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THE PROPERTIES OF SURFACTANTS SYNTHESIZED BY *Acinetobacter calcoaceticus* IMV B-7241 ON REFINED AND SUNFLOWER OIL WASTE

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The aim of the work was to compare the antimicrobial and anti-adhesive activity (including the ability to destroy biofilms), as well as the effect on oil degradation of the surfactants synthesized by the culture of *Acinetobacter calcoaceticus* IMV B-7241 on refined and sunflower waste.

The microbial surfactants were extracted from supernatant of cultural liquid by mixture of chloroform and methanol (2:1). The number of attached cells and the degree of biofilm destruction were analyzed spectrophotometrically. Antimicrobial properties of the surfactants were determined by index of the minimal inhibiting concentration. The concentration of oil in water was analyzed by the gravimetric method after extraction with hexane.

It was shown that microbial surfactants synthesized in medium with 2% of both refined and fried oil were characterized by high antimicrobial (MIC with respect to bacterial test cultures 0.8–29 μg/ml, *Candida albicans* D-6 26 — 58 μg/ml) and anti-adhesive (decreasing number of bacterial and fungal cells of test cultures attached to abiotic surfaces by 35–70%, destruction of biofilms by an average of 40–44%) activity. Increasing concentration of waste oil in the medium to 4% was accompanied by the formation of surfactants with low antimicrobial activity, in the presence of which the degree of oil destruction in water (3–6 g/l) was 80–88% in 20 days, which is 10–16% higher than when using surfactants synthesized in a medium with 2% oil.

Obtained data indicate on the need for studies on the effect of cultivation conditions of producer on the properties of synthesized surfactants for the production of final product with stable predetermined properties, depending on the field of practical application.

Key words: microbial surfactants, sunflower oil waste, antimicrobial and anti-adhesive activity.

Biodegrading and nontoxic microbial surfactants (MS) are multifunctional due to their surfactant emulsifying properties, antimicrobial and anti-adhesive activities. MS are a worthwhile alternative to the chemical surfactants for various industrial and medical purposes, and in nature conservation technologies [1–5].

Nowadays, however, MS are industrially produced by only a few companies [6], although worldwide production of synthetic surfactants is 15 billion tons per year, expected to increase to 24 billion tons annually [7]. This situation is caused by insufficiently efficient microbial technologies of producing MS, partly because of highly expensive biosynthesis process and low MS concentrations. One of approaches of decreasing the MS cost is using industrial waste such as waste oil [8–10].

Another disadvantage of MS is the possible changes of their properties depending on the producers cultivation conditions, because the MS are secondary metabolites synthesized as a complex of such substances, see our previous studies [11, 12]. In those studies we emphasized the fact that at present the influence of cultivation conditions on the properties of microbial surfactants escapes the

attention of researchers. In recent years the number of publications on the dependence of biological properties of MS on their chemical composition has increased [13–16]. However, the authors of these papers did not investigate the dependence between the biological properties of surfactant and the conditions of cultivating producers.

Earlier [17], we showed that replacing purified glycerol in the cultivation medium of *Acinetobacter calcoaceticus* producer IMV B-7241 with the biodiesel production waste (technical glycerol) was accompanied by a decrease in the antimicrobial and anti-adhesive activity of synthesized surfactants. In [17], it was suggested that this may be caused by the inhibiting effect of monovalent cations (components of biodiesel production) on the activity of $NADP^+$-dependent glutamate dehydrogenase, the key enzyme in the biosynthesis of aminolipids responsible for these properties of the surfactants complex.

In previous studies [18], we have established the conditions for the cultivation of *A. calcoaceticus* IMV B-7241 on processed (fried) sunflower oil of different quality, which provide maximum rates of synthesis of surfactants. The maximum concentration of surfactant (7.9–8.5 g/l) was achieved during the cultivation of the IMV strain B-7241 on the waste after frying meat (4%) and sunflower potato (6%) oil, respectively. However, it is known [19, 20] that waste oils from frying potatoes and meat differ in composition, particularly in toxic substances. Thus, the iron cations from meat increase the degree of oxidation and thermal degradation in the waste oil [19]. In addition, when frying meat, toxic heterocyclic amines are formed [19, 20], which can act as inhibitors of $NADP^+$-dependent glutamate hydrogen dehydrogenase. For example, in the presence of 10 mM 2,4-pyridinedicarboxylate (derivative of heterocyclic aromatic amine pyridine), the activity of that enzyme in *Aspergillus niger* NCIM 565 is reduced by 2–4 times [21, 22].

Hence, the aim of this work was to compare the antimicrobial and anti-adhesive activity (including the ability to destroy biofilms), as well as the impact on the destruction of oil pollution by surfactants synthesized during the cultivation of *A. calcoaceticus* IMV B-7241 on refined and fried sunflower oil.

Materials and Methods

Study objects. The main study object was strain of petrooxidizing bacteria isolated from oil-contaminated soil and identified as *Acinetobacter calcoaceticus* K-4 [23]. The strain K-4 is registered in Depository of microorganisms of D. K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine under the number IMV B-7241.

Bacterial strains of *Escherichia coli* IEM1-, *Bacillus subtilis* BT2- (grown from spore cells in 24 hr), *Staphylococcus aureus* BMC-1, *Pseudomonas* sp. MI-2, *Enterobacter cloacae* C-8; yeasts *Candida albicans* D-6 and fungus *Aspergillus niger* P-3 were used to assess the antimicrobial and anti-adhesive activity of MS. The cultures were taken from the collection of microorganisms of the Department of biotechnology and microbiology of National university of food technologies.

Medium composition and cultivation conditions. The strain *A. calcoaceticus* IMV B-7241 was grown in liquid mineral medium as follows (g/l): 0.35 $(NH_2)_2CO$, 1.0 NaCl, 0.6 $Na_2HPO_4 \cdot 12H_2O$, 0.14 KH_2PO_4, 0.1 $MgSO_4 \cdot 7H_2O$, distilled water up to 1 l, pH 6.8–7.0. Also, 0.5% yeast lysate (v/v) and 0.1% microelement solution (v/v), were added. The microelement solution contained (g/100 ml): 1.1 $ZnSO_4 \cdot 7H_2O$; 0.6 $MnSO_4 \cdot H_2O$; 0.1 $FeSO_4 \cdot 7H_2O$; 0.004 $CuSO_4 \cdot 5H_2O$; 0.03 $CoSO_4 \cdot 7H_2O$; 0.006 H_3BO_3; 0,0001 KI; 0.5 EDTA (Trilon b). In one version, the concentration of urea in the medium was increased to 0.7–1.0 g/l.

Refined sunflower oil "Oleina" (Dnipropetrovsk oil and extraction plant) and waste oil from frying potatoes and meat (McDonald's, fast food restaurants network, Kyiv) (2–5%, volumetric) were used as a source of carbon and energy.

As inoculum we used the cultures in the exponential growth phase, grown on the medium of the described composition containing 0.5% (v/v) of the respective oil. The inoculation material (10^4–10^5 cells/ml) amounted to 10% of the volume of the culture medium.

The bacteria were cultivated in flasks of 750 ml with 100 ml of medium on the shaker (320 rpm) at 28–30 °C for 120 hr.

Determination of the antimicrobial and anti-adhesive activity of MS. The MS under study were extracted from supernatant of the culture liquid with Folch's method (chloroform and methanol mixture, 2:1).

The antimicrobial properties of MS were analyzed by the index of minimum inhibitory concentration (MIC). The MIC was determined using the method of double serial dilutions in

meat-peptone broth (MPB) for bacteria and liquid yeast as described earlier [11].

The anti-adhesive properties of surfactant were determined as described in [11]. The number of adherent cells (degree of adhesion) was found spectrophotometrically as the ratio of the optical density of the suspension obtained from substrates (tile, steel, linoleum) treated with the MS preparations to the optical density of the control samples (without surfactant processing), expressed as a percentage.

The MS effect on the biofilm destruction was studied according to the method shown in our previous publication [11].

Assessment of biological destruction of oil. The oil pollution of water was modeled according to [9, 24]. The experiment was conducted for 20 days. The amount of oil was determined by weight method after triple extraction with hexane (ratio 1:1). To study the influence of surfactants on the decomposition of oil we used preparations in the form of a culture liquid (10%, volumetric).

Total number of living cells in water during the experiment (20 days) was determined by Koch's method on MPA.

The quantity of oil was determined by the weight method. For this, extraction of petroleum with hexane was carried out three times (ratio 1: 1). The organic extract was evaporated to a constant mass on a rotary evaporator IR-1M2 (Russia) at a temperature of 55 °C and an absolute pressure of 0.4 atm.

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 [24]. The differences between the means were considered significant at $P < 0.05$.

Results and Discussion

Antimicrobial activity of surfactants of A. calcoaceticus IMV B-7241. Table 1 includes data on the dependence of MIC index of synthesized surfactants on the conditions of cultivation of IMV strain B-7241 on oily substrates. It was showed that *A. calcoaceticus* IMV B-7241, cultured on the medium with concentration of fried sunflower oil of different quality increased by 4–5%, produced surfactants with extremely low antimicrobial activity: the MIC for the bacterial test cultures was > 600–900 μg/ml, and in the case of yeast > 1200–1700 μg/ml (see Table 1). In these studies, the concentration of the carbon source in the medium increased simultaneously with the content of the nitrogen source to maintain

Table 1. **The influence of sunflower oil concentration and nitrogen source in the culture medium of *A. calcoaceticus* IMV B-7241 on the antimicrobial activity of surfactants**

Oil as substrate	Concentration in medium		Minimum inhibiting concentration (μg/ml) against				
	oil, %	Nitrogen source, g/l	*Escherichia coli* IEM-1	*Bacillus subtilis* BT-2 (spores)	*Staphylococcus aureus* BMC-1	*Pseudomonas* sp. MI-2	*Candida albicans* D-6
Refined	2	0.35	0.8	51.2	1.6	0.8	25.6
	4	0.7	15	64	32	16	130
Waste of frying meat	4	0.7	> 670**	> 670**	> 670**	> 670**	> 1340**
	4	1.0	> 850**	> 850**	> 850**	> 850**	> 1700**
Waste of frying potatoes	2	0.35	0.9*	28,8*	0.45*	0,45*	57.6*
	4	0.7	> 600**	> 600**	> 600**	> 600**	> 1200**
	4	1.0	> 750**	> 750**	> 750**	> 750**	N.d.
	5	0.7	> 850**	> 850**	> 850**	> 850**	N.d.
	5	1.0	> 900**	> 900**	> 900**	> 900**	N.d.

Note. Here and after: the statistical error of the analysis of minimum inhibitory concentration did not exceed 5%. N.d. — not determined.

* — $P \leq 0.05$ compared to control (minimum inhibitory concentration of MS, synthesized on medium with 2% (v/v) refined oil).

** — $P \leq 0.05$ compared to control (minimum inhibitory concentration of MS, synthesized on medium with 4% (v/v) refined oil).

the carbon/nitrogen ratio at the optimal level for synthesis of surfactants.

At the same time, increase concentration of refined oil to 4 % in the culture medium of IMV B-7214 strain was accompanied by MS synthesis, antimicrobial activity of those MS (MIC 15–130 μg/ml) was higher in comparison with that established for surfactants obtained on the fried oils of similar concentration (MIC > 600–1700 μg/ml) (Table 1). These data confirm our assumption that the composition of fried oils includes the synthesis inhibitors in the MS complex, responsible for the antimicrobial properties of *A. calcoaceticus* IMV B-7241.

Each year, the number of publications on the antimicrobial properties of MS and the potential use of these products of microbial synthesis in medicine is constantly increasing [25, 26]. However, there are only a few reports of the antimicrobial properties of surfactants synthesized on the oil-containing industrial waste [27–29].

Thus, the rhamnolipid of *Thermus thermophilus* HB8 [27], synthesized on such substrates, inhibited the growth of *Micrococcus lysodeikticus*. The growth of bacteria of the genus *Streptococcus* decreased by 35–65% in the presence of Rufisan (6–12 mg/l), synthesized by *Candida lipolytica* UCP0988 on soybean oil wastes (6%), while that of the representatives of the genus *Staphylococcus* was inhibited by only 15–18% [28]. It should be noted that Rufisan did not exhibit antimicrobial activity on *Candida albicans*.

In [29] it was showed that rhamnolipid synthesized by *Pseudomonas aeruginosa* LBI on soybean oil wastes inhibited the growth of *Bacillus cereus* and *Mucor miehei* at concentration of 64 μg/ml, and the growth of *Neurospora crassa*, *Staphylococcus aureus* and *Micrococcus luteus* was inhibited at 256 μg/ml.

In previous studies [30, 31] we showed the dependence of the antimicrobial activity of *Nocardia vaccinii* IMV B-7405 on the quality of the frying oil and the duration of the process. The strongest antimicrobial effect on the phytopathogenic bacteria of the genera *Pseudomonas*, *Xanthomonas* and *Pectobacterium* was caused by the MS solutions synthesized by *N. vaccinii* IMV B-7405 on the fried potato waste oil (reducing the survival of phytopathogenic bacteria by 50–95%), as well as by IMV B-7405 strains cultivated for 7 days on all oil-containing substrates (minimum inhibitory concentrations of 7–40 μg/ml, which is several times lower than that of MS synthesized in 5 days) [30].

The antibacterial (against *B. subtilis* BT2-, *E. coli* IEM1-, *Proteus vulgaris* PA12-, *S. aureus* BMC1-, *Pseudomonas* sp. MI-2, *E. cloacae* C-8, *Erwinia aroid*eae H-3) and antifungal (against *Candida tropicalis* PE-2 and *C. albicans* D-6) MIC of MS synthesized by *N. vaccinii* IMV B-7405 on fried potato waste oil were lower in 2.5 to 8 times than those of MS produced on media with fried meat waste oil [31]. Longer cultivation (for 7 days instead of 5 days) of *N. vaccinii* IMV B-7405 on waste oil after frying potatoes was accompanied by the synthesis of surfactants, MIC of which against test cultures was lower by 1.4–4 times. The MIC indexes of *N. vaccinii* IMV-7405 surfactants correlated with the presence of aminolipids in the synthesized MS, and the activity of $NADF^+$-dependent glutamate dehydrogenase enzyme, which is the key enzyme for their biosynthesis [31].

Note that in [30, 31], *N. vaccinii* IMV B-7405 was cultured on a medium containing 2% (volumetric) of fried oil.

Comparing the MIC of MS synthesized for 5 days on a medium with 2% fried potato oil revealed higher antimicrobial activity of the MS of *A. calcoaceticus* IMV B-7241 relative to the tested test cultures (0.4–58 μg/ml, Table 1) than that of MS of *N. vaccinii* IMV B-7405 (22–176 μg/ml) [31].

Anti-adhesive properties of MS. Since a 4–5% increase of the concentration of fried oil in *A. calcoaceticus* IMV B-7241 cultivation medium was accompanied by the formation of MS with low antimicrobial activity (Table 1), the anti-adhesive properties were determined for MS synthesized on a medium with 2% fried potatoes waste oil (Table 2).

According to the data in the Table 2, the adhesion of the tested test cultures to abiotic materials treatment with MS solutions was practically the same for MS synthesized on both refined and treated oils. Thus, the quantity of *B. subtilis* BT-2 cells attached to different surfaces was 27–48%, that of *S. aureus* BMS-1 cells was 25–58%, and that of *C. albicans* D-6 cells was 40–65%. Importantly, that significantly reduced adhesion of test cultures to materials treatmented with MS was achieved at extremely low concentrations of surfactants of *A. calcoaceticus* IMV-7241 (1.25–2.5 μg/ml).

Earlier [32] were found that the adhesion of *B. subtilis* BT-2, *E. coli* IEM-1 and *C. albicans* D-6 decreased by 30–60% on abiotic surfaces treated with solutions of MS synthesized by

Table 2. **The influence of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on refined or fried sunflower oil on the adhesion of microorganisms to abiotic surfaces**

Test culture	Oil substrate	Surfactant concentration, μg/ml	Adhesion,%			
			polystirene	tile	steel	linoleum
Bacillus subtilis BT-2	Refined	2.5	34	36	41	33
		1.25	27	47	48	41
	Fried	2.5	32*	34*	48*	34*
		1.25	29**	49**	44**	44**
Staphylococcus aureus BMC-1	Refined	2.5	57	58	47	25
		1.25	46	81	56	36
	Fried	2.5	56*	54*	45*	25*
		1.25	53**	79**	51**	37**
Candida albicans D-6	Refined	2.5	46	52	34	47
		1.25	42	49	47	65
	Fried	2.5	51*	51*	40*	44*
		1.25	n.d.	48**	43**	51**
Aspergillus niger P-3	Refined	2.5	60	N.d.	N.d.	n.d.
		1.25	77	N.d.	N.d.	N.d.
	Fried	2.5	54*	N.d.	N.d.	N.d.
		1.25	51**	N.d.	N.d.	N.d.

Notes. The medium contained 2% (v/v) oil after frying potatoes. Statistical error of determination of adhesion did not exceed 5 %. N.d. — not determined.

* — $P \leq 0.05$ compared to control (adhesion after treatment with 2.5 μg/ml surfactant, synthesized on the medium with 2 % of refined oil).

** — $P \leq 0.05$ compared to control (adhesion after treatment with 1.25 μg/ml surfactant, synthesized on medium with 2 % of refined oil).

N. vaccinii IMV B-7405 on refined and fried sunflower oil, at an order of magnitude higher concentrations of surfactants (10–40 μg/ml).

It should be noted that the publications of the anti-adhesive properties of surfactants, synthesized on oil-containing substrates, are not numerous. In [28] it was reported that Rufisan synthesized by *C. lipolytica* UCP0988 on waste products of soybean oil reduced the adhesion of bacteria of the genus *Streptococcus* and *Lactobacillus* on polystyrene plates.

For example, at the minimum investigated concentration of MS (0.75 mg/l), the adhesion of test cultures was 61–91%. With increased MS concentration in a solution up to 12 mg/l, Rufisan reduced the number of attached *E. coli* and *C. albicans* cells by 21–51%.

The probiotic strain *Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674 in cultures on various substrates including waste sunflower oil synthesized MS, which at a concentration of 10 mg/ml reduced the amount of *E. coli* cells attached to plastic by 13% and *Staphylococcus aureus* by 37% [33].

The data in Table 2 show that *A. calcoaceticus* IMV B-7241 on refined and processed oils synthesized MS, which were more effective as anti-adhesive agents than Rufisan and MS produced by *P. freudenreichii* subsp. *freudenreichii* PTCC 1674, because they caused the 35–75% decrease of the adhesion of test cultures at a concentration of only 1.25–2.5 μg/ml.

The role of MS of A. calcoaceticus IMV B-7241 in biofilm destruction. The data presented in Table 3 indicate that the degree of destruction of bacterial biofilms in the presence of surfactant depended on the quality of the oil used as substrate (refined, fried), the concentration of MS and the type of test culture. For example, the degradation of *B. subtilis* BT-2 biofilm for all studied concentrations of surfactant (29–233 μg/ml) obtained on fried sunflower oil was 6–15% higher compared to the values established

Table 3. **The influence of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on refined and fried sunflower oil on the destruction of bacterial biofilms**

Test culture	Oil substrate	Destruction (%) of biofilms treated with MS in concentrations (μg/ml)			
		29	58	116	233
Bacillus subtilis BT-2	Refined	5	7	37	48
	Fried	11*	19*	47*	63*
Staphylococcus aureus BMC-1	Refined	52	53	53	55
	Fried	36*	38*	57*	66*
Escherichia coli IEM-1	Refined	50	54	67	75
	Fried	83*	42*	33*	25*
Enterobacter cloacae C-8	Refined	24	29	52	54
	Fried	32*	47*	53*	55

Notes. The producer was grown on medium with 2% (v/v) oil after frying potatoes.
The statistical error of determination of the biofilm destruction did not exceed 5 %.
* — $P \leq 0.05$ compared to control (biofilm destruction after treatment with solutions of MS synthesized on medium with 2% refined sunflower oil).

for MS synthesized on the refined substrate. The degree of destruction of the *S. aureus* BMS-1 biofilm in the presence of MS formed on fried oils was 4–11% higher compared with the effect of MS obtained on refined oil, only at higher concentrations of such surfactants (116–233 μg/ml). At the same time, the maximum destruction of *E. coli* IEM-1 biofilm (83%) was achieved at the minimum the concentration (29 μg/ml) of surfactants synthesized on the fried oil. The degree of destruction of *E. cloacae* C-8 biofilm in the presence of 116–233 μg/ml of surfactant, was similar for MS synthesized on refined and fried oil (52–55%). However, at lower concentrations (29–58 μg/ml), MS obtained on fried oils destroyed the biofilm of this test culture better.

Hence, based on the data presented in Table 3, it can be concluded that replacing the refined oil with the fried oil in the cultivated medium of *A. calcoaceticus* IMV B-7241 is accompanied by the synthesis of surfactant which at low concentrations effectively destroys bacterial biofilms.

Notably, there are practically no reports about destruction of biofilms in the presence of surfactants synthesized in oil-containing substrates, although there are many publications on the effect of microbial surfactants on the degree of their destruction. Such data were summed up in the review [34]. The same publication presents data on the destruction of biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 on traditional substrates (ethanol, purified glycerin, hexadecane). Unlike MS derived from waste oils, surfactants produced by the IMV strain V-7241 on traditional substrates efficiently destroyed biofilms at higher concentrations (up to 1.28 mg/ml) [34].

In 2017, a lipopeptide synthesized by the actinobacteria strain *Nesterenkonia* sp. MSA31, isolated from the marine sponge *Fasciospongia cavernosa* (Lendenfeld, 1889), was reported to affect the destruction of *S. aureus* biofilm [35]. The MSA31 strain was grown in medium with 10% olive oil, the lipopeptide was extracted with organic solvents (ethyl acetate, methanol, petroleum ether, dichloromethane) from a supernatant pre-acidified to pH 2.0. Surfactant solutions in the range of concentrations of 25 to150 μg/ml were used to determine the effect of the lipopeptide in destroying the biofilms. It was established that the maximum destruction of *S. aureus* biofilms (90%) was achieved in the presence of lipopeptide at a concentration of 125 μg/ml [35].

Effect of MS on the destruction of oil pollution in water. Previously [36], we have shown that MS synthesized by *A. calcoaceticus* IMV B-7241 on traditional substrates intensified the decomposition of oil in water and soil, even in the presence of heavy metals. One of the determined mechanisms of intensification of oil decomposition

Table 4. **Oil destruction in water and quantitative changes in microbiota in the presence of culture liquid after the cultivation of the A. *calcoaceticus* IMV B-7241 strain on refined and fried oil**

Oil as substrate	Oil concentration in medium,%	Degree of oil destruction (%) at initial concentration (g/l)		Microorganisms in water (CFU/ml) at oil concentration (g/l)	
		3.0	6.0	3.0	6.0
Refined	2	71*	70*	$(1.9 \pm 0.09) \cdot 10^6$**	$(2.2 \pm 0.11) \cdot 10^6$**
Fried	2	72*	70*	$(2.0 \pm 0.10) \cdot 10^6$**	$(2.2 \pm 0.11) \cdot 10^6$**
	4	88*	80*	$(3.1 \pm 0.15) \cdot 10^6$**	$(2.9 \pm 0.14) \cdot 10^6$**

Notes. The producer was grown on medium with 2% (v/v) oil after frying potatoes.
The statistical error of determination of the oil destruction did not exceed 5 %.
* — $P \leq 0.05$ compared to control (8% — degree of oil destruction in water without the culture liquid).
** — $P \leq 0.05$ compared to control ($2.6 \cdot 10^4$ CFU/ml — initial number of cells in water before the addition of oil and culture liquid).

is the activation of natural oil-oxidizing microbiota under the influence of microbial surfactants.

However, high antimicrobial activity of microbial surfactants can be a significant barrier to their application in environmental technologies. For example, in some cases, highly concentrated MS can reduce the rate of purification process, adversely affecting destructor microorganisms. Whang et al. [37] note that surfactin at a concentration of 40 mg/l negatively affected the remediation of soils from diesel fuel, and at a concentration of 400 mg/l completely inhibited it. It should be noted that there are no data on the correlation of surfactant's antimicrobial activity and its role in the degradation of oil pollution.

Hence, the effect of surfactant *A. calcoaceticus* IMV B-7241 with different antimicrobial activity on decomposition of oil in water was investigated at the next stage of our study (Table 4).

It was established that the degree of oil destruction in water (both in concentrations of 3.0 and 6.0 g/l) and the total number of microorganisms which survived to the end of the experiment in the presence of MS, synthesized in medium with 4% of fried oil, were 10–16% and in 1.3–1.5 times higher than the values established for the MS, obtained in a medium with a lower (2%) concentration of the substrate. This can be explained by the fact that increasing the concentration of waste oil up to 4% in the *A. calcoaceticus* IVB-7241 culture medium induces formation of the surfactants with low antimicrobial activity (Table 1). As can be seen from the data in Table 4, such surfactants are promising for the purification of the environment from xenobiotics, since they do not have a negative effect on natural destructor microorganisms.

Hence, it was found that the replacement of refined oil (2%) with a similar concentration of fried waste oil in the culture medium of *A. calcoaceticus* IMV B-7241 was accompanied by the synthesis of surfactants with high antimicrobial and anti-adhesive activity (including the ability to destroy biofilms).

Increasing the concentration of waste oil to 4–5% allows the production of surfactants with low antimicrobial activity, which can be used in environmental technologies for the destruction of xenobiotics.

The presented data confirm the necessity of research on the influence of culture conditions on the properties of synthesized surfactants in order to obtain a target product with stable predetermined properties depending on the field of practical application.

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ВЛАСТИВОСТІ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН, СИНТЕЗОВАНИХ *Acinetobacter calcoaceticus* ІМВ В-7241 НА РАФІНОВАНІЙ І ВІДПРАЦЬОВАНІЙ СОНЯШНИКОВІЙ ОЛІЇ

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Метою роботи було порівняти антимікробну та антиадгезивну активність (зокрема й здатність до руйнування біоплівок), а також дослідити вплив на деструкцію нафтових забруднень поверхнево-активних речовин, синтезованих у процесі культивування *Acinetobacter calcoaceticus* ІМВ В-7241, на рафінованій та відпрацьованій соняшниковій олії.

Поверхнево-активні речовини екстрагували із супернатанта культуральної рідини сумішшю хлороформу і метанолу (2:1). Кількість адгезованих клітин і ступінь руйнування біоплівки визначали спектрофотометричним методом, антимікробні властивості — за показником мінімальної інгібувальної концентрації (МІК). Концентрацію нафти у воді вимірювали ваговим методом після екстрагування гексаном.

Встановлено, що мікробні поверхнево-активні речовини, синтезовані у середовищі з 2% як рафінованої, так і відпрацьованої олії, характеризувалися високою антимікробною (МІК щодо бактерійних тест-культур 0,8–29 мкг/мл, щодо *Candida albicans* Д-6 — 26–58 мкг/мл) та антиадгезивною (зниження кількості прикріплених до абіотичних поверхонь клітин тест-культур бактерій і грибів на 35–70%, руйнування біоплівок у середньому на 40–44%) активністю. Підвищення концентрації відпрацьованої олії у середовищі до 4% супроводжувалося утворенням мікробних поверхнево-активних речовин з невисокою антимікробною активністю, за наявності яких ступінь деструкції нафти у воді (3–6 г/л) на 20-ту добу досягав 80–88%, що на 10–16% вище, ніж у разі використання поверхнево-активних речовин, синтезованих у середовищі з 2% олії.

Наведені дані свідчать про необхідність проведення досліджень впливу умов культивування продуцентів на властивості синтезованих мікробних поверхнево-активних речовин з метою одержання цільового продукту зі стабільними наперед заданими властивостями залежно від галузі практичного застосування.

Ключові слова: мікробні поверхнево-активні речовини, відпрацьована соняшникова олія, антимікробна та антиадгезивна активність.

СВОЙСТВА ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ, СИНТЕЗИРОВАННЫХ *Acinetobacter calcoaceticus* ИМВ В-7241 НА РАФИНИРОВАННОМ И ОТРАБОТАННОМ ПОДСОЛНЕЧНОМ МАСЛЕ

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Целью работы было сравнить антимикробную и антиадгезивную активность (в том числе и способность к разрушению биопленок), а также исследовать влияние на деструкцию нефтяных загрязнений поверхностно-активных веществ, синтезированных при культивировании *Acinetobacter calcoaceticus* ИМВ В-7241, на рафинированном и отработанном подсолнечном масле.

Поверхностно-активные вещества экстрагировали из супернатанта культуральной жидкости смесью хлороформа и метанола (2:1). Количество адгезированных клеток и степень разрушения биопленки определяли спектрофотометрическим методом, антимикробные свойства — по показателю минимальной ингибирующей концентрации (МИК). Концентрацию нефти в воде измеряли весовым методом после экстракции гексаном.

Установлено, что поверхностно-активные вещества, синтезированные в среде с 2% как рафинированного, так и отработанного масла, характеризовались высокой антимикробной (МИК по отношению к бактериальным тест-культурам 0,8–29 мкг/мл, *Candida albicans* Д-6 — 26–58 мкг/мл) и антиадгезивной (снижение количества прикрепленных к абиотическим поверхностям клеток тест-культур бактерий и грибов на 35–70%, разрушение биопленок в среднем на 40–44%) активностью. Повышение концентрации отработанного масла в среде до 4% сопровождалось образованием поверхностно-активных веществ с невысокой антимикробной активностью, в присутствии которых степень деструкции нефти в воде (3–6 г/л) на 20-е сутки достигала 80–88%, что на 10–16% выше, чем при использовании поверхностно-активных веществ, синтезированных в среде с 2% масла.

Приведенные данные свидетельствуют о необходимости проведения исследований влияния условий культивирования продуцентов на свойства синтезированных поверхностно-активных веществ для получения целевого продукта со стабильными заданными свойствами в зависимости от области практического применения.

Ключевые слова: микробные поверхностно-активные вещества, отработанное подсолнечное масло, антимикробная и антиадгезивная активность.