BIOLOGICAL SURFACE ACTIVE COMPOUNDS APPLICATION POSSIBILITIES AND SELECTION OF STRAIN WITH EMULSIFYING ACTIVITY

BIOLOĢISKO VIRSMAS AKTĪVO SAVIENOJUMU IZMANTOŠANAS IESPĒJAS

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Abstract. *The biological oil- polluted soil treatment method is used widely; however it is not effective enough because the microorganism's metabolism is affected by seasonal temperature fluctuation. Besides, soil remediation processes are very slow because of low solubility of oil and oil products in water. Biological surface active compounds show promising results in oil-polluted soil bioremediation. Three hydrocarbons degrading microorganisms strains, showing high emulsification activity were selected.*

Keywords: *surface active compounds, biosurfactants, bioremediation*

Introduction

There is a public demand for environmental practices that reduce pollution and a growing demand for restoration of contaminated sites. A variety of chemical, physical and biological methods are used for oil- polluted soil remediation. The biological oil- polluted soil treatment method is used widely because it is cheap and relatively effective. However, it is not always easy to achieve desired results because microorganisms are dependent on seasonal temperature fluctuation [1], big oil concentration in oil- polluted soil [2] and on bioavailability of pollutants. The spontaneous soil remediation processes are very slow because of low solubility of oil and oil products in water [3, 4].

Surface active compounds (SAC's) are widely used wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. Surfactants are organic molecules that usually consist of a hydrophobic and hydrophilic part. The hydrophilic part makes surfactant soluble in water, while the hydrophobic part makes them tend to concentrate at interfaces. The presence of surfactant molecules at air- water interface results in a reduction of the surface tension of aqueous solutions and leads to the stabilization of foams. Another characteristic of surfactants is the formation o micelles, small agregates of surfactant molecules. Depending on the nature of the hydrophilic group, four types of surfactants can be distinguished: anionic, cationic, zwiterionic and non-ionic surfactants. Surfactants can be produced chemically (synthetic surfactants) and biologically (biosurfactants). The most common hydrophobic parts of synthetic surfactants are parafins, olefins, alkylbenzenes, alkylphenols and alkohols; the hydrophilic goup is usually a sulphate, sulphonate or a carbxylate group (anionic surfactants), a quaternary ammonium group (cationic surfactants), polyoxelene, sucrose or polypeptide group (non-ionic). The major classes of biosurfactants include glycolipids, phospholipids, fatty acids, lipopeptides/ lipoproteins, and biopolymeric biosurfactants. Surfactants are produced on large scale and have numerous applications, e.g., as additives in cleaning agents, food, cosmetics, in mining and road construction, detergents, fabric softeners, emulsifiers, paints, adhesives, inks, foaming agents, soil bioremediation etc. Some applications of biological surface active compounds are shown in Table 1.

Table 1.

Biosurfactant uses and effects [5]

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 S	olubilize 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

Surface active compounds improve the bioavailability of highly hydrophobic pollutants. They reduce surface tension and therefore improve the solubility of oil and oil products in water and availability for the microorganisms. It is frequently observed that the rate of removal of compounds from soil is very low even though the compounds are biodegradable. Biodegradation of hydrophobic compounds may take place only in the aqueous phase. The rate at which a particular organic compound dissolves in water is critical to its biodegradability, as this governs the rate of transfer to the organism. The application of surfactants to release hydrophobic pollutants, with the objective of increasing their bioavailability and biodegradability, has mixed results [6]. In particular, surfactants vary greatly in their toxicity to humans and ecotoxicity, and their resistance to biodegradation may lead to the secondary pollution. This is one of the major barriers to the development of the technique. For soil remediation systems, another technology inhibiting observation is that the addition of surfactants to soil can form highly viscous emulsions that are difficult to remove. Large quantities of surfactants are also required [7] and in a soil system, large quantities of aqueous chemicals can ruin soil permeability. Many synthetic surfactants inhibit PAH-degrading microorganisms [7]. 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 the surfactants on large scale in detergents, the toxicity of these compounds has been tested relatively well. The toxic effect of surfactants on bacteria can be explained by two main factors: disruption of cellular membranes by interaction with lipid components and reactions of surfactant molecules with protein essential to the functioning of the cell.

Surfactants biodegradability is a factor that can have negative and positive effects in the use of surfactants for bioremediation. Negative effects can be caused by: depletion of minerals and oxygen; toxicity of surfactant intermediates, which are often more toxic than the parent compounds; or preferential degradation. Moreover, the degradation of the surfactant will reduce any bioavailability enhancing effects. 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. Another positive effect is that a degradable surfactant might be used as a primary substrate when the pollutant is degraded co-metabolically. Biologically produced surfactants accur 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 sunthetic surfactants, a lower toxicity can be expected from most biosurfactants, although some biosurfactants can be as toxic as synthetic ones. The biological oil- polluted soil washing by biological surfactants is used more and more widely *in situ* and *ex situ* soil remediation. Biosurfactants may offer several advantages over synthetic surfactants; they are very effective surfactants, having about a 10 to 40 fold- lower critical micelle concentration than synthetic surfactants. Their production by microbes is widespread: in the last decade at least 13 new glycolipid producers and 13 new lipopeptide producers have been reported [7]. Biological surface active compounds are produced by a wide variety of microorganisms: Pseudomonas sp., Rhodococcus sp., Acinetobacter sp., Azotobacter vinelandii [8]. The low molecular mass biosurfactants are generally glycolipids or lipopeptides, the high molecular mass biosurfactants are polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex of these biosurfactants [9]. Various biosurfactants, produced by different microorganisms are listed in Table 2.

Table 2.

Various biosurfactants produced by microorganisms [5]

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

The individual rhamnolipids are able to lower the surface tension of water from 72 mN m^{-1} to 25-30 mN m^{-1} at concentrations of 10-200 mg l^{-1}. After initial testing, rhamnolipids seem to have potential applications in combating marine oil pollution, removing oil from sand and in combating zoosporic phytopathogens [9].

The aim of this study is selection of microorganisms, producing biosurfactants, showing best results in emulsifying hydrophobic compounds.

Materials and methods

Microorganisms. Forty two strains of hydrocarbon degrading microorganisms were used for the investigation. The microorganisms were isolated from the petroleum hydrocarbon polluted environment and are maintained in the cultures collection of JSC Biocentras.

Media and cultivation conditions. Nutrient broth (*Oxoid*, United Kingdom) was used for preparation of the innoculum. The cultures were grown in the broth for 16-18 h at 30 °C in a rotary shaker (*Innova* 43, New Brunswick Scientific Co. Inc, USA) at 200 rpm. The submerged cultures were used as innoculum at the 2 % (v/v) level. For emulsification studies, microbial strains were cultivated in medium with following compositinion (g/l): NH_4NO_3- 2.2, KCl- 0.1, KH_2PO_4- 0.5, K_2HPO_4- 0.1, $CaCl_2$- 0.01, $MgSO_4 \cdot 7H_2O$- 0.5, $FeSO_4 \cdot 7H_2O$- 0.05, Yeast extract – 0.1 and n-tridecane- 20.0. The final pH of medium was 7.2. Cultivations were performed in 750 Erlenmeyer flasks containing 100ml medium at 30 °C in the rotary shaker at 200rpm for 48 h.

Emulsification measurement. Emulsification activity was measured by adding 5 ml n-hexane to 5 ml of 10 % suspension of culture liquid in 0.1 M Na_2CO_3 and hand shaking for 30 s. The mixture was kept still 1 h prior to measurement. The emulsification activity was calculated by dividing the measured height of the emulsion layer by the total height and multiplying by 100.

Results and discussion

The results are shown in Table 3. It is clearly seen three strains showing high emulsification activity: 21, Gr2 and N3 (68.2, 52.2 and 49.5 %, respectively) (Table 3.). The highest activity towards n- tridecane has strain 21 (68.2 %). These strains are selected to the future soil bioremediation technology using biological surface active compounds.

Table 3.

Emulsification activity of culture liquid from hydrocarbon degrading microorganisms

Strain	*Emulsification activity, %*	*Strain*	*Emulsification activity, %*
C1	27.3	23	20.0
KM	18.7	NJ5	22.0
SM	22.4	NJ9	39.4
M1	25.6	S10	0.0
M2	20.4	S20	0.0.
Maz1	0.0	NJ12	20.1
L2	0.0.	S3	10.7
J1	12.0	S7	10.0
98	10.0	D1	0.0
V1	0.0	NJ1	0.0
N1	19.3	NJ11	9.7
Sv1	0.0	NJ13	0.0
Gr2	52.2	NJ2	0.0
Gr5	0.0	NJ15	12.3
K3	15.0	Isp13	19.2
E2	0.0	Isp71	22.9
T	14.7	Isp91	20.7
21	68.2	Kl1	21.0
PP	29.9	Isp51	24.5
P2	25.5	Isp52	22.0
N3	49.5	Z2	18.5

Conclusions

- SAC's are applied widely in different areas, such as textile, food, inks, environment cleanup etc. Biological surfactants are more advantageous in comparison with synthetic because of lower toxicity and thus can be applied more widely.
- The method for selection of microorganisms having emulsification activity was selected and applied.
- Three strains, having high emulsification activity were selected from 42 hydrocarbons degrading microorganisms strains: 21, Gr2 and N3. The highest emulsification activity has strain 21- 68.2 **%.**

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