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USE OF BIOSURFACTANT PREPARATION OF *RHODOCOCCUS ERYTHROPOLIS* IMV Ac-5017 FOR REMEDIATION OF WATER POLLUTED WITH OIL AND HEAVY METALS

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Abstract. *The results of present work showed that the oil destruction degree in water treated with surfactant preparation of Rhodococcus erythropolis IMV Ac-5017 have increased significantly in presence of 0.01 mM* Cu^{2+} *(55–70 %) compared to the samples without copper cations (30 %). It was determined that the addition of 0.05–0.1 mM of* Cu^{2+} *into the media with fried sunflower oil and glucose or with n-hexadecane led to increasing surfactant synthesis by strain IMV Ac-5017 by 40 % compared to the medium without metal. It was shown that the activity of alcanehydroxylase of IMV Ac-5017 strain increased by 1.5 and 2 fold in the presence of 0.05 and 0.1 mM* Cu^{2+}*, respectively, in the reaction mixture.*

Key words: *water remediation, surfactant, copper cations, Rhodococcus erythropolis IMV Ac-5017*

Introduction. The world demand for oil in 2008 was 85.62 million barrels per day and now it increased by 1.5 fold [2, p. 231]. The global transport and use of both petroleum and its derivatives have made petroleum hydrocarbons major contaminants in both prevalence and quantity in the environment [1, p. 62; 2, p. 232].

Another acute problem facing humanity is environmental pollution by heavy metals. Metals are quite persistent in the environment, what significantly complicates their disposal. Heavy metals get into the environment with industrial (metallurgy, mining and engineering industry) and domestic wastewaters, as a result they accumulate in soils, and groundwater, and after that get to the drinking water [1, p. 61; 3, p. 610]. Lead, copper, cadmium, nickel, cobalt, mercury and others are most common metals in contaminated ecosystems.

It is known [3, p. 613] that in polluted ecosystems are often present both crude oil and metals, that's why it is important to search for the remediation methods which would help to remove such complex pollution. Currently the biological methods are most effective. They are based on the use of microorganisms and their metabolites, such as surfactants [3]. In the previous work the oil-oxidizing bacteria identified as *Rhodococcus erythropolis* IMV Ac-5017 were isolated from the oil-polluted samples of soil. The ability of the strain to synthesize the metabolites with surface-active and emulsifying activity during the cultivation on different hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (glucose, ethanol) substrates was determinded [4, p. 473]. It was shown that the addition of Cu^{2+} (up to 0.05 mM) into the nutrient medium for *R. erythropolis* IMV Ac-5017 cultivation at the exponential growth phase accompanied with increasing surfactant synthesis for 36 % compared to the cultivation of bacteria on the medium without copper ions. The oil degradation degree in the presence of 0.01 mM of Cu^{2+} increased for 25–45 % compared to the variant without copper. Control of water microflora showed the 1–2-fold increase of the total number of microorganisms in all samples treated with surfactant. The oxidation of *n*-hexadecane in IMV Ac-5017 strain, as in most of the genus *Rhodococcus*, is catalized by three-component alcanehydroxylase complex, as previously established [5, p. 604]. This complex contains the soluble NADH-rubredoksynreductase, soluble redoksyn and membrane bound monooxygenase (or alcanehydroxylase). Since it is known [6, p. 15] that copper cations are activators of monooxygenase, we have assumed that increasing oil degradation degree in the presence of surfactants and copper cations could be caused by the activating influence of Cu^{2+} on the alcanehydroxylase activity – the first enzyme of hydrocarbons catabolism.

The aim of present work – investigation of copper cations influence on alcanehydroxylase activity of *R. erythropolis* IMV Ac-5017 and surfactant synthesis during strain cultivating on hydrophobic substrates; studding of strain IMV Ac-5017 surfactants role in protection of water indigenous microflora from the negative influence of copper cations, and investigation of oil degradation in water, containing the mixture of toxic metals, treated with surfactant preparation.

Materials and methods. Bacteria were grown up on the liquid mineral medium (g/L distilled water): $NaNO_3$ – 1.3, NaCl – 1.0, $Na_2HPO_4{\cdot}12H_2O$ – 0.6, KH_2PO_4 – 0.14, $MgSO_4{\cdot}7H_2O$ – 0.1, $FeSO_4{\cdot}7H_2O$ – 0.001, pH 6.8–7.0. *n*-Hexadecane and fried sunflower oil were used as the carbon and energy sources in concentration of 2 vol. %. Glucose (0.1 vol. %) was added into the medium with fried oil at the beginning of cultivation. The inoculum – culture from the middle of exponential growth phase (48 h) cultivated on the medium of aforesaid composition with 1 vol. % of substrate. The cultivation of *R. erythropolis* IMV Ac-5017 was carried out in the 750 ml flasks, containing 100 ml of medium, on a shaker (320 rpm) at 30 °C during 120 hours. We added 0.01–0.5 mM of Cu^{2+} into the nutrient medium at the beginning of cultivation, in the middle of exponential and at the beginning of stationary growth phase for studding of copper cations influence on surfactant synthesis. The activity of alcanehydroxylase (EC 1.14.15.3) was determined spectrophotometrically (by the NADH oxidizing at 340 nm with the use of *n*-hexadecane as electron donor) in the cell-free extracts, obtained after *R. erythropolis* IMV Ac-5017 cultivation on the medium with *n*-hexadecane. The 0.01, 0.05 and 0.1 mM of copper cations were added into the reaction mixture.

The post fermentative cultural liquid was used as surfactant preparation for oil degradation. The 0.01 mM Cu^{2+}, Cd^{2+} and Pb^{2+} as 1 M solutions ($CuSO_4{\cdot}5H_2O$, $CdSO_4{\cdot}8H_2O$ and $Pb(CH_3COOH)_4$, respectively), as well as crude oil (2.0 g/L) were added into the water for modeling complex pollution. Then samples were treated with surfactant preparation (50 mg/L of water).

Studding of surfactants role in protection of water indigenous microflora from the negative influence of copper cations was carried out in bacterial suspension with sterile water (control, without surfactant) and sterile supernatant of cultural liquid (with surfactant). After the treatment of suspensions with Cu^{2+} the quantity of viable cells was determined.

Results and discussion. Conducted enzymatic analysis confirmed the activation alcanehydroxylase by copper cations. It was shown that the activity of alcanehydroxylase of IMV Ac-5017 strain increased by 1.5 and 2 fold in the presence of 0.05 and 0.1 mM Cu^{2+}, respectively, in the reaction mixture.

It was determined that the addition of 0.05–0.1 mM of Cu^{2+} into the media with fried sunflower oil and glucose or with *n*-hexadecane led to increasing surfactant synthesis by 40 % compared to the medium without metal. It should be noted that during the cultivation of IMB Ac-5017 strain on the medium with *n*-hexadecane and Cu^{2+} the increasing surfactant production by 110 % was obtained compared to the indexes of surfactant synthesis on ethanol containing medium. It can be explained by stimulation of alcanehydroxylase activity by cations Cu^{2+}.

We have isolated two bacterial strains in our previous investigations of crude oil biodegradation with surfactant preparation. The quantity of these bacteria increased significantly during the bioremediation process. It was determined that 100 % of these bacterial cells survived in presence of surfactant after addition of copper (0.01–0.05 mM), while in the samples without surfactant almost all of the cells died.

The results of remediation of crude oil contaminated water, containing the mixture of heavy metals (Cu^{2+}, Cd^{2+} and Pb^{2+}) are presented in the table 1.

Due to the data presented in the table the oil degradation degree was substantially lower in the samples without copper cations compared to the variants with Cu^{2+}. These data show that cations Cu^{2+} act as activators of alcanehydroxylases of indigenous microflora (similar to cells of IMV Ac-5017 strain), which results in intensification of crude oil assimilation.

Table 1.

Degradation of crude oil by surfactant preparation* in water containing toxic metals

Mixture of metal cations**	Concentration of residual oil, g/L	Oil degradation degree, %
Cu^{2+}+ Cd^{2+}+Pb^{2+}	0.60±0.03	70±3.5
Cu^{2+}+ Cd^{2+}	0.90±0.04	55±2.6
Cu^{2+}+Pb^{2+}	0.76±0.03	62±3.1
Cd^{2+}+Pb^{2+}	1.40±0.07	30±1.5
Without metals	1.01±0.05	50±2.5

** The experiment duration was 20 days. Oil degradation degree in control variant (without surfactant and Cu^{2+}) was 9 %.*

*** Concentration of each cation in mixture was 0.01 mM.*

Conclusion.

So, the results of present work showed that the oil destruction degree have increased significantly in presence of Cu^{2+} and surfactant preparation of IMV Ac-5017 strain. We assumed that surfactants made oil water soluble and increased its bioavailability for indigenous oil-oxidizing microflora. Biosurfactactants also protect microbial cells from the Cu^{2+} toxic effect. The oil destruction intensification in presence of Cu^{2+} could be caused by Cu^{2+} positive influence on alcanehydroxylase activity in IMV Ac-5017 strain, as well as in indigenous microflora. The positive influence of Cu^{2+} (0.05 and 0.1 mM) on alcanehydroxylase activity confirms the intensification of surfactant synthesis (by 40 %) while *R. erythropolis* IMV Ac-5017 growth on medium with *n*-hexadecane and copper cations. Besides, the highest oil degradation degree (up to 70 %) was obtained in the variants containing mixture of metals with copper cations and treated with surfactant preparation, while only 30 % of oil degraded in the variants, which contained Cd^{2+} and Pb^{2+}.

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References

1. *Kavamura V.N., Esposito E.* Biotechnological strategies applied to the decontamination of soil polluted with heavy metals // Biotechnol. Adv. – 2010. –V. 28. – P. 61 – 69.

2. *Tyagi M., Fonseca M.M.R, Carvalho C.C.C.R.* Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes // Biodegradation. – 2011. – V. 22. – P. 231 – 241.

3. *Gadd G.M.* Metals, minerals and microbes: geomicrobiology and bioremediation // Microbiol. – 2010. – V. 156. – P. 609 – 643.

4. *Pirog T.P., Shevchuk T.A., Volishina I.N., Karpenko E.V.* Production of surfactants by *Rhodococcus erythropolis* EK-1, grown on hydrophilic and hydrophobic substrates // Prikladnaya Biokhimiya i Mikrobiologiya. – 2004. – V. 40. – P. 544−550. [Applied Biochemistry and Microbiology (Engl. Transl.). – 2004. – V. 40. – P. 470−475].

5. *Pirog T.P., Shevchuk T.A., Klimenko Yu.A.* Intensification of surfactant synthesis in *Rhodococcus erythropolis* EK-1 cultivated on hexadecane // Prikladnaia biokhimiia i mikrobiologiia. – 2010. – V. 46. – P. 651–658. [Applied Biochemistry and Microbiology (Engl. Transl.). – V. 46. – P. 599–606].

6. *Van Beilen J.B., Funhoff E.G.* Alkane hydroxylases involved in microbial alkane degradation // Appl. Microbiol. Biotechnol. − 2007. − V. 74. − P. 13–21.

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