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Microbiological aspects of surfactant use for biological soil remediation

F. Volkering[1], A.M. Breure[2,*] & W.H. Rulkens[3]
[1] *MTI Environmental Technology, P.O. Box 444, 6500 AK Nijmegen, The Netherlands;* [2] *Laboratory for Ecotoxicology, National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands, e-mail: ton.breure@rivm.nl;* [3] *Department of Environmental Technology, Wageningen Agricultural University, P.O. Box 8192, 6700 EV Wageningen, The Netherlands (* author for correspondence)*

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Abstract

Biodegradation of hydrophobic organic compounds in polluted soil is a process involving interactions among soil particles, pollutants, water, and micro-organisms. Surface-active agents or surfactants are compounds that may affect these interactions, and the use of these compounds may be a means of overcoming the problem of limited bioavailability of hydrophobic organic pollutants in biological soil remediation. The effects of surfactants on the physiology of micro-organisms range from inhibition of growth due to surfactant toxicity to stimulation of growth caused by the use of surfactants as a co-substrate. The most important effect of surfactants on the interactions among soil and pollutant is stimulation of mass transport of the pollutant from the soil to the aqueous phase. This can be caused by three different mechanisms: emulsification of liquid pollutant, micellar solubilisation, and facilitated transport. The importance of these mechanisms with respect to the effect of surfactants on bioavailability is reviewed for hydrophobic organic pollutants present in different physical states.

The complexity of the effect of surfactants on pollutant bioavailability is reflected by the results in the literature, which range from stimulation to inhibition of desorption and biodegradation of polluting compounds. No general trends can be found in these results. Therefore, more research is necessary to make the application of surfactants a standard tool in biological soil remediation.

Introduction

Soil pollution with hydrophobic organic compounds (HOCs) is a world wide environmental problem. Many studies on biodegradation of such compounds in soil have shown that their slow release from the soil matrix to the aqueous phase is often the rate-limiting step in the process (e.g. Beck & Jones 1995, Erickson et al. 1993, Luthy et al. 1994, Weissenfels et al. 1992, Würdemann et al. 1995). This phenomenon is referred to as limited bioavailability, a term that is also used with a somewhat different meaning by toxicologists. The definition for limited bioavailability that will be used in this study is: ‘A pollutant has a limited bioavailability when its uptake rate by organisms is limited by a physicochemical barrier between the pollutant and the organisms’. For a better insight into the processes that play a role in bioavailability, it is essential to understand the interactions between the soil matrix, the pollutant, and the micro-organisms. These interactions are dependent on (i) the type and physicochemical state of the pollutant, (ii) the type and physicochemical state of the soil, (iii) the type and state of the micro-organisms, and (iv) external factors, such as the temperature and presence of nutrients and / or oxygen.

The first two factors determine the form in which contaminants occur in soil. The different physical forms possible for organic contaminants are illustrated in Figure 1 (Volkering 1996). They can be dissolved in pore water, adsorbed onto soil particles, absorbed into soil particles, or be present as a separate phase, which can be a liquid or a solid phase. As bioavailability is dependent on mass transfer rates, transport of the

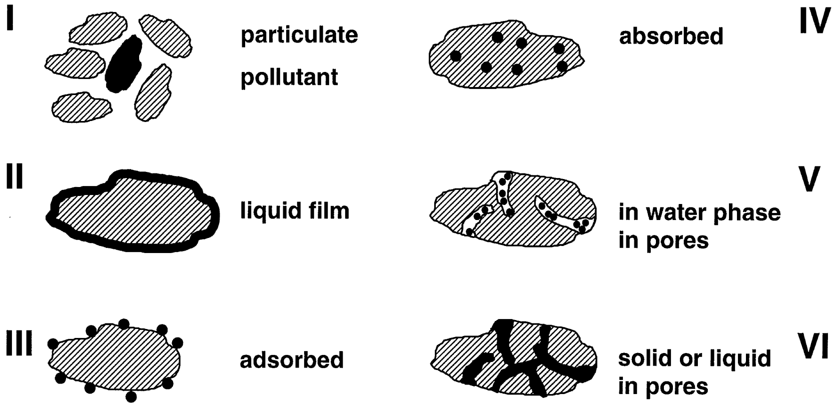

Figure 1. Different physical forms of organic pollutants in soil: I: solid particles; II: liquid film; III: adsorbed onto soil; IV: absorbed into soil; V: in soil macropores; VI in soil micropores.

pollutant to the aqueous bulk phase is the key process governing the bioavailability of the contaminant. A review on the subject of bioavailability was published by Mihelcic et al. (1993).

Of the possible technological solutions to limited pollutant bioavailability, a very promising one is the use of surface-active agents, usually called surfactants, to mobilise the pollutant (Morris & Pritchard 1993). For *in situ* remediation, other solutions are either generally too expensive, such as increasing the soil temperature, or are still in the developing phase, such as the application of acoustic techniques and the use of organic solvents, chemical oxidants, and fungi or fungal enzymes. For *ex situ* remediation, increasing temperature, mechanical reduction of the size of soil aggregates, and improving the mixing conditions provide realistic options.

The first research on the application of surfactants in soil was performed in the petroleum industry. Using the dispersive and surface-active action of surfactants, the objective was to improve the recovery of oil from oil wells by flushing with surfactant solutions. It is, therefore, not surprising that the first study on the use of surfactant for clean-up of contaminated soils was performed by the American Petroleum Institute (1979). In this article and in many other articles on the use of surfactants for soil remediation, the washing of polluted soil with surfactant solutions is addressed. These papers deal mainly with the effect of soil-surfactant and soil-pollutant interactions on the 'solubilisation' of HOCs and on the transport of HOCs through soil. Less has been published on the use of surfactants in biological soil remediation. The use of surfactants is somewhat controversial, as the mechanism of their function is not well understood. The aim of this paper is to give an overview of the present knowledge on the use of surfactants in soil bioremediation, focusing on the microbiologically important aspects, such as surfactant-micro-organism interactions and the bioavailability of pollutants.

Physicochemical properties of surfactants

Surfactants are organic molecules that usually consist of a hydrophobic part and a hydrophilic part. The hydrophilic part makes surfactants soluble in water, while the hydrophobic part makes them tend to concentrate at interfaces. The presence of surfactant molecules at air-water interfaces results in a reduction of the surface tension of the solution and leads to the stabilisation of foams. Many surfactants can reduce the surface tension of aqueous solutions from 72 mN/m (the value for water) to 30 ± 5 mN/m, depending on type and concentration of the surfactant.

Another characteristic of surfactants is the formation of micelles, small aggregates of surfactant molecules. At low concentrations in aqueous solutions, single molecules are present. Beyond a certain concentration, called the critical micelle concentration (CMC), the surfactant molecules cluster together, forming aggregates of 20-200 molecules. The CMC is temperature-dependant and different for every surfactant. At soil temperatures, the CMC typically ranges from 0.1 to 10 mM. In simple terms, micelles can be seen as little (2–4 nm) drops with a hydrophobic core and a hydrophilic shell. The presence of micelles leads to an increase in the apparent solubility of HOCs; this is also referred to as 'solubilisation', a phenomenon

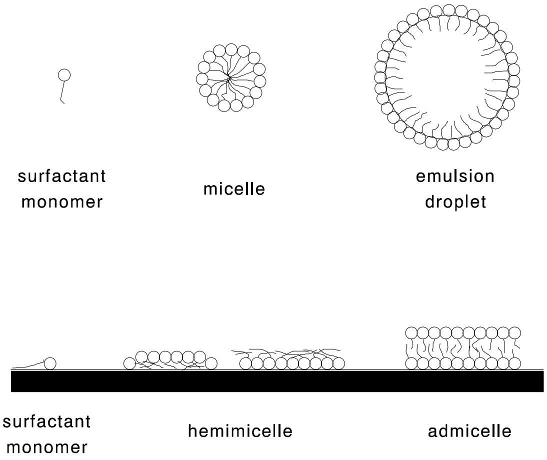

Figure 2. Schematic representation of different surfactant forms in soil (adapted from West and Harwell (1992)).

discussed later in this paper. At higher surfactant concentrations, other types of surfactant aggregates may occur. These high concentrations are not interesting for soil remediation processes and are not addressed here.

In the presence of a non-aqueous phase liquid (NAPL), surfactants concentrate at the liquid-liquid interface, reducing the interfacial tension. This may cause dispersion of NAPL droplets and stabilisation of emulsions. A thermodynamically stable emulsion with a droplet diameter less than 1 μm is called a micro-emulsion, while all other emulsions are called macro-emulsions.

Concentration of surfactant molecules at solid surfaces also leads to a reduction of the interfacial tension, which may improve the contact between the aqueous and solid phases. In high concentrations, surfactant sorption onto solid surfaces leads to the formation of single (hemimicelles) or double (admicelles) layers of surfactant molecules at the interface. The surfactant concentration at which the latter phenomenon occurs is called the critical admicelle concentration; this concentration is usually lower than the CMC (West & Harwell 1992; Nayyar et al. 1994). Figure 2 gives a schematic representation of the most important forms in which surfactants may occur in soil.

Depending on the nature of the hydrophilic group, four types of surfactants can be distinguished: anionic, cationic, zwitterionic, and non-ionic surfactants. Surfactants can be produced chemically (synthetic surfactants) and biologically (biosurfactants). The most common hydrophobic parts of synthetic surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols; the hydrophilic group is usually a sulphate group, a sulphonate group, or a carboxylate group (anionic surfactants), a quaternary ammonium group (cationic surfactants), polyoxyethylene, sucrose, or polypeptide (non-ionic). The major classes of biosurfactants include glycolipids, phospholipids, fatty acids, lipopeptides/lipoproteins, and biopolymeric surfactants. Surfactants are produced on large scale and have numerous applications, e.g., as additives in cleaning agents, food, and cosmetics, and in mining and road construction. An extensive overview of the composition and physicochemical properties of surfactants is given by Rosen (1989).

Surfactant - micro-organism interactions

Surfactant toxicity

Introduction of a surfactant in the environment will always lead to contamination with this surfactant and is, therefore, of little use when the compound itself gives rise to environmental concern. Consequently, the toxicity of the surfactant and its potential degradation products is one of the most important criteria for the selection of surfactant in soil clean-up. Because of the use of surfactants on large scale in detergents, the toxicity of these compounds has been tested relatively well. An overview of the toxicity of the different surfactant types towards numerous species is given by De Oude (1992). The toxic effect of surfactants on bacteria can be explained by two main factors (Helenius & Simons 1975): (i) disruption of cellular membranes by interaction with lipid components and (ii) reactions of surfactant molecules with proteins essential to the functioning of the cell. At a pH of 7 and higher, cationic surfactants are the most toxic ones, while anionic surfactants display the most toxic behaviour at lower pH values. Non-ionic surfactants are in general less active against bacteria than ionic surfactants. Biologically produced surfactants occur naturally in soil, and the use of these surfactants in bioremediation processes may be more acceptable from a social point of view. In comparison with synthetic surfactants, a lower toxicity can be expected from most biosurfactants, although some biosurfactants can be as toxic as synthetic surfactants (Lang & Wagner 1993). For micro-organisms degrading hydrophobic hydrocarbons, the presence of surfactants, especially in concentrations above the CMC, surfactants have often had an inhibiting effect (Laha & Luthy

1991, 1992; Roch & Alexander 1995; Tsomides et al. 1995). Although this phenomenon may be partly explained by the reduced availability of micellar hydrocarbon (Guha & Jaffé 1996a, 1996b, Volkering et al. 1995), inhibition was also observed for hydrophilic substrates. As yet, the reason for this effect of the presence of micelles is not fully understood.

Surfactant biodegradation

Surfactant biodegradability is a factor that can have negative and positive effects in the use of surfactants for bioremediation. Negative effects can be caused by: (i) depletion of minerals or oxygen; (ii) toxicity of surfactant intermediates, which are often more toxic than the parent compounds (Holt et al. 1992); or (iii) preferential degradation of the surfactant, slowing the pollutant degradation (Tiehm 1994). Moreover, the degradation of the surfactant will reduce any bioavailability enhancing effects (Holt et al. 1992; Oberbremer et al. 1990). The most obvious positive effect of surfactant degradation is the removal of the surfactant from the polluted site. Furthermore, the presence of a degradable surfactant may enhance the uptake rate of hydrocarbons. This was found, for example, by Miller & Bartha (1989), who proved that the degradation of long-chain n-alkanes was limited by transport of the substrate over the bacterial membrane. They showed that ‘solubilisation’ of the alkanes in liposomes, micelles constructed of phospholipids, could overcome this limitation. The authors inferred that this was caused by fusion of the liposomes with the cell membrane. More evidence for this mechanism is given by Bury & Miller (1993), who found that uptake of micellar *n*-decane and *n*-tetradecane was stimulated by a biodegradable surfactant, resulting in higher growth rates. This was probably caused by direct uptake of the hydrocarbons along with the micelles. Another positive effect is that a degradable surfactant might be used as a primary substrate when the pollutant is degraded co-metabolically. Some evidence for this is given by Lee et al. (1995), who found that the anaerobic degradation of non-ionic surfactants coincided with zones in which high removal of carbon tetrachloride was observed. Extensive information on the biodegradability of numerous surfactants has been collected by Swisher (1987); a recent update on the aerobic degradation of surfactants is given by Van Ginkel (1996).

Surfactant absorption to cell membranes

Biological membranes consist largely of phospholipid molecules, which are very similar to surfactants. Hence, these membranes are very effective in absorbing surface-active compounds (Sikkema et al. 1995). Apart from the toxic effect and the possibility of fusion with micelles, which have been discussed above, two more consequences of this absorption have been described. First, the aqueous concentration of the surfactant will be lowered, a process similar to the adsorption of surfactant to soil (Swisher 1987). Second, it may have an effect on the permeability of the membrane for HOCs, (Mihelcic et al. 1993; Van der Werf et al. 1995), thus causing enhanced uptake of hydrophobic substrates and possibly the leaching of intermediates (Lantz et al. 1995).

Bacterial mobility

It is well known that biosurfactants play an important role in the interaction of bacteria with interfaces (Neu 1996). Consequently, the addition of surfactants to soil can be expected to influence these interactions and therewith the mobility of bacteria in soil. Marchesi et al. (1991) found that anionic sulfate surfactants can cause attachment to sediment of bacteria degrading these surfactants. The addition of pyruvate or of non-biodegradable surfactants did not induce bacterial attachment. The authors implied that the adherence was related to the bacterial uptake of the sorbed surfactant. Jackson et al. (1994) observed the opposite effect, namely a decrease in the attachment of *Pseudomonas pseudoalcaligenes* to clay when an anionic sulphate surfactant was present. Three possible explanations for the effect were given. First, the surfactant could cause a decrease in the reversible adsorption of the bacteria due to a change in charge density. Second, the surfactant could prevent flocculation, thus increasing the transport of bacteria. The last explanation involved the dissolution of extracellular polymers, which are responsible for the irreversible adsorption. Since attachment can alter the environment and activity of micro-organisms (Van Loosdrecht et al. 1990), this effect of surfactants can be of importance for soil remediation. This is especially true for *in situ* bioremediation, in which transport of bacteria can be an essential process.

Surfactants and pollutant bioavailability

The bioavailability of hydrophobic organic pollutants can be enhanced by surface-active compounds through three main mechanisms:

1. Emulsification of non-aqueous phase liquid pollutant. As mentioned above, surfactants can decrease the interfacial tension between an aqueous and a non-aqueous phase. This may lead to the formation of micro-emulsions or, with energy input, to the formation of macro-emulsions. This results in an increase in the contact area, enabling improved mass transport of the pollutant to the aqueous phase and in mobilisation of sorbed liquid-phase pollutant;
2. Enhancement of apparent solubility of the pollutant. This so called ‘solubilisation’ is caused by the presence of micelles. Hydrophobic organic compounds will dissolve mainly in the core of the micelles, whereas more hydrophilic molecules, such as mono-aromatic compounds, may be present in the core and the shell of the micelles. The transport of micellar hydrocarbon to the aqueous phase can be very rapid due to the small size of the micelles (Almgren et al. 1979), but it is not clear whether ‘solubilised’ hydrocarbons are directly available to the degrading micro-organisms (Grimberg et al. 1995; Guha & Jaffe 1996a, 1996b; Roch & Alexander, 1995; Volkering et al. 1995). If the HOC molecule is not available, then the micelles can reduce bioavailibility and slow biogradation.
3. Facilitated transport of the pollutant. This term covers several different processes, such as the interaction of a pollutant molecule with single surfactant molecules, the interaction of surfactants with separate-phase or sorbed hydrocarbons (both as single surfactant molecules and as micelle-like aggregates at surfaces), mobilisation of pollutant by swelling of the organic matrix, and mobilisation of pollutant trapped in soil ganglia caused by lowering the surface tension of the pore water in soil particles (e.g. Deitsch & Smith 1995, Yeom et al. 1996).

These three mechanisms may all cause enhanced mass transport and are therefore often hard to distinguish. The relative contribution of the mechanisms depends strongly on the physical state of the pollutant, as will be discussed below. It should be noted that enhancing the mobility of pollutants *in situ* might be a double edged sword, as it may result in spreading of contaminants to previously uncontaminated areas.

Along with positive effects, surfactants also can have negative effects on the bioavailability of HOCs. In addition to segregating the HOC (item 2 above), surfactants can inhibit bacterial attachment, disperse soil colloids causing clogging of pores, or interfere with the natural interactions of micro-organisms with the pollutant. In the following sections, the positive and negative effects mentioned above are discussed for the case of non-aqueous liquid phase pollutant, solid pollutant, and sorbed pollutant. Pollutant present in porewater is hard to distinguish from sorbed pollutant and, therefore, is not dealt with separately.

Non-aqueous phase liquid pollutant

Microbial uptake mechanisms

In the literature, three mechanisms for the microbial uptake of liquid hydrocarbons have been proposed (Bury & Miller 1993; Hommel 1990): (i) uptake of hydrocarbon dissolved in the aqueous phase; (ii) direct uptake of hydrocarbons from the liquid-liquid interface; (iii) uptake of "pseudo-solubilised" hydrocarbons. The first mechanism is usually found for compounds with a relatively high aqueous solubility (Goswami et al. 1983), although Yoshida et al. (1971) found that the uptake of C_{16} to C_{18} alkanes by the yeast *Candida tropicalis* proceeded via the aqueous phase. Direct uptake of NAPLs, involving bacterial attachment to the liquid-liquid interface, has often been described for poorly soluble substrates (e.g. Efroymson & Alexander 1994; Prokop et al. 1971; Wodzinski & Larocca 1977). Shreve et al. (1995) and Zhang & Miller (1994) showed that cell hydrophobicity and production of cell-wall-associated biosurfactants play an important role in the attachment. The presence of bacterial emulsifiers and of the bacteria themselves at the interface lowers the interfacial tension, thus causing enhanced dispersion of the NAPL. This results in an increase in surface area and with it an increase in biodegradation rates. Pseudosolubilisation of NAPLs by microbial excretion products has been studied extensively by Goswami et al. (1983) and Reddy et al. (1982). Depending on the organism, the hydrocarbon, and the environmental conditions, uptake may take place through either one or a combination of the above mechanisms.

Effect of surfactants

In a system with two liquid phases, the addition of surfactant can have two consequences. First, it may improve dispersion and pseudosolubilisation of the NAPLs, as was demonstrated by Breuil & Kushner (1980), who showed that C_{16} and C_{18} fatty acids, lipids, and the synthetic surfactants Triton X-100, FL-70, and Brij 35 stimulated growth of *Acinetobacter lwoffi* and of *Pseudomonas aeruginosa* on hexadecane. Second, the presence of surfactants at the liquid-liquid interface may inhibit the bacterial attachment to this interface, thereby reducing the degradation rate. This was first shown by Aiba et al. (1969) for the degradation of *n*-dodecane and *n*-tetradecane in the presence of Tween 20 and for *n*-hexadecane by Mimura et al. (1971). In a recent study, Efroymson & Alexander (1994) reported that the presence of Triton X-100 in shaking cultures inhibited the growth of an *Arthrobacter* sp. on *n*-hexadecane, dissolved in the inert NAPL heptamethylnonane. They showed that, in the presence of the surfactant, the number of bacteria in the aqueous phase was higher and concluded that the surfactant prevented the attachment to the liquid-liquid interface and thereby inhibited the hydrocarbon degradation. In contrast, Triton X-100 stimulated growth of the same organism on naphthalene. In conclusion, it can be said that the best results of the application of surfactants can be expected for NAPL pollutants that are not degraded via direct uptake of hydrocarbons from the liquid-liquid interface.

NAPLs in soil

When a pollutant is present as a NAPL-phase in soil, basically the same effects of surfactants as described above can be expected. Additionally, NAPLs trapped in soil pores may be mobilised by the reduction of surface tension and interfacial tension. An overview on the literature concerning the biodegradation of NAPLs in soil in the presence of surfactants is presented in Table 1.

Rittmann & Johnson (1989) found the dispersant Corexit 7664 to have no effect on the biodegradation of lubricating oil in soil in microcosm and reactor experiments. Testing synthetic non-ionic and anionic surfactants in microcosms, Rasiah & Voroney (1993) found that 2 out of 6 surfactants (1 non-ionic and 1 anionic) were significantly effective in enhancing biodegradation of oil from a polluted soil. The best results were obtained with the most biodegradable surfactant tested. In column experiments with sandy soil, Ducreux et al. (1993) used a non-ionic surfactant to enhance oil biodegradation. It was found that a 0.05% (w/v) surfactant solution increased the removal of oil by 50% when compared to experiments without surfactant. Column experiments with diesel oil showed that RESOL 30, a mixture of biodegradable non-ionic and anionic surfactants, improved oil mobilisation and biodegradation (Ducreux et al. 1995). In bioreactor experiments with a 10% soil slurry amended with 1.35% of a hydrocarbon mixture, performed by Oberbremer et al. (1990), microbiologically produced glycolipids promoted the onset and increased the extent of biodegradation. Using a surface-active compound produced by *Pseudomonas aeruginosa* UG2, Jain et al. (1992) showed that, at the highest concentration used (100 mg. g^{-1} soil), the surfactant significantly enhanced the disappearance of tetradecane, pristane, and hexadecane, but not of 2-methylnaphthalene. Addition of the surfactant-producing bacteria at densities up to $10^8 \times g^{-1}$ soil had no effect on the hydrocarbon disappearance. Since the hydrocarbons used were not radioactively labelled, and since no measure for bacterial growth was presented, it is not certain whether the disappearance of the hydrocarbons was caused by biodegradation.

Solid pollutant

Microbial uptake mechanisms

Direct uptake via attachment of bacteria to solid hydrocarbon surfaces is possible, as described by Goswami et al. (1983) for growth on sterols, by Zilber et al. (1980) for the growth of a marine pseudomonad on *n*-tetracosane, and by Guerin & Jones (1988) for the growth of a *Mycobacterium* sp. on phenanthrene. More often, however, no bacterial attachment on the surface of solid substrate is found (Bouchez et al. 1995; Cameotra et al. 1983; Chakravarty et al. 1972; Mulder et al. 1998; Stucki & Alexander 1987; Thomas et al. 1986; Volkering et al. 1992; Wodzinski & Bertolini 1972; Wodzinski & Coyle 1974). If the bacteria are not attached to the solid surface, the substrate has to dissolve before it can be taken up. The effects of dissolution of solid substrate on bacterial growth kinetics have been described by Chakravarty et al. (1972), Volkering et al. (1992, 1993), and Mulder et al. 1998. The first authors presented a mathematical model in which the biodegradation of *n*-eicosane by a *Pseudomonas* sp. was limited by the dissolution rate of the substrate. In this model, the authors

Table 1. Literature review on the effect of surfactants on the biodegradation of NAPLs in soil

Authors	Rittmann & Johnson 1989	Oberbremer et al. 1990	Jain et al. 1992	Ducreux et al. 1993, 1995	Rasiah & Voroney 1993
Soil type	silty clay loam	unknown	silt loam (2.1% o.c.)	silica sand	clay loam (1.2% o.c.)
Contamination	lubricating oil	mixture of alkanes and aromatics	oily mixture of alkanes and methylnaphthalene	diesel oil	oil refinery waste
Microorganisms	enriched mixed culture	mixed culture	*Pseudomonas aeruginosa*	mixed culture	enriched indigeneous population
Setup	microcosm and slurry reactor	slurry reactor	microcosms	columns	microcosms
Surfactant type	commercial dispersant Corexit 7664	several biosurfactants	biosurfactant	nonionic; mixture of nonioni and anionic	3 nononic and 3 anionic
Surfactant concentration	5% (microcosm) or 0.11 and 0.22% (reactors) of added nutrient solution	200 mg.L^{-1}	25 or 100 μg.g^{-1} soil	0.05% w/w; 1.9 and 10.5 g/L	0.35 μl.g^{-1} soil.week^{-1}
Effect on mobilization	N.D.	N.D.	N.D.	stimulation	N.D.
Effect on biodegradation	no effect	stimulation	stimulation for alkanes, no effect for methylnaphthalene	stimulation	stimulation (best result with most degradable surfactant)
Explanation	insufficient mixing	emulsification	–	emulsification	oil emulsification, surfactant used as cosubstrate

N.D.: not determined; o.c.: organic carbon.

assumed that growing cells produce a metabolite that helps the solubilisation of the substrate. Unfortunately, a synthetic emulsifier was used in the experiments performed to validate the model, making the interpretation of the data very difficult. In a later study of the same group (Cameotra et al. 1983), evidence is presented that the uptake of *n*-docosane by the yeast *Endomycopsis lipolytica* YM and the uptake of *n*-tetradocosane by *Pseudomonas* PG-1 occurred primarily through pseudosolubilisation by microbial excretion products. In our own studies (Volkering et al. 1992, 1993, Mulder et al. 1998), we found that the degradation of naphthalene by a *Pseudomonas* sp. was limited by the substrate dissolution rate and that no solubility-enhancing excretion products were involved. Using phenanthrene, similar results were obtained by Bouchez et al. (1995).

Effect of surfactants

The effect of surfactant addition on the biodegradation of solid hydrocarbons has been described in a limited number of articles. In contrast to liquid phase pollutants, no dispersion can occur, excluding the first mechanism of bioavailability enhancement. When bacterial attachment occurs, the effect of surfactant can be the same as described above for liquid hydrocarbon, i.e. inhibition of biodegradation by inhibition of attachment. This has been reported by Goswami et al. (1983), who found that the synthetic alkylphenol polyglycol ether HYOXYD AAO prevented attachment of *Arthrobacter* 317 to b-sitosterol and strongly inhibited the degradation of the sterol. In contrast, other synthetic surfactants, such as Tween 40, Tween 80, and Unisperse-P, had a slightly increasing effect on the growth on b-sitosterol.

When the micro-organisms use substrate in the dissolved state only, surfactant can enhance the bioavailability in two ways. First, the dissolution rate can be increased by partitioning of the hydrocarbon into micelles. We inferred earlier (Volkering et al. 1993, 1995) that this effect is of little importance in the dissolution-limited growth phase (bioavailability-

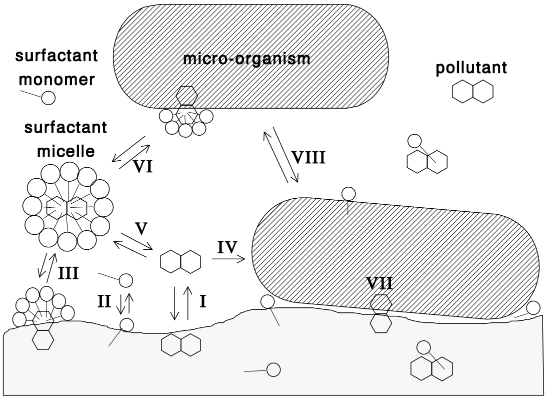

Figure 3. Schematic overview of the interactions between microorganisms, soil, pollutant, and surfactants. I sorption of pollutant, II sorption of surfactant molecules onto soil, III solubilisation of pollutant, IV uptake of pollutant from the water phase by micro-organisms, V partitioning of pollutant between the water phase and the micelles, VI sorption of micelles to micro-organisms, VII direct uptake of pollutant from the solid phase by micro-organisms, VIII sorption of micro-organisms onto soil.

limited phase), as the aqueous hydrocarbon concentration will be very low in this phase. This is confirmed by experiments with *n*-octadecane performed by Zhang & Miller (1992), in which they found that the addition of 300 mg.L^{-1} of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027 increased the solubility of the alkane by a factor 10^4, whereas the *n*-octadecane mineralization rate by the same organism was only increased by a factor 4. Second, the surfactant can affect the dissolution process by interaction with the surface of the substrate, as suggested by Gerson (1993). A possible mechanism for this interaction is the formation of micelle-like structures at the solid surface (Nayyar et al. 1994). This will affect the maximal dissolution rate and may increase microbial growth in the dissolution-limited phase. Grimberg et al. (1996) and Volkering et al. (1995) showed that, under dissolution-limited conditions, the stimulating effect of surfactants on the biodegradation of solid PAH was not based on micellar solubilisation.

Tiehm (1994) found that different synthetic nonionic surfactants stimulated the growth of mixed cultures on several polycyclic aromatic hydrocarbons, but from the data presented it cannot be concluded whether this stimulation was caused by growth on micellar substrate, pseudosolubilised during the first period of the experiment, or by an increased maximal dissolution rate. Similar results were found by Hunt et al. (1993) for the biodegradation of phenanthrene in the presence of a rhamnolipid biosurfactant and by Lantz et al. (1995) for the biodegradation of fluoranthene in the presence of Triton X-100.

Solid pollutant in soil

Although less frequently occurring than NAPLs, pollutant may occur in soil as solid particles (e.g. Vogel et al. 1995, Weissenfels et al. 1992). The mechanisms of the action of surfactants can be expected to be essentially the same as for solid pollutant in the absence of soil. In addition to this, the wetting properties of surfactants may improve the contact between the pollutant and the aqueous phase.

Sorbed pollutant

Microbial uptake mechanisms

Most studies on the biodegradation of sorbed compounds have been performed with artificially contaminated soils or other matrices. The majority of these studies revealed that micro-organisms can only take up dissolved substrate (e.g. Alvarez-Cohen et al. 1993, Ogram et al. 1985, Rijnaarts et al. 1990, Scow & Alexander 1992) . There are, however, publications showing that sorbed compounds are to some extent available for micro-organisms without prior desorption (Gordon & Millero 1985; Guerin & Boyd 1992; Hermannson & Marshall 1985). Nevertheless, even when sorbed substrates were found to be directly degradable, they were less bioavailable than aqueous-phase substrates (Gordon & Millero 1985). Therefore, desorption plays an essential role in the microbial growth on sorbed substrates.

Effect of surfactants on sorption processes

The use of surfactants in soil bioremediation leads to complex interactions among surfactant, soil, pollutant, and micro-organisms. A simplified schematic overview of these interactions is shown in Figure 3. In reality, the interactions are more complex due to the heterogeneity of the soil.

The adsorption of surfactant molecules onto soil is dependant on the properties of the soil and the surfactant (Edwards et al. 1991, 1992, 1994a). Although surfactant adsorption is an unfavourable process for micellar solubilisation (West & Harwell 1992), it may play an essential role in the facilitated transport of sorbed substrate.

Table 2. Literature overview on the effect of surfactants on biodegradation of sorbed hydrophobic organic compounds

Authors	Viney & Bewley 1990	Aronstein et al. 1991, Aronstein et al. 1992, Aronstein & Alexander 1993	Laha & Luthy 1991, Laha & Luthy 1992	Dohse & Lion 1994	Volkering et al. 1995	Providenti et al. 1995a,b
Matrix	sand	sand (0.4% o.c.), silt loam (7.6% o.c.), muck (32.9% o.c.)	silt loam (1.5% o.c.)	sand (0.05% o.c.)	porous resins XAD-4 and XAD-7	sandy loam
Contamination	polychlorinated biphenyls	phenanthrene, biphenyl	phenanthrene	phenanthrene	naphthalene	phenanthrene, creosote
Microorganisms	several isolates and pure strains	mixed culture	mixed culture	mixed culture	*Pseudomonas* sp.	*Pseudomonas aeruginosa*
Setup	microcosm	shaking soil slurries and column experiments	shaking soil slurries	shaking soil slurries	shaking cultures,	shaking soil slurries
Surfactant type	alkylphenol ethoxylates	alcohol ethoxylates	several nonionic surfactants	microbial polymers	Triton X-100, Brij 35	rhamnolipid biosurfactants
Surfactant concentration	5 g.L^{-1}	sub-CMC	varying	30 or 100 mg TOC.L^{-1}	varying	100-400 mg.L^{-1}
Effect on desorption	stimulation	no effect	stimulation of partitioning	stimulation	stimulation	N.D.
Effect on biodegradation	inhibition	stimulation	inhibition at high surfactant conc.	no effect	stimulation	mixed
Explanation	surfactant toxicity	–	– (no toxicity)	–	–	

N.D.: not determined; o.c: organic carbon.

The effect of surfactants on the equilibrium desorption or partitioning of HOCs between the soil and the aqueous phase, which is important in soil washing, has been studied by many researchers for synthetic surfactants (e.g. Edwards et al. 1991, 1992, 1994a, 1994b; Liu et al. 1992a, 1992b; Park & Jaffé 1993; Sun & Boyd 1993; Vigon & Rubin 1989) and biosurfactants (e.g. Harvey et al. 1990; Juwarker et al. 1993; Scheibenbogen et al. 1994; Van Dyke et al. 1993a; 1993b). Generally, sorbed surfactant increases the sorbing capacity of the soil, whereas solute surfactant increases the aqueous solubility of HOCs. The net effect from these two mechanisms is dependant on the surfactant concentration. At concentrations well above the CMC, a decrease in the soil-water partitioning coefficient of the pollutant can be expected, especially for very hydrophobic pollutants. At surfactant concentrations below or near the CMC, the effect on the partitioning is dependant on soil and surfactant properties (Haigh 1996). It should be noted that the CMC of the surfactant is based on the surfactant concentration in the aqueous phase. This implies that the presence of a sorbing agent, such as soil, will lead to an increase in the apparent CMC (Edwards et al. 1994b). In contrast, cationic surfactants can be used to promote sorption of HOCs to more hydrophilic surfaces, even at high concentrations (Wagner et al. 1994).

For assessing the effect of surfactants on the mobilisation of sorbed substrate, desorption kinetics are more important than equilibrium desorption data. Batch experiments can be used for studying desorption kinetics, but continuous desorption experiments ("leaching experiments") provide more useful data. Most of the studies published on this subject demonstrate that the desorption and mobilisation of hydrophobic pollutants from soil can be enhanced by the addition of surfactants (e.g. Abdul & Gibson 1991; Abriola et al. 1993; Deitsch & Smith 1995; Dohse & Lion 1994; Duggan et al. 1994, Pennell et al. 1993, 1994; Roy et al. 1994; Yeom et al. 1996). Deitsch &

Smith (1995) and Yeom et al. (1996) concluded the enhancement to be caused by micellar solubilisation and facilitated transport, the latter process being the most important.

Effect of surfactants on pollutant biodegradation

The effect of surfactants on the biodegradation of sorbed HOCs has been the subject of several studies. A summary of some of the results is given in Table 2. Due to the complexity of the interactions described above, many different effects of surfactant in this system can be envisioned, depending on the circumstances used.

Laha & Luthy (1991, 1992) found for various surfactants that the presence of these compounds in concentrations above the CMC inhibited the mineralisation of phenanthrene by a mixed culture in a soil-water system. At concentrations below the CMC, no inhibition was observed and dilution of the solutions to a surfactant concentration below the CMC removed the inhibitory effect. The mineralisation of glucose in mineral medium and in a soil-water system was not significantly affected by surfactant, and the use of glucose as a co-substrate inhibited the degradation of phenanthrene only temporarily. The authors concluded that the inhibition was not caused by toxicity or by preferential degradation of the surfactant, but could not give an explanation for the inhibition. For polychlorinated biphenyls Viney & Bewley (1990) found that the non-ionic surfactants Triton X-100 and Tensoxid S50 stimulated the equilibrium desorption from soil, but inhibited the degradation of all PCB congeners by several isolates.

Dohse & Lion (1994) studied the desorption and mineralisation of phenanthrene sorbed onto a low-carbon sand in the presence of several different microbial polymers. Of the 28 polymers tested, 24 decreased the soil-water partitioning coefficient of phenanthrene. One polymer was used for column experiments in which the breakthrough of phenanthrene was investigated. The mobility of phenanthrene was increased by the polymer. In mineralisation experiments, however, no significant effect of the polymer on the biodegradation of sorbed phenanthrene was found.

In two research articles published by the group of Alexander (Aronstein et al. 1991; Aronstein & Alexander 1992), the effect of the non-ionic surfactants, Alfonic 810-60 and Novel II 1422-56, in concentrations below the CMC on the desorption and mineralisation of phenanthrene and biphenyl in batch systems was documented. Although the surfactants at these low concentrations did not result in an appreciable change in the partitioning of the hydrocarbons between the soil and the aqueous phase, the mineralisation by indigenous and introduced bacteria was markedly enhanced. The authors noted that the extent of desorption is of less importance than the desorption rate, but presented no experiments that could explain the observed effect. Moreover, the procedure used to introduce the substrate into the soil may lead to the formation of hydrocarbon crystals. Thus, not only desorption, but also dissolution may have occurred. Another study by the same group (Aronstein & Alexander 1993) concerned the biodegradation of phenanthrene and biphenyl in soil columns through which solutions with low concentrations of the surfactant Novel II 1412-56 were pumped. Mineralisation of both hydrocarbons was stimulated by the presence of the surfactant, but since no desorption experiments with the soil columns under sterile conditions were performed, it is not possible to draw conclusions on the mechanism of the stimulation. A promising aspect of the study was the fact that the presence of surfactant did not cause an increase in the effluent concentrations of the two hydrocarbons and their degradation products.

Experiences with polluted soil

In the biodegradation studies with soil discussed in the sections above, the pollutant was introduced into the soil in the laboratory prior to the experiments. However, in the real-world situation, pollutants have often been present in the soil for several decades. Considering that the bioavailability of organic pollutants in soil decreases significantly during ageing (Hatzinger & Alexander 1995), the effect of surfactants on aged pollutants may be more explicit than on fresh pollutants.

Laboratory scale

Table 3 gives an overview of relatively well-documented publications that address the application of surfactants in polluted soil at the bench scale. A number of these studies were performed as pre-investigation for a full scale bioremediation (Balba et al. 1993; Bewly et al. 1989; Ellis et al. 1990, 1991). In

Table 3. Literature overview on the effect of surfactants on the remediation of contaminated soil and sludge

Authors	Bewley et al. 1989	Ellis et al. 1990, Balba et al. 1993	Ellis et al. 1991	Marks et al. 1992	Belkin et al. 1994	Ghosh et al. 1995
Soil type	complex	unknown	sandy clay, clay loam	refining sludge and petrochemical sludge	unknown	MGP soil with 75% o.c.
Contamination	coal tar (PAHs)	refinery oil	creosote	PAHs	PAHs	PAHs
Microorganisms	mixed culture	strains isolated from indigenous population	*Pseudomonas sp.*	mixed culture	indigenous population	enriched mixed culture
Setup	*in situ* treatment and landfarming	microcosms, *in situ* treatment and landfarming	microcosms, columns	continuous slurry reactors	columns	batch
Surfactant type	unknown	Cyanamer P70	Ethylan CD 916	Triton N-100, Triton X-100	unknown	Triton X-100
Surfactant concentration	unknown	unknown	0.5% w/v (desorption); 200 μg.g^{-1} (biodegradation)	1 g.L^{-1}	unknown	0.2-2.5 g.L^{-1}
Effect on desorption	stimulation		stimulation of solubilization and leaching	possible stimulation of partitioning	not determined	stimulation
Effect on biodegradation	possible stimulation of biodegradation	stimulation in microcosms, possible stimulation in full scale treatment	possible stimulation of biodegradation	possible stimulation of biodegradation	decrease in effluent genotoxicity	no effect
Explanation	no blanks, several parameters changed	no blanks, several parameters changed	two parameters changed	no blanks, several parameters changed	none	surfactant concentration to low?

N.D.: not determined.

these studies, an optimised treatment, including surfactant addition, is compared with a non-optimised treatment. It is therefore uncertain whether the enhanced pollutant biodegradation observed is actually caused by the surfactant addition. Most other studies with polluted soil are also difficult to interpret. This is illustrated by the results obtained by Ghosh et al. (1995) with a weathered manufactured gas plant soil with 75% organic matter. In well-designed gas purge desorption experiments, adding 5.5 g/L Triton X-100 to the aqueous phase increased the effective PAH diffusion coefficient by 4 orders of magnitude. However, this facilitated transport was not observed in much less well-designed biodegradation experiments described in the same publication, where Triton X-100 in concentrations up to 2.5 g/L was found to have no significant effect on the biodegradation. Belkin et al. (1994) reported that the use of an unknown surfactant changed the course of the toxicity of the leachate from columns filled with PAH-polluted soil in which biodegradation occurred. At the beginning of the treatment, a toxicity increase was observed, but at the end of the experiment the toxicity of the surfactant-amended soil was lower than the control soil without surfactant. Unfortunately, no data on PAH-concentrations and on biodegradation were presented. You et al. (1995) and Zappi et al. (1995) investigated the use of surfactant for enhancing the clean-up of TNT-contaminated soil. Although the mechanism remained unclear, some evidence suggested that the surfactants used, Triton X-100 and Tween 80, stimulated the co-metabolic transformation of TNT.

Overall it can be said that, although the interpretation is often difficult, more positive results of surfactant application have been found in experiments with polluted soils than with artificially polluted soils. This is consistent with the insight that bioavailability-limitations are more important for aged pollutants then for fresh pollutants.

Field scale

Little detailed information is available on the use of surfactants in field studies. The published results of application of surfactants in field scale bioremediation processes are often limited to the statement that surfactants were used to overcome bioavailability limitations without further evidence for beneficial effects of the surfactant addition (e.g. Balba et al. 1993; Bewley et al. 1989; Marks et al. 1992; Rhodes et al. 1995; Ross et al. 1995; Yu & Ward 1995). An exception is formed by the oleophilic fertiliser Inipol EAP 22, a product in which both nutrients and surfactants are present. This product was used on a large scale to stimulate biological clean-up of shorelines contaminated with oil originating from the Exxon Valdez oil spill (Glaser 1991). Compared to addition of other fertilisers, the addition of Inipol EAP 22 was more successful in stimulating oil degradation. It was not investigated whether this stimulation was caused by a more efficient nutrient addition or by enhanced bioavailability of the oil due to the surface-active properties of Inipol EAP 22. Recent work by Churchill et al. (1995, 1995a) and Croft et al. (1995), however, showed that Inipol EAP 22 also stimulated hydrocarbon degradation under conditions without N- or P-limitation, and the authors suggested that the positive effect of the fertiliser on the biodegradation of the oil spilled by the Exxon Valdez may have been significantly caused by its surface-active properties.

Selection of surfactants

For successful application of surface-active compounds in bioremediation, the choice of the surfactant is of crucial importance. Of all the aspects mentioned earlier in this review, surfactant toxicity is the only one that is always unfavourable. The other aspects can have positive and negative effects on the clean-up of soil, depending on the external conditions. These conditions can be different for every case and include the following factors:

- type and physicochemical state of the pollutant (NAPL, solid, sorbed);
- type and physicochemical state of the soil (pH, presence of oxygen, moisture content, presence of nutrients, permeability, etc.);
- type and state of the degrading micro-organisms;
- mixing conditions.

It is important to have at least a global impression of these factors before deciding to apply surfactants in a biological soil treatment process. Several parameters can be used to characterise surfactants, the most important of which are listed below:

- Hydrophile-lipophile balance or HLB number, an empirical parameter that describes the relative contribution of the hydrophilic moiety to the weight of the surfactant molecule. HLB numbers are supplied by most surfactant manufacturers. Surfactants with an HLB number of 3-6 are lipophilic and can be used to form water in oil emulsions, whereas surfactants with an HLB number in the range of 10 to 18 are more hydrophilic and can be used to form oil in water emulsions. The surfactants that are most successful in the washing of oil-contaminated soils usually have an HLB higher than 10. The relation between HLB and solubilising properties of alkyl ethoxylates has been studied by Diallo et al. (1994).
- Reduction of the surface tension. Surface tension reduction has been identified as the most important parameter for the effectiveness of surfactants in oil recovery (Hill et al. 1973).
- Reduction of the interfacial tension. When a pollutant is present as a NAPL, its mobilisation is largely determined by the interfacial tension. The interfacial tension reduction can be evaluated by making a Winsor diagram, which is based on the emulsification of the pollutant (West & Harwell 1992).
- Emulsifying activity and solubilisation efficiency. These parameters give a measure for the amount of a specific NAPL (usually an aliphatic hydrocarbon) that can be solubilised or emulsified by an amount of surfactant. Several different methods for quantifying these parameters have been applied (e.g. Banat 1993, Barakat and Gracia 1983, Van Dyke et al. 1993b, Vigon & Rubin 1989).

Although these parameters have been used in several publications, there is no general rule as to which parameter is the best selection criterion. For sorbed pollutant, it can be inferred that facilitated transport in soil is the most important effect of surfactants in bioremediation; thus, the surface and interfacial tension reduction are probably the best parameters for selection of surfactants for biological soil remediation when the pollutant is present in a sorbed state. When the pollutant is present as a NAPL, interfacial tension reduction and emulsifying activity are likely to be the most suited parameters.

In addition to this, it is advisable to perform simple standardised laboratory experiments in which the effect of the surfactant on the mobilisation and biodegradation of the pollutant is determined, e.g., Bury and Miller 1993, Grimberg et al. 1996, and Volkering et al. 1995.

Future prospects

Many different surfactants have been tested with many different types of soil and pollutants. Although surfactants have been found to stimulate the degradation of hydrophobic pollutant, no general trends can be found using these results. Therefore, more laboratory research using simple model systems is necessary. It is likely that the insights to be obtained from such research can be used to understand the more complicated processes in field situations.

Since the application of surfactants is one of the few realistic technological solutions for the problem of limited pollutant bioavailability, the use of surfactants as a tool in the biological clean-up of soil has promising possibilities. This is especially true for *in situ* techniques. The effect of surfactants in landfarming may be limited by a lack of mixing, although application in intensive landfarming could be used to enhance the clean-up process. The application of surfactants in aerobic bioreactors could shorten the retention time and therewith the volume of the reactor, but may also cause unwanted foaming (Oberbremer & Müller-Hurtig 1989).

From an economic and scientific point of view, the best results are to be expected when surfactants are used at low concentrations, with the possible exception of pollutants present as a non-aqueous liquid phase. Dispersion of this type of pollutant may be an effective way of increasing its bioavailability, but may require rather high surfactant concentrations.

Biosurfactants have the advantage over synthetic surfactants in that they are socially more acceptable and generally are less toxic (Banat 1995). Francy et al. (1991) found that bacteria from hydrocarbon contaminated zones, and especially those from biostimulated zones, displayed higher biosurfactant production than bacteria from pristine zones. Moreover, biologically produced surfactants may be especifically effective for the growth substrate of the surfactant-producing micro-organism (Falatko & Novak 1992). Hence the stimulation of the biosurfactant production by the pollutant-degrading organisms (*in situ* or on site) is a promising option that is likely to be cost-effective and excludes possible interference with the natural interactions among the degrading population and the pollutant.

In conclusion, much more knowledge is required before the application of surfactants can be used as a standard technique in soil remediation. Criteria will have to be developed for making decisions to use surfactants. These criteria may include simple desorption and/or biodegradation experiments. To make this possible more insight is required. Therefore laboratory studies, field studies, and especially *in situ* studies on the use of surfactants with proper control experiments are essential.

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