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USE OF CELLS AND SURFACTANTS OF *NOCARDIA VACCINII* K-8 IN BIOREMEDIATION PROCESSES

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Abstract. *The possibility of use of Nocardia vaccinii K-8 cells as well as their metabolites for remediation of oil polluted ecosystems was studdied. It was shown that the highest oil destruction degree (94–98 %) in polluted water (2.6 g/L) was achieved in the case of treatment with suspension of N. vaccinii K-8 cells ($9.8 \cdot 10^7$ CFU/mL) after 30 days, while surfactant preparation of post fermentative cultural liquid (100–300 mL/kg) was more effective for remediation (destruction of 74–83 % of oil) of oil polluted soil (20 g/kg). Furthermore, the introduction of 30 ml of these preparation to the oil polluted sand (0.1 mL of oil/1 g of sand) resulted in detachment of 90 % of oil.*

Key words*: Nocardia vaccinii K-8, surfactants, indigenous microflora, oil pollution*

Introduction. Microbial surfactants (biosurfactants) are widely used in different fields of industry that is why the demand on synthetic surfactants is increasing constantly. At the same time the pace of biotechnology development and rising of attention to environment safety caused a great interest of scientist to microbial surfactants as alternative to chemical ones [1].

Biosurfactants application for the oil pollution degradation gives numerous ecological advantages [2, 5]. They don't increase the petroleum products toxicity, partly emulsify oil and increase its availability to microorganisms. Their use provide low operating costs, simple maintenance, well treatment that leads to almost complete degradation of organic compounds to oxides of carbon, nitrogen, etc. In contrast of microbial surfactants, the use of chemicals causes great damage to ecosystems and slows their recovery. Variety of options for the use of microbial surfactants in remediation processes are offered: the use of microorganisms-producers of surfactants for oil and oil products processing, treatment of contaminated cites with the solutions of surfactants for hydrocarbons solubilization, which stimulates the growth of the indigenous microflora; remediation of the most polluted areas with the use of bioreactors in which polluted soil recovers by surfactant solution [3, 4].

Oil-oxidizing bacteria *Nocardia vaccinii* K-8 were isolated from the oil-polluted samples of soil. It was shown that these bacteria had ability to synthesize surfactant. The regularities of surfactant synthesis on the medium with glycerol were determined previously, the medium composition was optimized with the use of mathematical methods of experiment planning [6].

The aim of present work was to investigate the possibility of *N. vaccinii* K-8 cells and extracellular metabolites with surface active and emulsifying properties use for microbial destruction of crude oil in water, soil and sand.

Materials and methods. The *N. vaccinii* K-8 cells grown on glucose-potato agar (GPA) for 48 h were washed with sterile tap water for cell suspension preparation. The quantity of living cells was determined by Koch method. The postfermentative cultural liquid, obtained after K-8 strain cultivation on the medium with glycerol, and supernatant were used as surfactant preparations.

The crude oil (2.6 g/L) was added into the water (2 L), then it was treated by suspension of *N. vaccinii* K-8 cells. The soil or sterile sand (1 kg) were polluted with 20 mL of crude oil

and treated with surfactant preparation (100–300 mL). Diammonium phosphate (0.01 %) was added into polluted samples as source of biogenic elements, which are required for oil-oxidizing bacteria vital activity. Oil polluted water, soil and sand without surfactant treatment were used as control variants in all experiments. The concentration of residual oil was determined by weight method after triple extraction with hexane (1:1). The obtained organic extract was evaporated at 55 °C and pressure of 0.4 atm.

Results and discussion. At the first stage of investigation we studied the possibility of oil polluted water remediation with suspension of *N. vaccinii* K-8 cells. During the first week of visual monitoring of water reservoirs the significant changes on the water surface weren't observed. Later oil rapidly degraded and changed its initial structure. The film lost oiliness, turned into a cluster of small dry flakes, lower part of which was on the water, and the rest settled on the bottom of the reservoirs after mixing. The transparent mucoid biomass was clearly visible on the oil flakes in reservoirs treated with *N. vaccinii* K-8 suspension on the 25th day of experiment.

It was shown (table 1) that the highest oil degradation degree (up to 94 %) was obtained after the water treatment with the K-8 strain cell suspension of higher concentration ($9.8 \cdot 10^7$ CFU/mL). With decreasing concentration of cells in suspension at 2 times the percentage of degradation of oil decreased by 34%.

Table 1

Dependence of the oil degradation degree on the concentration of N. vaccinii K-8 cells in suspension

Cell concentration in suspension, CFU/mL	Residual oil concentration*, g/L	Oil degradation degree, %
$9.8 \cdot 10^7$	0.16±0.008	94±4.2
$4.9 \cdot 10^7$	1.04±0.052	60±3.0
Control (without treatment with cell suspension)	2.52±0.130	3±0.2

* The initial oil concentration was 2.6±0.13 g/L.

The results of microbial control of the total quantity of microorganisms, that was conducted during the experiment, showed that the concentration of living cells of indigenous microflora increased significantly on 25th day. Such results could be explained by *N. vaccinii* K-8 ability to crude oil assimilation and surfactant synthesis, which transfer oil in available form for other microorganisms. The native microflora of reservoirs begins to proliferate due to the formation of available hydrocarbon substances.

On the next stage the effectiveness of oil degradation in soil at presence of surfactant preparation was studied (table 2). Thus, the highest oil degradation degree (84 %) was obtained after polluted soil treatment with post fermentative cultural liquid (300 mL). In the case of supernatant use the oil degradation degree was lower (55–72 %). The obtained results may indicate that not only surfactants participate in oil degradation as activators of indigenous soil microflora, but also *N. vaccinii* K-8 cells which have oil oil-oxidizing ability.

For the more complete evaluation of the possibility of *N. vaccinii* K-8 surfactant use for bioremediation technologies we studied the oil degradation in sterile sand. The sterile sand was used to avoid the indigenous microflora impact on oil degradation. However, in this case under the influence of surfactant preparations of K-8 strain a small amount of oil degraded (about 10%). It is known that sand has a large contact area, so it well absorbs oil on the surface, due to

the ability to reduce surface and interphasial tension surfactants transferred it to the dissolved state and contribute to oil detachment. Oil detachment properties of cultural liquid of *N. vaccinii* K–8 were determined by the quantity of oil absorbed on the sand after the treatment with surfactant preparation (table 3).

Table 2

The crude oil* degradation in soil treated with surfactant preparations of N. vaccinii K–8

Surfactant preparation	Surfactant preparation concentration, mL/kg of soil	Residual oil concentration, g/kg of soil	Oil degradation degree, %
Cultural liquid	100	5.9±0.18	71±2.1
	200	5.3±0.16	74±2.3
	300	3.4±0.10	84±2.5
Supernatant	100	9.2±0.28	55±1.7
	200	7.9±0.24	61±1.8
	300	5.6±0.17	73±2.2
Control (without treatment with preparations)	0	19.4±0.97	3±0.2

** The initial oil concentration was 20.0±1.0 g/kg of soil.*

Table 3

Oil detachment properties of N. vaccinii K–8 surfactants

Volume of preparation, mL	Residual oil quantity*, mg	Percentage of detached oil, %
10	12.6±0.63	65±3.3
20	7.9±0.40	78±3.9
30	3.0±0.15	92±4.6

** Residual oil quantity in the control variant was 36±1.8 mg.*

Thus, the percentage of detached oil in the polluted sand treated with surfactant preparation of K-8 strain (30 mL) was 92 %, which demonstrates their high oil detachment properties.

Therefore, the results of this work show the possibility of effective use of cells and surfactants preparations of *N. vaccinii* K-8 in environmental technologies for bioremediation of oil polluted ecosystems.

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