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INDUSTRIAL WASTE BIOCONVERSION INTO SURFACTANTS BY *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 AND *Nocardia vaccinii* IMV B-7405

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The aim of the work is to realize an alternative processing of toxic industrial waste into surfactants by strains *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 for remediation of environment.

The studied strains were grown in liquid media containing such sources of carbon as waste (fried) sunflower oil, technical glycerol (by-product of biodiesel production), and aromatic compounds. The synthesis of surfactants was evaluated by emulsification index, conditional concentration of surfactants and concentration of extracellular surfactants, which was determined gravimetrically after their extraction from supernatant by the mixture of methanol and chloroform. The concentration of oil in water and soil was analyzed by gravimetric method after extraction with hexane.

It was shown that with increasing concentration of the inoculum up to 10–15% and two times increase of nitrogen source content in medium containing 7–8% (v/v) of crude glycerol, concentration of surfactants synthesized by *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 was 3.4; 5.0 and 5.3 g/l, respectively, that is 1.6–1.7 times higher as compared with values on basal medium with the same content of substrate. The maximum concentration (3.9–4.3 g/l) of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on fried sunflower oil (4%) was achieved by using the inoculum grown on refined oil. The ability of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 to decompose aromatic compounds (phenol, naphthalene, toluene, hexachlorobenzene, benzoic and N-phenylanthranilic acid) with simultaneous synthesis of extracellular metabolites with surface-active and emulsifying properties was established. In the presence of surfactants in the form of culture liquid (5–10%), the degree of degradation of complex oil with heavy metal (Cu^{2+}, Cd^{2+}, Pb^{2+}, 0.01–0.5 mmol) pollution in water (3–6 g/l) and soil (20 g/kg) after 20 days was 82–92%.

Bioconversion of industrial waste into surfactants for environmental technology allows recycling toxic waste, reducing costs of microbial surfactants and provides double effect of environmental purification, which is achieved in the production and use of microbial surfactants.

Key words: industrial waste, microbial surfactants, environment remediation.

Until recently there has been no doubt that the environment, air, land and water, always effectively "recycle" domestic, industrial and agricultural waste. Now we know that it is not so. Humanity faces two fundamental problems: management of waste which are constantly generated in huge quantities, and degradation of toxic compounds accumulated for decades in landfills, water and soil. It should be noted that the danger lies not only in waste containing toxic substances (such as phenol and its derivatives) but also in waste that enter the environment in uncontrolled quantities, such as oil containing (waste from oil and fat production, fried oil used in catering, etc.).

Biofuel, including biodiesel, is one of the most promising substitutes for fossil fuels. In the last decade, biodiesel production increased significantly [1, 2]. For example, the expected

annual increase in biodiesel production is 8–10% [2]. However, due to rapidly growing demand for biodiesel, there is a problem of recycling its byproduct, glycerol. This glycerol fraction contains a wide variety of impurities, making impossible its use in many traditional areas of application, and storage, disposing of it is a serious environmental problem because of its increased alkalinity and the content of methanol [1, 2].

As a result of increased industrial activity, mono- and polyaromatic hydrocarbons and their derivatives which are toxic, carcinogenic and resistant to external factors more and more pollute the environment that is a significant danger to human health and the biosphere as a whole [3–5]. At the turn of XX–XXI centuries, biotechnological methods of environmental remediation from aromatic compounds attract the interest due to their safety, low cost and high capacity of bacterial destructors [6, 7].

Progressive rates of oil pollution necessitate the development of environmentally friendly and economically reasonable purification methods aimed at intensifying processes of hydrocarbons decomposition. Such a method of soil and water treatment is bioremediation, based on natural potential of microorganisms. It is known that in presence of heavy metals, efficiency of oil degradation can be reduced, so today it is important to find environmental remediation methods for such complex environmental pollution [8].

In recent decades, microbial surfactants (MS) have become the subject of intense theoretical and applied researches driven by their possible practical use in various industries, as well as nature conservation technologies to clean the environment [9–11].

In our previous studies from oil-contaminated soil samples we isolated strains of oil-oxidizing bacteria identified as *Rhodococcus erythropolis* EK-1, *Acinetobacter calcoaceticus* K-4, and *Nocardia vaccinii* K-8 [12]. The strains are registered in Depository of microorganisms of Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the numbers IMV Ac-5017, IMV B-7241, and IMV B-7405 respectively. The strains *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 have been shown to synthesize metabolites with surface-active and emulsifying properties when grown on hydrophilic (glucose, ethanol, glycerol) and hydrophobic (liquid paraffins, *n*-hexadecane) substrates [13].

The purpose of this work is to realize alternative processing of toxic industrial waste into surfactants by *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 for environmental bioremediation.

Materials and Methods

Study objects. Study objects were strains of oil-oxidizing bacteria isolated from oil-contaminated soil and identified as *Rhodococcus erythropolis* EK-1, *Acinetobacter calcoaceticus* K-4, and *Nocardia vaccinii* K-8 [12]. The strains are registered in Depository of microorganisms of Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the numbers IMV Ac-5017, IMV B-7241, and IMV B-7405 respectively.

Culture medium content and cultivation conditions. R. erythropolis IMV Ac-5017 was grown in liquid mineral medium (g/l): $NaNO_3$ — 1.3, $MgSO_4 \cdot 7H_2O$ — 0.1, NaCl — 1.0, Na_2HPO_4 — 0.6, KH_2PO_4 — 0.14, $FeSO_4 \cdot 7H_2O$ — 0.001, pH 6.8–7.0.

For cultivation of *A. calcoaceticus* IMV B-7241, the following medium was used (g/l): $(NH_2)_2CO$ — 0.35, $MgSO_4 \cdot 7H_2O$ — 0.1, NaCl — 1.0, Na_2HPO_4 — 0.6, KH_2PO_4 — 0.14, pH 6.8–7.0. Also to the medium were added yeast autolysate — 0.5 (v/v) and micronutrients solution — 0.1 (v/v).

N. vaccinii IMV B-7405 strain was grown in liquid mineral medium (g/l): $NaNO_3$ — 0.5, $MgSO_4 \cdot 7H_2O$ — 0.1, $CaCl_2 \cdot 2H_2O$ — 0.1, KH_2PO_4 — 0.1, $FeSO_4 \cdot 7H_2O$ — 0.001, yeast autolysate — 0.5% (v/v).

In one version of the experiment, the concentration of nitrogen nutrition source in the culture medium of studied strains was increased twice.

As the source of carbon and energy we used refined sunflower oil "Oleyna" (Dnepropetrovsk oil extraction plant), waste oil after frying potatoes and meat (the McDonald's network of fast food restaurants, Kyiv), and unrefined sunflower oil (4%, v/v).

Also as a source of carbon and energy, refined (> 99.5%) glycerol was used in concentration 1.0–1.5% (v/v). To modify the average composition of technical glycerol, NaCl or KCl at concentration of 2.5%, and 0.3% methanol or ethanol (v/v) were added into a mineral medium with refined glycerol. This substrate is hereinafter called "modified glycerol". Also as a source of carbon and energy we used technical glycerol which is a

waste of biodiesel production (Zaporizhzhia biofuel plant, Zaporizhzhia, Ukraine). Concentration of technical glycerol in culture medium was 2–10% (v/v). When using technical glycerol as substrate, its content in the medium was calculated as equimolar by carbon concentration to the purified glycerol, taking into account the average content in the glycerol fraction (70%).

As the sole source of carbon and energy in a medium, phenol, hexachlorobenzene, naphthalene, benzoic, sulfanilic and N-fenylantranilic acids were also used at a concentration of 0.3–1.5% (w/w), and benzene and toluene at a concentration of 0.3–1.5% (v/v). Phenol and sulfanilic acid were dissolved in distilled water and sterilized in autoclave for 40 min at 120 °C, and weights of hexachlorobenzene, naphthalene, benzoic and N-fenylantranilic acids were sterilized with UV light for 30 min.

As inoculum we used the cultures from the exponential growth phase, grown on the respective liquid medium containing 0.5–1% (v/v) of substrate. The quantity of inoculation material (10^4–10^5 cells/ml) was 5–10% of the volume of the culture medium. The bacteria were cultivated in 750 ml flasks containing 100 ml of medium on the shaker (320 oscillations per min) at 28–30 °C for 120 h.

Indexes of growth and surfactants synthesis. Biomass was determined by optical density of the cell suspension with the following determination of dry biomass by calibration curve.

The ability to synthesize MS was evaluated on the following parameters: surface tension (σ_s) of the cell-less culture liquid, measured with semi-automatic tensiometer (LAUDA TD1C, Germany); conditional surfactant concentration (MS*, dimensionless); the amount of extracellular synthesized surfactants (g/l), and emulsification index (E_{24}).

For rapid determination of the quantitative content of surfactants in the culture liquid the index of conditional MS concentration (MS*) was used, defined as the dilution rate of cell-less culture liquid (supernatant) to the point of CMC (critical micelle concentration). Then, plot of surface tension σ_s against the logarithm of supernatant dilution was made. Abscissa of the inflection point of the curve corresponds to the MS*.

The amount of extracellular synthesized MS (g/l) was determined gravimetrically after extraction from supernatant of culture liquid using modified Folch mixture. To obtain supernatant, culture liquid was centrifuged at 5,000 g for 20 min. Isolation of extracellular MS was performed as described below.

In a cylindrical separatory funnel (500 ml), 100 ml of the supernatant and 20 ml of 1 M HCl solution were added, funnel was then closed with polished stopper and shaken for 3 min, then another 15 ml of 1 M HCl solution and 65 ml of chloroform and methanol (2:1) mixture were added and stirred for 5 min (lipid extraction). The extracted liquid was left in separating funnel to separate phases and subsequently the lower fraction (organic extract 1) was removed and the water phase was reextracted. The second extraction was carried out adding 35 ml of 1 M HCl solution and 65 ml of chloroform and methanol (2:1) mixture to the water phase, extracting lipids for 5 min. After phase separation, the lower fraction was isolated as organic extract 2. At the third cycle of extraction, 100 ml of chloroform and methanol (2:1) mixture was added to the water phase, and organic extract 3 was obtained as described above. Then extracts 1–3 were combined and evaporated on a rotary evaporator IP-1M2 (Russia) at 50 °C and 0.4 bar absolute pressure to constant weight.

Emulsification index for the 50-fold diluted culture liquid was determined as follows: to 2 ml of culture liquid diluted with distilled water, 2 ml sunflower oil (a substrate for emulsification) were added and shaken for 2 min. Definition of emulsification index (E_{24}) was carried out after 24 h, as the ratio of height of emulsion layer to the total height of liquid in a test tube, expressed as a percentage.

Study of oil biodegradation in water and soil. To simulate soil contamination with oil and metal cations, 1 kg of soil, 25 ml oil, surfactant preparations as post-fermented culture liquid (100–200 ml), and 0.01% diammonium phosphate as a source of nutrients were placed in a plastic container and mixed. In simulation of complex pollution with oil and metal cations, such substances were added to soil (singly or combined): 0.01–0.1 mmol Cu^{2+}, Cd^{2+}, Pb^{2+} as 1M solutions of $CuSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 8H_2O$, and $Pb(CH_3COOH)_4$ salts respectively. Samples were stirred every 3 days to improve aeration, and were moisturized with sterile water. The duration of the experiment was 20 days.

To simulate water contamination with oil and metals, 2 l of pumped water covered with 6–15 ml of oil in a plastic container were treated with surfactant preparations in concentration of 5–10% (v/v) and 0.01–0.5 mmol Cu^{2+}, Cd^{2+}, and Pb^{2+} separately and in various combinations. Diammonium phosphate

(0.01%) was used as a source of nutrients. During the experiment (20 days) total viable cell counts in pumped water were performed by Koch method on MPA.

The amount of oil was evaluated gravimetrically. Oil extraction with hexane (1:1) was performed 3 times. The organic extract was evaporated to constant weight on a rotary evaporator IP-1M2 (Russia) at 55 °C and 0.4 bar absolute pressure.

All experiments were thrice replicated; the number of parallel determinations in the experiments was 3 to 5. The statistical treatment of the experimental data was carried out as described previously [6, 16]. The differences between the means were considered significant at $P < 0.05$.

Results and Discussion

Surfactants synthesis on biodiesel waste. At the first phase we studied the influence of technical glycerol components (methanol, ethanol, potassium and sodium) on surfactants synthesis by strains *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017, and *N. vaccinii* IMV B-7405, and the possibility of using biodiesel waste for the synthesis of surfactants.

The study showed that in medium containing pure glycerol (1%, v/v) and glycerol fraction components (K and Na salts — 2.5%, methanol and ethanol — 0.3%) the conditional concentration of MS increased by 11–68% for *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017, and *N. vaccinii* IMV B-7405 compared to the values on medium without these salts and alcohols. On medium with technical glycerol (2.2%) obtained directly from the producer of biodiesel (Zaporizhzhia biofuel plant), strains synthesized extracellular MS with concentration twice higher than that for the purified substrate (Table 1). Note that the used concentrations of modified and technical glycerol were equimolar by carbon to 1% purified glycerol, and that modified glycerol was obtained by adding 2.5% NaCl and 0.3% methanol to purified substrate.

Taking into account the volume of biodiesel production in the world, and the amounts of technical glycerol obtained as a byproduct [1, 2], it is clear that the effective use of this waste as a substrate in biotechnological processes needs as high as possible its content in the culture medium for producers of economically valuable microbial metabolites.

Thus, our next step was to find the cultivation conditions for *R. erythropolis* IMVAc-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 in medium with the highest possible concentration of technical glycerol that ensures high rates of surfactants synthesis.

Increased concentrations of inoculum to 10–15% and twice increased (compared to the basal medium) of nitrogen source content allowed to carry out the processes of synthesis of surfactants by strains IMV Ac-5017, IMV B-7241 and IMV B-7405 on medium with 7–8% technical glycerol (v/v). Under these conditions, the concentration of extracellular MS synthesized by studied strains amounted to 3.4–5.3 g/l, which is 1.4–3,0-fold higher than at the basal medium with same concentration of substrate (Table 2).

Published data indicate that concentrations of MS synthesized by different producers on technical glycerol usually are low [14–18]. For example, the strain of *Bacillus subtilis* LSFM-05 in the culture medium with 5% technical glycerol synthesized 1.36 g/l of surfactin [14]. *P. aeruginosa* MSIC02 when grown on the medium with pre-hydrolyzed (treatment

Table 1. **Synthesis of extracellular MS by IMB B-7405, IMB Ac-5017 i IMB B-7241 grown on medium with varying glycerol types**

Strain	MS (g/l) if grown on glycerol		
	purified	modified	technical
IMV B-7405	1.8±0.09	2.5±0.12*	3.5±0.18*
IMV Ac-5017	0.5±0.03	0.7±0.03*	1.0±0.05*
IMV B-7241	2.4±0.12	3.2±0.16*	4.7±0.23*

Note. * — $P \leq 0.05$ compared to control (concentration of MS produced by strains grown on purified glycerol).

Table 2. **The influence of the nitrogen source concentration in the medium with technical glycerol on MS synthesis by IMV B-7241, IMV Ac-5017, and IMV B-7405**

Strain	Concentration of glycerol, %	Nitrogen source concentration, g/l	MS, g/l
R. erythropolis IMV Ac-5017	6	1.3	2.4±0.12
		2.6	2.9±0.14*
	7	1.3	1.7±0.08
		2.6	2.9±0.14*
	8	1.3	1.5±0.07
		2.6	3.4±0.17*
A. calcoaceticus IMV B-7241	5	0.35	3.8±0.19
		0.7	4.6±0.23*
	6	0.35	3.9±0.19
		0.7	4.3±0.21*
	7	0.35	3.5±0.17
		0.7	5.0±0.25*
N. vaccinii IMV B-7405	6	0.5	2.9±0.14
		1.0	5.0±0.25*
	7	0.5	2.0±0.10
		1.0	5.0±0.25*
	8	0.5	1.8±0.09
		1.0	5.3±0.26*

Note. * — $P \leq 0.05$ compared to control (concentration of MS produced by strains IMV Ac-5017, IMV B-7241, and IMV B-7405 on basal medium containing 1.3, 0.35 and 0.5 g/l of nitrogen source respectively).

with sulfuric acid) technical glycerol (5% v/v) synthesized 1.27 g/l of rhamnolipidis, while on medium containing not-hydrolyzed substrate the synthesis level was several times lower [15].

The strain *Starmerella bombicola* ATCC 22214 synthesized up to 6.6 g/l of sophorolipids on medium containing 15% technical glycerol (v/v) and/or 10% sunflower oil (v/v) [16]. Higher concentrations (nearly 9 g/l of glycolipids) have been observed after cultivation of *Ustilago maydis* for 12 days on medium with 50 g/l of technical glycerol [17]. The authors upped the MS concentration to 32 g/l by adding some amino acids, B vitamins, ammonium citrate, mannose and erythritol (20 g/l) into the medium containing 50 g/l of technical glycerol [17]. In [18] it has been established that strain *P. aeruginosa* WAE synthesizes 2.6 g/l of MS if grown on biodiesel production waste.

Note that in most papers, concentration of technical glycerol in the culture medium for MS producers has been 5% (v/v) or 50 g/l [14–17]. In [17] the authors investigated the influence of higher concentrations of substrate on synthesis of glycolipids. However, increasing the concentration of technical glycerol to 80 g/l resulted in amount of synthetized glycolipids reduced twice.

Thus, the rate of MS synthesis by *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMB B-7405 on biodiesel production waste is not only comparable to that described in the literature, but also surpasses those for many well-known producers.

Fried sunflower oil as a substrate for surfactant synthesis. Table 3 shows the results of MS synthesis by *A. calcoaceticus* IMV B-7241 cultivation on various sunflower oil-containing substrates.

Experiments showed that use of the inoculum grown on molasses resulted in 1.7–2.7 times less amounts of MS if strain was cultivated on fried and unrefined sunflower oil compared with those on purified (refined) substrate (Table 3). At the same time, emulsification index of 50-fold diluted culture liquid changed but slightly. However, if in the inoculum preparing medium molasses was replaced with refined sunflower oil, increased

Table 3. **Synthesis of MS by IMV B-7241 cultured on medium with sunflower oil (4%)**

Carbon source in medium for inoculum	Sunflower oil for MS biosynthesis	MS, g/l	E_{24}, %
Molasses	Refined	4.0±0.20	56
	Unrefined	2.3±0.12*	50**
	Waste after frying potatoes	1.5±0.08*	49**
	Waste after frying meat	2.8±0.14*	54**
Refined sunflower oil	Refined	3.4±0.17	51
	Unrefined	3.3±0.16*	47**
	Waste after frying potatoes	3.9±0.19*	52**
	Waste after frying meat	4.3±0.21*	54**

Note. * — $P \leq 0.05$ compared to control (concentration of MS synthesized by strain IMV B-7241 on refined sunflower oil). ** — $P \leq 0.05$ compared to control (emulsification index for cultivation of strain IMV B-7241 on refined sunflower oil). The measurement error for emulsification index did not exceed 5%.

synthesis of microbial surfactants has been observed for fried and unrefined oil compared with the values for refined substrate. Note that using of inoculum grown on molasses and sunflower oil was not accompanied by significant changes in the emulsification index (Table 3).

In literature sources there is enough information on the use of oil-containing substrates for synthesis of microbial surfactants [19–23]. However, in most cases, producers of MS are cultivated mainly on refined plant oils or oil production wastes (sludge).

Much less researches are dedicated to synthesis of MS on fried oil. For example, *Pseudomonas fluorescence* MFS03 cultured on 2% fried plant oil synthesized 4.2 g/l of MS [20]. Cultivation of *P. aeruginosa* PB3A on the medium containing fried oil (1%) was accompanied by synthesis of 0.3–0.6 g/l of MS [21]. *P. aeruginosa* ATCC 9027 grown on over-fried sunflower oil (initial concentration of 15 ml/l, followed by introduction of 20 ml/l at 72nd hour of growth) produced rhamnolipids in concentration of 8.5 g/l [23].

Transformation of aromatic compounds in surfactants. Data on the synthesis of surfactants by *R. erythropolis* IMV Ac-5017 grown in medium with different concentrations (0.5–1.5%) of aromatic substrates is shown in Table 4.

The results showed that the strain IMV Ac-5017 is able to use phenol and toluene at concentration of 0.5% as sources of carbon and energy for biosynthesis of surfactants (conditional surfactant concentration 3.3 and 1.3, respectively). Higher concentrations of phenol and toluene appeared to be toxic to *R. erythropolis* IMV Ac-5017. Benzene and naphthalene even in low concentrations inhibited the biosynthesis of MS (MS* did not exceed 0.6). Given that the aromatic compounds in concentrations above 0.5% inhibited the synthesis of surfactants by strain IMB As-5017, in further studies *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMB B-7405 were grown in medium with lower concentrations of substrates (0.3–0.5%). Utilization of aromatics by *N. vaccinii* IMV B-7405 was accompanied by the formation of extracellular metabolites with surface-active and emulsifying properties. The maximum rates of MS synthesis (MS* 2.3–2.6% and E_{24} 70–75%) were observed when strain IMV B-7405 had been cultivated in medium containing 0.5% naphthalene, N-phenylantranilic acid and phenol.

The strain *A. calcoaceticus* IMV B-7241 is able to synthesize MS when grown on wider variety of aromatic substrates than the strains *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 (Table 5). The highest conditional concentration of MS and E_{24} (up to 75%) were observed after cultivation of strain IMV B-7241 on medium with 0.5% phenol and benzoic acid.

Analysis of literature [24–26] has showed that some microorganisms under the cultivation conditions on aromatic substances are able to synthesize metabolites with surface-active and emulsifying properties. For example, *Brevibacillus* sp. PDM-3 [24] and *Pseudomonas* sp. USTB-RU [25] produced surfactants on phenantrene, and a member of *Acinetobacter* genus (strain USTB-X)

Table 4. **Synthesis of surfactants by *R. erythropolis* IMV Ac-5017 on medium containing aromatic substances**

Substrate	Concentration,%	Conditional concentration of MS	E_{24},%
Phenol	0.5	3.3±0.16*	43**
	1.0	0.8±0.04*	56**
	1.5	0.3±0.01*	40**
Naphthalene	0.5	0.6±0.03*	48**
	1.0	0.3±0.01*	40**
	1.5	0.3±0.01*	40**
Benzene	0.5	0.5±0.02*	48**
	1.0	0.3±0.01*	45**
	1.5	0	0
Toluene	0.5	1.3±0.06*	40**
	1.0	0.3±0.01*	42**
	1.5	0	0
Hexadecane (control)	2.0	4.8±0.2	70

Note. * — $P \leq 0.05$ compared to control (conditional concentration of MS produced by strain IMV Ac-5017 on hexadecane). ** — $P \leq 005$ compared to control (emulsification index for strain IMV Ac-5017 on hexadecane). The measurement error for emulsification index did not exceed 5%.

Table 5. **Synthesis of surfactants by *A. calcoaceticus* IMV B-7241 grown in medium with aromatic substances**

Substrate	Concentration, %	Conditional concentration of MS	E_{24},%
Phenol	0.3	3.2±0.16*	65**
	0.5	3.6±0.18*	75**
Benzene	0.3	1.6±0.08*	50**
	0.5	1.5±0.08*	50**
Toluene	0.3	1.7±0.09*	55**
	0.5	1.2±0.06*	50**
Benzoic acid	0.3	2.1±0.10*	55**
	0.5	2.8±0.14*	52**
N-phenylanthranilic acid	0.3	1.9±0.09*	45**
	0.5	2.0±0.10*	50**
Naphthalene	0.3	1.1±0.05*	45**
	0.5	0	0
Sulfanilic acid	0.3	1.0±0.05*	40**
	0.5	0	0
Hexachlorobenzene	0.3	1.5±0.08*	45**
	0.5	1.7±0.09*	53**
Ethanol (control)	0.3	0.8±0.04*	40**
	0.5	1.0±0.05*	43**

Note. * — $P \leq 0.05$ compared to control (conditional concentration of MS produced by strain IMV B-7241 on ethanol). ** — $P \leq 0.05$ compared to control (emulsification index for strain IMV B-7241 on ethanol). The measurement error for emulsification index did not exceed 5%.

synthesized MS on pyrene [26]. In [27] it has been shown that cultivation the strain *P. aeruginosa* NY3 on medium with mixture of polycyclic aromatic compounds (5 ml/l fluorene, anthracene, phenanthrene, pyrene and fluoranthene) was accompanied by their decomposition by 10–20% within 24 h. The strain NY3 was shown to be able to synthesize rhamnolipids, although the authors studied the chemical composition of MS growing *P. aeruginosa* NY3 on glucose and glycerol [27].

The authors of [24–27] indicate that the ability to synthesize such extracellular metabolites greatly facilitates the assimilation of aromatic substrates by microorganisms.

Effect of surfactants on degradation of complex oil and heavy metal pollutions in water and soil. For now purifying of water and soil from oil is mostly done with biological products that are lyophilizated biomass (or paste) of oil-oxidizing bacteria [11]. However, microorganisms introduced into oil contaminated ecosystems need some time to adapt to new conditions. Thus it would be more effective (compared to bio-augmentation) to implement another purification method, biostimulation (involving introduction of various substances, nutrients etc., which stimulate autochthonous, natural microbiota). Effective stimulants of natural oil-oxidizing microbiota are microbial surfactants [4, 10, 11, 28, 29], and the best removal of hydrocarbons is achieved by the use of microorganisms able to assimilate the oil and simultaneously synthesize surfactants. Hence, as preparations for treatment of the oil pollution we used culture liquid containing the cells of oil-oxidizing bacteria and the MS they produced.

Table 6 shows data on destruction of complex pollution of oil and heavy metals in the presence of MS of *A. calcoaceticus* IMV B-7241. After culture liquid was added to water containing 3 g/l of oil and a mixture of cations of three heavy metals (Cu^{2+}, Cd^{2+}, and Pb^{2+}), the level of oil degradation was 90–92%, and in the case of increased concentration of oil in water (up to 6 g/l) the level of its degradation decreased slightly (to 85–88%). Intensification of oil degradation in the presence of surfactants is caused by the activation of natural oil-oxidizing microbiota that is evidenced by its increase in 100–1000 times by the end of the experiment.

Further experiments showed the possibility of using *R. erythropolis* IMB Ac-5017 the culture liquid for the destruction of complex with heavy metals oil pollutions in soil, at that copper cations stimulated the degradation of oil in the presence of surfactants (Table 7). Similar patterns were found during the study of the effect of *A. calcoaceticus* IMV B-7241 surfactants on the purification of oil and heavy metals contaminated water (Table 6).

Results given in Tables 6 and 7 support our previous findings about the role of MS of *N. vaccinii* IMV B-7405 in degradation of complex oil and heavy metal pollutions in water and soil [30]. Here it is also established that

Table 6. **Effect of culture liquid of *A. calcoaceticus* IMV B-7241 on oil degradation in water in the presence of Cu^{2+}, Cd^{2+} and Pb^{2+}**

Cation concentration in water, mmol			Level of oil degradation (%) at the initial concentration of (g/l)	
Cu^{2+}	Pb^{2+}	Cd^{2+}	3.0	6,0
0.5	0.1	0.1	92.7±4.6*	88.5±4.4**
0.5	0.1	0.5	92.0±4.6*	87.7±4.4**
0.5	0.5	0.1	90.2±4.5*	85.2±4.3**
0.5	0.5	0.5	90.0±4.5*	85.1±4.3**
0	0,5	0,5	65.5±3.2*	53.2±2.7**
0,5	0	0,5	93.6±4.7*	89.1±4.4**
0,5	0,5	0	85.1±4.3*	81.4±4.0**
0	0	0	76.2±3.8	69.4±3.5

Note. * — $P \leq 0.05$ compared to control (degradation of 3 g/l oil in water in the presence of MS without metal cations). ** — $P \leq 0.05$ compared to control (degradation of 6 g/l oil in water in the presence of MS without metal cations). Duration of experiment was 20 days.

Table 7. **Degradation of complex oil and heavy metal pollutions in soil (20 g/kg) in the presence of *R. erythropolis* IMV Ac-5017 culture liquid (5%, v/v)**

Concentration of cations in soil, mmol			Concentration of residual oil, g/kg	Oil degradation,%
Cu^{2+}	Cd^{2+}	Pb^{2+}		
0	0	0	11.8±0.59	41±2.0
0.1	0.01	0.01	6.6±0.33*	67±3.3**
0.1	0.01	0	2.0±0.10*	90±4.5**
0.1	0	0.01	1.6±0.08*	92±4.6**
0	0.01	0.01	13.4±0.67*	33±1.6**

Note. * — $P \leq 0.05$ compared to control (concentration of residual oil in soil in the presence of MS without metal cations); ** — $P \leq 0.05$ compared to control (oil degradation in soil in the presence of MS without metal cations).

copper cations show a stimulating effect on oil degradation.

We suppose that one of the mechanisms causing increased oil degradation in the presence of low concentrations of copper cations may be Cu^{2+} stimulating the activity of alkane hydroxylases (the first enzymes of hydrocarbon catabolism) of both MS-producing strains and natural oil-oxidizing microbiota.

Our results are consistent with published data on metagenomic analysis of oil-contaminated soil and water which has showed that after the oil pollution, an induction of AlkB genes, responsible for synthesis alkane hydroxylases, is observed in these ecosystems [31–33]. The literature [34] and our own results [35] indicate that alkane hydroxylases are activated by copper cations.

Thus, it is established the possibility of bioconversion of toxic industrial waste (technical glycerol, fried sunflower oil, aromatic compounds) into surfactants by strains *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405. An important advantage of microbial surfactants of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 is that they can be produced from industrial waste and subsequently used for the degradation of oil in water and soil, including in the presence of heavy toxic metals. Thus, during the production and use of these surfactants the effect of "double" environmental remediation is achieve.

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БІОКОНВЕРСІЯ ПРОМИСЛОВИХ ВІДХОДІВ У ПОВЕРХНЕВО-АКТИВНІ РЕЧОВИНИ ШТАМАМИ *Rhodococcus erythropolis* IMB Ac-5017, *Acinetobacter calcoaceticus* IMB B-7241 ТА *Nocardia vaccinii* IMB B-7405

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Метою роботи було здійснити альтернативну переробку токсичних промислових відходів у поверхнево-активні речовини штамами *Rhodococcus erythropolis* IMB Ac-50171, *Acinetobacter calcoaceticus* IMB B-7241 та *Nocardia vaccinii* IMB B-7405 для біоремедіації довкілля.

Штами вирощували у рідких середовищах, що містили як джерело вуглецю відпрацьовану (пересмажену) соняшникову олію, технічний гліцерол (відхід виробництва біодизеля) та ароматичні сполуки. Синтез поверхнево-активних речовин оцінювали за індексом емульгування, умовною концентрацією та концентрацією позаклітинних поверхнево-активних речовин, яку визначали ваговим методом після екстракції з супернатанта сумішшю метанолу і хлороформу. Концентрацію нафти у воді та ґрунті визначали ваговим методом після екстракції гексаном.

Показано, що у разі збільшення концентрації інокуляту до 10–15%, підвищення у 2 рази вмісту джерела азотного живлення у середовищі з 7–8% (об'ємна частка) технічного гліцеролу концентрація синтезованих *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 і *N. vaccinii* IMB B-7405 поверхнево-активних речовин становила 3,4; 5,0 і 5,3 г/л відповідно, що у 1,6–1,7 разів вище порівняно з показниками на базовому середовищі з такою самою концентрацією субстрату. Максимальна концентрація поверхнево-активних речовин (3,9–4,3 г/л), синтезованих *A. calcoaceticus* IMB B-7241 на відпрацьованій соняшниковій олії (4%) досягалася за використання інокуляту, вирощеного на рафінованій олії.

Встановлено здатність *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 і *N. vaccinii* IMB B-7405 розкладати ароматичні сполуки (фенол, нафталін, толуол, гексахлорбензол, бензойна та N-фенілантранілова кислоти) з одночасним синтезом позаклітинних метаболітів з поверхнево-активними та емульгувальними властивостями. За присутності поверхнево-активних речовин у вигляді постферментаційної культуральної рідини (5–10%) ступінь деструкції комплексних з важкими металами (Cu^{2+}, Cd^{2+}, Pb^{2+}, 0,01–0,5 мМ) нафтових забруднень у воді (3–6 г/л) і ґрунті (20 г/кг) через 20 діб становив 82–92%.

Біоконверсія промислових відходів у поверхнево-активні речовини для природоохоронних технологій дає змогу утилізувати токсичні відходи, знизити собівартість мікробних ПАР і забезпечити подвійний ефект очищення довкілля, який досягається під час виробництва і використання мікробних поверхнево-активних речовин.

Ключові слова: промислові відходи, мікробні поверхнево-активні речовини, ремедіація довкілля.

БИОКОНВЕРСИЯ ПРОМЫШЛЕННЫХ ОТХОДОВ В ПОВЕРХНОСТНО-АКТИВНЫЕ ВЕЩЕСТВА ШТАММАМИ *Rhodococcus erythropolis* IMB AC-5017, *Acinetobacter calcoaceticus* IMB B-7241 И *Nocardia vaccinii* IMB B-7405

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Целью работы было осуществить альтернативную переработку токсичных промышленных отходов в поверхностно-активные вещества штаммами *Rhodococcus erythropolis* IMB Ac-5017, *Acinetobacter calcoaceticus* IMB B-7241 и *Nocardia vaccinii* IMB B-7405 для биоремедиации окружающей среды.

Штаммы выращивали в жидких средах, содержащих в качестве источника углерода отработанное (пережаренное) подсолнечное масло, технический глицерол (отход производства биодизеля) и ароматические соединения. Синтез поверхностно-активных веществ оценивали по индексу эмульгирования, условной концентрации и концентрации внеклеточных поверхностно-активных веществ, которую определяли весовым методом после экстракции из супернатанта смесью метанола и хлороформа. Концентрацию нефти в воде и почве определяли весовым методом после экстракции гексаном.

Показано, что при увеличении концентрации инокулята до 10–15%, повышении в 2 раза содержания источника азотного питания в среде с 7–8% (по объему) технического глицерола концентрация синтезированных *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 и *N. vaccinii* IMB B-7405 поверхностно-активных веществ составляла 3,4; 5,0 и 5,3 г/л соответственно, что в 1,6–1,7 раза выше по сравнению с показателями на базовой среде с такой же концентрацией субстрата. Максимальная концентрация поверхностно-активных веществ (3,9–4,3 г/л), синтезированных *A. calcoaceticus* IMB B-7241 на отработанном подсолнечном масле (4%) достигалась при использовании инокулята, выращенного на рафинированном масле. Установлена способность *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 и *N. vaccinii* IMB B-7405 ассимилировать ароматические соединения (фенол, нафталин, толуол, гексахлорбензол, бензойная и N-фенилантраниловая кислота) с одновременным синтезом внеклеточных метаболитов с поверхностно-активными и эмульгирующими свойствами. В присутствии поверхностно-активных веществ в виде постферментационной культуральной жидкости (5–10%) степень деструкции комплексных с тяжелыми металлами (Cu^{2+}, Cd^{2+}, Pb^{2+}, 0,01–0,5 мМ) нефтяных загрязнений в воде (3–6 г/л) и почве (20 г/кг) через 20 сут составляла 82–92%.

Биоконверсия промышленных отходов в поверхностно-активные вещества для природоохранных технологий позволяет утилизировать токсичные отходы, снизить себестоимость микробных поверхностно-активных веществ и обеспечить двойной эффект очистки окружающей среды, который достигается при производстве и использовании микробных поверхностно-активных веществ.

Ключевые слова: промышленные отходы, микробные поверхностно-активные вещества, ремедиация окружающей среды.