Biosurfactant-enhanced Remediation of Oil-contaminated Environments†

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ABSTRACT: The development of new efficient cleaning technologies for the bioremediation of the environment and its cleaning from oil products has become a subject of great interest. Surfactants influence the bioremediation of oil and other organic contaminants through increasing their availability. Since surfactant products of a microbial nature are highly efficient in this respect, they have important advantages over synthetic materials. Being biodegradable, they are ecologically safe.

A new washing bioremedy based on surfactant products synthesized microbially from the bacterial strain *Pseudomonas sp. PS-17* has been obtained. It contains an unique biocomplex of a glycolipid biosurfactant and an alginate biopolymer and possesses a high surface and emulsifying activity. The washing and antisorptional capacity of this bioremedy has been tested on objects of various natures, viz. bird feathers, animal skins and furs. We have obtained positive results in the remediation of sea sands, birds and animals suffering from ecological catastrophy. It was shown that the bioremedy has mild properties and does not change the structure of the feathers, skins and furs studied. The bioremedy remains active over the temperature range 15–90°C.

INTRODUCTION

At the start of the third millennium, the technological pressure on natural ecosystems continues to grow at a rapid rate. Oil and oil-related products are extremely dangerous pollutants, both in volume and effect. They have a considerable influence on oceanic ecosystems. Annually, the seas and oceans receive from 5 up to 10 million tonnes of oil and oil-related products which arise from sea transport, coastal dumping, losses during production on oil-field shelves and spills caused by tanker scuttlings. Such spills lead to the death of a large number of sea organisms (both zoo- and phytoplankton which form the basis of all nutritional chains in oceanic ecosystems), sea mammals and birds, and also cause an infringement of the natural processes occurring in the ecosystem. Thus oil spills result in an infraction of the ecosystem balance, leading to its degradation or complete destruction. Hence, the design of new effective washing agents is urgent with the priority now being the application of ecologically safe and economically profitable washing remedies using new surfactants obtained by biotechnological means.

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Biosurfactants, which are the products of microbial synthesis with surface active and emulsifying properties, can exceed the performance of their synthetic equivalents in terms of efficiency. Microbial surfactants have specific advantages in that they are biodegradable, non-toxic and effective under conditions of extreme temperature, pH and salt concentration (Elysseev and Kutcher 1991). Their synthesis can be based on cheap substrates including industrial waste products. The wide range of physical, chemical and biological properties exhibited by microbial surfactants allow the possibility for the creation of new ecologically safe technologies.

A wide range of microorganisms capable of producing effective surfactants has been created in the Biotechnology Laboratory at the Department of Physical Chemistry, Lviv Institute NAS. Thus, glycolipid, peptidolipid and polysaccharide surfactants as well as extracellular biosurfactant complexes of these lipids and biopolymers have been obtained from cultures of microorganisms such as *Bacillus subtilis C-14* (Elysseev *et al.* 1990a,b; Shulga *et al.* 1993a,b), *Rhodococcus erythropolis AP 25* (Shulga *et al.* 1990), *Candida lipolytica Y-917* (Lesik *et al.* 1989a,b), *Cryptococcus albidus Y-78* (Kutcher *et al.* 1990) and the carotin-synthesizing strain *Phaffia rodozyma Y-2274* (Kutcher *et al.* 1991). One such prospective culture is the bacterial strain *Pseudomonas sp. PS-17* (Karpenko *et al.* 1996; Pochmurskiy *et al.* 1997).

The main aim of the work described here was the research of new, effective and environmentally safe methods for the removal of oil pollution. The major direction of the research has been the study of surface-active biogenic substances potentially capable of cleaning up oil from polluted coastal sand, marine bird feathers and marine animal furs. Such substances should be effective both in fresh and sea water, be non-toxic, harmless to living beings and be easily degradable both in their natural form as well as in the form of oil emulsions.

EXPERIMENTAL

Microorganism

A strain of *Pseudomonas sp. PS-17* originally isolated from soil samples in the vicinity of an oil refinery (Shulga *et al.* 1996) was used throughout this work.

Cultivation conditions

The optimized medium used for *Pseudomonas sp. PS-17* growth and biosurfactant production was the same as that described previously (Shulga *et al.* 1996), the carbon source being glycerol or rape oil (30 g/l). *Pseudomonas sp. PS-17* was grown in 250 ml conical flasks containing 50 ml of a sterile medium at a pH value of 7.0. The culture was maintained at 30°C on a reciprocating shaker at 140 rev/min for 96–144 h. The biomass concentration was determined by the method described by Rapp *et al.* (1979) and the Wilhelmy method with a platinum plate was used for measurements of the surface and interfacial tensions (Abramson 1988). The concentration of rhamnolipids was measured by the anthron method (Hodge and Hofreter 1962) after extraction.

Isolation and preparation of biosurfactants

After cultivation, the cells were separated by centrifugation. The supernatant was acidified with an aqueous 10% HCl solution to a pH value of 2.0 and extracted with ethyl acetate. After removal of the solvent by rotary evaporation, the residue was analyzed by thick layer chromatography on Silica Gel 60 (No. 5745, E. Merck AG, Darmstadt, Germany) using solvent systems containing chloroform, methanol, acetone and acetic acid mixtures in a 90:10:6:1 (vol/vol) ratio.

Study of the washing ability of biosurfactants

The complex model method was employed in experiments concerning shore oil pollution removal and in the cleaning of birds and animals. Samples of sea sand, feathers and fur were polluted with crude oil to enable their subsequent clean-up with a complex of the *Pseudomonas sp. PS-17* strain. For such cleaning experiments, various aqueous solutions of the complex up to a concentration of 1% were employed.

In experiments modelling the removal of shore pollution, sea sand samples of 5 g weight polluted with oil were used. Model experiments on the remediation of marine animals involved the use of gull feathers and coypu and musk rat fur polluted with crude oil of a density equal to 1200 kg/m^3. For cleaning up the various samples, the following preparation variants were used: (1) 1% solution of the PS-17 complex in fresh water; (2) 1% solution of the PS-17 complex in sea water; (3) 1% solution of the synthetic detergent 'Lotus' in fresh water; and (4) 1% solution of the synthetic detergent 'Lotus' in sea water solution. Samples of the synthetic detergent 'Lotus' were obtained from Vremia, Kharkov, Ukraine.

In a typical experiment, samples of polluted sand were poured into flasks, 50 ml of a given preparation added and the flasks shaken for 15 min. After that, the liquid was decanted, the samples washed with water and dried. Polluted feathers and fur samples were cleaned with tampons moistened in the bioremedy and then washed with water and dried. The cleaned samples of sand, feathers and fur were weighed and the quantity of unremoved oil determined by extraction with an organic solvent mixture (hexane + chloroform + isopropanol in the ratio 2:1:1). The rate of pollution removal was then calculated from the formula:

$$P = \frac{m_p - m_o}{m_p - m_r} \times 100\% - \Delta\%$$

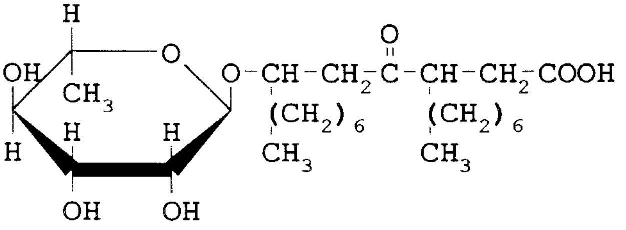
where m_p is the weight of the polluted sample, m_o is the weight of the original sample, m_r the weight of the treated sample and $\Delta\%$ is the natural fat content of the fur and/or feathers.

RESULTS AND DISCUSSION

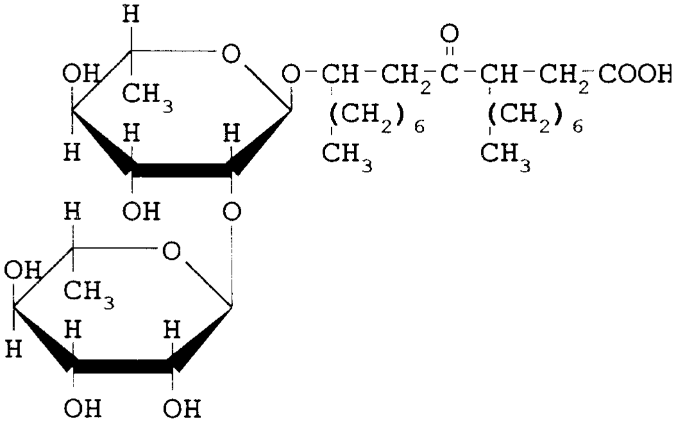
The bacterial strain *Pseudomonas sp. PS-17* was capable of synthesizing extracellular surface-active substances which possess the unique natural composition of a biosurfactant and a biopolymer, both of which are capable of causing a considerable decrease in the surface and interfacial tensions to values of 29.0 and 0.01–0.07 mN/m, respectively, and are capable of forming stable highly dispersed emulsions. The biosurfactant/biopolymer complex is also capable of forming crystalline structures.

The biosurfactants are rhamnolipids which consist of molecules of rhamnose and β-oxidecanic acid, respectively (see Figure 1). The low parameters for their surface and interfacial tensions, as well as their critical concentrations for micelle formation (CMC), indicate their high surface activity (see Table 1). The extracellular biopolymer synthesized by the *Pseudomonas sp. PS-17* strain is a polysaccharide of an alginate nature with a molecular weight in the range 3–4 × 10^5. The results of experiments involving the cleaning of oil-polluted samples of sea sand, fur and feathers are listed in Tables 2–4, respectively.

The data listed in these tables indicate that, both in fresh water and sea water, the extent of removal of oil pollution by the PS-17 complex from the samples studied was quite high, being 82–95% in fresh water and 78–92% in sea water. The residual, insignificant quantities of oil remaining in sand are incapable of harm to living beings on the coast while the oil remaining on the bird



RL -1



RL -2

Figure 1. Rhamnolipids RL-1 and RL-2 produced by *Pseudomonas sp. PS-17.*

TABLE 1. Surface-active Properties of Extracellular Rhamnolipids of the *Pseudomonas sp. PS-17* Strain

Substance	CMC (mg/l)	Surface tension (mN/m)	Interfacial tension in n-heptane (mN/m)	Concentration in cultured liquid (g/l)
RL-1	50	29.5	0.020	2.8
RL-2	20	28.8	0.014	4.2

feathers and animal furs also had no essential influence on their viability. In addition, it was found that the PS-17 complex had a significant advantage over synthetic detergents. Thus, its activity in sea water was much higher than that of the detergent, with the extent of removal of pollution with the complex being 2–2.5-times higher than that observed for the ‘Lotus’ detergent.

Most known detergents lose their surface activity in mineralized water. Thus, the extent of removal of pollution by the ‘Lotus’ detergent with sea water (relative to fresh water) decreased by ca. 50%. In contrast, the PS-17 complex exhibited a decrease in activity of only 3–5% in sea water. This property of the complex is especially important since it allows the removal of oil pollution from oceans and seas which are the most endangered of all.

TABLE 2. Relative Efficiency in Cleaning Up Sand Polluted with Oil

Washing agent	Pollution removal (%)
1% solution of PS-17 complex in fresh water	95.0
1% solution of PS-17 complex in sea water	92.3
1% solution of 'Lotus' detergent in fresh water	94.5
1% solution of 'Lotus' detergent in sea water	35.0
Control I — fresh water	0.7
Control II — sea water	1.2

TABLE 3. Relative Efficiency in Cleaning Up Feathers Polluted with Oil

Washing agent	Pollution removal (%)
1% solution of PS-17 complex in fresh water	85.1
1% solution of PS-17 complex in sea water	77.4
1% solution of 'Lotus' detergent in fresh water	86.0
1% solution of 'Lotus' detergent in sea water	40.2
Control I — fresh water	2.1
Control II — sea water	2.4

TABLE 4. Relative Efficiency in Cleaning Up Fur Polluted with Oil

Washing agent	Pollution removal (%)
1% solution of PS-17 complex in fresh water	82.3
1% solution of PS-17 complex in sea water	78.0
1% solution of 'Lotus' detergent in fresh water	83.1
1% solution of 'Lotus' detergent in sea water	45.1
Control I — fresh water	0.5
Control II — sea water	0.8

Visual as well as microscopic observations of the feather and fur samples showed that their treatment with the PS-17 complex had no effect on their structures. In addition, it was demonstrated that the complex had no harmful effect on the skins of birds and animals. Hence, the PS-17 complex can be used successfully for cleaning up oiled birds and mammals.

For these various reasons, the extracellular complex PS-17 appears to be highly effective for the removal of oil pollution from fresh water and, in particular, sea water thereby allowing it to be recommended for use in the treatment of water and shore oil as well as for the remediation of marine birds and animals from the effects of such pollution. The total lack of toxicity of the complex, its ability to undergo complete biodegradation and its high activity makes the PS-17 complex ideal for such purposes.

New efficient technologies for the purification of the environment are essential for countries with an extensive oil industry, allowing both periodic cleaning and a rapid treatment for use in oil tanker accidents or other types of water pollution.

CONCLUSIONS

1. The bacterial strain *Pseudomonas sp. PS-17* is an effective producer of an unique natural biocomplex consisting of a biosurfactant and a biopolymer.
2. It has been shown that the PS-17 complex exhibits considerable efficiency in the removal of oil pollution from fresh and salt water and in effecting its removal from shore areas.
3. Use of the PS-17 complex could save the lives of hundreds of thousands of marine birds and animals suffering from oil pollution, many of which constitute endangered or disappearing species.
4. The know-how for producing the PS-17 complex is simple and quite cheap, so that no significant capital outlays are necessary prior to commencing production.
5. The bioremedy should also have a high efficiency in removing oil pollution from soil. If this can be demonstrated, the PS-17 complex could be considered a universal preparation for oil pollution removal.

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