumulate on precisely defined domains of the bacteria surface and serve to enrich the plants in specific types of micro-organisms in the rhizosphere or to make these usable for mobilizing otherwise barely available substrates. Like the antibodies in the animal kingdom, plant lectins are also highly specific and only react with quite specific structures on the surfaces of specific groups of bacteria. Figure 4 shows the accumulation of bacteria on a fluorescence-labeled model activator.

With the aid of the lectin-like activator, hydrocarbon-degrading bacteria can dock specifically onto the oil-surfactant micelles formed as in the plant rhizosphere and are thus enriched on the surface of the suspended micro particles. As a result of the improved contact between the micelle surface and the bacteria, the natural degradation process of the hydrocarbons can be additionally accelerated.

6   Performance of nature-related surfactants

The performance of a close-to-nature surfactant composition from BioVersal Deutschland GmbH was studied as part of a pilot remediation at DB Netz AG. A highly contaminated area of a still-active locomotive parking area on the Halle site was selected as a suitable remediation area. The remediation area of 10 m^2 was located in the immediate vicinity of the track and was surrounded by similarly loaded areas which served as a control area. In addition to the many years of use and the corresponding ageing of the incorporated oils, the advantage of the selected track areas for studying pollutant mobilization was that an enclosed body of soil occurred

here which was bounded from below by an approximately 10 cm thick band of clay. Any discharge of pollutants into deeper layers of soil was thus excluded from the start.

The remediation was carried out on the basis of an ENA (enhanced Natural Attenuation) approach retaining the natural soil structure. The degradation of the hydrocarbons was tracked by gas-chromatographic analysis in accordance with DEV H53. In addition, the composition of the high-boiling hydrocarbon-fraction was studied by GC analysis after an acid hydrolysis.

The analysis of the initial concentrations at the site shows an hydrocarbon loading with strong vertical zoning where a free phase (about 100 g hydrocarbons per kg of soil) was found in an approximately 10 cm wide strip of the ballast area. The average loading of the site (three samples each from the remediation area and from the adjacent control area) can be seen from Figs. 5 and 6. As a result of the long pre-usage, the contamination studied already exhibited a high degree of ageing with high fractions of polymerized and highly branched-chain compounds. Thus, before the beginning of the tests suitable specialists for the degradation of tertiary branched-chain alkanes and steroids were isolated from the autochthonous flora of the site and pre-cultivated as inoculation culture.

The measured initial loading was used as the basis to estimate the average hydrocarbon content of the soil as approximately 35 kg hydrocarbons per m^2 of soil area and the necessary treatment quantities for oxygen and nutrients were then calculated. The original N and P content of the site was negligible relative to the quantity of organic substances. Thus, the required ratio of 5% N and 1% P relative to the total organic load was adjusted by adding a conventional commercial fertilizer. As a result of the high loading of the soil, the oxygen dosing was carried out using hydrogen peroxide.

Figure 5.

Elimination of petroleum hydrocarbons by optimizing the natural degradation in the track region. Hydrocarbon content in the test area recorded from July 2000 to October 2001.
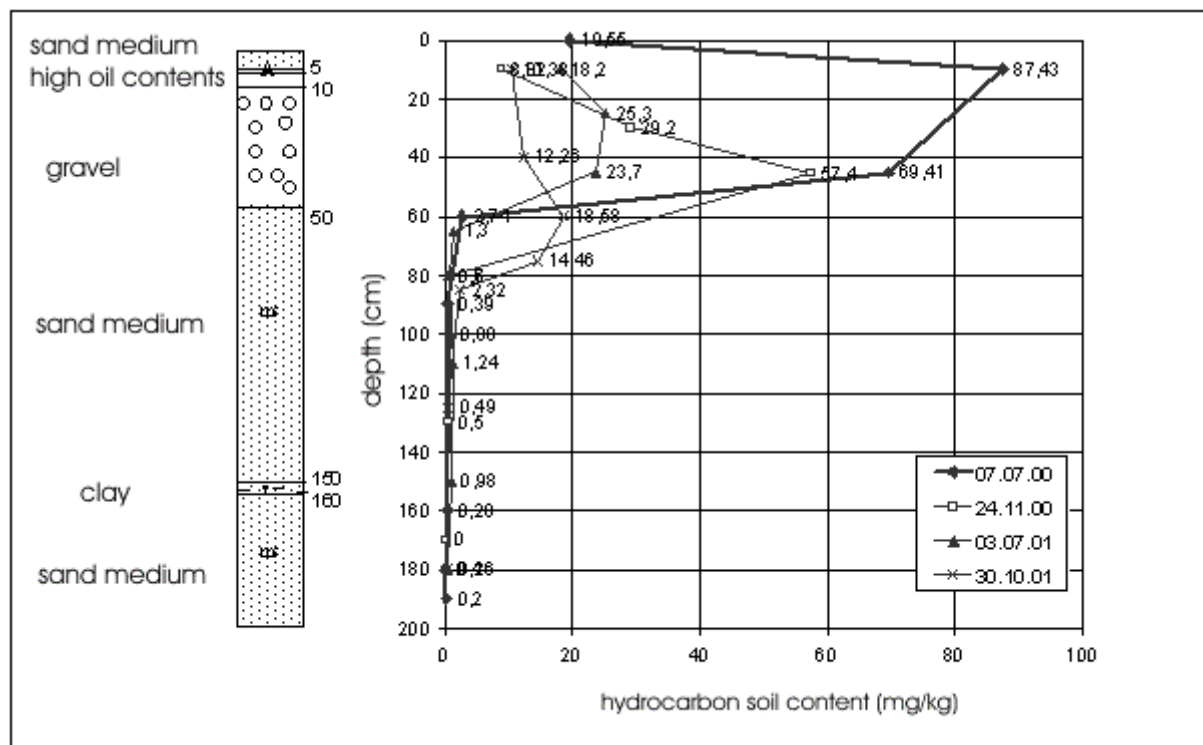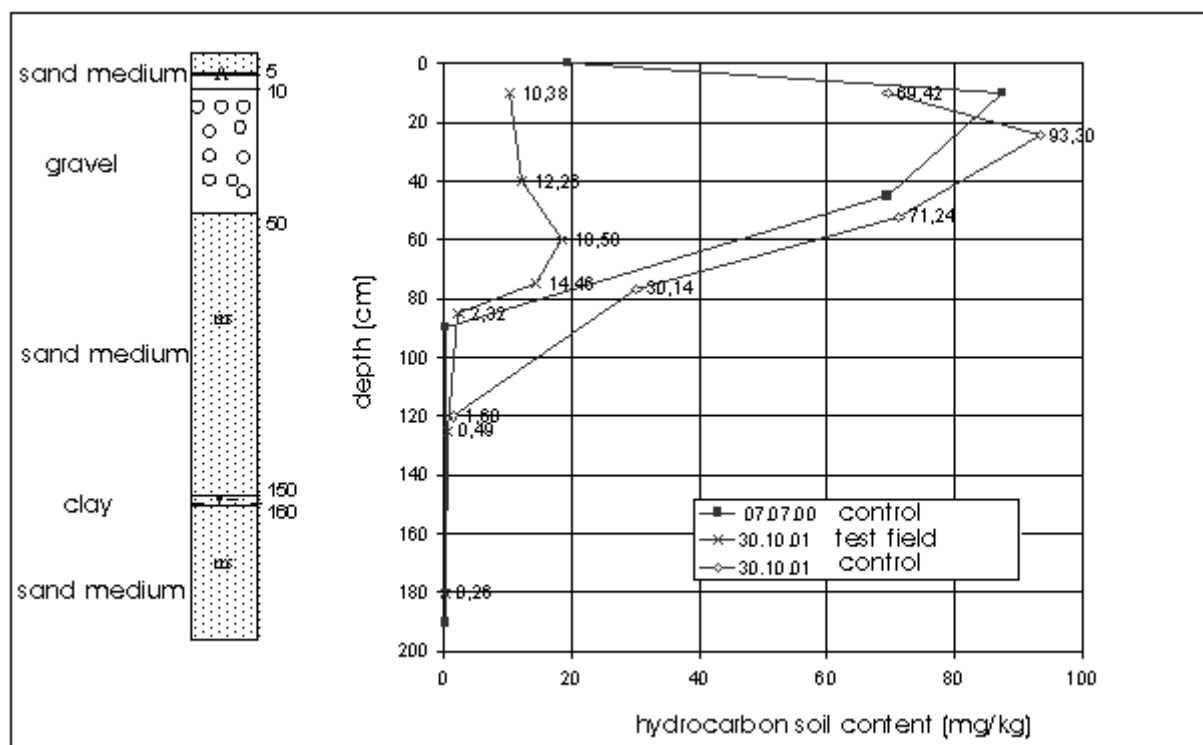
Figure 6

Change in the hydrocarbon content in the untreated track region between July 200 and October 2001 (reference area).

In detail the following additives were supplied into the soil (calculated for 10 m^2 of remediation area) to optimize the natural degradation potential:

- 0.5 L BioVersal FW in 10 L of water (to mobilize the pollutants);

- then 20 L of pre-cultivated micro-organism suspension distributed uniformly over the area, nutrients (corresponding to 200 g/m^2 total N as a first dose) distributed in solid form on the soil;

- then 8 L/m^2 of 1% H_2O_2 solution uniformly distributed and micro-organisms and nutrients slowly flushed into the soil,

The treatment was repeated three times (in November 2000, May 2001 and October 2001). In this case however, only BIOVERSAL FW was added for pollutant mobilization and hydrogen peroxide was used as the oxygen supplier.

The results achieved can be seen from Figs. 5 and 6. It can be clearly seen that despite unfavorable weather conditions (very low temperatures from November 2000 to May 2001) the existing very high loading was reduced by approximately 80% in the test period of 15 months. The displacement of pollutants into deeper soil layers was only small. Strong loading peaks of up to 100 g/kg at the beginning of remediation were only slightly distributed during the remediation process. As a result of the method used, there is thus little risk to the deeper layers of soil caused by pollutant displacement when the pollutant mobilization and degradation were suitably coordinated.

It is worth noting that with the technology used by means of a specific combination of biocompatible surfactants and suitable specialists from the site's own bacteria population, extensive mineralization of the hydrocarbons was achieved without the otherwise characteristic accumulation of bacterial degradation products of a steroid nature. As has been shown with this pilot experiment, this procedure can be used even with very high and severely aged contamination. In the present case almost 50 g hydrocarbons per kg of soil was eliminated over a period of 15 months in some areas of the soil.

With reference to the composition, it can be assumed that the residual contamination still present at the end of the experiment will continue to be degraded subsequently under suitable conditions [13]. Further sampling to track the natural degradation is envisaged for this purpose.

7   Discussion

Despite certain uncertainties in the balancing of natural degradation and retention process, Natural Attenuation (NA) or Enhanced Natural Attenuation (ENA) processes following an extensive elimination of the contamination source are increasingly recognized or even recommended by many specialists as remediation strategies for petroleum hydrocarbon contaminated sites [5]. The limited natural self-cleaning potential should be taken into account particularly in cases of high pollutant concentrations. Small quantities of mineral oils can be mineralized or incorporated into the soil matrix (e.g. by humification) within a few weeks by the site's own micro-organism flora.

For larger pollutant loads (above 5 g/m^2) it must be anticipated that the site's natural resources will be overstressed. In such cases it is necessary to adapt the natural resources to the extent of the contamination as part of ENA. In particular pollutant mobilization and degradation must be matched to one another. Only those surfactants which are themselves completely biodegradable, have no or only minor ecotoxic properties and are compatible with the site's own micro-organisms, are suitable for the ENA approach. If these conditions are not satisfied, some impairment of the biological degradation or even damage to the micro flora must be expected and the mobilized pollutants can disseminate uncontrollably.

Contamination that has existed in high concentration for fairly long periods or has accumulated over a fairly long period is particularly problematical for the ENA approach. In such cases oil ageing occurs. Additional measures are needed to eliminate such aged contamination such as the selective cultivation and use of the site's own suitable micro-organisms. As has been demonstrated for a real contaminated site, the ENA approach can be used in principle for extreme hydrocarbon concentra-

tions with suitable optimization provided that the frequently high-boiling and severely resinified substances can be mobilized by suitable surface-active substances.

The performance of nature-related surfactants has recently been demonstrated in other studies and using other surfactant containing products. For example, IABG mbH has successfully eliminated mineral oils giving a maximum contamination of 14 000 mg/L by using an ENA approach [14]. Similarly good initial results can be expected for a cable oil remediation in the Berlin area [15].

The continual fresh supply of mineral oils is found to be a problem with the ENA approach in track areas. Although the freshly supplied oil is barely aged and thus biologically more easily degradable, the natural self-cleaning potential rapidly becomes overstressed again in terms of nutrients and oxygen. In technical usage a regular determination of the site situation and after treatment to eliminate fresh loadings would thus be indicated.

References

[1]   *BIA Report 5/99,* Hauptverband der gewerblichen Berufsgenossenschaften (HVGB), Sankt Augustin **1999.**

[2]   W. Fritsche *Environmental biology,* Gustav Fischer Verlag, Jena **1998.**

[3]   M. Martienssen, *Research report of the Saxony State Ministry for Environment and Geology, FA No.* 13-8802.3523/101, **2001.**

[4]   F. Vandecasteele, J.P. Monot, F. Fayolle, *Appl. Microbiol. Biotechnol.* **2001,** 56, 339.

[5]   H. Rügner, G. Teutsch, P. Grathwohl, W. Kohler, *Altlastenforum Baden-Württemberg e.V. Series of communications, Vol. 5,* Stuttgart **2001.**

[6]   W. Babel, Research Project FE-50003 of the UFZ Leipzig-Halle *"Xenobiotics and micro-organisms",* Leipzig **1995.**

[7]   H. Mühle, in *Research and development report 1994 of the UFZ Environmental Research Centre Leipzig-Halle*, Leipzig **1994.**

[8]   D. Hunkeler, D. Jorger, K. Haberli, P. Höhener, J. Zeyer, *J. Contam. Hydrol.* **1998**, 32 (1-2), 41.

[9]   *Römpp Chemie Lexikon - CD Version 1.0*, Georg Thieme Verlag, Stuttgart **1995.**

[10]   J. Danzer, M. Herbert, , *Altlastenforum Baden-Württemberg e.V. Series of communications, Vol. 5,* Stuttgart **2000.**

[11]   F. Volkering, A. M. Breure, W. H. Rukens, *Biodegradation* **1997**, 8, 401.

[12]   R. Dallinger, Expert Report for BioVersal Deutschland GmbH, Leipzig **2000**.

[13]   M. Martienssen, *Internal Research Report,* DB Netz AG, Halle/Salle **2001.**

[14]   J. Uhde, J.-H. Hein, *Internal remediation report,* DB Netz AG, Halle/Salle **2002.**

[15]   A. Dahn, verbal communication.