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MICROBIAL SURFACTANTS IN ENVIRONMENTAL TECHNOLOGIES

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It was shown literature and own experimental data concerning the use of microbial surface active glycolipids (rhamno-, sophoro- and trehalose lipids) and lipopeptides for water and soil purification from oil and other hydrocarbons, removing toxic heavy metals (Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}), degradation of complex pollution (oil and other hydrocarbons with heavy metals), and the role of microbial surfactants in phytoremediation processes.

The factors that limit the use of microbial surfactants in environmental technologies are discussed. Thus, at certain concentrations biosurfactant can exhibit antimicrobial properties and inhibit microorganisms destructing xenobiotics. Microbial biodegradability of surfactants may also reduce the effectiveness of bioremediation. Development of effective technologies using microbial surfactants should include the following steps: monitoring of contaminated sites to determine the nature of pollution and analysis of the autochthonous microbiota; determining the mode of surfactant introduction (exogenous addition of stimulation of surfactant synthesis by autochthonous microbiota); establishing an optimal concentration of surfactant to prevent exhibition of antimicrobial properties and rapid biodegradation; research both in laboratory and field conditions.

Key words: microbial surfactants, bioremediation.

Surfactants are widely used in different fields of industry that is why the demand for their synthetic analogues is continually increases. At the same time the modern development of biotechnology and growing attention to environment defense are the source of interest to microbial surfactants as an alternative way of chemical analogues. Due to environmental safety, the ability to emulsify hydrophobic compounds and increase the efficiency of microbial decomposition of xenobiotic, surfactants are promising for use in environmental technologies.

Over the past decades, the environment has got a huge amount of industrial waste, agricultural chemicals and products of their partial transformations that are unique or rare in nature. This resulted in a negative impact on all levels of organization of living matter and that balance breaks the balance that was created over millions of years, so that there is a real threat to the sustainable functioning of ecosystems.

Today oil is the main source of energy worldwide, while the probability of getting it into the environment, leading to negative consequences [1]. Since 1992 the world has held more than 20 oil spills [http:/www.endgame.org/oilspills.htm], which led to economic losses and violations of ecological balance. To eliminate the consequences of such accidents physical and mechanical methods are usually used, but they are not always effective enough. According to the Office of Technology Assessment (Office of Technology Assessment, USA) after large-scale disaster, it is possible to remove only 10–15% of oil by mechanical methods [1].

To eliminate oil spill there are some promising biological methods, based on direct introduction of petrooxidizing microorganisms (bioaugmentation) or using various substances that stimulate the natural (autochthonous) microbiota (biostimulation) such as microbial surfactants [2, 3]. For the first time the possibility of using microorganisms for the biodegradation of oil in marine sediments was described by Jones et al. 1983 [4]. In the future, scientists expanded the knowledge of oil destructors and conditions of their application [5–8].

Recently, interest in microbial surfactants is increasing [9–14], due to significant progress

in the development of technologies for their cheaper production [15, 16] and the rapid trend development to protect the environment [17].

Due to environmental safety, the ability to emulsify hydrophobic compounds, form complexes with heavy metals and improve the effectiveness of some xenobiotics microbial degradation of surfactant may acquire wide application in environmental technologies [18–24]. In addition, these metabolites are unique because they can be obtained from industrial waste and then be used for destruction of pollutants, i.e. during the production and application of microbial surfactants, there is an effect of "double" environment cleaning [25, 26].

The mechanisms of oil destruction

The literature describes several mechanisms of oil degradation under surfactants, one of which is associated with desorption, mobilization or solubilization of organic pollutants that leads to increasing their bioavailability to microorganisms, and another is related with increased cell surface hydrophobicity of most destructors [27, 28]. According to the first mechanism, the removal of hydrophobic xenobiotics from the soil can occur in two ways, depending on the concentration of surfactant. For concentrations of surfactants, lower the critical micelle concentration (CMC), there is the phenomenon of mobilization, resulting in reduced capillary and interfacial forces that hold the oil in the soil, and it is observed the increase of contact angle of the surfactant with the system oil/soil [19]. If the concentration of surfactant is at CMC level and above, the process of solubilization takes place. In this case, oil is placed in micelles, which hydrophobic ends are placed inside and hydrophilic — outside. It provides solubility of hydrophobic contaminant [29, 30]. It was carried out the electron microscopy study of the absorption of *n*-alkanes by *Pseudomonas aeruginosa* and it was found that by its nature the phenomenon is similar to pinocytosis [31].

There is another mechanism, which was described in [32]. It was noted the chemical and structural changes of surface structures of *P. aeruginosa* in case of introduction of the rhamnolipids and hexadecane. It was shown that in the presence of surfactants in very low concentrations it is observed the release of cell wall lipopolysaccharide from pseudomonas cell wall, which leads to an increase in surface hydrophobicity. There are reports [33] that rhamnolipids do not affect the cell wall lipopolysaccharide, but can change the composition of the outer membrane proteins.

Mohanty et al. [34] compared the mechanisms of naphthalene adsorption by *Burkholderia multivorans* (NG1) in the presence of Triton X-100 and JBR-515 rhamnolipids. Synthetic surfactants increased naphthalene bioavailability due to its emulsification and maintaining interphase contact with the cell while microbial surfactants solubilized the organic pollutant.

Water and soil purification from oil and other hydrocarbons

It was offered a variety of options using microbial surfactants for cleaning: using of their microorganisms-producers to utilize oil and oil products; treating the most contaminated sites with surfactant solutions in bioreactors; treating contaminated areas with these solutions to solubilize hydrocarbons. The last one stimulates the development of natural petrooxidizing microbiota [35]. It was demonstrated the ability of *Pseudomonas* sp. LP1 to metabolize oil and diesel fuel [36], the degree of destruction was 92.34% and 95.29% respectively. There are reports [37] about the ability of *Brevibacterium* sp. PDM-3 to degrade phenanthrene and other polyaromatic hydrocarbons (93.92%). It was noted high rates of degradation of oil, aliphatic and aromatic hydrocarbons (85–97%) in case of introduction of sophorolipids [38]. Das et al. [39] showed the effectiveness of the introduction of *Bacillus subtilis* DM-04, *P. aeruginosa* M and *P. aeruginosa* NM in oil contaminated soil: on the 120th day the general level of petroleum hydrocarbons fell from 84 to 21–39 g/kg soil, while in the control variant (without introduction of microorganisms), the figure was 83 g/kg. Fan et al. [40] found that in case of introduction of *Candida tropicalis* SK21, that able to synthesize surfactants in soil contaminated by oil (16.3 g/kg), on the 180th day 83% of hydrocarbons were degraded, while without yeast introduction this figure was only 61%. It was described the ability to use cells of *Pseudomonas cepacia* CCT6659, glycolipids synthesized by this strain and surfactant mixtures with the cell producer for bioremediation of soils from oil [41]. Oil degradation by 83% was observed after 10 days of common use of glycolipids and cells of *P. cepacia* CCT6659. It was shown the use of *Acinetobacter* sp. D3-2, which synthesizes lipopeptides, for oil destruction (82%) at 30 °C in the presence of 3% NaCl [42]. Indian scientists [43] purified and identified the strain *Planomicrobium chinense* B6,

which is capable of degradation of diesel fuel containing linear C_{14}–C_{36} *n*-alkanes, thanks the synthesis of emulsifier. Resistance of B6 strain to high temperatures, toxic metals and moderate salt concentration makes it promising for use in bioremediation processes of soils in a tropical climate. Diab et al. [44] established the possibility of using the supernatant *P. aeruginosa* SH 29 containing surfactant (index emulsification is 62%) for the cleaning of oil tanks. In subsequent studies, the authors showed that after treatment the soil with the supernatant of surfactant producers *Bacillus* spp. SH 20 and SH 26 (10 ml/100 g) the degree of degradation of oil sludge was 34 and 28% respectively.

In another study [45] authors showed that the use of culture fluid and corresponding supernatant of *P. aeruginosa* RS29, containing monorhamnolipids removed respectively 85 and 55% of hydrocarbons from the sludge.

It was described the theoretical possibility about using of surfactants (10 mg/ml) from probiotic strain *Lactococcus lactis* in the process of remediation of oil-contaminated water (10 ml/40 ml) [46]. Recently, it has been reported about the isolation and identification of *Rhodococcus* sp. JZX-01 that is capable of surfactant synthesis and degradation of oil in water (65.27% in 9 days), even at low temperatures and high salinity [47]. In addition, this strain is able to utilize long-chain hydrocarbons (C_{31}–C_{38}) and branched alkanes. Jain et al. [48] reported about the potential use of alkaliphilic bacteria *Klebsiella* sp. RJ-03 in the process of remediation due to their ability to synthesize surfactants from cheap raw materials (corn flour) and their stability over a wide range of pH and temperature. It was shown the possibility of using *Serratia marcesencs* L-11 for the degradation of polyaromatic hydrocarbons due to the strain ability to synthesize surfactants and catechol-1,2-dehydrogenase [49].

It was found that the use of synthetic dodecyl sulfate and rhamnolipids resulted in the removal of oil from the soil by 44–46%, whereas saponin (surfactants of vegetable origin) provided around 27% [50]. In case of introduction of 10 g/l sophorolipids to the soil contaminated by 2-methylnaphtalene it was removed 30% of xenobiotics [38].

There are some data that efficiency of microbial surfactants is not inferior, and in some cases superior compared with synthetic Span 20/80/85. Lai et al. [19] compared the efficiency of rhamnolipids and the microbial surfactant with synthetic surfactants Tween 80 and Triton X-100 for cleaning the soil from oil (oil concentration 9.3 g/kg).

The maximum degree of oil degradation (62–63%) was observed in case of the treatment with microbial surfactants, while using synthetic surfactants provide only 35–40%. It was determined the degree of soil laundering from the diesel fuel by lipopeptides from *B. subtilis* SPB1 and their synthetic analogues — SDS and Tween 80 [51].

It was established that microbial surfactants not inferior to the efficiency of synthetic analogues and thanks their use it was removed up to 87% of fuel for 24 hours at 30 °C. In another study [12] the authors showed that using glycolipids from *Candida sphaerica* UCP0995 it was possible to get the degree of laundering sand from motor oil equal 95%. That makes the use of surfactants of this strain perspective in the process of bioremediation.

Complex technology of cleaning soil from oil was described in articles of Kildisas et al. [52] and Baskys et al. [53]. This technology consisted of several stages: laundering soil from oil using surfactants, destruction of residual oil by bioaugmentation and phytoremediation. As a result, at the pilot plant area of 340 m^2 which contained 1,000 m^3 of soil, the concentration of oil was reduced from 180–270 to 3.2–7.3 g/kg.

Moldes et al. [54] investigated the possibility of using surfactants from *Lactobacillus pentosus* CECT-4023 T (ATCC-8041) in the remediation of soil from octane. It was shown that introduction of surfactants led to activation of natural soil microbiota and after 15 days, it was observed destruction of 59 octane and 63% of its initial concentration by 700 and 70 000 mg/kg, respectively. In control variant (without the addition of surfactants), it was degraded 1 and 24% of octane respectively. In subsequent studies, this group of scientists compared the effectiveness of the use of surfactants from *L. pentosus* CECT-4023 T (ATCC-8041) and dodecyl sulfate for the purification of soil from octane (7,000 mg/kg) [55]. It was found that in case of using microbial surfactants (2.65 mg/l) for 15 days the degree of octane degradation was 65.1%, while the presence of synthetic surfactants — only 37.2%.

Introduction of rhamnolipids, sophorolipids and trehalosolipids led to the increase of efficiency for 30–50% and accelerated the soil purification from halogen derivatives for 8 days compared with the data obtained with surfactants [56]. With the addition of rhamnolipids from *P. aeruginosa* SP4 at

a concentration of 250 mg/l, the degree of pyrene decomposition in soil (500 mg/kg) was 84.6%, while in the absence of surfactants — 59.8% [57].

Scientists from Latvia [58] suggested using mathematical methods of planning the experiment to predict the efficiency of microbial surfactants for cleaning the environment. The object of the study were surfactants of glycolipid nature with the commercial name AURA-PURE ST (manufacturer AURAVIA LATVIA SIA, Latvia).

It was taken into account such factors as temperature, contact time of surfactants, the concentration of surfactants in the solution. It was established that at the critical value of all conditions (35 °C, 15 min, 0.3%, mass fraction) up to 99.32% of the oil was removed.

It was shown the possibility of using untreated surfactants and solutions of treated surfactants (0.8 and 0.16 g/l) from *C. sphaerica* UCP 0995 for the destruction of diesel oil in different types of soil [59]. In addition, the authors studied the laundering properties of studied metabolites. It was shown that by introducing 200 ml of preparation, the degree of oil decomposition was 75, 92 and 50% in clay, muddy and sandy soil respectively (100 g/kg soil). Preparations surfactants possessed effective laundering properties: at the concentration of 0.16 g/l, they removed 88% of the engine oil from the soil. Treatment with untreated surfactants of the cotton fabric tissue contaminated with oil (3 g of oil for 25 m^2 of fabric tissue), resulted in removal of 41% removal of hydrocarbons.

Cerqueira et al. [60] studied the ability of 45 isolates to use oil sludge as the sole source of carbon and energy. Basing on the analysis of 16S rRNA among the studied bacteria, it was selected and identified five strains (*Stenotrophomonas acidaminiphila* BB5, *Bacillus megaterium* BB6, *Bacillus cibi, P. aeruginosa* and *Bacillus cereus* BS20), which can be promising for use in environmental technology for its ability to synthesize surfactants and resistance to high concentrations of oil sludge (up to 30%).

The rapid development of biodiesel involves the search for effective methods of cleaning the environment under conditions of accidents. Authors [61] studied the mixture of diesel and biodiesel (B20), that it received from areas contaminated by hydrocarbons/ethers and consortium of microorganisms that was able to the decomposition of pollutants. According to the results of enzymatic analysis and capacity of surfactant synthesis, it was elected four bacterial isolates — *B. megaterium, B. pumilus, P. aeruginosa* and *Stenotrophomonas maltophilia*, which are planned to use for cleaning the polluted environment. In other studies [62], rhamnolipid adding under aeration conditions increased effectiveness of decomposition B20. However, it made no influence on the rate of destruction of diesel fuel obtained from oil.

Our research has shown that the presence of surfactant preparations from *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 and *Nocardia vaccinii* IMB B-7405 as cultural liquid and a supernatant resulted in the degree of oil degradation in the water 80–94% (Table 1), and in the soil — 67–86% (Table 2).

Generalizing data on the degradation of xenobiotic surfactants are given in Table 3.

Removal of toxic metals

It is known that heavy metals are not biodegradable, but the bacteria can change their mobility and toxicity due to chemical reactions (oxidation-reduction reactions, alkylation, etc.), the formation of complexes with synthesized metabolites or intracellular accumulation [78]. To extract metals *ex situ*, contaminated soil is collected in glass tubes and washed with surfactants (it is called "soil washing"). To do it *in situ* — create drainage pipe and trench, which are used for injection and pumping of surfactants ("soil flushing") [79, 80]. There are technologies that help to separate complexes of heavy metals and surfactants by centrifugation followed by the removal of these metals [80].

The principle of removing metals from soil is based on the fact that affinity of microbial anionic surfactants to metals is stronger than metal connection with soil, so complex "surfactant-metal" is easily washed away. Removal of metals from soil with cationic surfactants is based on the principle of ion exchange [27, 79, 81, 82]. Scheme of extracting metals from the soil using surfactants is shown in Fig 1.

Juwarkar et al. [84] showed that 0.1% dirhamnolipids from *P. aeruginosa* BS2 effectively removed metals from the soil in the following sequence $Cd^{2+} = Cr^{2+} > Pb^{2+} = Cu^{2+} > Ni^{2+}$. In addition, anionic surfactants synthesized by marine organisms were effective in soil remediation to remove Pb^{2+} and Cd^{2+} [85].

Table 1. **Indicators of water purification from oil using surfactant preparations from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017 and *N. vaccinii* IMB B-7405**

Strain-producer of surfactants	Surfactant preparation	Concentration of surfactant preparation,%	Concentration of remaining oil, g/l	The degree of oil destruction *,%
A. calcoaceticus IMB B-7241	Cultural liquid	5	0.20 ± 0.010	92.3
		10	0.32 ± 0.015	87.7
		15	0.36 ± 0.018	88.2
	Supernatant	5	0.44 ± 0.022	83.1
		10	0.32 ± 0.015	85.7
		15	0.38 ± 0.019	85.4
R. erythropolis IMB Ac-5017	Cultural liquid	5	0.18 ± 0.009	93.1
		10	0.17 ± 0.008	93.5
		15	0.17 ± 0.008	93.5
	Supernatant	5	0.42 ± 0.021	83.9
		10	0.40 ± 0.020	84.6
		15	0.39 ± 0.020	85.0
N. vaccinii IMB B-7405	Cultural liquid	5	0.15 ± 0.007	94.2
		10	0.29 ± 0.014	88.8
		15	0.40 ± 0.020	84.6
	Supernatant	5	0.45 ± 0.022	82.8
		10	0.54 ± 0.027	79.0
		15	0.55 ± 0.027	79.5

Note. In calculating the degree of oil degradation, the error does not exceed 5% . Time of exposure is 30 days. Initial concentration of oil in the water is 2.6 g/l. The degree of oil degradation in control (not treated with the surfactants) is 3.5% . Here and after all meanings* — $P < 0.05$ vs. control.

Table 2. **The influence of cultural liquid of *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017 and *N. vaccinii* IMB B-7405 on the efficiency of soil purification from oil**

Surfactant producer	Concentration of cultural liquid, ml/kg of soil	Concentration of remaining oil, g/kg	The degree of oil destruction *,%
A. calcoaceticus IMB B-7241	100	4.1 ± 0.21	80.8
	200	3.9 ± 0.19	81.8
	300	3.8 ± 0.19	82.2
R. erythropolis IMB Ac-5017	100	7.0 ± 0.35	67.3
	200	5.7 ± 0.28	73.4
	300	2.9 ± 0.14	86.4
N. vaccinii IMB B-7405	100	6.2 ± 0.31	71.0
	200	5.6 ± 0.28	74.0
	300	3.5 ± 0.17	83.5

Note. Initial concentration of oil in the soil is 21.4 g/kg. The degree of oil degradation in control (not treated with the surfactants) is 5.8% .

Table 3. **Using microbial surfactants in bioremediation**

Type of surfactant	Contaminant	Initial concentration of contaminant, g/l	Destructor	Efficiency of destruction, %	Destruction period, days	References
1	2	3	4	5	6	7
Rhamnolipids	Phenantrhrene	10	Monoculture of *Sphingomonas* sp.	99	10	[63]
Rhamnolipids	Anthracene	0.025	Monoculture of *Sphingomonas* sp. and *Pseudomonas* sp.	52	18	[64]
Monorhamnolipids	Hexadecane	0.5	Monoculture of *C. tropicalis*	93	4	[65]
Rhamnolipids, emulsan, surfactants of native microbiota	Pyrene	0.05	Monoculture of *P. fluorescens*	98	10	[66]
Rhamnolipids	Polycyclic aromatic hydrocarbons	12.85 g/kg of soil	Alfalfa, arbuscular mycorrhiza consortium	61	90	[67]
Monorhamnolipids	Phenol	0.5	Monoculture of *C. tropicalis*	99	30 hours	[68]
Rhamnolipids	Petroleum hydrocarbons	0.823	Autochthonous marine microbiota, biogenic elements	59	5	[69]
Rhamnolipids	Petroleum hydrocarbons (C_{19}–C_{34} alkanes)	5	Autochthonous marine microbiota	96	18	[70]
Rhamnolipids	Phenantrhrene	500 mg/kg of soil	Monoculture of *Pseudomonas putida* ATCC 17484	91	10	[71]
Rhamnolipids	Phenantrhrene	200 mg/kg of soil	Monoculture of *Sphingomonas* sp.	47	70	[72]
Rhamnolipids	Mixture of diesel and biodiesel	15	Consortium	77	7	[73, 74]
Sophorolipids	Mixture of hydrocarbons	6 mg/g of soil	Autochthonous soil microbiota	97	6	[38]
Not identified	Oil sludge	–	Mixture of bacterial cultures	91% (linear hydrocarbons) and 52% (aromatic hydrocarbons)	40	[75]
Not identified	Hydrocarbons of diesel lubricants	450 mg/l	Autochthonous soil microbiota	77	15	[76]
Not identified	Pyrene	–	Monoculture of *B. subtilis* and *P. aeruginosa*	32–48	4	[77]

Note: «–» — data not shown.

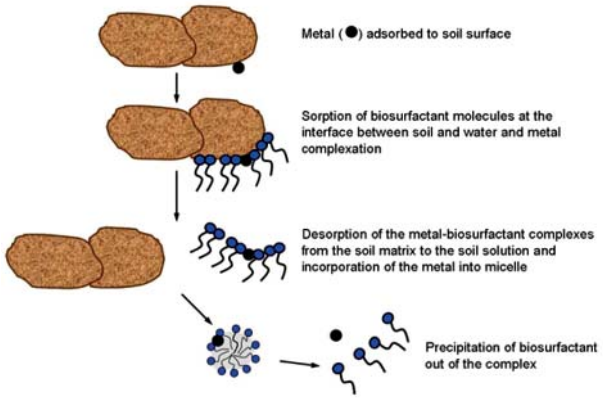

Fig. 1. **Mechanism of metal purification from soil using surfactants [83]**

Liu et al. [86] found that soil washing with dirhamnolipids (0.02 M) accompanied by the removal of 15.35% Cu^{2+} and 14.42% Pb^{2+}. With the presence of saponins (3 g/l) and biodegradable S, S-ethylenediamine acid (10 mM) it was possible to remove 99.8% Pb^{2+}, 85.7% Cu^{2+} and 45.7% PCBs [87].

The efficiency of extracting metals from contaminated soil essentially depends on further introduced substances. Thus, the number of copper and nickel cations, which were removed by rhamnolipids, was significantly increased in the presence of 1% NaOH [88]. It is believed that the addition of alkali results in dissolution of the organic fractions, which contain metals in bound form. As a result, cations become more available.

Wang and Mulligan [89] compared the efficiency of surfactants as foam and supernatant (traditional method) to remove Cd^{2+} and Ni^{2+} from the soil. The authors found that the use of surfactants as foam allows to remove 73.2% and 68.1% of Cd^{2+} and Ni^{2+} respectively, while the traditional way — only 61.7% and 51%. Similar results were described in [90]; it was shown the possibility of using saponins from soapberry and surfactant from *B. subtilis* (BBK006) as a foam to remove copper (6511 mg/kg), lead (4955 mg/kg) and zinc (15,090 mg/kg) from the soil. It was established that due to surfactant addition, the degree of removal of Pb^{2+}, Cu^{2+} and Zn^{2+} from soil after 48 hours was 1–2, 16–17 and 21–24% respectively. After saponin treatment (0.15 g/l), it was removed from the soil 98% Pb^{2+}, 95% Cu^{2+} and 56% Zn^{2+} at pH 4 and 30 °C for 72 hours.

Sorption of metals from soil greatly depends on its chemical composition. It is known that one of the components of sand soil is quartz, so in [90, 91] it was selected for the research. It was established that the use of 25 mM of rhamnolipids removed up to 91.6% of Cd^{2+} and 87.2% of Zn^{2+} from the quartz.

It was compared the efficiency of using sulfur bacteria *Acidithiobacillus* sp. and their mixture with the surfactant (producers: *B. subtilis* PCM 2021 and *B. cereus* PCM 2019) for the extraction of zinc (initial concentration in the mineral was 12471.4 mg/kg), copper (41237.3 mg/kg), lead (14129.9 mg/kg), nickel (13244.7 mg/kg) cadmium (1.9 mg/kg) and chromium (72.4 mg/kg) [92]. Using the second modified method (a mixture of bacteria and surfactants) was 2 times more effective at leaching chromium (23%). The degree of removal of zinc and cadmium was the same as in case of sulfur bacteria, and their mixture with surfactant, and was 48 and 93% respectively. Maximum figures of extraction of nickel and copper (48.5 and 53% respectively) were observed for the traditional method of using only bacteria.

The destruction of complex pollutants

It is known that the presence of heavy metals can decrease of effectiveness of oil degradation. That is why an important task is to find methods of environmental purification from such complex contaminants [93]. One way to reduce the toxic effects of metals on cell-destructors is their binding to calcium carbonate, phosphates, chelating agents, clay minerals, and surfactants [94–97].

Thus, it was described the possibility of using rhamnolipids, which bind metal in the complex and facilitate its removal using *Burkholderia* sp. through the release of lipopolysaccharide [98]. It was observed improved phenanthrene degradation by *P. aeruginosa* in the presence of cadmium cations with addition of rhamnolipids [99].

Singh et al. [100] showed that during the washing of soil contaminated with oil by the mixture of surfactant and phengicin from *B. subtilis* A21 it was possible to remove hydrocarbon 64.5% and 44.2% cadmium, cobalt 35.4%, 40.3% lead, 32.2% nickel, 26.2% copper and 32.07% zinc. Mechanisms of resistance to toxic metals, factors that affect the bioavailability of metals and

methods of determining the impact of metals on bioremediation processes of complex pollution as well as the methods of cleaning were thoroughly described in [93].

In addition to metal, the complex contamination may contain other compounds that are toxic to microorganisms-destructors [100]. Thus, Chrzanowski et al. [101] used rhamnolipids linking homologues of chlorophenols in micelles. It reduced the toxicity towards destructor *P. putida* DOT-T1E. Subsequently, the authors applied this technique to improve the cleaning of wastewater polluted with oil and chlorophenol by consortium of microorganisms [102]. Nievas et al. [103] investigated bilge water — complex waste like oil fuel, composed of *n*-alkanes and insoluble branched, cyclic and aromatic hydrocarbons. Introduction of consortium, which synthesizes an emulsifier, provided the degree of decomposition of waste equal 58–85%, depending on their nature and accessibility for destructors.

Our data indicate that the degradation of oil in water containing various concentrations of copper cations, by surfactants from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017 and *N. vaccinii* IMB B-7405 are significantly higher than for variants only with Cu^{2+} (Table 4).

Table 4. **Influence of copper cations on the oil destruction in water with the presence of cultural liquid of *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017 and *N. vaccinii* IMB B-7405**

Cu^{2+} concentration in water, mM	Oil destruction * (%) after treatment with surfactants from the strain		
	IMB B-7241	IMB Ac-5017	IMB B-7405
0	75.7	73.7	74.0
0.01	98.2	95.3	98.6
0.05	98.0	80.5	98.4
0.1	95.4	N.d.	98.4
0.5	90.8	N.d.	89.0
1.0	87.6	N.d.	N.d.

Note. Period of exposure is 20 days. Initial concentration of oil in water — 3.0 g/l. The degree of oil destruction in the water that is non-treated with surfactants and copper cations (control) — 2.5%. N.d. — not determined.

The next step examined the possibility of using the surfactant from the strain IMB B-7405 in a cultural liquid to clean the water containing oil and cations of several toxic metals (Table 5). It was established that the lowest efficiency degradation of oil (65%) was observed in the presence of cadmium and lead cations in oil-contaminated water. In the presence of copper cations in the mixture, the degree of oil decomposition in water was increased. We assume that one of the mechanisms that cause the increase of oil degradation in the presence of low concentrations of copper cations may be Cu^{2+} stimulation of alkan hydrolase activity (initial enzymes of hydrocarbon catabolism) of both: strain-producers of surfactant and natural oil-oxidizing microbiota.

Another mechanism underlying high efficiency of oil degradation by surfactant preparations from *N. vaccinii* IMB B-7405 in the presence of metal cations may be the presence of protective functions in surfactants. Our experiments have shown that the removal of surfactants accompanied by death of all cells of *N. vaccinii* IMB B-7405 in the presence of Cu^{2+}, Cd^{2+} and Pb^{2+} (0.05–0.5 mM), whereas in the surfactant presence under similar conditions up to 60–70% of cells were alive.

Table 5. **Influence of cultural liquid of *N. vaccinii* IMB B-7405 (10%, volume fraction) on the oil destruction (6.0 g/l) in water in the presence of Cu^{2+}, Cd^{2+} and Pb^{2+}**

Concentration of cations in water, mM			Oil destruction*,%	The number of cells*, CFU/ml
Cu^{2+}	Cd^{2+}	Pb^{2+}		
0	0	0	76	$(4.9 \pm 0.24)\cdot10^5$
0	0.5	0.5	65	$(2.1 \pm 0.10)\cdot10^5$
0.5	0	0.5	78	$(6.5 \pm 0.32)\cdot10^5$
0.5	0.5	0	81	$(7.1 \pm 0.35)\cdot10^5$
0.5	0.5	0.5	82	$(7.2 \pm 0.36)\cdot10^5$

Note. The number of cells in the original water (before introduction of oil, surfactants and metal cations, — control) was $(2.4 \pm 0.12)\cdot10^3$ CFU/ml. The degree of oil destruction in the water untreated with surfactants and metal cations (control) — 2.0%.

Period of exposure — 25 days.

In calculating the degree of oil degradation, the error does not exceed 5%.

Phytoremediation

Many current reviews generalized data on the use of phytoremediation to clean the environment [104–107]. It is believed that the interaction of "plant-microorganism" significantly affects the biotransformation of organic substances. As for microbial surfactants, the effectiveness of phytoremediation can be increased by adding surfactant-synthesizing microorganisms resistant to heavy metals. Thus, it was studied the effect of cadmium-resistant *Bacillus* sp. J119 on the purification of contaminated soil using canola, corn, Sudan grass and tomatoes [108]. It was established that the studied strain colonized roots of all plants, but increased synthesis of biomass and uptake of cadmium was observed only for tomato. The authors concluded that the efficiency of phytoremediation depends on the type of plant and microorganism. Details of particular use of surfactant producers in phytoremediation were described in [109]. It is believed that the most promising for phytoremediation are microorganisms capable of synthesis of surfactant, resistant to metals, as well as those that have been isolated from the rhizosphere of plants [110]. In a series of their investigations, Becerra-Castro et al. [111, 112] reported on the isolation of the metal-resistant microorganisms from the roots of *Alyssum serpyllifolium*, and 15–20% of them were able to synthesize surfactants.

Limitations and prospects of microbial surfactant using

Most current reviews and experimental papers highlight the positive effects of using microbial surfactants for bioremediation processes, but the critical highlight certain negative aspects of their use will allow us to employ such biotechnology in practice.

The reason of the limited use of microbial surfactants in environmental technologies is that most studies carried out under laboratory conditions, which allow only demonstrate the applicability of certain fundamental approaches [3].

It has to be noted that surfactants using is not always accompanied with enhancing efficiency of remediation: in some cases, positive effects of surfactants were not observed [113, 114]. In addition, the range of studied surfactants is very limited according to their chemical nature and mainly represented by rhamnolipids (Table 3).

The impact of the surfactant concentrations on bioremediation processes is still insufficiently studied. Most scientists believe that microbial surfactants are non-toxic [95, 96], but on the other hand, they are able to detect antimicrobial properties [115]. In addition, in some cases, the high concentration of surfactant can reduce the speed of the cleaning process, adversely affecting the microorganisms-destructors.

Thus, Whang et al. [116] noted that surfactant at a concentration of 40 mg/l had negative influence on soil remediation of diesel fuel, and at the concentration of 400 mg/l completely inhibited it.

Another important issue of surfactant use in environmental technologies is their biodegradation. On the one hand, this property of microbial surfactants is their advantage over synthetic analogues [95, 96], but on the other — they can be decomposed in the environment quicker than reveal their effect.

Thus, Lin et al. [117] reported that the effectiveness of the remediation significantly increased at the beginning of the experiment, after surfactant introduction, but at the end, results were the same as the controls (without surfactants). It was described phenomenon when rhamnolipids were decomposed faster than diesel fuel, and cleaning did not occur [62, 118].

The scientists associated the solution of this problem with the use of destructors that are not able to use surfactants as a source of carbon [119–121].

In most studies, the scientists use a monoculture of microorganisms-destructors, but more attention is paid to consortia [122]. This is due to the fact that the survival of microorganisms in the composition of consortia is higher compared to monocultures [123]. It was shown that using of two consortia (composed of the genera *Pseudomonas* and *Bacillus*), which form the surfactant, the degree of decomposition of oil in the ground (2%) at the roots of wheat (*Triticum aestivum*) and mustard (*Brassica juncea*) was rather high and amounted to 73–75% after 150–180 days [124].

Applying consortia in the purification process usually involves bioaugmentation but it depends on many factors: the compatibility of used strains, the ability of strains to synthesize surfactants, etc. In some cases, the addition of the described microorganisms-producers of surfactants had no effect on the cleaning process [125], and competition of few strains for carbon source led to antagonism.

On the other hand, surfactants can contribute to the interaction of microorga-

Table 6. **Stages of development of surfactant biotechnology for environment cleaning [3]**

Design step	Relevant step	Criteria
1	2	3
I. Initial characterization of the polluted area	1. Initial recognition of pollutants	Establishment of either single or multi-contaminant type pollution
	2. Assessment of the target pollutants concentration range	Determination of readily bioavailable, potentially bioavailable and unavailable pollutant fractions
	3. Analysis of relevant environmental factors	Range of temperature, pH, redox potential, moiety, soil properties, etc.
	4. Evaluation of nutrient levels	Potential limitation due to insufficient microelements, electron acceptors, etc.
	5. Analysis of autochthonous microflora	Screening for native microbial consortia with the ability to either remove or mobilize the pollutant by producing biosurfactants
II. Laboratory scale experiments	1. Selection of appropriate bioremediators for conducting the bioremediation process	Microorganisms or plants which exhibit high tolerance toward target pollutants and distinct remediation potential (relevant catabolic genes, hyperaccumulative properties, etc.)
	2. Selection of additional amendments	Nutrients, co-inoculants, plant growth promoting microorganisms, arbuscular mycorrhizal fungi, etc.
A. The economic rationale for the addition of exogenous microbial surfactants (*ex situ* method)	1. Selection of a biosurfactant and biosurfactant-producing microorganisms	Previous studies related to the topic or the native habitat of biosurfactant-producing microorganisms
	2. Assessment of potential biosurfactant-induced toxicity	EC50 values for relevant bioremediators towards biosurfactant only as well as biosurfactant-pollutant combinations; Analysis of microbial community dynamics as a response to the presence of biosurfactants
	3. Evaluation of efficiency for biosurfactant-amended remediation	Increase in pollutant bioavailability, increased removal rate, short-term stimulation, enhanced biomass growth for the bioremediator
A. The economic rationale for the addition of exogenous microbial surfactants (*ex situ* method)	4. Determination of biosurfactant degradability	Determination of the period needed for biostimulation of autochthonous microbiota, and the period of surfactant action before its biodegradation
	5. Establishment of an optimal biosurfactant production method	Cheaper technologies for synthesis of surfactants using waste, the definition of necessity for purification surfactant preparations
B. The economic rationale for the stimulation of surfactant synthesis by autochthonous microbiota (*in situ* method).	1. Selection of appropriate biosurfactant-producers	The choice of destructors from native microbiota that are capable of synthesis of surfactant (native soil microbiota, marine microbiota and rhizobacteria). Using recombinant microorganisms capable of synthesis of surfactants or microbial consortia with high potential of bioaugmentation (relationship between consortium representatives and autochthonous microorganisms)
	2. Evaluation of biocompatibility between biosurfactant producers and the biofactor relevant for the treatment process	The absence of antagonistic relations, simultaneous growth, increasing bioavailability of pollutants, etc.
	3. Selection of an introduction method	Introduction of culture fluid or immobilized cells of surfactant producer
	4. Initial bioaugmentation tests	Absence of negative impact of the producer of surfactants on the balance in the ecosystem and on the native microbiota

Table 6. End

1	2	3
	5. Long-term ability to produce biosurfactants	Definition of indicators of surfactant synthesis after introduction, identification of genes responsible for synthesis of surfactants (after a certain period of time)
III. Field scale feasibility study	1. Environmental response towards biosurfactants or biosurfactant-producers	Influence of surfactants and their producers to the microbial balance in the population; surfactant toxicity towards native microbiota, adaptability and survival of the surfactant producer after surfactant introduction, etc.
	2. Efficiency of treatment	Short-term and long-term removal of target pollutants in biosurfactant-amended treatment compared to control; duration
	3. Evaluation of treatment feasibility	Justification of each treatment step; Potential Evaluation of treatment feasibility efficiency enhancement vs. additional costs associated with biosurfactant-supplementation

nisms under natural conditions, enhancing the process of xenobiotic degradation (e.g. polyaromatic hydrocarbons). More about this interaction is described in review [113].

It was shown that in case of rhamnolipid introduction, the degree of decomposition of oil containing long-chain *n*-alkanes and polyaromatic hydrocarbons (PAHs) with a complex structure, by consortium increased by 5.63% [126]. There were no positive effects of surfactants on the efficiency of degradation of oil hydrocarbons in the presence of alkanes with short chains and simple PAHs.

In addition to the introduction of consortia in polluted environment, there is an interesting approach, which involves the selection of autochthonous microbiota and creating recombinant surfactant producers by genetic engineering, followed by the introduction of microorganisms in the ecosystem. However, it is unlikely.

Henry et al. [127] proposed an approach to biostimulation, which means the surfactant introduction in encapsulated form for phenanthrene degradation, but these drugs were less effective than usual.

Concomitant use of bioaugmentation and biostimulation was described in [128, 129]. Ángeles et al. [130] studied the decomposition of oil (32 mg/kg) in sterile and non-sterile soil in case of the introduction of *P. putida* CB-100, capable of synthesis of rhamnolipids. It was found that the introduction of CB-100 strain led to maximum oil degradation in non-sterile soil (61.1%) in the presence of nutrients.

Bioaugmentation was also more effective compared with biostimulation in field studies. Szulc et al. [131] studied the role of rhamnolipids in the degradation of diesel fuel in the soil during 365 days by a consortium consisting of *Aeromonas hydrophila*, *Alcaligenes xylosoxidans*, *Gordonia* sp., *Pseudomonas fluorescens*, *P. putida*, *Rhodococcus equi*, *Stenotrophomonas maltophilia*, *Xanthomonas*.

Llad et al. [132] compared different approaches to improve the efficiency of bioremediation: introduction of mineral substances, rhamnolipids, consortium, destructor fungus *Trametes versicolor*. It was established that the maximum degree of degradation of hydrocarbons in the soil (50%) was noted after 200 days by adding *T. versicolor,* which intensified autochthonous microbiota presented by Gram-positive bacterial groups of *Firmicutes* and *Actinobacteria*.

It should be noted that some authors consider activating autochthonous microbiota ineffective and lengthy process [133].

Efficiency of ecosystem cleaning often depends on the concentration of the limiting factors, including nitrogen, phosphorus and trace elements. It was shown that using mixed cultures, rhamnolipids and additional introduction of nutrients, scientists could reach the degree of decomposition of oil sludge equal 98% for 8 weeks. If nutrients were not used, this figure was only 52% [134]. Similar results were obtained in the case of *Lactobacillus delbrueckii* [25, 135].

The important problem of remediation is definition of final products of oil degradation. Some authors used *Pseudomonas* sp., which synthesized surfactants for oil destruction in marine environment (10% volume fraction)

using GC-MS analysis for the definition of the final products of oil degradation [136]. The schematic representation of possible degradation pathway of crude oil is given at Fig.2.

As for phytoremediation, it remains little known surfactant impact on the plants. In [137] the authors noted that to remove copper, cadmium and lead, the combined use of *Lolium perenne*, amino poly carboxylic acid, rhamnolipids and citric acid was quite effective, but surfactants enhanced phytotoxicity. Research of Marecik et al. [138] confirmed that rhamnolipids highly specifically act on plants, reducing the growth of biomass. Some authors [139] showed that to remove cadmium, phytotoxicity and efficiency of rhamnolipids greatly depend on their concentration. In particular, the concentration of more than 4.4 mmol/kg of surfactants adversely affect the corn and sunflower used in the purification process; and the concentration of 0.02–1.4 mg/kg were ineffective because of sorption in soil matrix.

On the other hand, the success of surfactant using in environmental technologies depends on many factors: pollutant nature, peculiarity of surfactants interaction with the microorganism-destructor or autochthonous microbiota, biodegradation, surfactant toxicity etc. Stages of development of surfactant biotechnology for environment cleaning are presented in Table 6 [3, 140–142].

So, surfactants of different chemical nature and their producers can be used in the processes of soil and water remediation from polyaromatic carbohydrates, petrol carbohydrates, diesel fuel and toxic heavy metals and complex contamination (carbohydrates and metals). Development of effective technologies using microbial surfactants should include the following steps: monitoring of contaminated sites, definition of the nature of pollution and analysis of the autochthonous microbiota; the choice of the microorganism-destructor (according to bioaugmentation process) compatible with natural microbiota; determining the mode of surfactant introduction (exogenous addition of stimulation of surfactant synthesis by autochthonous microbiota); establishing an optimal concentration of surfactant to prevent exhibition of antimicrobial properties and rapid biodegradation; research both in laboratory and field conditions.

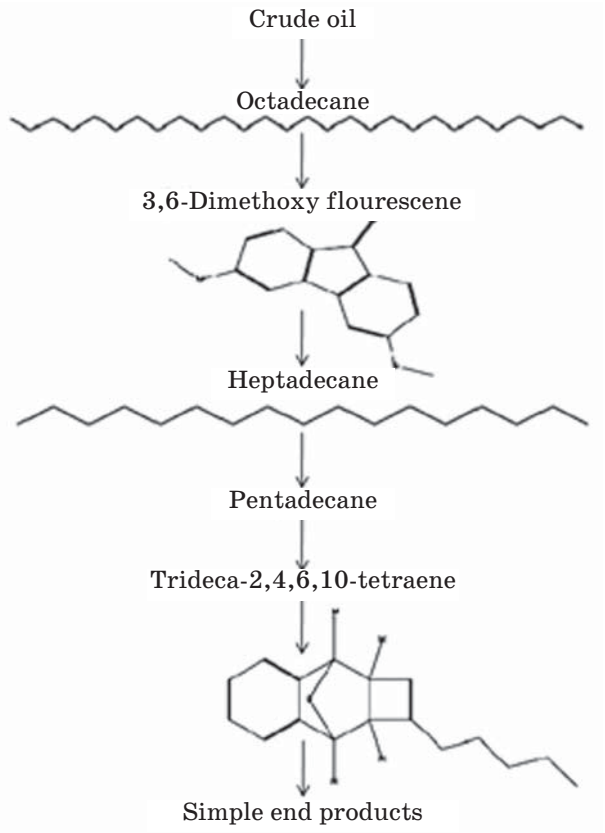

Fig. 2. **Degradation pathway of crude oil till simple end products [136]**

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МІКРОБНІ ПОВЕРХНЕВО-АКТИВНІ РЕЧОВИНИ У ПРИРОДООХОРОННИХ ТЕХНОЛОГІЯХ

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В огляді наведено дані літератури та власних експериментальних досліджень авторів щодо використання мікробних гліколіпідів (рамно-, софоро- і трегалозоліпідів) та ліпопептидів для очищення води і ґрунту від нафти та інших вуглеводнів, вилучення токсичних важких металів (Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}), деструкції комплексних забруднень (нафта й інші вуглеводні з важкими металами), а також висвітлено роль мікробних поверхнево-активних речовин у процесах фіторемедіації.

Обговорюються чинники, що обмежують використання мікробних поверхнево-активних речовин у природоохоронних технологіях. За певних концентрацій поверхнево-активні речовини можуть виявляти антимікробні властивості та інгібувати мікроорганізми-деструктори ксенобіотиків. Біодеградабельність таких речовин може також знижувати ефективність біоремедіації.

Розроблення ефективної технології з використанням мікробних поверхнево-активних речовин має включати такі етапи: моніторинг забрудненої ділянки, з'ясування характеру забруднення і аналіз автохтонної мікробіоти; визначення способу введення поверхнево-активних речовин (екзогенне внесення, стимуляція їх синтезу автохтонною мікробіотою); встановлення оптимальної концентрації для уникнення вияву антимікробних властивостей, а також швидкої біодеградації; проведення досліджень як у лабораторних, так і в польових умовах.

Ключові слова: мікробні поверхнево-активні речовини, біоремедіація.

МИКРОБНЫЕ ПОВЕРХНОСТНО-АКТИВНЫЕ ВЕЩЕСТВА В ПРИРОДООХРАННЫХ ТЕХНОЛОГИЯХ

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Представлены данные литературы и собственных экспериментальных исследований авторов о применении микробных поверхностно-активных гликолипидов (рамно-, софоро- и трегалозолипидов) и липопептидов для очистки воды и почвы от нефти и других углеводородов, удаления токсичных тяжелых металлов (Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}), деструкции комплексных загрязнений (нефть и другие углеводороды с тяжелыми металлами), а также освещена роль микробных поверхностно-активных веществ в процессах фиторемедиации.

Обсуждаются факторы, ограничивающие использование микробных поверхностно-активных веществ в природоохранных технологиях. При определенных концентрациях поверхностно-активные вещества могут проявлять антимикробные свойства и ингибировать микроорганизмы-деструкторы ксенобиотиков. Биодеградабельность сурфактантов микроорганизмами также может снижать эффективность биоремедиации.

Разработка эффективной технологии с использованием микробных поверхностно-активных веществ должна включать следующие этапы: мониторинг загрязненного участка, определение характера загрязнения и анализ автохтонной микробиоты; определение способа введения поверхностно-активных веществ (экзогенное внесение, стимуляция их синтеза автохтонной микробиотой); установление оптимальной концентрации для предотвращения проявления антимикробных свойств, а также быстрой биодеградации; проведение исследований как в лабораторных, так и в полевых условиях.

Ключевые слова: микробные поверхностно-активные вещества, биоремедиация.