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in subsurface environments

THE INFLUENCE OF SURFACTANTS AND BIOSURFACTANTS ON THE BIOAVAILABILITY OF HYDROPHOBIC ORGANIC POLLUTANTS IN SUBSURFACE ENVIRONMENTS

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Key words: surfactant, biosurfactant, rhamnolipids, solubilization, bioavailability, biodegradation, bioremediation, adsorption, desorption

ABSTRACT

Due to their low aqueous solubility and high affinity for organic matter, hydrophobic organic compounds (HOC) tend to sorb onto soil and stay immune to microbial activity. The accumulation and persistence of organic pollutants in soil can result in their slow and continuous leaching to groundwater, limiting the efficiency of restoration technologies like pump and treat or *in-situ* bioremediation. Synthetic surfactants have proved effective in mobilizing organic contaminants sorbed onto soil, and are widely used in soil washing/flushing restoration technologies. Nonetheless, the toxic nature of synthetic surfactants can prevent further microbial interaction with the pollutants, once they are incorporated to the micellar pseudo-phase. Biosurfactants (specifically rhamnolipids) have been tested as effective carriers of organic pollutants in soil, and have proved also to enhance (or at least not inhibit) the microbial up-take of the contaminants after their micellar solubilization. Bioavailability of HOC in subsurface environments can therefore only be enhanced by surfactants capable to promote the transport of the pollutants from the soil matrix to the micellar phase (desorption), and once there be able to ease the contact pollutant-microorganisms (biodegradation).

Palabras clave: surfactantes, biosurfactantes, rhamnolípidos, solubilidad, biodisponibilidad, biodegradación, bioremediación, adsorción, desorción

RESUMEN

Dada su baja solubilidad y gran afinidad hacia la materia orgánica, los compuestos orgánicos hidrofóbicos (COH) tienden a adsorberse al suelo y a mantenerse inmunes a los microorganismos potencialmente capaces de biodegradarlos. Como consecuencia de esto, la acumulación y la persistencia de este tipo de contaminantes orgánicos genera filtraciones lentas y continuas en acuíferos y limita el empleo de tecnologías clásicas para la recuperación de suelos contamina-dos, como el "lavado y tratado con agua" o la biorremediación *in situ*. Los surfactantes sintéti-

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cos, muy usados en tecnologías de lavado de suelos ("soil washing/flushing"), son capaces de movilizar contaminantes orgánicos fuertemente adsorbidos al suelo. No obstante, los surfactantes sintéticos tienden a inhibir la actividad microbiológica sobre las moléculas del contaminante que han sido incorporadas a su fase micelar. Se ha comprobado que los biosurfactantes (especificamente los rhamnolípidos), además de ser efectivos en la solubilización de contaminantes orgánicos adsorbidos en suelos, son capaces de promover (o al menos no inhibir) su biodegradación. La biodisponibilidad de los COH puede incrementarse usando surfactantes que promuevan tanto el transporte de contaminantes de la matriz del suelo a la fase micelar (desorción), como el contacto entre los microorganismos y los contaminantes incorporados a la fase micelar (biodegradación).

INTRODUCTION

There is considerable concern regarding the existence and fate of organic pollutants in soil because of: (1) the potential for adverse impacts on microbial communities which are necessary for a healthy soil environment, and (2) the potential for transport of contaminants in groundwater, and hence, toxicological impacts on human health. The mobility, as well as chemical and biological transformations of pollutants in soils (e.g. pesticides) are controlled in large measure by their adsorption onto soil particles. A major limitation in current *in situ* soil remediation technologies is the difficulty in delivering the pollutant to the water phase for treatment. For example, "pump and treat" technologies typically do not attain cleanup goals within the predicted time frame, due to "tailing" or flushing of pollutants from soil into groundwater. Also, *in situ* biodegradation rates are frequently observed to be slower than expected compared to rates of biodegradation in aqueous or liquid culture.

In recent years, *in situ* bioremediation has been considered to be a panacea for the remediation of contaminated soils. Bioremediation strategies based on the use of indigenous microbial populations (i.e. intrinsic) and/or augmentation (i.e. addition of microorganisms, nutrients and/or carbon and electron sources) have been the focus of considerable research and technology development. For example, land farming, i.e., the application of contaminated soils to agricultural land, is currently being considered by many states as a viable approach for managing contaminated soils at agrochemical handling sites. The justification for this technology is that pesticides applied to soil are susceptible to chemical, photochemical, and more importantly, biological transformations; although rates can be highly variable. The success of bioremediation strategies is clearly dependent on the presence of appropriate pollutant/pesticide-degrading microorganisms as well as environmental conditions which are conducive to microbial metabolism. However, even when appropriate microbial strains are present and environmental conditions are adequate, the extent of biodegradation may still be severely limited by the availability of hydrophobic organic pollutants (HOC) to the microorganisms. It is generally accepted that only the pollutants in soil solution/groundwater (soluble) are available for microbial degradation (Scow and Hutson 1992). HOC are generally known for their low aqueous solubility and great tendency to stay sorbed in soil, and therefore unavailable for any possible microbial activity. Moreover, several experiments have proved that "aged" pollutants as compared to freshly added ones, can diffuse deep into soil micropores that are inaccessible to microorganisms (Zhang and Miller 1995).

Bioavailability plays a major role in limiting the degree to which soil can be decontaminated via either intrinsic or augmented bioremediation. Depending on the relative solubility, a significant fraction, if not the majority, of pollutant may be sorbed to soil surfaces, thereby reducing the soluble concentration. Bioavailability is influenced by a complex set of factors, including: (1) cell-surface interactions, (2) cell-contaminant interactions, and (3) contaminant-surface interactions. Surfactants can greatly enhance the aqueous solubility of HOC and have been successfully used in conjunction with remediation technologies such as soil washing/flushing and pump and treat. However, synthetic surfactants have proved to prevent the microbial action over the pollutants once they are solubilized in the micellar phase, either by limiting the contact pollutant-microorganism, or interfering with substrate transport into cells by damaging the membrane structure (Rouse *et al.* 1994). Being toxic to most of microorganisms and having considerable affinity for soil surface, synthetic surfactants tend to accumulate and pollute subsurface systems (Lee *et al.* 1995, Chin *et al.* 1996).

Recent studies have proved that microbial surfactants can be as good as their synthetic counterparts in enhancing aqueous solubility of alkanes, polycyclic aromatic hydrocarbons (PAH), aromatics, and chlorinated biphenyls sorbed in soil (Van Dyke *et al.* 1993a, b, Scheibenbogen *et al.* 1994, Thangamani and Shreve 1994, Kanga *et al.* 1997, Kommalapati *et al.* 1997, Zhang *et al.* 1997). Being microbially produced biosurfactants are expected to be non-toxic and biodegradable as compared to synthetic surfactants.

Though surfactants and biosurfactants can effectively mobilize organics sorbed in soil phase, their adverse or beneficial effects on bioavailability of pollutants are poorly understood and have lead frequently to contradictory conclusions. Laha and Luthy (1992), for example, verified that some nonionic surfactants enhanced phenanthrene desorption from soil slurries, but inhibited its mineralization. Jain *et al.* (1992) and Zhang and Miller (1992) have observed increased biodegradation of linear hydrocarbons and PAH in soil in the presence of biosurfactants. Falatko and Novak (1992), have noted either an inhibition or no improvement of gasolines biodegradation following addition of biosurfactants.

Bioavailability is influenced by a complex set of interactions between the pollutant, the soil, the surfactant micellar phase, the true aqueous phase and the microorganisms. The purpose of this work is to review such interactions and ponder the importance of each one in the process of bioavialability of HOC in subsurface environments when a micellar phase is present.

Surfactants and biosurfactants; their uses in soil remediation

Surfactants can create aggregates called micelles when the critical micelle concentration (CMC) of the surfactant is exceeded. Micelles consist of arrangements of surfactant molecules that create a hydrophobic pseudophase with a hydrophilic exterior. Below the CMC, surfactants in solution exist as individual molecules or monomers. Above the CMC a constant monomer concentration is maintained in equilibrium with the micelles. The formation of micelles allows for micellar solubilization of important amounts of a HOC above its standard aqueous solubility. The value of CMC depends on the nature of the surfactant molecule (the more hydrophobic surfactants may form micelles at lower concentrations than the more hydrophilic), and can be affected by changes in temperature or by the presence of chemical compounds such as electrolytes and other HOC.

The application of surfactants can enhance soil remediation essentially in three ways:

1. Increasing contaminant mobility and solubility to improve pump-and-treat performance
2. Decreasing the mobility of contaminants to prevent their migration
3. Speeding the rate of biodegradation of contaminants in soil.

Surfactants increase contaminant mobility either by enhancing the apparent solubility of the contaminant in water, or by reducing interfacial tension between the water and non-aqueous phase liquids (NAPL) (U.S. Environmental Protection Agency 1995). Cationic surfactant mol-ecules can easily attach to negatively charged moieties of clay surfaces creating a new hydrophobic coat where HOC can be strongly sorbed and immobilized. Further research has proven that besides mobilizing pollutants sorbed in soils, surfactants and specifically biosurfactants can improve the rate of their in situ biodegradation (US Environmental Protection Agency 1995).

Surfactant washing is an *ex situ* process where soil is excavated, and later either heaped on plastic liners and irrigated with surfactant solution, or washed in tanks or pits in a continuous or batch process. *In situ* surfactant flushing involves the delivery of surfactant solution to the contaminated medium in place by irrigation and/or injection wells; the contaminant-laden surfactant solution is then pumped up for treatment (and possible recycle) by recovery wells. The range of applicability of surfactant flushing/washing depends on three main conditions:

1. The contaminant must be hydrophobic and preferentially non-volatile. Suitable compounds include most of pesticides, polychlorinated biphenyl (PCB), semi-volatiles, petroleum products, chlorinated solvents, and aromatic solvents.
2. It must be able to deliver the surfactant solution to the contaminant. Soils with very low permeability are not qualified for soil flushing and an *ex-situ* washing technique may be needed.
3. The complete recovery of contaminant-laden surfactant solution is desirable. At some sites geological factors may make this an uncertain or expensive matter, in which case one must either use the *ex situ* technique or employ a different technology.

Nonionic surfactants like Triton X-100 were initially preferred for soil remediation mainly because their CMC are much lower than those of anionic ones, and can work as more effective carriers. Yet, some present workers are switching to anionic surfactants like Sodium Dodecyl Sulfate (SDS), mainly because contaminant-laden surfactant solutions can be recovered easily by gentle extraction of the contaminant with hexane or mineral oil, whereas nonionic surfactants present serious problems upon recovery and reuse (US Environmental Protection Agency 1995).

Microbial surfactants are complex molecules synthesized by bacteria, yeasts, and fungi, which can include peptides, glycolipids, glycopeptides, fatty acids and phospholipids. Being amphipatic molecules, biosurfactants possess both lipophilic (commonly a fatty acid chain) and hydrophilic moieties (carboxylate groups, amino groups, phosphate groups, or carbohydrates). Microorganisms can produce either extra-cellular or cell wall bound biosurfactants. While extra-cellular biosurfactants cause the emulsification or solubilization of the substrate, cell wall-associated ones are in charge of promoting the

penetration of organic compounds into the cell (Mercade and Manresa 1994). Some microorganisms produce biosurfactants during their growth exclusively on hydrophobic substrates (like *Rhodoccocus* and *Corynebacterium*), while others produce them either on water-soluble or hydrophobic substrates (*Pseudomonas aeruginosa* and *Torulopsis. bombicola*). The latter microorganisms are the most adapted for growing and producing biosurfactants in subsurface environments (Rouse *et al.* 1994)

The largest market for environmental use of biosurfactants is the petroleum industry where they are used in microbial enhanced oil recovery (MEOR), and remediation of sites contaminated by oil spills. Both *ex-situ* or *in situ* application of biosurfactants can be chosen depending on environmental and economical factors. In general, *ex situ* techniques are more expensive than *in situ* ones, but *in situ* techniques are sometimes impossible to perform because of environmental conditions that limit the growth of the microorganisms and the production of adequate amounts of biosurfactant.

Biosurfactants may be used to enhance the solubilization and biodegradation of toxic organic compounds in addition to those found in the petroleum industry such as PCB, PAH, and pesticides (Zhang and Miller 1992, Mata-Sandoval 2000).

Rhamnolipids; properties, production, and uses in the environmental field

Rhamnolipids are glycolipid extra-cellular biosurfactants produced by several strains of *Pseudomonas* species, growing on diverse carbon substrates such as long chain hydrocarbons, carbohydrates, glycerol, and vegetal oils (Guerra-Santos *et al.* 1986, Linhardt *et al.* 1989, Van Dyke *et al.* 1993b, Mercade and Manresa 1994, Arino *et al.* 1996, Mata-Sandoval *et al.* 1999). There are four types of rhamnolipid molecules whose hydrophobic and hydrophilic moieties are formed by one or two molecules of ••hydroxydecanoic acid••and one or two molecules of rhamnose, respectively (Lang and Wagner 1987). Other types of rhamnolipid with different fatty acid chains ($C_{10}C_8$, $C_{10}C_{12}$, and $C_{12}C_{12}$) are reported as produced in minor proportions (Zhang and Miller 1994, Champion *et al.* 1995, Mata-Sandoval *et al.* 1999). The $RhC_{10}C_{10}$ (monorhamnolipid m.w. = 504) and the $Rh_2 C_{10}C_{10}$ (dirhamnolipid m.w. = 650) are the varieties more abundantly produced by *Pesudomonas* strains (Lang and Wagner 1987, Linhardt *et al.* 1989, Zhang and Miller 1994, Arino *et al.* 1996, Mata-Sandoval *et al.* 1999) (**Fig. 1**).

The presence of a carboxylic group in rhamnolipids confer them acid-base properties. A pKa of 5.6 has been reported for the $RhC_{10}C_{10}$ variety (Zhang and Miller 1992). Therefore, pH conditions are very important for rhamnolipids application in the mobilization of organic compounds in subsurface environments. At pH above 5.6 rhamnolipids
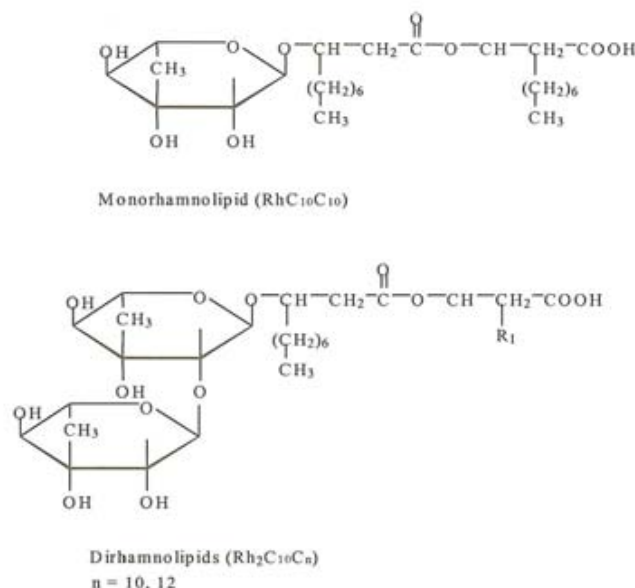

Monorhamnolipid ($RhC_{10}C_{10}$)

Dirhamnolipids ($Rh_2C_{10}C_n$)
n = 10, 12

Fig. 1. Rhamnolipids produced by *Pseudomonas aeruginosa* UG2. R1 = C_7H_{15}, C_9H_{19}, or C_9H_{17} (dehydro species) (Mata-Sandoval *et al.* 1999)

will be preferentially in their ionic form and will tend to stay soluble in the aqueous phase, while at pH values below the pKa, the neutral form of the molecule will become more hydrophobic.

A biosurfactant with good properties for use in the remediation of contaminated soils should have:

1. Small average minimum surface tension,
2. High average emulsifying activity,
3. Low CMC to enhance the mobilization of great amounts of pollutant through micellar transport, and
4. High affinity for hydrophobic compounds but still be hydrophilic enough to minimize partition into soil.

Experiments performed by Van Dyke *et al.* (1993a, b), with several biosurfactants showed that rhamnolipids produced by the *P. aeruginosa* strains (specially the ones by UG2 strain) are among the most effective ones when applied for the removal of HOC from contaminated soils. They posses the lowest average minimum surface tension, fairly high average emulsifying activity, fairly low CMC, and high affinity for hydrophobic organic molecules (see **Table I**).

Vegetal oils are among the most widely used substrates for research on biosurfactants production. Higher conversion yields and production of rhamnolipids have been reported using corn oil as sole carbon substrate when compared to other hydrophobic and hydrophilic substrates (**Fig. 2B**) (Linhradt *et al.* 1989, Mata-Sandoval *et al.* 2000a). Morever, current studies with residual lipidic
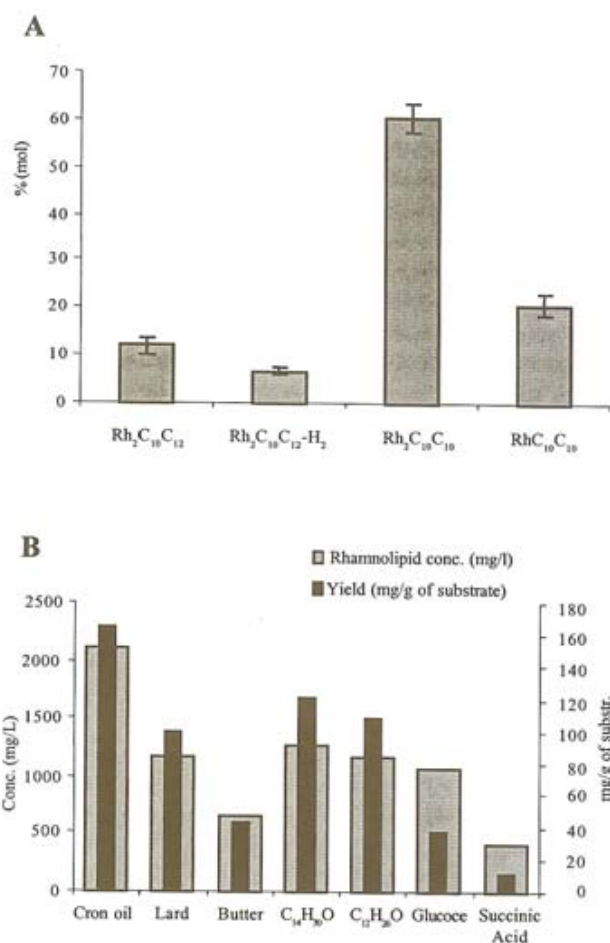
TABLE I. COMPARISON OF BIOSURFACTANTS PRODUCED BY SEVERAL MICROORGANISMS (Van Dyke *et al.* 1993b)

Strain	Type of Surfactant	Carbon Source	Emulsifying Activity (U/mL filtrate)	Minimum. Surf. Tension (mN/m)	% HCB (recovered in Aq. Phase)
B. Subtilis ATCC21331	Lipopeptide	Glucose	12.7	28.6	13.1
P. aeruginosa PG201	Rhamnolipid	Glucose	10.4	30.5	38.9
P. aeruginosa UG2	Rhamnolipid	Glucose	15.5	31.4	48.0
P. aeruginosa ATCC101145	Rhamnolipid	Glucose	4.1	30.6	15.5
T. bombicola ATCC 22214	Sophorolipid	GlucoseCorn oil	15.8	34.0	8.2
A. calcoaceticus RAG-1	lipopoly-saccharide	Hexadecane	22.0	54.4	41.9
B. licheniformis JF-2	Lipopeptides	Glucose-hexadecane	0.2	40.0	2.7

wastes from the oils and fats processing industry indicate that they are able to support microbial growth and fuel rhamnolipid production when supplied as the sole carbon

A

B

Fig. 2. A: Average composition of obtained rhamnolipid mixtures produced *by P. aeruginosa* UG2 on different substrates. B: Total rhamnolipid culture concentration and yield by *P. aeruginosa* UG2 grown on different carbon sources

source (Mercade and Manresa 1994). The use of cost-free or cost-credit feedstocks may lead to a cost effective *in situ* or *ex situ* rhamnolipid production that can compete with that of traditional synthetic surfactants.

Interactions between surfactants and soil

There are important environmental and economical aspects concerning the sorption of surfactants onto soil. Being toxic for most of the indigenous microorganisms, synthetic surfactants tend to accumulate in soil, and their sorption contributes even to prevent the possible microbial action from specific degraders over them. Potential losses of surfactant through sorption into soil is a point of consideration when analyzing economically the feasibility of soil washing/flushing technologies, and the adequate type of surfactant to be selected.

According to Laha and Luthy (1992), partitioning into soil organic matter is the predominant mechanism of surfactant sorption, and it can occur in three ways: (1) through hydrophobic surface interactions between the hydrocarbon chains of surfactant molecules and the hydrophobic regions of humic matter; (2) through hydrogen bonding between surfactant polar groups (like ethoxylate or hydroxyl) and polar groups of humic matter (e.g., hydroxyl and phenolic groups); and (3) through partitioning of the surfactant into bulk organic matter. Nevertheless, results of other studies have found that clay surfaces can play an important roll in the process of sorption of surfactants in soils. A comparative study after the sorption of Triton X-100 and rhamnolipids into two silt loams, Beltsville A (OC = 4.13%, Clay = 16.0%) and Hagerstown A (OC = 3.11%, Clay = 27.0%), and a clay soil Hagerstown B (OC = 0.35%, Clay = 59.5%), showed that both surfactants observed a higher rate of sorption with the soil of high clay content Hagerstown B (Mata-Sandoval 2000) (Fig. 3).

It is generally accepted that surfactant monomers present at concentrations below the CMC are responsible for most of surfactant interaction with soil. Once the CMC is attained, the sorption of micelles onto surfaces directly
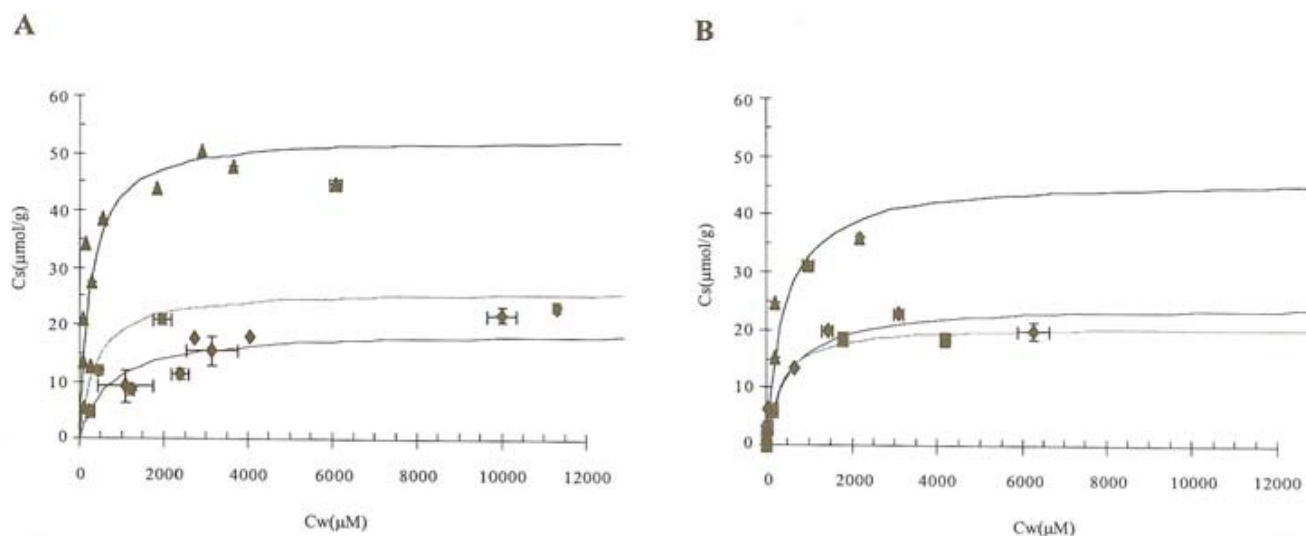
A

B

Fig. 3. Langmuir isotherms for the sorption of Rhamnolipid mixture by *P. aeruginosa* UG2 (A), and Triton X-100 (B) onto Beltsville A (◆), Hagerstown A (■), and Hagerstown B (▲) soils

from bulk solution has been reported to be insignificant (Laha and Luthy 1992, Chin *et al.* 1996). Laha and Luthy (1992), report a CMC value of 0.2% (v of surfactant/v of water) for Tween 80 in a 1:8 (g/ml) soil-water system, whereas the CMC reported for Tween 80 in pure water is 0.0013% v/v. Therefore, the difference between the surfactant dose necessary to attain the CMC in a soil-water system and a clean water system represents the amount of surfactant (monomer) sorbed onto soil.

Interactions between surfactants, soil, and sorbed organic compounds

Solubilization enhancement of HOC by aqueous surfactant solutions is a micellar phenomenon (Rosen 1989). Therefore in the presence of soil, surfactant solutions will only be able to solubilize sorbed pollutants if the micellar phase is still attained after monomers had reached a sorption equilibrium with the soil. Studies by Chin *et al.* (1996), showed that the sorption of 2-methyl-naphthalene into soil at Triton X-100 concentrations below the CMC was

enhanced, while the formation of micelles above the CMC increased the sorbate aqueous-phase concentration. Triton X-100 nonionic surfactant can significantly enhance the mobility of organic pollutants if applied in sufficiently large quantities (above CMC). At equilibrium concentrations below the CMC, however, the surfactant will sorb to the soil phase, increasing its hydrophobicity and retarding the movement of organic pollutants. Studies by Edwards *et al.* (1994), shows similar interactions between Triton X-100, a sandy soil (Lincoln fine sand), and phenanthrene.

So far, most of the desorption studies in soils are performed using soils spiked with fresh pollutants. In these cases, the organics are bound almost exclusively to the soil particle surface (adsorption) and desorption can be tested after a short period of time. Extensive research has proven that this does not happen when pollutants have been in contact with the soil for extended periods of time. In several experiments, aged pollutants such as simazine pesticide, phenanthrene, and 4-nitrophenol, showed slow desorption rates compared to freshly added pollutants (**Table II**).

TABLE II. CALCULATED PARTITION COEFFICIENTS (K_d) FOR FRESH VERSUS AGED PESTICIDES

Pesticide	Fresh Pesticide K_d (mL/g)	Aged Pesticide K_d (mL/g)	Aged K_d / Fresh K_d	Reference
EDB	1.49-2.08	170-300	100-150	Steinberg *et al.* 1987
Simazine	1.5	26	17	Scribner *et al.* 1992
Atrazine	1.32-2.17	4.0-29	3-13	Pignatello and Huang 1991
Metolachlor	0.77-2.96	27-49	16-35	Pignatello and Huang 1991
Atrazine	1.6-1.8	16-21	10	Pignatello *et al.* 1993
Metolachlor	2.6-2.8	15-17	6	Pignatello *et al.* 1993

The most accepted explanation for this phenomenon, is that aged pollutants in soils are likely partitoned deeply in organic matter or entrapped in soil micropores that are inaccesible to microbial degraders. Slow intra-particle diffusion can control desorption rates, and consequently bioavailability is prevented (Scribner *et al.* 1992, Hatzinger and Alexander 1995). There is a large body of evidence that indicates that sorption is irreversible. Results by Pignatello and Huang (1991), Scribner *et al.* (1992) and Pignatello *et al.* (1993), showed that desorption Kd's increase as a function of the sorption equilibration time and this depends on substrate structure.

Experiments were conducted by Mata-Sandoval (2000) to study the performance of Triton X-100 in the desorption of fresh and aged trifluralin from three soils: Beltsville A (BVA), Hagerstown A (HTA), and Hagerstown B (HTB) (**Fig 4**). The on-set of trifluralin desorption in HTB clay soil took place at higher concentrations of Triton X-100 (compared to BVA, and HTA) due to the high capacity of this soil to sorb the surfactant (**Figs 3** and **4**). Triton X-100 could desorb trifluralin more efficiently from HTA soil (OC = 3.11%) than from BVA soil (OC = 4.13%), because the last one contains higher organic carbon content that competes with the surfactant micelles for the distribution of the pesticide between the soil and the micellar phase. In similar desorption studies rhamnolipids proved less effective in desorbing trifluralin from the same type of soils (results not shown). Solubilization studies of trifluralin in rhamnolipids and Triton X-100 solutions, proved that at concentrations above the CMC, Triton X-100 (K_{supra} = 95.2 mmol trifluralin/mol surf) could almost double the capability to solubilize trifluralin, compared to rhamnolipids (K_{supra} = 48.5 mmol trifluralin/mol surf) (Mata-Sandoval *et al.* 2000b). The less efficient performance of rhamnolipids in desorbing trifluralin can be explained by its higher affinity for soil (specifically in Hagerstown B) and its lower solubilization enhancement of the pesticide compared to Triton X-100.

Differences below 3.5% were obtained between the amounts of trifluralin desorbed from aged and fresh soils using Triton X-100. The results showed that somehow the surfactant micelles are able to access the interior of soil particles and partially mobilize even the deeply sorbed pesticide as well as the one tightly bound to the soil organic matter (SOM).

Effects of surfactants on the bioavailability of organic compounds

In order to promote bioavailability of HOC in subsurface environments, surfactants must not inhibit microbial activity upon the pollutants once they are incorporated to the micellar pseudo-aqueous phase. The molecular structure of both surfactant and contaminant,
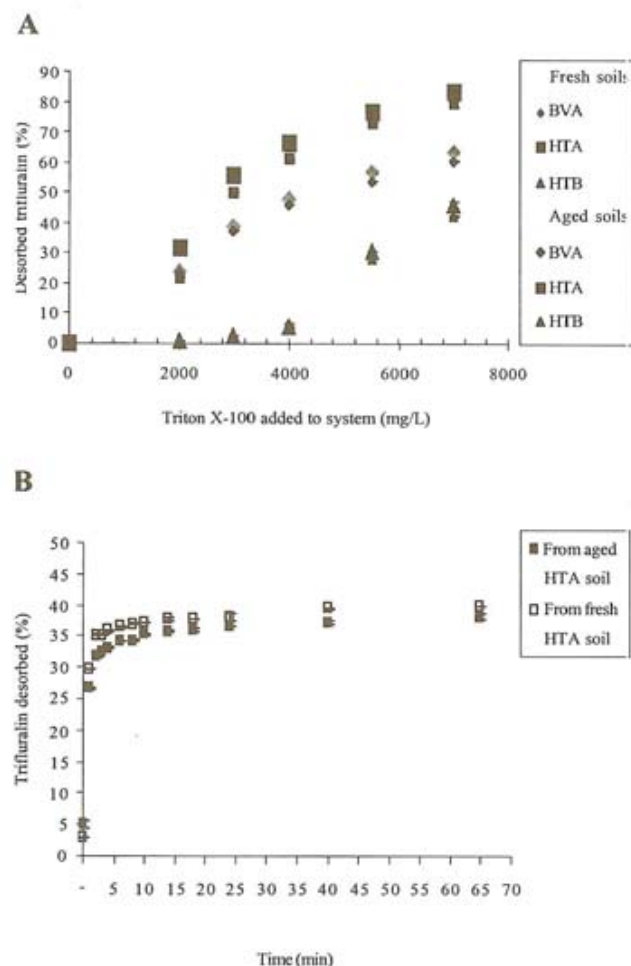

Fig. 4. A: Desorption of aged and fresh trifluralin (in percentage of total originally adosrbed to soil) from Beltsville A (BVA) (◆), Hagerstown A (HTA) (■), and Hagerstown B (HTB) (▲) soils, at different concentrations of Triton X-100 added to the system. B: Desorption (and micellar solubilization) of aged trifluralin (■)and fresh trifluralin (☐) from Hagerstown A soil into a 2000 mg/L Triton X-100 solution. Error bars represent standard deviation from duplicates

as well as the nature of the microbial membrane and/or cell wall are important factors that can influence the rate of contaminant uptake by microorganisms.

By the nature of their envelope, microorganisms can be hydrophobic or hydrophilic with respect to organic molecules. The first ones possesses a cell wall and membrane that permit direct transport of organics through them, while the second ones require an amphipathic molecule that could ease the transport of organics through the membrane. In the absence of synthetic surfactants, some hydrophilic microorganisms can produce their own biosurfactants that can ease the uptake of hydrophobic substrates.
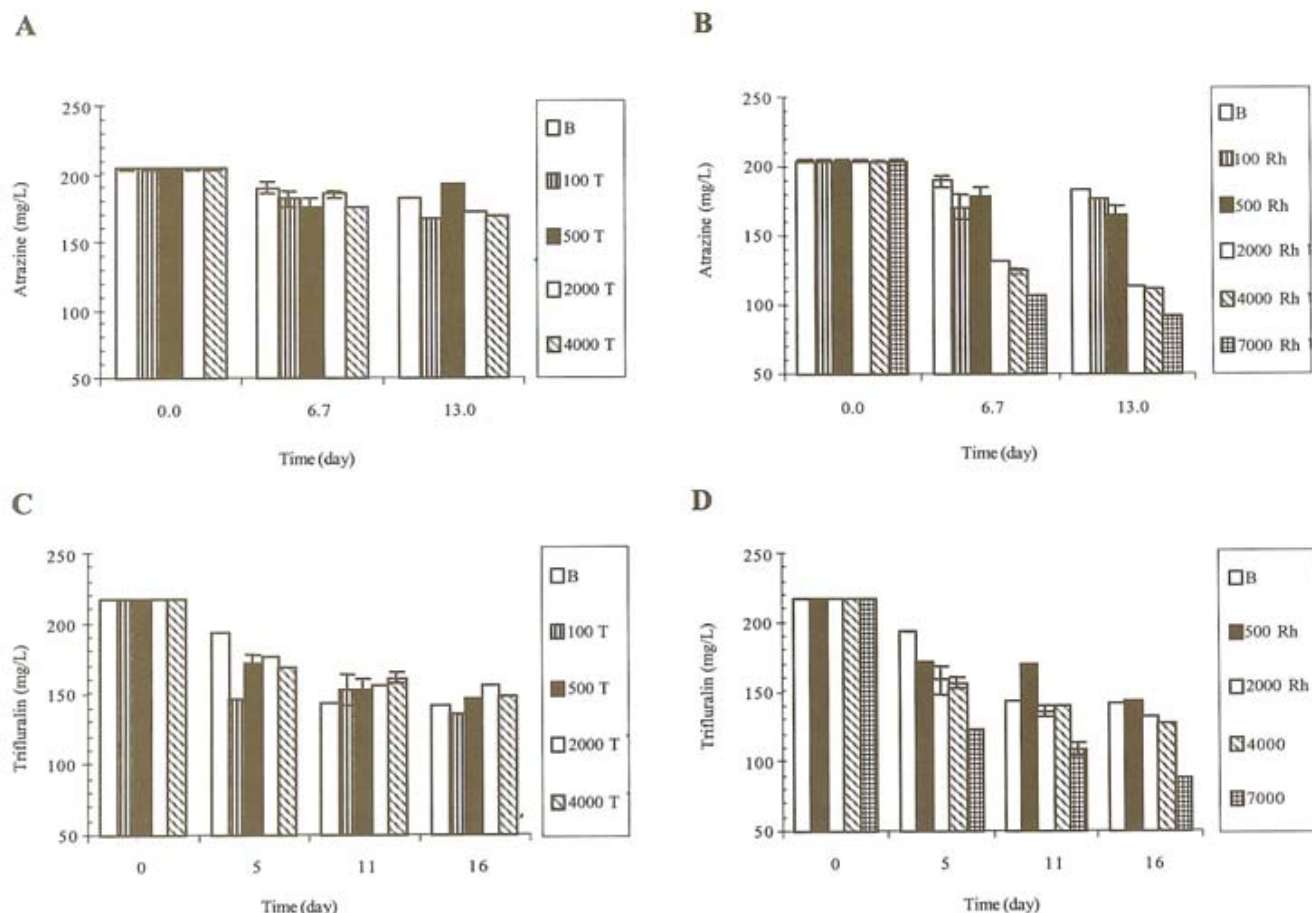
A

B

C

D

Fig. 5. Biodegradtion of atrazine (A, B) and trifluralin (C, D) in liquid media by *Streptomyces* PS 1/5 in the presence of Triton X-100 (T) and Rhamnolipid mixture (Rh) produced by *P. aeruginosa* UG2. (concentration of surfactants in mg/L). Error bars represent standard deviation from duplicates

Due to their molecular structure, surfactants at supra-CMC can inhibit biodegradation either by blocking the contact microorganism/pollutant, or causing toxic effects on the degraders. Apart from creating physical or electrostatic barriers between the pollutant and the microorganisms, surfactants can interfere with substrate transport into cells by damaging irreversibly the membrane structure (denaturalizing enzymes or washing out cell membrane material). It has been proven that even the compatibility of biosurfactants with cell membrane structures are sometimes specific to the microorganisms that produce them, and even though biosurfactants are essentially biodegradable, they can produce negative effects on the membranes of other microorganisms (Fry and Istok 1994).

Some researchers have confirmed either no positive effect, or a strong inhibition of HOC rates of biodegradation in the presence of synthetic surfactants at concentrations above their CMC (Laha and Luthy 1992). More recent research agrees that certain portion

of contaminants trapped into the micelles of some surfactants, are available for biodegradation, and that this depends upon the nature of the surfactant (Guha and Jaffé 1996a, b).

A study by Mata-Sandoval (2000) was conducted to test the effect of Triton X-100 and rhamnolipids on the biodegradation in liquid media of trifluralin and atrazine by *Streptomyces* PS 1/5 (Shelton *et al.* 1996), and coumaphos by a coumaphos degrading consortia isolated from contaminated cattle dips (Shelton and Somich 1988, Karns *et al.* 1995). The results showed that rhamnolipids at concentrations above 2000 mg/L were able to enhance the extent of trifluralin biodegradation up to a 75% whereas Triton X-100 didn't have any affect on the extent of biodegradation (35-45% similar to the control) (**Figs. 5C** and **5D**). Similar effect was obtained for atrazine, rhamnolipids at concentrations above 2000 mg/L, enhanced the extent of biodegradation up to 55%, while Triton X-100 gave similar results to that of the control (only 17%) (**Figs. 5A** and **5B**). In the case of coumaphos,

rhamnolipids retarded the on-set of its biodegradation on the first days, but at the end of the process 95% of the pesticide was degraded (**Fig. 6B**). Triton X-100 did not enhanced the extent of coumaphos biodegradation at concentrations below the CMC (125 mg/L). At concentrations above the CMC, Triton X-100 inhibited almost completely the microbial action over the pesticide (**Fig 6A**).

Modeling the behavior of surfactants in contaminated subsurface environments

A first approximation to modeling the partition of pollutants in subsurface systems is to assume that sorption/desorption reaches instantaneous equilibrium. The fraction of organic pollutant in the water phase (*fw*) can be determined using soil/water distribution coefficient, (K_d), and the bulk density (ρ) and porosity (θ) of the soil with the equation:

$$fw = \frac{1}{1 + K_d \frac{\rho}{\theta}}$$

Such a model can provide a first estimate of pollutant bioavailability. It assumes that the rates of adsorption and desorption are fast, relative to biodegradation rates and that sorption is completely reversible. Now, considering a pollutant (*C*) that is degraded microbially with a first order rate constant (k_b):

$$\frac{dC}{dt} = -fw \cdot k_b \cdot C$$

This simple model demonstrates that the rate of biodegradaiton is significantly influenced by the fraction of pollutant in the aqueous phase. While this simple model is often used to determine the mobility of pesticides in soils, some studies have proved that in real secenarios sorption can require several months to reach equilibrium (Karickoff and Morris 1985, Ball and Roberts 1991), and desorption is normally slower than sorption (Pavlostathis and Jaglal 1991, Locke 1992,). A large number of studies have indicated that K_d's obtained from desorption, as opposed to adsorption experiments, increase as a function of the sorption equilibrium time (aging process) (Steinberg et al. 1987, Pignatello et al. 1993).

Experimental results have showed that the use of surfactants may help to accelerate the desorption of HOC from soils even when they have undergone aging
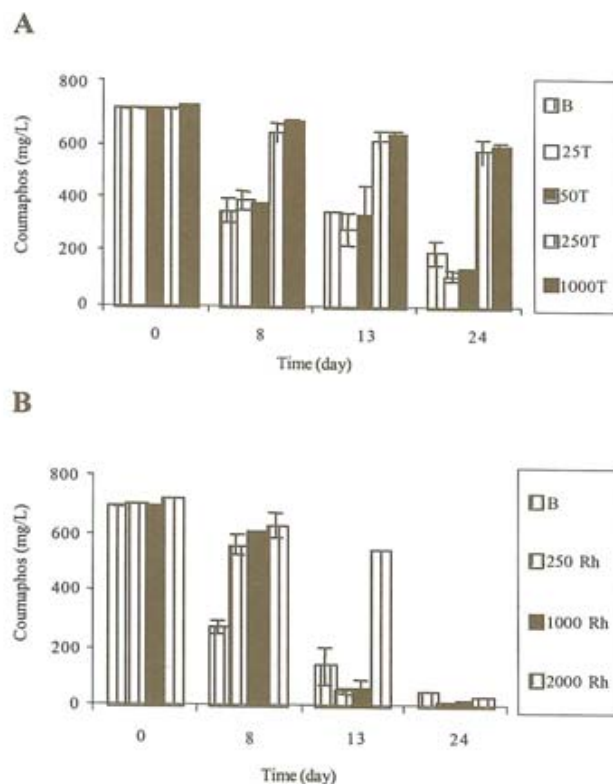
A

B

Fig. 6. Biodegradation of coumaphos in liquid media by coumaphos degrading consortium in the presence of Triton X-100 (T) and Rhamnolipid mixture (Rh) produced by *P. aeruginosa* UG2 (concentration of surfactants in mg/L). Error bars represent standard deviation from duplicates

processes (Mata-Sandoval 2000) (**Fig. 4B**). In this way it is possible to reach much faster equilibirum of desorption of pollutants. New *Kd's* of desorption in the presence of surfactants can be incorporated into biodegradation kinetic models, since desorption and biodegradation occur sequentially.

CONCLUSIONS

So far, the source of information regarding this topic indicates that no single factor can be absolutely reliable to predict the effects that surfactants and biosurfactants can have on the microbial degradation of organic compounds in soils. Solubilization enhancement of HOC by surfactants is an important issue, but even when some results indicate that biosurfactants proved better in enhancing solubilization of hydrocarbons, PAH's, and chlorinated by-phenyls (Thangamani and Shreve 1994, Van Dyke et al. 1993a, b, Zhang et al. 1997), synthetic surfactants like Triton X-100 have proved more effective than rhamnolipids in promoting solubilization of pesticides

like trifluralin or coumaphos (Mata-Sandoval *et al.* 2000b).

Surfactants with low affinity for soils (low CMC) are preferred, but the results of adsorption studies are still not conclusive between the performance of synthetic surfactants and biosurfactants. While Thangamani and Shreve (1994), proved less affinity of rhamnolipids to soil compared to linear dodecyl-benzene sulfonate (SDS), Mata-Sandoval (2000) found a stronger soil affinity of rhamnolipids compared to Triton X-100.

Extensive results have proved that biosurfactants can promote the biodegradation of HOC once they are attached to the biosurfactant micellar aggregates (Jain *et al.* 1992, Zhang and Miller 1992, Falatko and Novak 1992, Mata-Sandoval 2000). The most accepted explanation is that biosurfactants molecular structures are compatible with the structures of cell wall and cell membranes, and this permit the direct transport of the pollutant molecules from the micellar phase to the inside of the cells through the formation of a hemimicellar structure between the micelle and the cell membrane/ cell wall (Guha and Jaffé 1996a, b). Reports by other authors like Laha and Luthy (1992) showed that at concentrations above the CMC synthetic surfactants inhibit biodegradation of pollutants, either because the polar groups facing the outside core of the micelles form a physical barrier between the degraders and the pollutant, or because surfactant molecules can have toxic effects on the microorganisms.

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