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IMPROVEMENT OF THE TECHNOLOGY FOR SURFACTANT SYNTHESIS BY *Acinetobacter calcoaceticus* IMV B-7241

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The aim of the work was to intensify the synthesis of surfactants by *Acinetobacter calcoaceticus* IMV-7241 cultivated on ethanol and other carbon substrates. *A. calcoaceticus* IMV-7241 was grown in the medium with mono- (ethanol, glycerol, liquid paraffin, *n*-hexadecane, glucose) and mixed substrates in the presence of organic acids or heavy metal cations (0.1–2.0 mM Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}). The synthesis of surfactants was evaluated by emulsification index of cultural liquid, conditional concentration and concentrations of extracellular surfactants, which were determined gravimetrically after their extraction from supernatant with the mixture of methanol and chloroform. It was shown that addition of citrate and fumarate (0.01%) at the end of exponential growth phase of *A. calcoaceticus* IMV B-7241 in the medium with ethanol (2%) and the maintenance of neutral pH increased the surfactants' concentration in 3.5 times (up to 6.0 g/l). The quantity of extracellular surfactants synthesized by the strain IMV B-7241 in the medium containing mixture of *n*-hexadecane and glycerol (molar ratio 1:7) and C/N 30 was increased in 2.6–3.5 times in comparison with cultivation on corresponding monosubstrates. Addition of 2.0 mM Cu^{2+} at the stationary growth phase of *A. calcoaceticus* IMV B-7241 in medium with liquid paraffin and *n*-hexadecane led to the increase of surfactants' synthesis in 2.3–2.5 times compared with those in the medium without Cu^{2+}.

Approaches to intensification of surfactants' synthesis by *A. calcoaceticus* IMV B-7241 (including addition of biosynthesis precursors and cultivation on the mixture of substrates) can be used to increase the efficiency of microbial technologies.

Key words: surface-active substances, intensification of biosynthesis, precursor of biosynthesis, mixture of substrate, heavy metals.

The unique properties of microbial surfactants determine their use in various industries instead of chemically synthesized analogues [1]. Surfactants of microbial origin are used to solve a number of practical problems: environmental problems (pollution of soil and water by xenobiotics that can lead to the ecological disaster), the search for alternative antimicrobial agents against resistant microorganisms [1–3]. Most existing technologies for the synthesis of microbial surfactants include the cultivation of producers on high-cost hydrophobic substrates (hydrocarbons), because under these conditions the synthesis of the desired product is the highest [3–6]. To reduce the cost of microbial surfactants technology individual hydrocarbons are replaced by liquid paraffins (the waste of oil refining industry). However, a significant disadvantage of using liquid hydrocarbons is their ability to crystallization at the temperature of about 20 °C. This makes great complications for the efficient work of the service equipment [http://www.ngpedia.ru/id503849p3.html]. Replacement onto water-soluble carbon source (for example, ethanol) can greatly simplify the technology of microbial surfactants. As for the biosynthesis of surfactants can be used synthetic ethanol (its cost is the same as liquid paraffin) the cost of the final product obtained on this substrate will be low. Previous studies had established the ability of *Rhodococcus erythropolis* IMB Ac-5017 to synthesize surfactants on ethanol, but the indicators of such synthesis were low [7].

Earlier from oil-contaminated soil samples we had selected oil-oxydizing strain of bacteria, identified as *Acinetobacter calcoaceticus* K-4 [8], deposited in the depository of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine with number IMB B-7241. It was shown that the mentioned strain was able to

synthesize the complex of neutral, amino- and glycolipids (presented by trehalose mycolates) under cultivation on the ethanol [8].

It is known that the most studied producers of trehalose mycolates are representatives from *Rhodococcus*, synthesizing glycolipids surfactants on hydrocarbon substrates [6, 9, 10]. Most members of the genus *Acinetobacter* synthesize macromolecular emulsifiers, their chemical nature can be considered as a complex of extracellular polysaccharides and proteins [11, 12]. These compounds do not possess surface active properties. Only in 2009 the first report appeared about the ability of member genus *Acinetobacter* to synthesize low molecular weight surfactants, but only on hydrophobic substrates [13]. Subsequently, it was given more information about the surfactants synthesis by representatives of *Acinetobacter*. Thus, Chen et al. [14] isolated from oil-contaminated soil surfactant-synthesizing microorganisms and studied their properties. Among the studied microorganisms *Acinetobacter* sp. YC-X 2 was the most productive. It was able to synthesize (on the optimized media with *n*-hexadecane, peptones and meat extract) surfactants that were stable in a wide range of pH (5-11), temperature (up to 121 °C) and high concentrations of Na^+ and Ca^{2+} (up to 18%, w/v). Other researchers have shown that the isolated strain of *Acinetobacter* sp. D3-2 synthesized surfactants having lipopeptide nature in concentration 0.52 g/l. These surfactants reduced the surface tension to 26.3 mN/m [15]. The authors [16] studied the properties of lipopeptides synthesized by *Acinetobacter bayli* ZJ2, purified from oil-contaminated soil in China. It was shown, that surfactants from strain ZJ2 reducing the surface tension of water solutions to 35 mN/m, were stable at 8% of salt concentration, pH 4–9 and helped to increase oil recovery by 28%. The ability of *Acinetobacter calcoaceticus* to synthesize glycolipids was described in [17]. Formed surfactants can be considered as a complex of mono- and dirhamnolipids and showed effective emulsifying properties. American scientists have identified strains-producers of rhamnolipids such as *A. calcoaceticus* NRRL B-59190 and 59191 NRRL B. These producers synthesized 2.0–2.2 g/l surfactants on the medium with glycerol [18].

So, the unique property of isolated by us the strain of *A. calcoaceticus* IMV B-7241 is its ability to produce uncharacteristic for *Acinetobacter trehalose* mycolates on ethanol (non-traditional substrate for synthesis of surfactants). Under these conditions of cultivation the amount of synthesized surfactants was higher than in case of *R. erythropolis* IMV Ac-5017, but remained low compared to the amount obtained on hydrophobic substrates. Taking into consideration all above mentioned, the aim of this work was the intensification of surfactants' synthesis by *A. calcoaceticus* IMV B-7241 using ethanol and other carbon substrates.

Materials and Methods

The object of research. As the object of research it was used selected from oil-contaminated soil the strain of *Acinetobacter calcoaceticus* K-4, deposited in the Depositary of microorganisms of the Zabolotny D. K. Institute of Microbiology and Virology NAS of Ukraine with number IMV B-7241 [8].

The composition of the medium and cultivation conditions. *A. calcoaceticus* IMV B-7241 was grown on Muntz liquid medium with our modifications [8] (g/l): $(NH_2)_2CO$ — 0.35, NaCl — 1.0, $Na_2HPO_4 \cdot 12H_2O$ — 0.6, KH_2PO_4 — 0.14, $MgSO_4 \cdot 7H_2O$ — 0.1, pH 6.8–7.0. The medium was supplemented with yeast autolysate, 0.5% (v/v) and the trace element solution, 0.1% (v/v) The solution of microelements contained (g/100 ml): $ZnSO_4 \cdot 7H_2O$ — 1.1; $MnSO_4 \cdot H_2O$ — 0.6; $FeSO_4 \cdot 7H_2O$ — 0.1; $CuSO_4 \cdot 5H_2O$ — 0.004; $CoSO_4 \cdot 7H_2O$ — 0.03; H_3BO_3 — 0.006; KI — 0.0001; EDTA (trilon B) — 0.5. In one variant, to maintain a certain value of C/N we used the following concentrations of $(NH_2)_2CO$: 0.32, 0.39, 0.42, 0.45 and 0.63 g/l.

As the source of carbon and energy we used the following monosubstrates: glycerol 0.5–1.3% (v/v), *n*-hexadecane — 0.5–2.0% (v/v), ethanol — 0.5–2.0% (v/v), glucose — 0.5–2.0% (w/v), liquid paraffines (C_{10}–C_{18}) — 2.0% (v/v); mixed substrates: a mixture of *n*-hexadecane with ethanol, glucose and glycerol, the concentration of each monosubstrates was 0.5 and 1.0%; mixture of *n*-hexadecane and glycerol in molar ratio 1:3; 1:4; 1:5; 1:6; 1:7 and 1:8 respectively. Sodium citrate (0.01–0.5%) and sodium fumarate (0.01–0.5%) were used as surfactant synthesis precursors. They were added into the medium as a 10% solution at the beginning of cultivation and at the late exponential growth phase.

In some experiments after 20–24 h of growth and before the addition of organic acids, pH level (7.0±0.2) was adjusted with 1N KOH (NaOH) During the cultivation it

was added Cu^{2+} (0.1–2.0 mM), Zn^{2+}, Cd^{2+} and Pb^{2+} (0.1–0.5 mM) from 1 M stock solutions of $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 8H_2O$, and $Pb(CH_3COO)_4$ correspondingly.

Inoculum (culture in the exponential growth phase) was grown on the above medium composition that contained monosubstrates as a carbon source in a concentration of 0.5–1.0% and the mixture of substrates at the concentration of 0.25% each. The concentration of inoculum (10^4–10^5 cells / ml) was 5% of the medium volume.

Cultivation was performed in 750 ml flasks with 100 ml of the medium on a shaker (220 rpm) at 30 °C for 24–120 h °C.

Indices of growth and surfactants synthesis. Biomass was determined using the optical density of the culture liquid at 650 nm (spectrophotometer KFK-3-01 (Russia)) with the following recalculation on absolutely dry mass according to calibration graphics or gravimetric method. Measurement of surface tension (σ_s) was performed on semi-automatic tensiometer LAUDA TD 1C (Germany). For rapid assessment of surfactant concentration in the culture liquid, we used indicator of "conditional surfactant concentration" (dimensionless value), which was defined as the degree of dilution of cell free culture liquid (supernatant) at the point of a sharp increase in surface tension. Then, we constructed a graph of dependence of surface tension σ_s on the value of logarithm of supernatant dilution. The abscissa of the inflection of the curve corresponds to the value of conditional surfactant concentration.

The concentration of extracellular surfactant (g/l) was determined gravimetrically after a threefold extraction of surface active lipids from the supernatant culture liquid by Folch mixture (chloroform:methanol, 2:1), followed by evaporation at 50 °C to constant weight as described previously [19]. The emulsification index (E_{24}, %) of culture liquid was evaluated as described in [20].

Capacity of surfactants produced by *A. calcoaceticus* IMV B-7241 to protect the producer from the effects of heavy metals was estimated as described in [21].

The enzymatic analysis. To recive cell-free culture liquid extracts, *A. calcoaceticus* IMV B-7241 was centrifuged (5,000 g, 20 min, 4 °C). The precipitate cells were washed twice from the medium by 0.05 M K^+ phosphate buffer (pH 7.0) and centrifuged (4,000 g, 15 min, 4 °C). The washed cells were resuspended in 0.05 M K^+ phosphate buffer (pH 7.0) and sonicated (22 kHz) three times for 20 s at 4°C on an UZDN-1 disintegrator. The resulting liquid was centrifuged (12,000 g, 30 min, 4 °C), the pellet was removed, and the supernatant was used as a cell-free extract.

Enzymes' activity was determined as described before [22]. The activity of isocitrate lyase (EC 4.1.3.1) was determined using the rate of the formation of phenylhydrazone glyoxylate at 324 nm. Activity of phosphoenolpyruvate (PEP)-synthase (EC 2.7.9.2) was determined by the rate of pyruvate formation, analyzing the oxidation of NADH at 340 nm for the coupled reaction with lactate dehydrogenase, PEP-carboxykinase (EC 4.1.1.49) activity was determined according to the formation of phosphoenolpyruvate and pyruvate in NADH oxidation process and glutamate dehydrogenase (EC 1.4.1.4) activity — according to the formation of glutamate during NADPH oxidation at 340 nm, PEP-carboxylase (EC 4.1.1.31) activity — according to the oxidation of NADH at 340 nm.

Activity of trehalose phosphate synthase (EC 2.4.1.15) [23] was measured by the formation of uridine diphosphate which was determined by the oxidation of NADH spectrophotometrically at 340 nm in the coupled reactions with pyruvate kinase and lactate dehydrogenase.

Enzyme activity was calculated in mmol of the product obtained for 1 min of the reaction and recalculated to 1 mg of the protein. Protein content in cell-free extracts was determined according to Bradford. Enzyme activity was analyzed at 28–30 °C. This temperature was optimal for the growth of *A. calcoaceticus* IMV B-7241.

All experiments were performed in three repetitions, the number of parallel measurements in the experiments was 3–5. Statistical analysis of experimental data was carried out by the algorithm described in [24]. Difference in average data considered as reliable at the significance level of $P < 0.05$.

Results and Discussion

The intensification of the synthesis of surfactant on ethanol in the presence of organic acids. One of the approaches to improve the efficiency of microbial technology is introduction of biosynthesis precursors into the medium [7, 19].

Taking into consideration the chemical composition of surfactants synthesized by *A. calcoaceticus* IMV B-7241 under optimal cultivation conditions on ethanol (complex of glyco-, amino and neutral lipids), we have

suggested the possibility of increasing their synthesis in case of adding citrate (regulator of lipid synthesis) and fumarate (gluconeogenesis precursor).

It was established that the maximum concentration of surfactants (5.0 g/l) was observed in case of simultaneous introduction of citrate and fumarate into the medium with ethanol at the late exponential growth phase of *A. calcoaceticus* IMV B-7241 (Table 1). Improving of surfactant synthesis in the presence of organic acids was caused by the increase in 1.2–7.0 times activity of enzymes of biosynthesis of glyco- (PEP-synthetase, trehalose phosphate syntase) and aminolipids ($NADP^+$-dependent glutamate dehydrohenase) compared compared with cultivation of IMV B-7241 strain on the ethanol without fumarate and citrate, as well as simultaneous functionning of glyoxylate cycle (activity isocitrate lyase 260 ± 13 nmol min^{-1} mg^{-1} protein) and PEP-carboxylase reaction (1 768 ± 88 nmol · $min^{-1}mg^{-1}$ protein).

During the cultivation of *A. calcoaceticus* IMV B-7241 on ethanol, pH level decreased to 4.0–4.5. In most bacteria salts of organic acids are transported into cells by symport with proton [25] and neutral pH is optimal for this process. So, in our experiments before addition of citrate and fumarate, we carried out neutralization of the culture medium. It was established that under such conditions surfactant concentration increased up to 6.0 g/l.

We had previously found that the simultaneous introduction of fumarate and citrate (0.01–0.02%) into the medium with glycerol (1% v/v) was accompanied by increasing concentration of *A. calcoaceticus* IMV B-7241 extracellular surfactants in 2.0–2.5 times as compared with cultivation of this strain in the medium without organic acids [19]. Our results, concerning the influence of organic acids on the synthesis of surfactants by the strain of IMV-B7241 grown on the ethanol, indicated the more significant effect of addition of citrate and fumarate in their equal concentration (0.01%).

Mechanisms of intensification of the synthesis of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on both glycerol and ethanol in the presence of organic acids are the same. They prove the simultaneous functionning of two anaplerotic ways, as well as the increased activity of biosynthetic enzymes of surfactant glyco- and aminolipides.

The established regularities concerning the influence of the biosynthesis precursors on the formation of surfactants by *A. calcoaceticus* IMV B-7241 are different from those previously obtained for the strain *R. erythropolis* EK-1 grown on ethanol [7]: the concentration of surfactant was increased almost in 3 times, while in case of the strain EK-1 — only in 2 times in the presence of 10-fold higher concentration of fumarate and citrate. It was earlier described the increase

Table 1. **Synthesis of surfactants under cultivation of *A. calcoaceticus* IMV B-7241 on ethanol in the presence of various concentrations of citrate and fumarate**

Organic acids,%	Surfactants, g/l	E_{24},% (1:49)
Citrate, 0.01	2.6 ± 0.1 *	91*
Citrate, 0.02	2.6 ± 0.1*	100*
Citrate, 0.1	1.9 ± 0.1*	87*
Fumarate, 0.01	2.8 ± 0.1*	100*
Fumarate, 0.02	2.5 ± 0.1*	87*
Fumarate, 0.1	2.1 ± 0.1*	88*
Citrate, 0.01 + Fumarate, 0.01	5.0 ± 0.3*	89*
Citrate, 0.02 + Fumarate, 0.02	3.2 ± 0.2*	79*
Citrate, 0.1 + Fumarate, 0.1	2.8 ± 0.1*	88*
Control (without organic acides)	1.7 ± 0.1	88

Note. In determining of the emulsification index error does not exceed 5%. Here and after: * — $P \leq 0.05$ relative to control (the concentration of surfactant and emulsifying index obtained on the medium without organic acids).

of the surfactant synthesis in the presence of citrate alone, added at the beginning of culture growth [26]. The optimum citrate concentration was 0.5–1.0%. At that concentration, citrate could be considered an additional growth substrate rather than a lipid synthesis regulator [26].

Using a mixture of growth substrates for synthesis intensification of surfactants. It was known that the cultivation of microorganisms on the mixture of substrates makes it possible to avoid the unproductive loss of carbon and energy. in case of monosubstrate and to increase the efficiency of transformation of carbon substrates into biomass and intensify the synthesis of secondary metabolites [7, 27].

It was established that under cultivation of *A. calcoaceticus* IMV B-7241 on the mixture of energy-excessive substrate (n-hexadecane) and energy-deficient C_2–C_6-substrates (ethanol, glycerol, glucose) the indices of surfactant synthesis were increased up to 125–430% as compared to those on the appropriate monosubstrates. These indices on the mixture of *n*-hexadecane and glycerol were maximal when inoculum was grown on *n*-hexadecane.

To achieve the maximum conversion of the substrate carbon into the final product it was necessary to determine the optimal molar ratios of monosubstrates' concentrations in the mixture [7]. This, in turn, makes necessary theoretical calculations of the energy required for surfactants and biomass synthesis on the energy-deficient substrate with subsequent determination of the concentration of the energy-excessive substrate which supplies energy for this process. To implement these theoretical calculations it was necessary to know the pathways of metabolism of the monosubstrates in surfactants producer. Our experiments have shown that the catabolism of glycerol to dihydroxyacetone phosphate in case of *A. calcoaceticus* IMV B-7241 takes place in two ways: through glycerol-3-phosphate and a dyhidroxy acetone [28]. To calculate the optimum ratio of the concentrations of *n*-hexadecane and glycerol we proposed the suppositious scheme of synthesis of trehalose monomycolate from glycerol [28]. According to this scheme, energy generation during trehalose monomycolate synthesis is 2.48 mol ATP/mol glycerol Taking into consideration the need of ATP for the synthesis of biomass (8 moles of ATP per 1 mol of glycerol) and the amount of energy generated during the synthesis of trehalose monomycolate from glycerol, at the expense of n-hexadecane it has to be received 5.52 moles of ATP which requires 0.145 moles *n*-hexadecane. Thus, the molar ratio of hexadecane and glycerol in the medium should be 0.145: 1 or 1: 6.9. As follows from Table 2, under experimental conditions, the maximal conditional concentration of surfactants (4.8) and emulsification index (55%) were observed for the theoretically calculated molar ratio of monosubstrates.

Table 2. **The surfactants' formation by *A. calcoaceticus* IMV B-7241 depending on the molar ratio of *n*-hexadecane and glycerol concentrations**

The molar ratio of *n*-hexadecane and glycerol mixed	C/N ratio	Conditional surfactant concentration	Index of emulsification, %
1:3	24	2.8 ± 0.1*	46*
	30	3.0 ± 0.2*	43*
1:4	27	3.8 ± 0.2*	40*
	30	3.9 ± 0.2*	45*
1:5	30	4.0 ± 0.2*	47*
1:6	33	4.2 ± 0.2*	43*
	30	4.2 ± 0.2*	43*
1:7	36	4.3 ± 0.2*	52*
	30	4.8 ± 0.2*	55*
1:8	39	4.1 ± 0.2*	42*
	30	4.2 ± 0.2*	43*

Note. Inoculum was grown on *n*-hexadecane. In determining the emulsification index of cultural liquid the error does not exceed 5%. * — $P \leq 0.05$ relative to control: conditional surfactant concentration (2.5) and emulsification index (40%) on monosubstrates.

Since the change in molar ratio of *n*-hexadecane and glycerol in the medium led to the change in the ratio of carbon/nitrogen, the next step was to investigate the synthesis of surfactant not only at the different molar ratio of monosubstrates in the medium, but at the constant value of C/N, equal to 30 (Table 2). Experiments have shown that under such cultivation conditions of IMV-B7241 strain the maximal values of surfactant synthesis was observed when the molar ratio of *n*-hexadecane/glycerol was 1:7.

To confirm the results about depending the synthesis of *A. calcoaceticus* IMV B-7241 surfactants on the ratio of the substrate concentrations and C/N, it was studied the formation of surfactants during cultivation of bacteria on the mixture of *n*-hexadecane and glycerol (ratio 1: 7) in a broader range of C/N. In these experiments extracellular surfactant concentration (g/l) determining gravimetrically after extraction with organic solvents, was used as a criterion of the synthesis It was established that 30 was the best ratio of carbon/nitrogen for the synthesis of surfactants. Under such conditions of *A. calcoaceticus* IMV B-7241 cultivation the amount of synthesized surfactants was 350–265% from the concentration obtained on monosubstrate glycerol and *n*-hexadecane respectively.

The increased level of *A. calcoaceticus* IMV B-7241 surfactant production on the mixture of *n*-hexadecane and glycerol is determined by the increase in the activities of enzymes responsible for the biosynthesis of surfactant glyco- (PEP-carboxykinase, PEP-synthetase) and aminolipids (NADP+-dependent glutamate dehydrogenase) and simultaneous functionning of two anaplerotic pathways (Table 3).

We have recently published the review [27] with literature data and results of our experimental studies concerning the use of the mixture of substrates for intensification technology of microbial synthesis of the valuable products of fermentation, primary and secondary metabolites, including surfactants. It was reviewed in detail glycolipid and lipopeptide synthesis on the mixture of hydrocarbon substrates and vegetable oils, as well as the necessity of determining not only the ratio of monosubstrates in the mixture, but also their concentrations. For correct interpretation of results, the amount of carbon contained in the mixture and in monosubstrates should be taken into account.

In [29, 30], the authors empirically established the monosubstrate concentrations in the mixture as well as the choice of substrates, and used extremely high concentration (100–200 g/l), resulting in the low degree of conversion of the used substrates into surfactants (10–15%).

In our research to estimate monosubstrate combination, we used Babel's energy classification [7]. In the study it was used the mixture of energy-excessive *n*-hexadecane with energy-deficient ethanol, glucose and glycerol, and their concentration in the medium were low (3.0–16.8 g/l). To determine the molar ratio of the concentrations of monosubstrates in the mixture we performed theoretical calculations of the energy that is needed for surfactant and biomass synthesis on energy-deficient glycerol with subsequent determination of the concentration of energy-excessive *n*-hexadecane that replenishes the energy consumption of the process. According to calculated optimal molar ratio of *n*-hexadecane and glycerol 1: 7, the yield of the surfactants from the substrate was higher than it was described in the literature [29, 30].

Thus, the results presented in this paper showed the advisability of using of the

Table 3. **Activities of the enzymes involved in anaplerotic pathways and surfactants biosynthesis in *A. calcoaceticus* IMV B-7241 grown on mono- and mixed substrates**

The concentration of substrate,%	Activity, nmol·min^{-1}mg^{-1} of the protein				
	Isocitrate lyase	$NADP^+$-dependent glutamate dehydrogenase	PEP-carboxylase	PEP-carboxy-kinase	PEP-synthetase
Glycerol 1.20 (control)	45 ± 2	322 ± 16	1608 ± 80	448 ± 22	2 894 ± 144
n-Hexadecane, 1.15 (control)	0	620 ± 31	2571 ± 129	667 ± 33	3 456 ± 172
n-Hexadecane, 0.5 + glycerol, 0.7	494 ± 24*	769 ± 38*	1 724 ± 86*	923 ± 46*	4 483 ± 224*

Note. * — $P \leq 0.05$ relative to control (activity of enzymes on monosubstrates). Mono- and mixed substrates are equimolar by carbon.

mixture of energetically unequal substrates for increasing the synthesis of surfactants and showed that high effectiveness of such mixed substrates can be achieved by both the correct choice of substrates and correct determination of molar ratio of their concentrations.

The influence of heavy metal cations on the synthesis of surfactants by A. calcoaceticus IMB B-7241 on non-carbohydrate substrates. It is known that for microorganisms one of adaptation mechanisms is the synthesis of extracellular protector compounds, including surfactants [31]. It was found in previous studies [32] that the addition of Cu^{2+} in low concentrations (0.01–0.5 mM) in exponential phase of *A. calcoaceticus* IMV B-7241 growth on hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (ethanol) substrates was accompanied by increasing conditional concentration of surfactant for 60–140% compared to the indices under cultivation in the medium without copper cations. The maximum intensification of the surfactant synthesis by *A. calcoaceticus* IMV B-7241 was observed in case of adding Cu^{2+} into the medium with hydrocarbons.

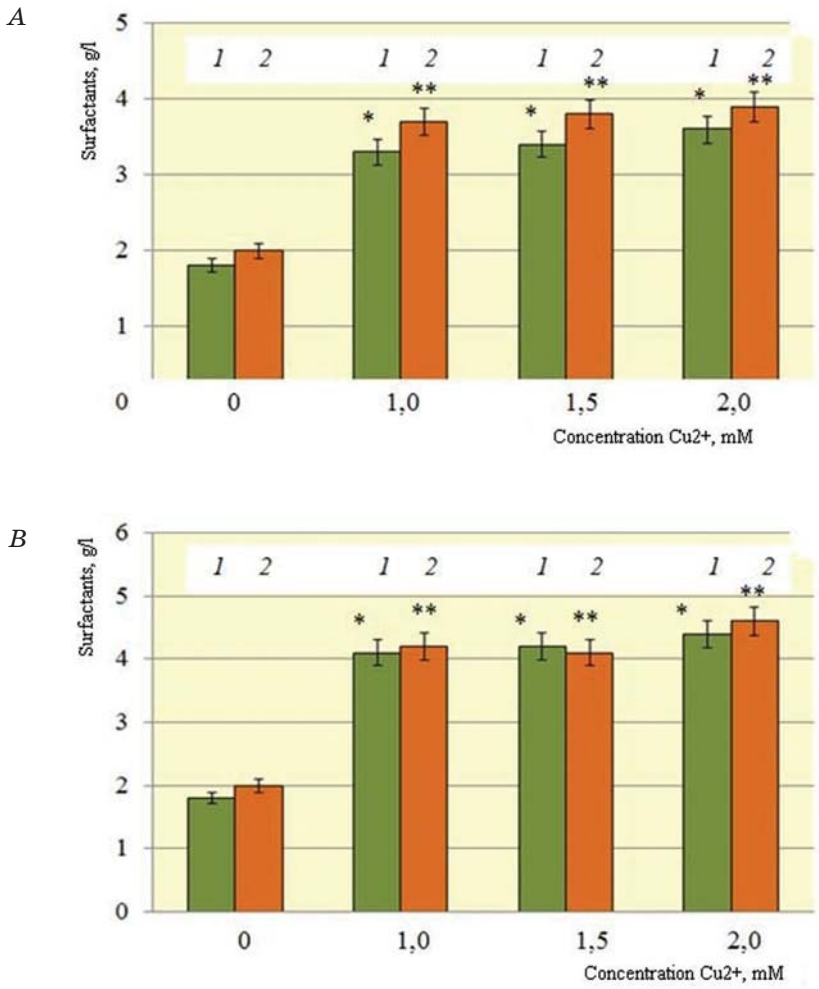
The increasing of surfactant synthesis in the presence of copper cations is determined by their activating effect on activity of alkane hydroxylase as well as 4-nitrozo-N,N-dimethylaniline-dependent alcohol dehydrogenase and enzymes of biosynthesis of surface active glyco-(PEP-synthetase) and aminolipids ($NADP^+$-dependent glutamate dehydrogenase) in *A. calcoaceticus* IMV B-7241 [32].

Since in the previous studies [32] the maximum intensification of surfactant synthesis in the presence of copper cations by *A. calcoaceticus* IMV B-7241 was observed on hydrophobic substrates, the next step was devoted to the study of effect of higher (2.0 mM) Cu^{2+}concentrations on the synthesis indices under cultivation of IMV B-7241 strain on *n*-hexadecane and liquid paraffin. It was established that independently of copper cations concentration and the time of their introduction (exponential and stationary phase) into medium with *n*-hexadecane and liquid paraffins the increasing synthesis of surfactant in 1.6–2.4 times as compared with the cultivation of

Table 4. **Effect of metal cations on the surfactants synthesis under cultivation of *A. calcoaceticus* IMB B-7241 on different substrates**

Cations	Adding cations (phase of growth)	The concentration of cations, mM	Surfactants, g/l		
			ethanol	glycerol	liquid paraffin
Cd^{2+}	Control	0	1.8 ± 0.09	2.1 ± 0.10	2.4 ± 0.12
	Lag-phase	0.1	1.2 ± 0.06*	1.6 ± 0.08*	1.7 ± 0.08*
		0.2	1.0 ± 0.05*	1.3 ± 0.06*	2.0 ± 0.10*
		0.3	0.7 ± 0.03*	1.0 ± 0.05*	2.3 ± 0.11*
	Exponential	0.1	2.3 ± 0.11*	2.8 ± 0.14*	2.9 ± 0.14*
		0.2	1.6 ± 0.08*	2.6 ± 0.13*	2.5 ± 0.12*
		0.3	1.0 ± 0.05*	2.5 ± 0.12*	2.2 ± 0.11*
	Stationary	0.1	0.6 ± 0.03*	2.0 ± 0.10*	1.6 ± 0.08*
		0.2	0.3 ± 0.01*	1.7 ± 0.08*	1.5 ± 0.07*
		0.3	0.2 ± 0.01*	1.3 ± 0.06*	2.0 ± 0.10*
Zn^{2+}	Control	0	1.7 ± 0.08	1.5 ± 0,07	2.2 ± 0.11
	Exponential	0.1	1.4 ± 0.07*	1.7 ± 0.08*	1.8 ± 0.09*
		0.5	1.2 ± 0.06*	1.5 ± 0.07*	1.6 ± 0.08*
	Stationary	0.1	1.6 ± 0.08*	1.9 ± 0.09*	2.1 ± 0.10*
		0.5	1.4 ± 0.07*	1.6 ± 0.08*	1.8 ± 0.09*
Cu^{2+} + Pb^{2+}	Control	0	N.d	N.d	2.1 ± 0.11
	Exponential	0.5 (Cu^{2+}) 0.1 (Pb^{2+})	N.d.	N.d	2.3 ± 0.12*
	Stationary		N.d	N.d	2.7 ± 0.13*
	Cu^2 + (Exponential) + Pb^2 + (Stationary)		N.d	N.d	3.0 ± 0.15*

Note: N.d — Not determined; * — $P \leq 0.05$ relative to control (the concentration of surfactant in the medium without metal cations).



Synthesis of surfactants under cultivation of *A. calcoaceticus* IMV B-7241 on *n*-hexadecane (*1*) and liquid paraffin (*2*) depending on the concentration of Cu^{2+}:

Addition of Cu^{2+} in the medium: *A* — exponential phase; *B* — stationary phase; * — $P \leq 0.05$ relative to control (the concentration of surfactant on *n*-hexadecane without adding Cu^{2+}); ** — $P \leq 0.05$ relative to control (the concentration of surfactant on liquid paraffin without adding Cu^{2+}).

IMB B-7241 strain without Cu^{2+} was observed (Figure). The maximum concentration of surfactants (up to 4.4–4.6 g/l) was achieved by adding of 2.0 mM Cu^{2+} in the stationary growth phase of A. calcoaceticus IMB B-7241.

It is known that microbial surfactants due to their ability to bind heavy metals can be used in environmental technologies to remove these contaminants [33]. In exeption of copper, the most frequently occured in the environment cations are cadmium, zinc, plumbum, etc. [33]. So, the next stage was to study the effect of metal cations on the the surfactant synthesis by *A. calcoaceticus* IMV B-7241 (Table 4). As it was found in previous studies (unpublished data), the introduction into cultivation medium of *A. calcoaceticus* IMV B-7241 the cations of Pb^{2+} accompanied by inhibition of both IMV B-7241 strain growth and surfactants biosynthesis. On the other hand, the addition of Cu^{2+} into the medium with liquid paraffin followed increasing synthesis of surfactant by IMV B-7241 strain. Thus, we suggested that the toxic effect of Pb^{2+} on cells of bacteria can be reduced in case of simultaneous introduction of lead and copper cations into into the medium (Table 4).

It was established that surfactant synthesis by *A. calcoaceticus* IMV B-7241 depended on the time of adding metal cations into the cultivation medium, their concentration and nature of the carbon substrate (Table 4).

So, the addition of 0.1 mM Cd^{2+} in the exponential phase of IMV-B-7241 strain growth on all the studied substrates the amount of synthesized surfactants was in 20–33% higher compared with that on the medium without metal cations. After addition

of zinc cations (0.1–0.5 mM) into medium with different carbon substrates, the surfactant concentration increased (13–20%) only under *A. calcoaceticus* IMV B-7241 cultivation on the glycerol. Maximal amount of synthesized surfactants (to 3.0 g/l) was achieved by adding 0.1 mM Cu^{2+} in the exponential phase of IMV B-7241 strain growth on liquid paraffins followed by the addition of 0.5 mM Pb^{2+} at the stationnary phase.

As in the case of addition of Cu^{2+}, one of the mechanisms of intensification of surfactant synthesis in the presence of metal cations may be the increase of their synthesis as protector compounds in response to unfavorable factors, which was confirmed by the study of protective functions of surfactants. Thus, after the removal of surfactants the survival of IMV B-7241 strain in the presence of 0.01–0.05 mM Cd^{2+} was decreased to 2–10%. Regarding to the influence of mixtures of metals, we can assume that the copper cations stimulate the synthesis of surfactants, and after the following introduction of lead cations, surfactants protect cells from toxic effects.

The role of exopolysaccharides in protecting of *Pseudoalteromonas* sp. SC SE425-7 and *Pseudoalteromonas* sp. SCSE709-6 from toxic effects of Cd^{2+} was described [34, 35]. Protecting role of surfactants from *Bacillus circulans* and *Bacillus cereus* NK1 against the influence of cations of cadmium, iron, lead, zinc and copper was described [36]. Neilson et al. [37] reported about the increased expression of genes rhlB/rhlC, responsible for the synthesis of rhamnolipides in the presence of 0.45 and 0.89 mM Cd^{2+}, and the influence of heavy metal cations on the qualitative composition of the synthesized surfactants. It is shown that in the presence of surfactant and Sd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Pb^{2+} cells of Bacillus cereus CM100B preserved their ability to divide, while without surfactants it was observed inhibition of peritrich flagella, that reduced the cell mobility [38].

The obtained are consistent with our previous data concerning the influence of heavy metal cations on the formation of surfactants from *R. erythropolis* IMV Ac-5017 [39], but the cell *A. calcoaceticus* IMV-B-7241 resisted to higher

Table 5. **Improved technology of surfactants synthesis by *A. calcoaceticus* IMV B-7241 on different substrates**

Substrate in accordance with the basic technology	Elements of improved technology	Increasing synthesis of surfactant relative to basic technology, %	The concentration of surfactant after improvement, g/l
Ethanol	Adding citrate and fumarate (0.01%) at the end of the exponential growth phase; Maintain a neutral pH level	350	6.0*
Glycerol	A mixture of *n*-hexadecane and glycerol in a molar ratio of 1: 7; C/N 30; Inoculum grown on *n*-hexadecane	350	2.5*
Hexadecane		265	
Liquid paraffin	Adding 2.0 mM Cu^{2+} in the stationary growth phase	230	4.6*
Hexadecane		245	4.4*
Ethanol	Adding 0.1 mM Cd^{2+} in the exponential growth phase	127	2.3*
Glycerol		133	2.8*
Liquid paraffin		121	2.9*
Glycerol	Adding 0.1 mM Zn2+ in the stationary growth phase	127	1.9*
Liquid paraffin	Adding 0.1 mM Cu^{2+} (exponential) + 0.5 mM Pb^{2+} (stationary phase)	143	3.0*

Note. In determining the increasing synthesis of surfactant relative to basic technology error does not exceed 5%.

* — $P \leq 0.05$ relative to control (the concentration of surfactants on the appropriate substrate in accordance with basic technology.

metal concentration (in ten times). In addition, these results confirmed our earlier suggestion that the concentration of metal cations and the time of their introduction into the cultivation medium may be the factor intensifying the synthesis of microbial surfactants [32].

Thus, as a result of the research it was proposed several approaches to improve surfactant synthesis by *A. calcoaceticus* IMV B-7241 both in the medium with ethanol and other substrates. Realisation of these approaches was accompanied by increasing extracellular surfactants concentration in 2.3–3.5 times as compared to the basic technologies (Table 5).

It was established that *A. calcoaceticus* IMV B-7241 synthesizes the maximal amount of surfactant (6.0 g/l) on the ethanol in the presence of precursor biosynthesis and under neutralization of the culture liquid. We have developed technology of surfactant synthesis by *A. calcoaceticus* IMV B-7241 which is not only better but also has advantages over the known in world the technologies of microbial surfactants on different carbon substrates [40]. Such advantages are: firstly using technological substrate (ethanol) instead of water-insoluble hydrophobic sources of carbon and the possibility of replacing expensive *n*-alkanes on this substrate; and secondly —a higher (in 1.2–3.8 times) yield of surfactants from substrate.

REFERENCES

1. *Marchant R., Banat I. M.* Biosurfactants: a sustainable replacement for chemical surfactants? *Biotechnol. Lett.* 2012, 34 (9), 1597–1605.
2. *Banat I. M., Franzetti A., Gandolfi I., Bestetti G., Martinotti M. G., Fracchia L., Smyth T. J., Marchant R.* Microbial biosurfactants production, applications and future potential. *Appl. Microbiol. Biotechnol.* 2010, 87 (2), 427–444.
3. *Banat I. M., Satpute S. K., Cameotra S. S., Patil R., Nyayanit N. V.* Cost effective technologies and renewable substrates for biosurfactants' production. *Front. Microbiol.* 2014, 5(697). doi: 10.3389/fmicb.2014.00697.
4. *Peng F., Liu Z., Wang L., Shao Z.* An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. *J. Appl. Microbiol.* 2007, 102 (6), 1603–1611.
5. *Franzetti A., Gandolfi I., Bestetti G., Smyth T. J. P., Banat I. M.* Production and applications of trehalose lipid biosurfactants. *Europ. J. Lipid Sci. Technol.* 2010, 112 (6), 617–627.
6. *Tuleva B., Christova N., Cohen R., Stoev G., Stoineva I.* Production and structural elucidation of trehalose tetraesters (biosurfactants) from a novel alkanothrophic *Rhodococcus wratislaviensis* strain. *J. Appl. Microbiol.* 2008, 104 (6), 1703–1710.
7. *Pidhorskyy V., Iutinska G., Pirog T.* Intensification of microbial synthesis technologies. *Kyiv: Nauk. Dumka.* 2010, 327 p. (In Ukrainian).
8. *Pirog T. P., Antonyuk S. I., Karpenko Ye. V., Shevchuk T. A.* The influence of conditions of *Acinetobacter calcoaceticus* K-4 strain cultivation on surface-active substances synthesis. *Appl. Biochem. Microbiol.* 2009, 45 (3), 272–278.
9. *Sadouk Z., Hacene H., Tazerouti A.* Biosurfactants production from low cost substrate and degradation of diesel oil by a *Rhodococcus* strain. *Oil Gas Sci. Technol.* 2008, 63 (6), 747–753.
10. *Tokumoto Y., Nomura N., Uchiyama H., Imura T., Morita T., Fukuoka T., Kitamoto D.* Structural characterization and surface-active properties of a succinoyl trehalose lipid produced by *Rhodococcus* sp. SD-74. *Oleo Sci.* 2009, 58(2), 97–102.
11. *Toren A., Navon-Venezia S., Ron E. Z., Rosenberg E.* Emulsifing activities of purified alasan proteins from *Acinetobacter radioresistens* KA53. *Appl. Environ. Microbiol.* 2001, 67 (3), 1102–1106.
12. *Dams-Kozlowska H., Mercaldi M.P., Panilaitis B.J., Kaplan D.L.* Modifications and applications of the *Acinetobacter venetianus* RAG-1 exopolysaccharide, the emulsan complex and its components. *Appl. Microbiol. Biotechnol.* 2008, 81 (2), 201–210.
13. *Zhao Z., Wong J. W. C.* Biosurfactants from *Acinetobacter calcoaceticus* BU03 enhance the solubility and biodegradation of phenanthrene. *Environ. Technol.* 2009, 30 (3), 291–299.
14. *Chen J., Huang P. T., Zhang K. Y., Ding F. R.* Isolation of biosurfactant producers, optimization and properties of biosurfactant produced by *Acinetobacter* sp. from petroleum-contaminated soil. *J. Appl. Microbiol.* 2012. 112 (4), 660–671.
15. *Bao M., Pi Y., Wang L., Sun P., Li Y., Cao L.* Lipopeptide biosurfactant production bacteria *Acinetobacter* sp. D3-2 and its biodegradation of crude oil. *Environ. Sci. Process Impacts.* 2014, 16 (4), 897–903.
16. *Zou C., Wang M., Xing Y., Lan G., Ge T., Yan X., Gu T.* Characterization and optimization of biosurfactants produced by *Acinetobacter baylyi* ZJ2 isolated from crude oil-contaminated soil sample towards microbial enhanced oil recovery applications. *Biochem. Eng. J.* 2014. http://dx.doi.org/10.1016/j.bej.2014.05.007.

17. *Hošková M., Ježdík R., Schreiberová O., Chudoba J., Šír M., Čejková A., Masák J., Jirků V., Řezanka T.* Structural and physiochemical characterization of rhamnolipids produced by *Acinetobacter calcoaceticus*, *Enterobacter asburiae* and *Pseudomonas aeruginosa* in single strain and mixed cultures. *J. Biotechnol.* 2015, V. 193, P. 45–51. doi: 10.1016/j.jbiotec.2014.11.014.
18. *Rooney A. P., Price N. P. J., Ray K. J., Kuo T.-M.* Isolation and characterization of rhamnolipid-producing bacterial strains from a biodiesel facility. *FEMS Microbiol. Lett.* 2009, 295 (1), 82–87.
19. *Pirog T. P., Shevchuk T. A., Shulyakova M. A.* The influence of organic acids on biosurfactant synthesis by strain *Acinetobacter calcoaceticus* IMV B-7241 culture on glycerol medium. *Biotekhnolohiia.* 2012, 5 (4), 88–95. (In Ukrainian).
20. *Cooper D. C., Goldenger B. G.* Surface-active agents from two *Bacillus* species. *Appl. Environ. Microbiol.* 1987, 53 (2), 224–229.
21. *Pirog T. P., Konon A. D., Pokora K. A., Shevchuk T. A., Iutinskaya G. A.* Influence of heavy metals on surfactants synthesis by *Nocardia vaccinii* IMV B-7405. *Mikrobiol. Zh.* 2014, 76 (4), 9–16. (In Russian).
22. *Pirog T. P., Korzh Yu. V., Shevchuk T. A., Tarasenko D. O.* Peculiarities of C_2-metabolism and intensification of the synthesis of surface active substances in *Rhodococcus erythropolis* EK-1 grown in ethanol. *Microbiology*. 2008, 77 (6), 665–673.
23. *Pan Y. T., Carroll J. D., Elbein A. D.* Trehalose-phosphate synthase of *Mycobacterium tuberculosis*. Cloning, expression and properties of the recombinant enzyme. *Eur. J. Biochem*. 2002, 269 (24), 6091–6100.
24. *Lakin G. F.* Biometrics. *Moskva: Vysshaya shkola.* 1990, 352 p. (In Russian).
25. *Ivanovskiy R. N.* Bioenergy and transport of substrates in bacteria. *Moskva: MAKSPress.* 2001, 46 p. (In Russian).
26. *De Roubin M. R., Mulligan C. N., Gibbs B. F.* Correlation of enhanced surfactin production with decreased isocitrate dehydrogenase activity. *Can. J. Microbiol.* 1989, 35 (9), 854–859.
27. *Pirog T. P., Shulyakova M. O., Shevchuk T. A.* Mixed substrates in environment and biotechnological processes. *Biotechnol. acta*. 2013, 6 (6), 28–44. (In Ukrainian).
28. *Pirog T. P., Konon A. D., Shevchuk T. A., Bilets I. V.* Intensification of biosurfactant synthesis by *Acinetobacter calcoaceticus* IMV B-7241 on a hexadecane–glycerol mixture. *Microbiology.* 2012, 81 (5), 565–572.
29. *Daverey A., Pakshirajan K.* Sophorolipids from *Candida bombicola* using mixed hydrophilic substrates: production, purification and characterization. *Colloids Surf. B. Biointerfaces*. 2010, 79 (1), 246–253.
30. *Konishi M., Morita T., Fukuoka T., Imura T., Kakugawa K., Kitamoto D.* Efficient production of mannosylerythritol lipids with high hydrophilicity by *Pseudozyma hubeiensis* KM-59. *Appl. Microbiol. Biotechnol*. 2008, 78 (1), 37–46.
31. *Borisova O. Iu., Gruber I. M., Egorova N. B., Ignatova O. M., Astashkina E. A.* Genetic characteristics of *Staphylococcus aureus* No 6. strain — producer of secreted protein-containing compounds possessing protective properties. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 2014, V. 6, P. 43–47. (In Russian).
32. *Pirog T. P., Konon A. D., Sofilkanich A. P., Shevchuk T. A., Parfeniuk S. A.* Effect of Cu^{2+} on synthesis of biosurfactants of *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017. *Mikrobiol. Zh.* 2013, 75 (1), 3–13. (In Russian).
33. *Mao X., Jiang R., Xiao W., Yu J.* Use of surfactants for the remediation of contaminated soils: a review. *J. Hazard. Mater.* 2015, V. 285, P. 419–435. doi: 10.1016/j.jhazmat.2014.12.009.
34. *Zhang H. O., Zhou W. Z., Ma Y. H., Zhao H. X., Zhang Y. Z.* FTIR spectrum and detoxication of extracellular polymeric substances secreted by microorganism. *Guang. Pu. Xue. Yu. Guang. Pu. Fen. Xi.* 2013, 33 (11), 3041–3043.
35. *Zhou W., Zhang H., Ma Y., Zhou J., Zhang Y.* Bio-removal of cadmium by growing deep-sea bacterium *Pseudoalteromonas* sp. SCSE709-6. *Extremophiles.* 2013, 17 (5), 723–731.
36. *Sriram M. I., Kalishwaralal K., Deepak V., Gracerosepat R., Srisakthi K., Gurunathan S.* Biofilm inhibition and antimicrobial action of lipopeptide biosurfactant produced by heavy metal tolerant strain *Bacillus cereus* NK1. *Colloids Surf. B. Biointerfaces* 2011, 85 (2), 174–181.
37. *Neilson J. W., Zhang L., Veres-Schalnat T. A., Chandler K. B., Neilson C. H., Crispin J. D., Pemberton J. E., Maier R. M.* Cadmium effects on transcriptional expression of rhlB/rhlC genes and congener distribution of monorhamnolipid and dirhamnolipid in *Pseudomonas aeruginosa* IGB83. *Appl. Microbiol. Biotechnol.* 2010, 88 (4), 953–963.
38. *Singh A. K., Dhanjal S., Cameotra S. S.* Surfactin restores and enhances swarming motility under heavy metal stress. *Colloids Surf. B. Biointerfaces* 2014, V. 116, P.26–31.doi:10.1016/j.colsurfb.2013.12.035.
39. *Pirog T. P., Shulyakova M. O., Shevchuk T. A., Sofylkanich A. P.* Biotechnological potential of bacteria of *Rhodococcus* strain and their metabolites. *Biotekhnolohiia*. 2012, 5 (2), 51–68. (In Ukrainian).
40. *Pirog T. P., Konon A. D.* Microbial surfactants. I. Glycolipids. *Biotechnol. acta*. 2014, 7 (1), 9–30. (In Ukrainian).

УДОСКОНАЛЕННЯ ТЕХНОЛОГІЇ СИНТЕЗУ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН *Acinetobacter calcoaceticus* ІМВ В-7241

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Мета роботи — інтенсифікація синтезу поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241 на етанолі та інших вуглецевих субстратах. *A. calcoaceticus* IMB B-7241 вирощували на моно- (етанол, гліцерол, рідкі парафіни, *н*-гексадекан, глюкоза) та змішаних субстратах за наявності у середовищі органічних кислот або катіонів важких металів (0,1–2,0 мМ Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}). Синтез поверхнево-активних речовин оцінювали за індексом емульгування культуральної рідини, умовною концентрацію та концентрацією позаклітинних поверхнево-активних речовин, яку визначали ваговим методом після екстрагування із супернатанта культуральної рідини сумішшю метанолу і хлороформу. Показано, що внесення цитрату і фумарату (0,01%) наприкінці експоненціальної фази росту та підтримання pH на нейтральному рівні дало змогу підвищити у 3,5 раза (до 6,0 г/л) концентрацію поверхнево-активних речовин під час культивування *A. calcoaceticus* IMB B-7241 на етанолі (2%). З використанням суміші *н*-гексадекану та гліцеролу (молярне співвідношення концентрацій 1:7) і C/N 30 кількість синтезованих позаклітинних поверхнево-активних речовин підвищувалась у 2,6–3,5 раза порівняно з кількістю на відповідних моносубстратах. У разі додавання 2,0 мМ Cu^{2+} у стаціонарній фазі росту *A. calcoaceticus* IMB B-7241 на рідких парафінах та *н*-гексадекані показники синтезу поверхнево-активних речовин збільшувалися в 2,3–2,5 раза порівняно з відповідними показниками на середовищі без Cu^{2+}.

Підходи до інтенсифікації синтезу поверхнево-активних речовин *A. calcoaceticus* IMB B-7241 (зокрема, внесення попередників біосинтезу та культивування на суміші ростових субстратів) можуть бути використані для підвищення ефективності мікробних технологій.

Ключові слова: мікробні поверхнево-активні речовини, інтенсифікація біосинтезу, попередники біосинтезу, змішані субстрати, важкі метали.

УСОВЕРШЕНСТВОВАНИЕ ТЕХНОЛОГИИ СИНТЕЗА ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ *Acinetobacter calcoaceticus* ИМВ В-7241

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Цель работы — интенсификация синтеза поверхностно-активных веществ *Acinetobacter calcoaceticus* ИМВ В-7241 на этаноле и других углеродных субстратах. *A. calcoaceticus* ИМВ В-7241 выращивали на моно- (этанол, глицерол, жидкие парафины, *н*-гексадекан, глюкоза) и смешанных субстратах при наличии в среде органических кислот или катионов тяжелых металлов (0,1–2,0 мМ Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}). Синтез поверхностно-активных веществ оценивали по индексу эмульгирования культуральной жидкости, условной концентрации и концентрации внеклеточных поверхностно-активных веществ, которую определяли весовым методом после экстракции из супернатанта культуральной жидкости смесью метанола и хлороформа. Показано, что внесение цитрата и фумарата (0,01%) в конце экспоненциальной фазы роста и поддержание pH на нейтральном уровне позволило повысить концентрацию поверхностно-активных веществ в 3,5 раза (до 6,0 г/л) при культивировании *A. calcoaceticus* ИМВ В-7241 на этаноле (2%). При использовании смеси *н*-гексадекана и глицерола (молярное соотношение концентраций 1:7) и C/N 30 количество синтезированных внеклеточных поверхностно-активных веществ штамма ИМВ В-7241 повышалось в 2,6–3,5 раза по сравнению с количеством на соответствующих моносубстратах. При добавлении 2,0 мМ Cu^{2+} в стационарной фазе роста *A. calcoaceticus* ИМВ В-7241 на жидких парафинах и *н*-гексадекане показатели синтеза поверхностно-активных веществ увеличивались в 2,3–2,5 раза по сравнению с соответствующими показателями на среде без Cu^{2+}.

Подходы к интенсификации синтеза поверхностно-активных веществ *A. calcoaceticus* ИМВ В-7241 (в частности, внесение предшественников биосинтеза и культивирование на смешанных ростовых субстратах) могут быть использованы для повышения эффективности микробных технологий.

Ключевые слова: микробные поверхностно-активные вещества, интенсификация биосинтеза, предшественники биосинтеза, смешанные субстраты, тяжелые металлы.