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BIOSURFACTANT OF *ACINETOBACTER CALCOACETICUS* IMB B–7241: INFLUENCE OF Cu^{2+} ON SYNTHESIS AND USE IN BIOREMEDIATION PROCESSES

Environmental problems worsened at the beginning of the XXI century cannot be solved in previously used traditional methods. Large-scale pollution caused mainly by oil, led to large anthropogenic stresses on the environment, and as a result, to disbalance in ecosystems. Nowadays, even with modern oil recovery, production, transportation and processing technologies from 1 to 16,5% of crude oil and processed products are lost. According to the preliminary estimates, the value of global losses of fuel is about 10^8 tons per year, of which approximately 25% got into the oceans and the rest - in soil and fresh water [1, p. 8452]. In addition, another problem is the environmental pollution by heavy metals. These substances enter and accumulate in the environment with emissions of the metallurgical, mining and engineering industry wastes [2, p.232]. Therefore, massive pollution of ecosystems occur not only in the general toxic effects of xenobiotics, resulting in the death of individuals or entire species, but also in inconspicuous at first glance embryotoxic, cytotoxic and mutagenic effects.

By now biological methods are the most effective for environment treatment. They are based on the activation of native microflora of contaminated water and soil or on using of microorganisms-destructors and their products [1, p.8453], in particular – of surface-active substances (SAS) . Microbial SAS are

are amphiphilic molecules that are produced from biological sources and tend to agglomerate to form micelles or concentrate at interfaces such as air/water, oil/water, or water/solid to reduce the interfacial and/or surface tension of a system. SAS of microbial origin have gained attention because of their biodegradability, low toxicity, ecological acceptability, and ability to be produced from renewable and less expensive substrates compared to their synthetic analogues. The biodegradability is the main advantage of biosurfactans that is why they do not cause environmental stress on ecosystems.

The mechanism of oil degradation is that microorganisms-destructors by synthesis of SAS partially emulsify oil, making it more soluble and accessible to native microflora [1, p.8453]. In addition, SAS can form complexes with heavy metal cations making them nontoxic that then accumulate in cells or precipitate [3, p.1008].

In the previous work the oil-oxidizing bacteria identified as *Acenitobacter calcoaceticus* K-4 were isolated from the oil-polluted samples of soil and deposited in the Depositary of microorganisms of the Institute of Microbiology and Virology of National Academy of Sciences of Ukraine at the number of IMV Ac-7241. Previously it was shown the ability of *A. calcoaceticus* IMB B-7241 to synthesize SAS under the conditions of growth on hydrophilic (ethanol, glucose) and hydrophobic (hexadecane) substrates [4, p.277].

The aim of this work was to investigate the influence of Cu^{2+} on the SAS synthesis by *A. calcoaceticus* IMB B-7241 and the ability of using of metabolites in the degradation of complex oil pollution of soil and water.

Cultivation of bacteria was carried out on the nutrient medium the composition of which was demonstrated previously [4, p.272]. To check the influence of heavy metals was used 1M solution of $CuSO_4 \cdot 5H_2O$ that was added in a medium in exponential and stationary phases of growth. The concentration of copper cations was 0,1 and 0,5 mM. Culture, obtained after 120 h of cultivation in the presence Cu^{2+}, was used as inoculum for seeding the medium without Cu^{2+}.

The quantity of synthesized surfactant was evaluated by conditional surfactant concentration (CSC*) in the cultural broth as described in the work [4, p.273].

For investigating the oil bioremediation processes in soil and water in the presence Cu^{2+} as surfactant preparations was used culture broth gained after cultivation of strain on ethanol using different inoculums: raised on ethanol (preparation 1), on ethanol in the presence of 0,1 mM Cu^{2+} (preparation 2) and on ethanol in the presence of 0,5 mM Cu^{2+} (preparation 3). For inoculum preparation copper cations were added in medium with ethanol in the early exponential growth phase. For the simulation of oil-contaminated ecosystems in the 2-L tanks of water added oil in concentration of 4 g/L (ρ=0,8 g/mL) or 20 mL/kg in the tanks with 2 kg of soil, then the samples were treated with SAS preparations in concentrations of 5% (v/v). For complex pollution simulation in addition to oil in soil was added Cu^{2+} in concentrations of 0,1 and 0,5 mM. Oil-contaminated soil not treated with preparations of SAS served as the control. Experiment lasted 30 days. Residual oil content in the studied samples was determined by dry weight measurement. Samples taken during the experiments were extracted three times with hexane (1:1). The organic extracts were vacuum-dried at 55◦C under the pressure of 0,4-0,5 kg/sm^2 to constant weight on rotary evaporator installation IR-1M2 (Russia).

From the literature it is known that for most microbial producers maximum levels of SAS synthesis are observed at pH close to neutral. Therefore, the first step of research checked the influence of Cu^{2+} cations on the SAS synthesis of *A. calcoaceticus* IMB B-7241 adjusting neutral pH. As a source of carbon and energy were used both hydrophilic (ethanol) and hydrophobic (oil paraffins and hexadecane) substrates.

It was found that during the cultivation of *A. calcoaceticus* IMB B-7241 on ethanol and hexadecane adding of 0,5 mM Cu^{2+} in exponential growth phase led to the increase in SAS synthesis level by 60% as compared to the strain growth on the medium without copper cations. Adding of 0,1 mM Cu^{2+} in exponential growth phase of IMB B-7241 on liquid paraffins accompanied by a significant

increase (by 140%) of CSC*. The maximum (by 80 – 245%) intensification of SAS synthesis of *A. calcoaceticus* IMB B-7241 was observed on the medium with the according substrate but without Cu^{2+}.

On the next step the degradation of complex pollution of soil and water (oil and copper cations) under the influence of preparations of SAS of strain IMB B-7241 was studied (table).

Table 1

Destruction of oil in soil and water under the action of different preparations of surfactants of IMB B–7241 in the presence of Cu^{2+}

Concentration of metal in soil or water, mM	Preparation of surfactant	A degree of oil destruction after 30 days, %	
		in soil	in water
0	preparation 1	84,1±4,1	86,6±4,3
	preparation 2	70,5±3,5	76±3,8
	preparation 3	80,0±4,0	89,2±4,6
0,1	preparation 1	55,6±2,6	52,7±2,2
	preparation 2	91,6±4,5	95,2±4,8
	preparation 3	90,0±4,4	91,6±4,5
0,5	preparation 1	60,3±3,0	70,7±3,4
	preparation 2	89,6±4,5	90,8±4,6
	preparation 3	74,8±3,6	86,8±4,4

Remarks. As control used oil polluted soil and water, where the degree of oil destruction after 30 days was 0%. As preparations of SAS used a cultural broth after cultivation of IMB B–7241 on ethanol with inoculum grown on ethanol (preparation 1), ethanol and the presence of 0,1 mM of Cu^{2+} (preparation 2) and ethanol and the presence of 0,5 mM of Cu^{2+} (preparation 3).

Maximal degree of destruction (90,0–95,2%) of complex contamination of

oil and copper cations (0,1 mM) in soil and water observed in the case of treatment with preparations 2 and 3. We suggest that the positive role of Cu^{2+} in oil biodegradation is caused by stimulant influence of copper cations on activity of alkane hydroxylases – the first enzymes of hydrocarbons catabolism both in IMB B–7241 and natural oiloxidazing microflora.

Consequently, as a result of the work it was shown that SAS of *A. calcoaceticus* IMB B–7241synthesized on the medium with Cu^{2+} intensify the process of oil destruction at presence of copper cations. This effect can be explained by influence of copper on activity of some enzymes of oil catabolism, in particular – alkane hydroxylases.

Thus, the results demonstrate the availability of application of preparations of SAS on the basis of cultural broth of *A. calcoaceticus* IMB B–7241 in the processes of bioremediation ekosystem polluted with oil and heavy metals.

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