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Biosurfactants and Their Application for Soil Bioremediation

*Naim Kosaric**

Professor Emeritus, University of Western Ontario, London, Ontario, Canada

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Dedicated to the memory of Professor Vera Johanides

Summary

Biosurfactants have been shown to promote biodegradation of hydrocarbons. A review of significant work is presented. Original data for biosurfactant assisted biodegradation of a selected herbicide metholachlor, chlorinated aromatics and naphthalene are also shown. Furthermore, pilot plant and large scale bioremediations of soil contaminated with polycyclic aromatic hydrocarbons (PAH) and heavy oil were performed. In the presence of selected biosurfactants, a preferential and significant (or complete) removal of PAHs was observed after only 22 days of bioremediation. Keeping in mind that bioremediation is generally a slow process, these results show a significant reduction of the time required to bioremediate contaminated sites. Based on the laboratory and pilot plant data, field bioremediation of several large contaminated sites was performed.

Key words: biosurfactants, bioremediation, soil, hydrocarbons

Introduction

Microbial surface active agents (biosurfacants) are important biotechnological products, with a wide range of applications in many industries. Their properties of interest are: (*i*) in changing surface active phenomena, such as lowering of surface and interfacial tensions, (*ii*) wetting and penetrating actions, (*iii*) spreading, (*iv*) hydrophylicity and hydrophobicity actions, (*v*) microbial growth enhancement, (*vi*) metal sequestration and (*vii*) anti-microbial action.

Most of the applications today involve the use of chemically synthesized surfactants. Production of surfactants in the United States and worldwide is estimated at $3.4 \cdot 10^9$ kg and $7 \cdot 10^9$ kg in 1989, respectively. The US surfactant industry shipments were $3.65 billion in 1989. The applications are very wide in a variety of industries as shown in Table 1.

There are many advantages of biosurfacants if compared to their chemically synthesized counterparts. Some of these are:

- *biodegradability*
- *generally low toxicity*
- *biocompatibility and digestibility* – which allows their application in cosmetics, pharmaceuticals and as functional food additives
- *availability of raw materials* – biosurfactants can be produced from cheap raw materials which are available in large quantities; the carbon source may come from hydrocarbons, carbohydrates and/or lipids, which may be used separately or in combination with each other
- *acceptable production economics* – depending upon application, biosurfactants can also be produced

* Corresponding author; E-mail: nkosaric@julian.uwo.ca

Table 1. Biosurfactant uses and effects

Use	Effect of surfactant
Metals	
Concentration of ores	Wetting and foaming, collectors and frothers
Cutting and forming	Wetting, emulsification, lubrication and corrosion inhibition in rolling oils, cutting oils, lubricants, *etc.*
Casting	Mold release additives
Rust and scale removal	In pickling and electrolytic cleaning
Plating	Wetting and foaming in electrolytic plating
Paper	
Pulp treatment	Deresinification, washing
Paper machine	Defoaming, color leveling and dispersing
Calender	Wetting and leveling, coating and coloring
Paint and protective coatings	
Pigment preparation	Dispersing and wetting of pigment during grinding
Latex paints	Emulsification, dispersion of pigment, stabilize latex, retard sedimentation and pigment separation, rheology
Waxes and polishes	Emulsify waxes, stabilize emulsions, antistat
Petroleum production/products	
Drilling fluids	Emulsify oil, disperse solids, modify rheological properties of drilling fluids for oil and gas wells
Worker of producing wells	Emulsify and disperse sludge and sediment in cleanout of wells
Producing wells	De-emulsify crude petroleum, inhibit corrosion of equipment
Secondary recovery	In flooding operations, preferential wetting
Refined products	Detergent sludge dispersant and corrosion inhibitor in fuel oils crank-case oils and turbine oils
Textiles	
Preparation of fibers	Detergent and emulsifier in raw wool scoring; dispersant in viscose rayon spin bath; lubricant and antistat in spinning of hydrophobic filaments
Dyeing and printing	Wetting, penetration, solubilization, emulsification, dye leveling, detergency and dispersion
Finishing of textiles	Wetting and emulsification in finishing formulations, softening, lubricating and antistatic additives to finishes
Agriculture	
Phosphate fertilizers	Prevent caking during storage
Spray application	Wetting, dispersing, suspending of powdered pesticides and emulsification of pesticide solutions; promote wetting, spreading and penetration of toxicant
Building and construction	
Paving	Improve bond of asphalt to gravel and sand
Concrete	Promote air entertainment
Elastomers and plastics	
Emulsion polymerization	Solubilization, emulsification of monomers
Foamed polymers	Introduction of air, control of cell size
Latex adhesive	Promote wetting, improve bond strength
Plastic articles	Antistatic agents
Plastic coating and laminating	Wetting agents
Food and beverages	
Food processing plants	For cleaning sanitizing
Fruits and vegetables	Improve removal of pesticides, and in wax coating
Bakery and ice cream	Solubilize flavor oils, control consistency, retard staling
Crystallization of sugar	Improve washing, reduce processing time
Cooking fat and oils	Prevent spattering due to super heat and water
Industrial cleaning	
Janitorial supplies	Detergents and sanitizers
Descaling	Wetting agents and corrosion inhibitors in acid cleaning of boiler tubes and heat exchangers.
Soft goods	Detergents for laundry and dry cleaning
Leather	
Skins	Detergent and emulsifier in degreasing
Tanning	Promote wetting and penetration
Hides	Emulsifiers in fat liquoring
Dyeing	Promote wetting and penetration

from industrial wastes and by-products and this is of particular interest for bulk production (*e.g.* for use in petroleum-related technologies)
- *use in environmental control* – biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil
- *specificity* – biosurfactants, being complex organic molecules with specific functional groups, are often specific in their action (this would be of particular interest in detoxification of specific pollutants): de-emulsification of industrial emulsions, specific cosmetic, pharmaceutical, and food applications
- *Effectiveness* – at extreme temperatures, pH and salinity

Most of the biosurfactants are high molecular-weight lipid complexes, which are normally produced under aerobic conditions. This is achievable in their *ex situ* production in aerated bioreactors.

The biosurfactant sources, classes and properties have been reviewed (*1-8*).

In general, biosurfactants can be classified as:
- glycolipids,
- hydroxylated and cross-linked fatty acids (mycolic acids),
- polysaccharide-lipid complexes,
- lipoprotein-lipopeptides,
- phospholipids,
- complete cell surface itself.

Table 2. Various biosurfactants produced by microorganisms

Microorganism	Type of surfactant
Torulopsis bombicola	Glycolipid (sophorose lipid)
Pseudomonas aeruginosa	Glycolipid (rhamnose lipid)
Bacillus licheniformis	Lipoprotein (?)
Bacillus subtilis	Lipoprotein (surfactin)
Pseudomonas sp. DMS 2847	Glycolipid (rhamnose lipid)
Arthrobacter paraffineus	Sucrose and fructose glycolipids
Arthrobacter	Glycolipid
Pseudomonas flurescens	Rhamnose lipid
Pseudmonas sp. MUB	Rhamnose lipid
Torulopsis petrophilum	Glycolipid and/or protein
Candida tropicalis	Polysaccharide-fatty acid complex
Corynebacterium lepus	Corynomycolic acids
Acinetobacter sp. HO1-N	Fatty acids, mono-and diglycerides
Acinetobacter calcoaceticus Rag-1	Lipoheteropolysaccharide (Emulsan)
Acinetobacter calcoaceticus 2CAC	Whole cells (lipopeptide)
Candida lipolytica	»liposan« (mostly carbohydrate)
Candida petrophilum	Peptidolipid
Nocardia erythropolis	Neutral lipids
Rhodococcus eryithropolis	Trehalose dimycolates
Corynebacterium salvonicum SFC	Neutral lipid
Corynebacterium hydrocarboclastus	Polysaccharide-protein complex

A list of various biosurfacants produced from different microbes is presented in Table 2.

Soil Bioremediation Methods

The accumulation and persistence of toxic materials in water and soil represents a major problem today. Various organics are generated as byproducts from various industries (*e.g.* petroleum and petrochemical, pulp and paper, chemical industries *etc.*), which may be released into the environment, or are accidentally spilled. Aromatics and their chlorinated derivatives, which are difficult to biodegrade and are toxic, are of primary concern. Aromatics and their chlorinated derivatives are generated in chlorine bleaching of cellulose pulp (*e.g.*, dioxins), pesticide and herbicides (*e.g.*, chlorophenols), moth repellents and air deodorant (*e.g.*, p-dichlorobenzene), petroleum and petrochemicals (*e.g.*, naphthalene), transformer oils (*e.g.*, polychlorinated biphenyls, PCB), chemical, plastics, iron and steel industries (*e.g.*, phenols), wood preservation (*e.g.*, pentachlorophenols, PCP), *etc*.

As the above chemicals are toxic and are proven carcinogens, their release to water and soil is prohibited. If, however, they do appear in industrial wastewaters, they must be treated and detoxified. Wastewater treatment is practised worldwide utilizing a combination of methods (chemical, physical, and biological). Biological methods show many advantages, and many organics can be efficiently degraded by aerobic and anaerobic processes. However, for degradation of calcitrant pollutants, special and/or adapted microbial cultures are needed, which can (*i*) survive in the contaminated environment and (*ii*) degrade the contaminant efficiently and completely.

While water treatment is relatively easy to perform, soil bioremedation is much more difficult and complex. The first problem arises due to difficulties in treating soil, especially when pollution is distributed over a large area. Thus, removal of soil from contaminated site becomes a costly undertaking, even though such *ex situ* treatment might be well established. This could be accomplished in two ways:

(*i)* Addition of nutrition to the soil in form of nitrogen, phosphorus and, if necessary, carbon compounds, which would allow the native microbial population to develop and augment, and thus provide more microorganisms for metabolism or cometabolism of the pollutant in question.

(*ii*) Produce *ex situ* a microbial population which is adapted to the pollutant and is capable to metabolize it efficiently, and then add this population, along with necessary nutrients, to the polluted soil. The added biomass would, under proper conditions, be able to survive in the soil and to further degrade objectionable organics.

Both methods are applied whereby method (*i*) seems to be more popular, but the strategy depends upon the type of the pollutant, the environmental soil conditions, and the availability of the adapted culture.

Bioremediation of soil contaminated with organic chemicals is a viable alternative method for clean-up and remedy of hazardous waste sites. The final objective

in this approach is to convert the parent toxicant product into a readily biodegradable one, which is harmless to human health and/or the environment. It is essential to eliminate and make harmless such hazardous wastes as they may either enter into plants thus contaminating this food source, or be leached into the ground-water, the purification of which becomes even more complex and difficult.

The biological remediation process can be performed (*i*) *in situ*, (*ii*) in a prepared bed, and (*iii*) in a slurry reactor system. *In situ* processes are usually accomplished by addition of microbial nutrients to the soil, which allows considerable growth of soil microbial indigenous population. Thus increased microbial biomass in the soil results in faster biodegradation of contaminating organics. The soil can also be dug-out and treated off-site in a similar way or it could be placed into a bioreactor to which water and nutrients are added and the biodegradation proceeds under continuous mixing, which enhances the biodegradation process.

The alternative is to selectively isolate and grow specific microbial cultures which are adapted to the toxicant and thus »trained« to degrade and utilize it as a substrate. Addition of surface-active agents, especially when biodegradation of non-polar compounds is encountered, helps in the uptake and metabolism of these compounds by the microbial population.

Comparing bioremedation with other available technologies for soil remediation, one can see a financial benefit when bioremediation is considered as shown in Table 3.

Table 3. Cost of soil treatment

Treatment	Cost per ton
Landfill disposal fees	\$140–200 + taxes + transportation
Mobile incineration	\$150–140
Stabilization/fixation	\$100–200
Bioremediation	\$15–70

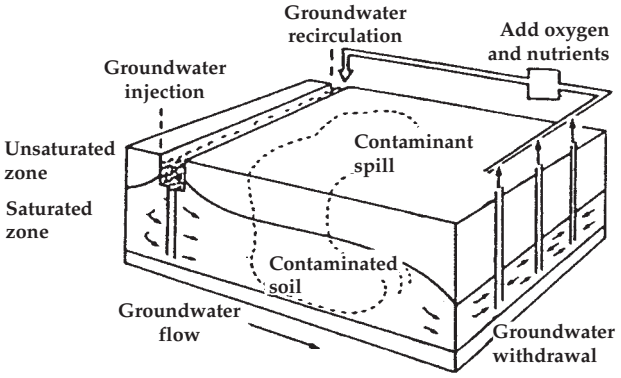

Fig. 1. Schematics of the *in situ* treatment of contaminated saturated soil

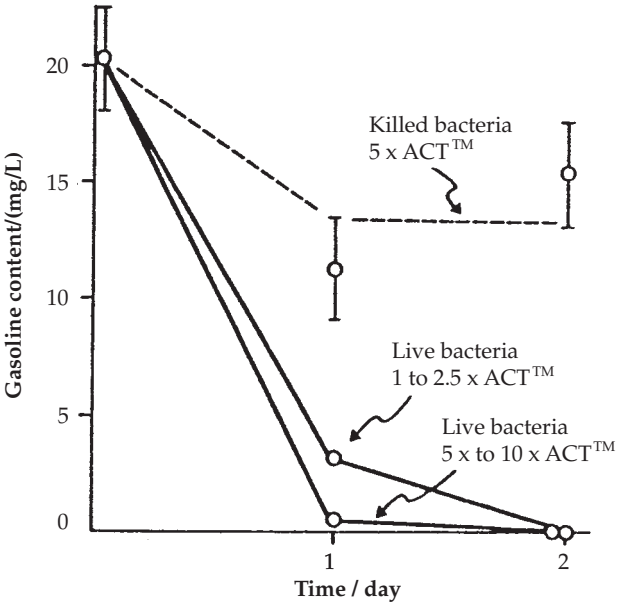

Fig. 2. Laboratory biodegradation of a gasoline contaminated soil

A typical *in situ* approach is shown in Fig. 1. In this approach, part of the ground-water can be collected at the underflow, pumped back onto the soil supplemented with nutrients and oxygen. For biodegradation of petroleum, about 3 kg oxygen are required for every kg of petroleum hydrocarbon degraded. Sparging with oxygen can deliver only 40 mg/L at the injection point while hydrogen peroxide can be dissolved and injected at concentrations > 500 mg/L and will gradually break down to oxygen during transport through the contaminated area.

Fig. 2 shows laboratory data when a proprietary microorganism mixture (ACT) was added to soil contaminated with gasoline and enriched with nutrients and oxygen. Compounds analyzed were benzene, toluene, ethylbenzene, xylenes and trimethylbenzene. The effect of live bacteria on the degradation of the hydrocarbons is evident.

When the soil was excavated and then treated according to the scheme in Fig. 3, biodegradation data as shown in Fig. 4 were obtained. Stimulation of microbial growth by added nutrients results in almost complete biodegradation in a relatively short period of time. The data are given as the sum of 18 major constituents of diesel fuel, analyzed by gas chromatography with flame ionization detection.

Bioremediation of petroleum-contaminated soils using microbial consortia as inoculum is the so-called

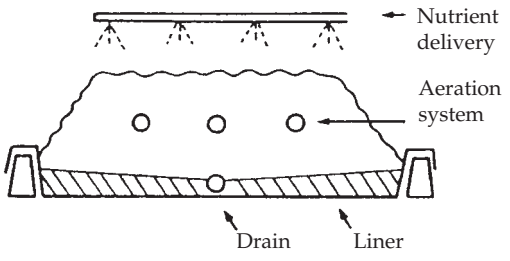

Fig. 3. On-site treatment method for excavated contaminated soil

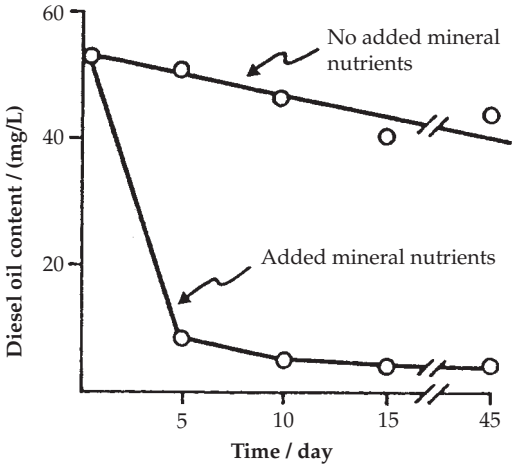

Fig. 4. Biodegradability of diesel fuel at the on-site soil pile

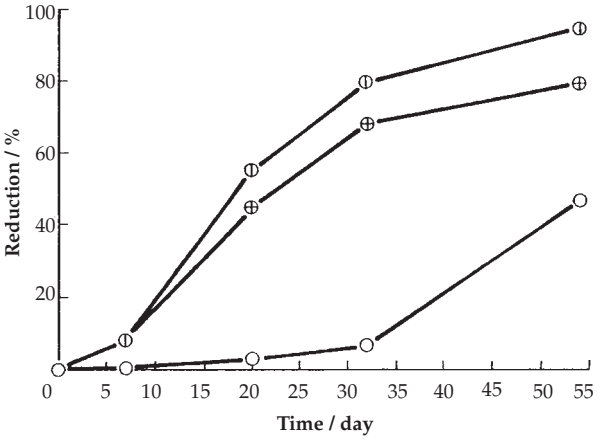

Fig. 5. Percent removal of benzene (⊕–⊕), toluene (⊕–⊕), **and xylene isomers** (○–○) **in nutrient-enriched groundwaters incubated anoxically with 500 mg NO_3^--N and 50 mg PO_4^{3-}-P**

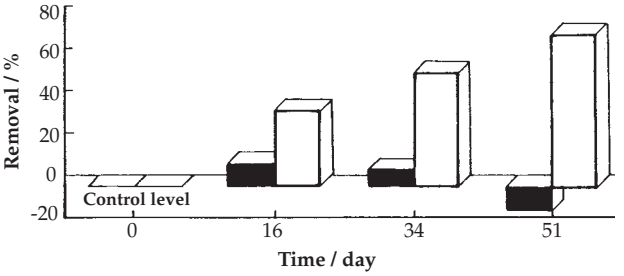

Fig. 6. Removal (%) of total BTX (benzene, toluene and xylene isomers) in groundwater incubated aerobically with 50 mg/L H_2O_2 (as an oxygen source) plus either nutrients (500 mg/L NO_3^--N and 50 mg/L PO_4^{3-}-P) or no nutrients. All removal values are calculated as compared to controls (no H_2O_2 and no nutrients) □ – 50 mg/L H_2O_2 with nutrients; ■ – 50 mg/L H_2O_2 without nutrients

bioaugmentation in soil. The term biostimulation refers to enhanced biodegradation by indigenous soil bacteria due to increase of their population by addition of nutrients. The nutrients include a nitrogen source, a phosphorous source, pH adjustment and trace minerals.

In general, biodegradation of the hydrocarbons at any given site will depend upon:

– indigenous soil microbial population,
– hydrocarbon variety and concentration,
– soil structure,
– nutrient availability,
– oxygen availability.

Soil microorganisms reported to degrade hydrocarbons under favorable conditions include *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Arthrobacter*, *Micrococcus and Acinetobacter*. Hydrocarbons with less than 10 carbon atoms tend to be relatively easy to degrade as long as the concentration is not too high to be toxic to the organisms. Benzene, xylene and toluene are examples of gasoline components that are easily degraded. Complex molecular structures, such as branched paraffins, olefins, or cyclic alkanes, are much more resistant to biodegradation.

Soil structure, which is the form of assembly of the soil particles, determines the ability of that soil to transmit air, water, and nutrients to the zone of bioactivity. Another major controlling factor is the variety and balance of nutrients in the soil. Nitrogen and phosphorous are the most common additives and one could roughly estimate that to degrade 1 L gasoline, the microorganisms would need about 44 g nitrogen, 22 g phosphorous and 760 g oxygen. Generally, optimum activity occurs when the soil moisture is 50–80 % of saturation (moisture holding capacity). When the moisture content falls below 10 % bioactivity becomes marginal.

Of particular interest, as mentioned earlier, is to what extent biodegradation of hydrocarbons can proceed at low or no oxygen. At low oxygen levels, denitrification will proceed if an alternate electron acceptor, such as nitrate, is available. When samples containing benzene, toluene, and xylene were incubated anoxically to which 500 mg/L NO_3^--N and 50 mg/L PO_4^{3-}-P were added, data as shown in Fig. 5 were obtained. The initial concentrations of benzene, toluene, and total xylene isomers were 13.3–13.7 mg/L, 33.7–33.9 mg/L, and 15.4–23.2 mg/L, respectively. The effect of nutrients in the above system, as compared to a system containing 50 mg/L H_2O_2 clearly shows the difference in biodegradation (Fig. 6).

Effect of Biosurfactants in Soil Bioremediation

Biodegradation of hydrocarbons in soil can also be efficiently enhanced by addition or *in situ* production of biosurfactants. It was generally observed that the degradation time, and particularly the adaptation time, for microbes was shortened.

Studies with chemical surfactants showed that the degradation of phenanthrene by an unidentified isolate could be increased by a nonionic surfactant based on ethylene glycol.

In oil-contaminated mud flats, the elimination of polycyclic aromatics from the crude oil Arabian light was due to wave action or to microbial degradation. The chemical surfactant Finasol OSR-5 doubled the initial content of aromatics and decreased the amount of aromatics removed after 6 months, whereas adding the bio-

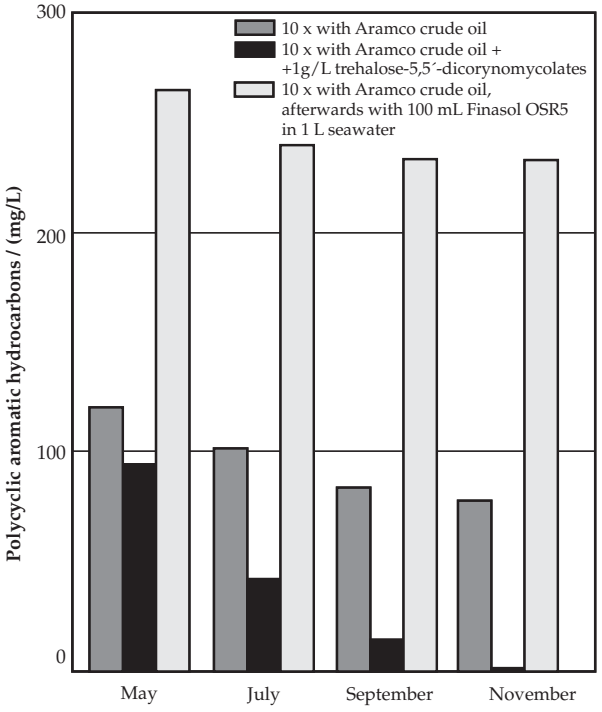

Fig. 7. Elimination of crude oil from flat tidal environments

surfactant trehalose-5,5′-dicorynomycolates caused complete elimination within the period (Fig. 7; (*9*)).

Recent data from the author's laboratory support other findings that sophorose lipids do enhance biodegradation of the hydrocarbons and their chlorinated derivatives in contaminated soil. As an example, the herbicide metholachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide], was significantly more degraded when sophorose lipids were added to a slurry bioreactor containing soil in suspension (Fig. 8).

As shown in Fig. 8a, the content of metholachlor in methanol extract of the soil slurry decreased rapidly after the first addition of sophorose lipids into the suspension, while its content in the aqueous phase remained almost unchanged (Fig. 8b). When sophorose lipids were added to the non sterile soil slurry at the 15th day, a drop in the metholachlor concentration also occurred but it was at a lower rate as compared to addition on day 2. This is to expect as the viable microbial populations responsible for biodegradation diminished during the 15 days exposure to the toxic metholachlor.

Another compound, 2,4-dichlorophenol (2,4-DCP), was also considerably more degraded when sophorose lipids were added to the soil slurry (Fig. 9). Decrease of 2,4-DCP in the suspensions was found to be generally a

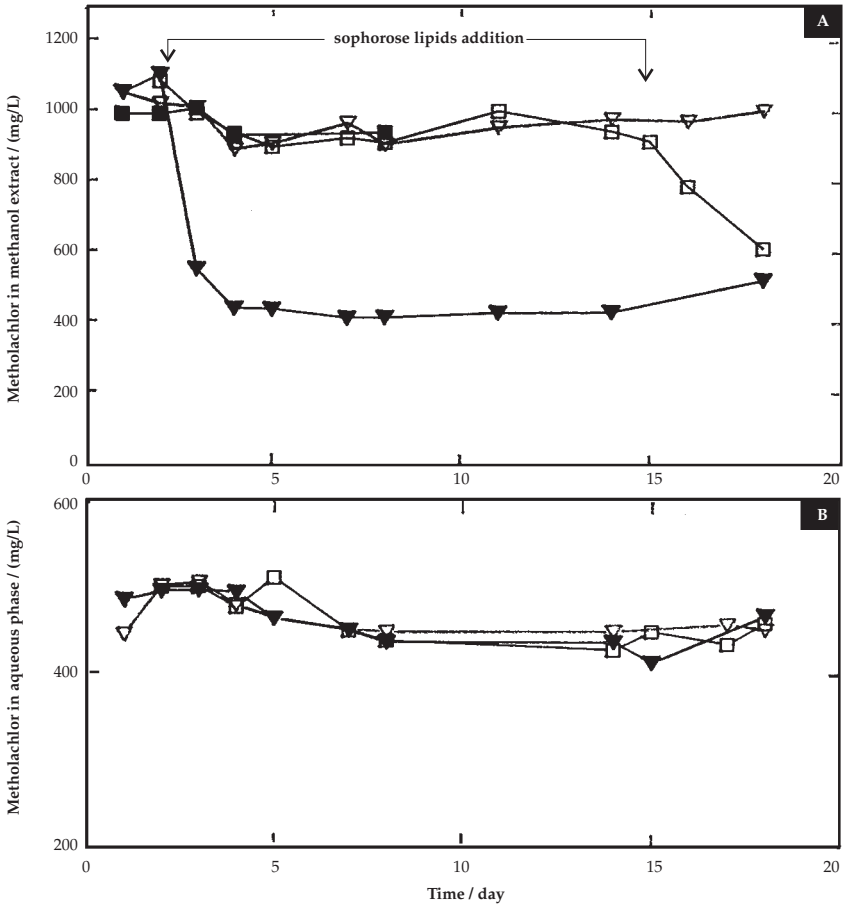

Fig. 8. Changes of metholachlor in: A) methanol extract of slurry and B) aqueous phase of soil suspension (soil 50 g, water 150 mL, metholachlor 262 mg, sophorose lipids 38 mg)
Δ–Δ sterilized soil without sophorose lipids; ▼–▼ non-sterilized soil with sophorose lipids; ■–■ sterilized soil with sophorose lipids; □–□ non-sterilized with sophorose lipids

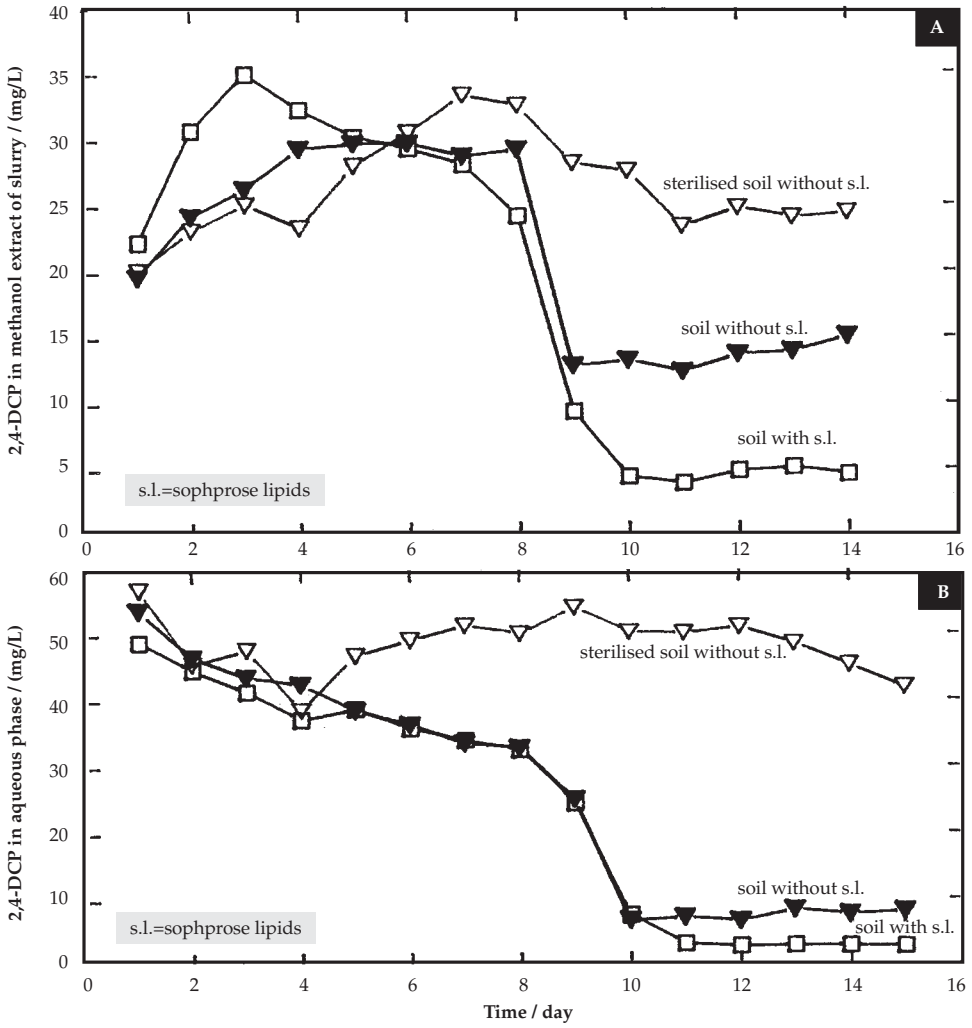

Fig. 9. Effect of sophorose lipids on 2,4-dichlorophenol in soil suspension; A) in methanol extract of slurry; **B) in aqueous phase** (soil 40 g; water 60 mL; 2,4-DCP 6 mg; sophorose lipids 38 mg)

slow process for the first 8 days. However, a sharp drop in the suspension was observed afterwards (Fig. 9a,b). In the presence of sophorose lipids, this drop was significantly deeper as compared to the sample without sophorose lipids.

Naphthalene biodegradation in soil slurry is shown in Fig. 10. When sophorose lipids were added to the slurry almost complete biodegradation was observed 5 days after the start of incubation. These experiments were performed in 250 mL shake flasks. In each flask 50 g of soil in 150 mL sterile deionized water were mixed with 35 mg of sophorose lipids. The following nutrients, in g/L were added (where applicable): 0.25 $MgSO_4 \cdot 7H_2O$; 1.00 Na_2HPO_4; 0.50 KH_2PO_4; 0.50 NH_4NO_3; 0.10 $CaCl_2 \cdot 2H_2O$; 0.20 citric acid; 0.20 glucose, 0.005 yeast extract and traces of B, Mn, Zn, Fe, Mo, and Cu.

Interesting data were also obtained when soil trays (about 20 % moisture) contaminated with polycyclic aromatic hydrocarbons (PAH) were incubated (at room temperature) for 22 days and more. Nutrients with sophorose lipids (added at time 0) were blended into the soil. Data are presented in Fig. 11. It is evident that many PAH were significantly removed. Even though PAH are most resistant to biodegradation they were degraded to a considerable degree in soil to which sophorose lipids were added. This removal was, however, dependant upon particular PAH.

On the basis of the experiments by Kosaric *et al*., the following conclusions can be drawn:

(i) Addition of sophorose lipids caused a sharp drop of metholachlor concentration in the methanol extract from soil slurry bioreactors.

(*ii*) Addition of sophorose lipids enhanced biodegradation of 2,4-DCP in the soil slurry reactors.

(*iii*) Naphthalene was significantly more eliminated in the soil slurry bioreactor in which sophorose lipids were present.

(*iv*) Some polycyclic aromatic hydrocarbons (PAH) were almost completely removed in 22 days, in the presence of sophorose lipids in trays containing soil, while some were resistant to biodegradation.

(*v*) There is a selectivity in biodegradation of PAH-s in soil.

Studies on biosurfactant-assisted bioremediation were also reported by other researchers. *P. aeruginosa*,

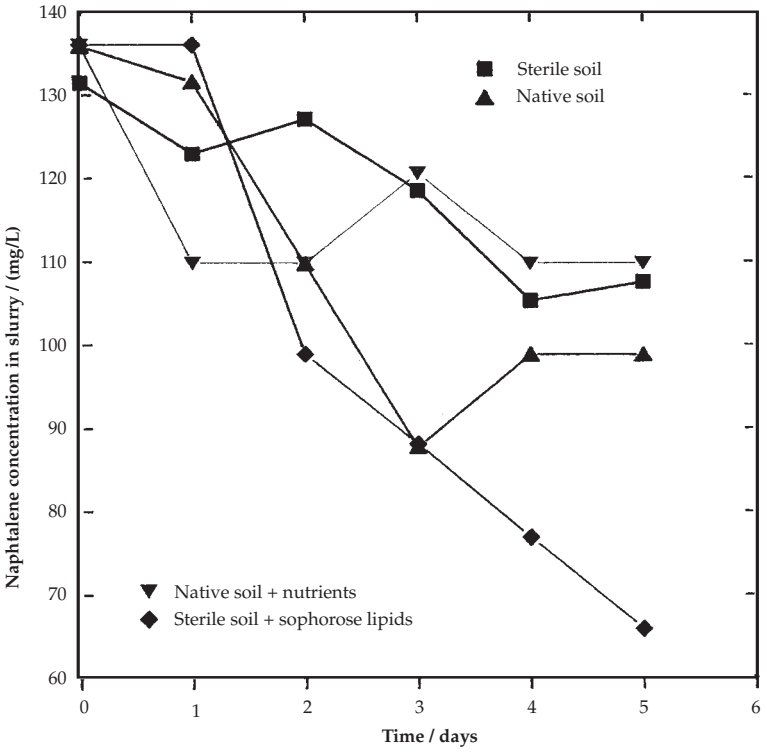

Fig. 10. Bioremediation of naphthalene-contaminated soil

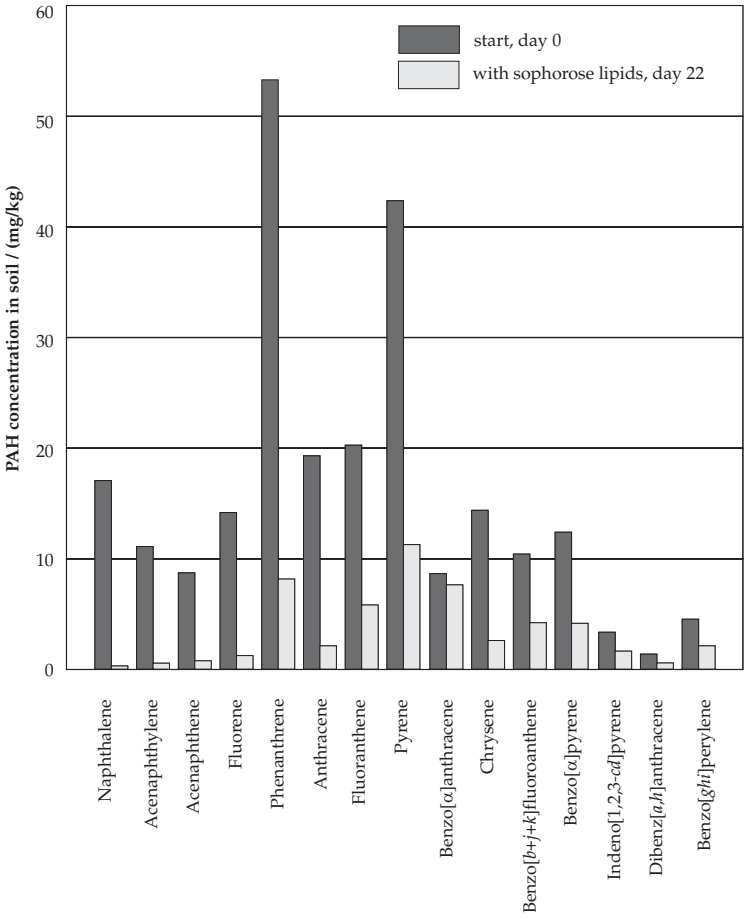

Fig. 11. Biodegradation of PAHs in soil

isolated from oil-polluted sea water, was able to degrade hexadecane, heptadecane, octadecane and nonadecane in seawater by up to 47, 58, 73 and 60 %, respectively, after 28 days of incubation (*10*). Presence of biosurfactants in the culture medium was shown by tensiometric measurements.

Jain *et al*. (*11*) added *Pseudomonas aeruginosa* UG2 biosurfactant to the soil contaminated with a hydrocarbon mixture of tetradecane, hexadecane, pristane and 2-methylnaphthalene. Enhanced degradation of all hydrocarbons, except 2-methylnaphthalene, was observed after 2 months incubation period.

In another experiment (*12*), contaminated soil was inoculated with *Pseudomonas ML2* or *Acinetobacter haemoliticus* and hydrocarbon degradations were compared with the same soil to which an ML2 biosurfactant product (at 41 or 82 μg/mL) was added. After 2 months of incubation, 39-71 % reduction of hydrocarbons was achieved by *A. haemoliticus*, while the *Pseudomonas* ML2 showed 11-71 % reduction. The treatment with the biosurfactant product gave the best results yielding 44-46 % reduction (when used at 41 μg/mL) and 32-34 % reductions (when used at 82 μg/mL). The results suggested that using cell-free biosurfactants, the degradation by indigenous microorganisms in the soil was stimulated.

Eliseev *et al.* (*13*) also reported the ability of a biosurfactant from *Bacillus* sp. to release oil from oily sand at a concentration of 0.04 mg/mL. Biosurfactants have also been demonstrated to successfully solubilize and remove hydrocarbon pollutants from contaminated soil. Examples are biosurfactant-containing broths from *Rhodococcus* ST-5 (*14*) and from the thermophilic *Bacillus* AB-2 (*15*).

Rhamnolipid biosurfactants from *Pseudomonas aeruginosa* were characterized for their ability to remove hydrocarbons from sandy-loam soil and silt-loam soil (*16*). The rhamnolipids at a concentration of 5 g/L were found to increase recovery of the hydrocarbons to 25-70 % in silt-loam soil and 40-80 % in sandy-loam soil.

Large scale field applications were also performed by Kosaric. Several contaminated sites in Canada and the Middle East were bioremediated with biosurfactant addition to the culture medium. These sites represented soil and sand contaminated by heavy hydrocarbons, primarily of industrial origin. Bioremediation was accelerated when glycolipid biosurfactants were added (0.5 kg/ton of soil), to the nutrient which was applied to the soil.

Machine-oil-contaminated soil has been shown to be remediated by microbial inoculation and by biosurfactant treatment. Successful bioremediation of oil-contaminated soil and groundwater from a US Army engineering plant, using natural surfactants produced by indigenous microorganisms, was demonstrated (*17*).

Biosurfactants from *Pseudomonas aeruginosa* SB30 were tested for their abilities to remove oil from the Exon Valdez Alaskan contaminated gravel in the laboratory (*18*). A 1 % biosurfactant solution was found to yield three times higher oil removal as compared to water controls. Bragg *et al.* (*19*) reported bioremediation on the Exon Valdez oil spill *in situ*. Other extensive bioremediation studies were successfully carried out on the oil-contaminated desert sand in Kuwait, both *in situ* and on site (*20*). In all these applications, indigenous microbial populations were utilized by introduction of specific nutrients (N and P) and oxygen to encourage biosurfactant production and hydrocarbon utilization (*21*).

The objective of most of the bioremediation studies is to eliminate contaminating hydrocarbons and their various derivatives. Here a study on bioremediation of metal-contaminated wastestreams reported by Miller (*22*) is also important to mention. Normally, whole cells or microbial exopolymers are used to concentrate and/or precipitate metals for their removal. Metal complexation by rhamnolipid biosurfactant, produced by *Pseudomonas aeruginosa* ATCC 9027, was proposed.

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Površinski bioaktivne tvari i njihova primjena u biološkom oporavku tala

Sažetak

Površinski bioaktivne tvari pospješuju biodegradaciju ugljikovodika, a ovdje su prikazani najvažniji radovi. Izneseni su i rezultati dobiveni primjenom površinski bioaktivnih tvari pri biodegradaciji herbicida metolaklora, kloriranih aromatskih ugljikovodika i naftalena. Nadalje, provedeni su radovi na biološkom oporavku zemljišta onečišćenih policikličkim aromatskim ugljikovodicima i teškim uljima u pilot-postrojenju i na poljima. U prisutnosti odabranih površinski bioaktivnih tvari, nakon 22 dana biološkog oporavka, iz zemlje su gotovo potpuno (ili potpuno) uklonjeni policiklički aromatski ugljikovodici. Znajući da je biološki oporavak tala općenito polagani proces, navedeni rezultati pokazuju da se vrijeme potrebno za provođenje ovog procesa može bitno skratiti. Na osnovi laboratorijskih podataka i onih dobivenih u pilot-postrojenju, proveden je biološki oporavak velikih onečišćenih površina.