Regulation of biological activity of surfactants under cultivation of *Acinetobacter calcoaceticus* IMB B-7241 on glycerol

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Abstract

Introduction. The aim of this research was to study the biological activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in the medium with glycerol of various degrees of purification and high content of calcium cations (activators of $NADH^+$-dependent glutamate dehydrogenase, which is a key enzyme of biosynthesis of surface-active aminolipids responsible for antimicrobial activity of surfactant complex).

Materials and methods. Cultivation of *A. calcoaceticus* was carried out in liquid mineral medium using as substrates refined glycerol and the waste from biodiesel production. The base medium did not contain calcium chloride, the content of $CaCl_2$ in the modified medium was 0.1 and 0.2 g/l. Surfactants were extracted from the supernatant of the culture luquid with a modified mixture of Folch. The number of adhered cells and the degree of biofilm destruction in the presence of surfactants was determined by spectrophotometric method, antimicrobial activity of surfactants - by the minimum inhibitory concentration (MIC).

Results and discussion. It was found that the additional introduction of 0.1−0.2 g/l of $CaCl_2$ into cultivation medium with refined glycerol was accompanied by the synthesis of surfactants, the MIC of which against bacteria (*Bacillus subtilis* BT-2, *Enterobacter cloacae* C-8, *Staphylococcus aureus* BMS-1) and yeast (*Candida albicans* D-6) were 1.01–21.3 μg/ml and were 1.4–29 times lower compared to the MIC of surfactants obtained in base medium (1.83−58.8 μg/ml). The adhesion of test cultures on abiotic materials treated with such surfactants was 8–13% lower, and the degree of biofilms destruction was 5–19% higher compared to the values, established for surfactants obtained on base medium. The increasing antimicrobial and anti-adhesive activity of surfactants synthesized on waste of biodiesel production was observed only when $CaCl_2$ was introduced into the medium at a concentration of 0.2 g/l. Surfactants synthesized in the presence of calcium cations in the medium with the waste of biodiesel production proved to be more effective destructors of bacterial biofilms in comparison with those obtained in the base medium only at low concentrations (0.7–5.5 μg/ml).

Conclusion. The results demonstrate the possibility of regulating the biological activity of *A. calcoaceticus* IMV B-7241 surfactants by changing in the composition of medium content of calcium cations - activators of $NADH^+$-dependent glutamate dehydrogenase (key enzyme of surface-active aminolipids biosynthesis). Surfactants synthesized under different cultivation conditions of *A. calcoaceticus* IMB B-7241 on glycerol are more effective biofilm destructors and antimicrobial and anti-adhesive agents compared to the known lipopeptides and rhamnolipids formed on glycerol.

Introduction

The disadvantages of technologies for obtaining microbial surfactants, which due to a combination of unique physicochemical and biological properties are metabolites of a wide range of applications, are changes in these properties in different cultivation conditions, as well as the high cost of such microbial synthesis products (Sharma and Sharma, 2021; Shu et al., 2021).

One of the ways to increase the efficiency of microbial surfactant technologies is using cheap industrial waste as a substrate, waste of biodiesel production, in particular (Crosse et al., 2019). Interest in waste of biodiesel production is due to the fact that the problem today is the need to dispose of large amounts of toxic industrial waste. The most effective way to dispose of such waste is to use them as substrates in biotechnological processes to obtain practically valuable products (Diamantopoulou et al., 2020).

There is no data in the literature about ways to regulate the biological activity of surfactants under the cultivation of the producer and notes that the main approaches to regulating the biological properties of microbial surfactants are their post-fermentation chemical modification and improvement of producer strains by metabolic and genetic engineering (Pirog et al., 2019).

It was previously established that *Acinetobacter calcoaceticus* IMV B-7241 synthesizes a complex of surfactant amino- and glycolipids on a wide range of carbon substrates, including glycerol of various degrees of purification. A study of the biological activity of surfactants synthesized on the waste of biodiesel production showed that such surfactants proved to be less effective antimicrobial agents compared to those formed on refined glycerol (Pirog et al., 2018).

Our previous studies have shown that one of the approaches to increase the antimicrobial and anti-adhesive activity of microbial surfactants is to increment the content of activators of key enzymes of aminolipid biosynthesis – the most effective antimicrobial agents (Pirog et al., 2018; 2019; 2021).

Activators of $NADH^{+}$-dependent glutamate dehydrogenase in strain *A. calcoaceticus* IMV B-7241 grown on refined glycerol are calcium cations. However, no activating effect of Ca^{2+} on the activity of this enzyme was detected during the cultivation of *A. calcoaceticus* IMV B-7241 on the waste of biodiesel production (Pirog et al., 2021)

In connection with the above, this work aimed to study the biological activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in a medium with glycerol of different degrees of purification and higher content of calcium cations (activators of $NADH^{+}$-dependent glutamate dehydrogenase – key enzyme of biosynthesis of surfactant aminolipids).

Materials and methods

Object of research

The main object of research was a strain of oil-oxidizing bacteria, identified as *Acinetobacter calcoaceticus* K-4 and isolated from an oil-contaminated soil sample. Strain *A. calcoaceticus* K-4 is registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the number IMV B-7241.

Bacterial strains (*Bacillus subtilis* BT-2, *Enterobacter cloacae* C-8, *Staphylococcus aureus* BMS-1) and yeast (*Candida albicans* D-6) from the collection of live cultures of the

Department of Biotechnology and Microbiology of the National University of Food Technology were used as test cultures in determining the antimicrobial and anti-adhesive activity of surfactants, as well as their role in the destruction of biofilms.

Medium composition and conditions of cultivation

Strain *A. calcoaceticus* IMV B-7241 was grown in the medium (g/l): $(NH_2)_2CO$ – 0.35; $MgSO_4 \cdot 7H_2O$ – 0.1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; pH 6.8–7.0. Yeast autolysate – 0.5% (v/v) and microelement solution – 0.1% (v/v) were additionally added into the medium. The micronutrient solution 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.

Modifications of the base medium:

1. Adding of $CaCl_2$ (0.1 g l),
2. Adding of $CaCl_2$ (0.2 g/l).

As carbon sources used (%, v/v) : refined glycerol – 3, waste of biodiesel production – 5. Concentrations of glycerol of different quality are equimolar on carbon.

As the inoculum was used culture in the exponential phase, grown in a medium of the above composition with 0.5% of the corresponding substrate. The inoculum with the number of bacteria 10^4–10^5 cells/ml was applied in an amount of 10% of the medium volume.

Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30°C for 7 days.

Determination of extracellular surfactant concentration

The amount of extracellular surfactants was determined using our modified method of Bly and Dyer (Bligh and Dyer, 1959) after extraction with a mixture of chloroform and methanol (2:1) from the supernatant of the culture liquid. To obtain the supernatant, the culture liquid was centrifuged at 5000 g for 20 minutes.

We modified the classical solvent system (Folch mixture - the known Bly and Dyer method used to isolate surfactants allows to isolate mainly nonpolar lipids) by adding 1 n HCl (chloroform-methanol – 1 n HCl = 4: 3: 2) because *A. calcoaceticus* IMV B-7241 synthesizes a complex of polar and non-polar lipids. This system allows to completely isolate both polar and non-polar lipids.

25 ml of supernatant was placed in a 100 ml cylindrical separation glass-stoppered funnel, added 1 n HCl solution to achieve a pH of 4.0-4.5 (about 5 ml). The funnel was shaken for 3 min, then added 15 ml of chloroform and methanol (2:1) and shaken again (lipid extraction) for 5 minutes. The mixture obtained after extraction was left in a separation funnel for phase separation, after which the lower fraction was drained (organic extract 1), and the aqueous phase was subjected to re-extraction. Upon re-extraction, 1 n HCl solution was added to the aqueous phase to achieve a pH of 4.0-4.5 (about 5 ml), 15 ml of a mixture of chloroform and methanol (2:1) and the lipids were extracted for 5 minutes. After phase separation, the lower fraction was drained to obtain organic extract 2. In the third step, 25 ml of a mixture of chloroform and methanol (2:1) was added to the aqueous phase, and extraction was carried out as described above for obtaining organic extract 3. Extracts 1-3 were combined and evaporated on a rotary evaporator IP-1M2 at 50ºC and an absolute pressure of 0.4 ATM to constant weight.

Obtaining surfactant preparations

Solutions of *A. calcoaceticus* IMV B-7241 surfactants with various concentrations were used as preparations in the researches. For this, the dry surfactant residue was dissolved in sterile phosphate buffer (0.1 M, pH 7.0) to the original volume (25 ml) and then diluted with this buffer to the required concentration. Surfactant solutions were sterilized in an autoclave at 112°C for 30 minutes.

Analysis of antimicrobial activity of surfactants

The antimicrobial activity of surfactants was analyzed by the minimum inhibitory concentration (MIC) (Chebbi et al., 2017). Determination of MIC was carried out by the method of double serial dilutions in meat-peptone broth (MPB) for bacteria and liquid wort for yeast. 1 ml of medium was added to 10 tubes, 1 ml of a surfactant solution of a certain concentration was added to the first tube, then mixed, after that 1 ml was taken and transferred to the next tube under sterile conditions. A similar dilution was performed for the next nine tubes. 1 ml was taken from the last tube. Thus, the final volume in each tube was 1 ml (MPB or wort and surfactant solution), and the surfactant concentration in each next tube was reduced by 2 times. As a control, 1 ml of MPB (for bacteria) or wort (for yeast) was used without the adding of surfactant solution. Next, 0.1 ml of test culture suspension (10^5–10^6 CFU/ml) was added to each tube and mixed. The tubes were incubated for 24 h at 28–30°C for bacteria and 24–26°C for yeast. The results were evaluated visually by the medium turbidity : (+) – tubes, medium turbidity was observed (growth of the test culture), (-) – there was no turbidity (no growth). The minimum inhibitory concentration of surfactant solution was defined as the surfactant concentration in the last tube where growth was absent.

Determination of anti-adhesive activity of surfactants

The anti-adhesive activity of surfactants was determined as described in (Rufino et al., 2011). Identical plates (1 cm^2) of test materials (tile, steel, glass) were pre-cleaned with detergent, rinsed with distilled water, air-dried and sterilized at 112°C for 30 minutes. After sterilization, the plates were treated with a surfactant solution (in the control version – sterile phosphate buffer) and kept at 30°C for 18-24 hours. Next, control and pre-treated surfactant materials were rinsed with sterile phosphate buffer or distilled water to remove residual preparations.

Test cultures of microorganisms were suspended in 100 ml of sterile tap water, pre-treated and untreated (control) materials were placed in the suspension and were kept for 2 h at 30°C. Control and pre-treated materials were rinsed with phosphate buffer to wash away non-adherent cells. Materials with adherent cells were left to air dry, after which the adherent cells were fixed by placing the material first in methanol (99%) for 15 min and stained in 1% gentian violet solution for 5 min. The plates of the material were rinsed with tap water and left at room temperature to dry. Next, the coloured adherent cells were washed from the surface of the materials with 1 ml of glacial acetic acid, made 9 ml of distilled water and measured the optical density of the resulting suspension spectrophotometrically using a wavelength of 540 nm.

The number of adherent cells was defined as the ratio of the optical density of the suspension obtained after treatment of materials with surfactants to the optical density of the suspension obtained after treatment of materials with phosphate buffer (control) and expressed as a percentage.

Study of the degree of the biofilm destruction under the action of surfactants

Determination of the effect of surfactants on the destruction of the biofilm was carried out as described in (Gomes et al., 2012). To obtain the biofilm, 180 µl of MPB or liquid wort and 20 µl of one-day test culture suspension were added to the polystyrene microplates, previously incubated for 24 h at the optimal temperature. Then the culture liquid was poured off and added another 180 µl of fresh MPB or wort and 20 µl of test culture suspension. Test culture suspensions were incubated for the next 24 hours. After 48 h, the culture liquid was poured off, and 200 µl solution with the different concentrations of surfactants was added to the microplate wells (with the biofilm of the test culture previously formed on them). In the control variants (wells) sterile tap water (200 µl) was added instead of surfactants. After exposure, the wells were washed three times with 200 µl of distilled water and the number of adherent cells was determined spectrophotometrically. The degree of biofilm destruction (%) was determined as the difference between cells adhesion in untreated and surfactant wells of the polystyrene plate.

Statistical analysis

All experiments were performed in 3 replicates, the number of parallel determinations in the experiments was 3-5. Statistical processing of experimental data was carried out as described in previous papers (Pirog et al., 2018). The differences in averages were considered reliable at the level of significance $p<0.05$.

Results and discussion

Effect of the concentration of activators of $NADH^+$-dependent glutamate dehydrogenase in the culture medium on antimicrobial synthesized surfactants

The table 1 shows the minimum inhibitory concentrations of the strain IMV B-7241 surfactants, synthesized in a medium with different concentrations of calcium chloride. The results of the studies showed that the cultivation of *A. calcoaceticus* IMV B-7241 in medium with refined glycerol and different concentrations of $CaCl_2$ was accompanied by the synthesis of surfactants with increased antimicrobial activity. Thus, the minimum inhibitory concentrations of such surfactants (1.01–21.3 µg/ml) were 1.4–29 times lower than the MIC surfactants obtained in the base medium (1.83−58.8 µg/ml).

However, the data in table 1, show that the antimicrobial activity of surfactants synthesized in the presence of 0.1 g/l $CaCI_2$ in the medium with the waste of biodiesel production was lower than the surfactants obtained on the base medium (MIC relative to the test cultures was 44.4 −355 and 29.7−59.5 µg/ml, respectively). In our opinion, one of the reasons for the lower antimicrobial activity of surfactants synthesized on waste of biodiesel production is the presence of potassium and sodium cations in these wastes, which may be potential inhibitors of $NADH^+$-dependent glutamate dehydrogenase *A. calcoaceticus* IMV B-7241.

At the same time, the adding of 0.2 g/l of calcium chloride into the medium with the waste of biodiesel production was accompanied by the synthesis of surfactants with higher antimicrobial activity compared with thoses synthesized on the medium with 0.1 g/l $CaCl_2$ (14.7−29.4 and 44.4−355 µg/ml, respectively, see Table 1). Moreover, the minimum inhibitory concentrations for *B. subtilis* BT-2 and *E. cloacae* C-8 surfactants synthesized in

the presence of 0.2 g/l of calcium chloride did not differ from those established for surfactants obtained on the base medium with the waste of biodiesel production (29.4-29.7 μg/ml), and the MIC for *S. aureus* BMS-1 and *C. albicans* D-6 such surfactants were even 4 times lower than the MIC of preparations synthesized in the base medium (14.7 and 59.5 μg/ml, respectively) (Table 1).

Table 1

Antimicrobial activity of *A. calcoac*eticus IMV B-7241 surfactants, synthesized in a medium with different concentrations of calcium cations

Carbon source	Concentration of $CaCl_2$ in the medium, g/l	Minimum inhibitory concentrations (μg / ml) relative			
		Bacillus subtilis BT-2 (spores)	*Entero-bacter cloacae* C-8	*Staphylo-coccus aureus* BMS-1	*Candida albicans* D-6
Waste of biodiesel production	0	29.7	29.7	59.5	59.5
	0.1	88.7	44.4	88.7	355
	0.2	29.4	29.4	14.7	14.7
Purified glycerol	0	3.67	1.83	58.8	29.4
	0.1	2.65	1.33	21.3	21.3
	0.2	1.01	1.01	2.03	4.06

Note. When determining the minimum inhibitory concentration, the error did not exceed 5%.

The detected dependence of the antimicrobial activity of surfactants synthesized on the waste of biodiesel production on the concentration of calcium cations in the medium can be explained for the following reasons. First, given the presence of monovalent cations - potential inhibitors of $NADH^{+}$-dependent glutamate dehydrogenase in biodiesel waste, may be required a higher concentration of calcium cations to activate this enzyme. Secondly, apparently, the strain IMV B-7241 has several glutamate dehydrogenases that are activated by different concentrations of calcium cations.

Our previous studies (Pirog et al., 2019) of the effect of different concentrations of calcium cations on the antimicrobial activity of surfactants synthesized during the cultivation of *A. calcoaceticus* IMV B-7241 on ethanol showed that the minimum inhibitory concentration against bacteria (*Escherichia coli* IEM-1, *B. subtilis* BT-2, *E. cloaceae* C-8, *Proteus vulgaris* PA-12) surfactants formed on a medium with 0.1 g/l $CaCl_2$, was 1.3−3.5 times lower, than MIC surfactants synthesized in the base medium (4−32 and 14−56 μg/ml, respectively).

Note that in the literature information on the antimicrobial activity of surfactants synthesized on refined glycerol are few and relate to lipopeptides synthesized by bacteria of the genus *Bacillu*s (Das et al., 2009; Singh et al., 2014), and rhamnolipids synthesized by representatives of the genus *Pseudomonas* and *Halomonas* (Alvionita and Hertadi, 2019; Buonocore et al., 2020). However, minimal inhibitory concentrations of surfactants *Bacillus circulans* (Das et al., 2009), *Bacillus amylofaciens* AR2 (Singh et al., 2014) and *Bacillus thuringiensis* pak2310 (Deepak and Jayapradha, 2015) against bacteria (*E. coli* NCIM 2931, *Micrococcus flavus* 2376, *P. vulgaris* NCIM 2857 and *S. aureus* MRS) and fungi (*Aspergillus niger*, *C. albicans, Fusarium solani* ATCC 36031, *Fusarium oxysporum* MTCC 7229,

Alternaria alternata MTCC 2724, *Alternaria citri* MTCC 4875, *Cladosporium cladisporo*rec*opular* ATCC 160 ATCC 58636, *Microsporum gypseum* MTCC 4522, *Trichophyton rubrum* MTCC 296) were in the range of 50–750 μg/ml, which is significantly higher than the values, established for our studied surfactants *A. calcoaceticus* IMV B-7241 (see Table 1). The MIC of rhamnolipids relative to bacterial (*S. aureus* 6538P, *Bacillus cereus, Listeria monocytogenes, Staphylococcus epidermidis, Stenotrophomonas maltophilia* 13637) and fungal (*T. rubrum, Trichophyton mentagrophytes*) test cultures were 3.13–433 μg/ml (Buonocore et al., 2020; Sen et al., 2020) and were higher than the minimum inhibitory indexes of surfactant *A. calcoaceticus* IMV B-7241 (see Table 1).

To date, we have found only one work in the available literature that investigated the antimicrobial activity of surfactants synthesized on biodiesel waste (Bharali et al., 2014). However, the authors of this work determined the antimicrobial activity of rhamnolipids synthesized by *Pseudomonas aeruginosa* JBK1 by the method of diffusion into agar, so compare their data with those given in table 1 does not seem possible.

Therefore, our results point out the possibility of regulating the antimicrobial activity of *A. calcoaceticus* surfactants IMV B-7241 synthesized in the medium with glycerol different degrees of purification by adding calcium cations and are consistent with the preliminary results of cultivation strain IMV B-7241 on medium with ethanol and different concentrations of Ca^{2+} (Pirog et al., 2019).

Influence of calcium cations on anti-adhesive activity of surfactants

The data shown in table 2 indicate that the additional introduction of calcium cations into the medium with refined glycerol was accompanied by the synthesis of surfactants with increased anti-adhesive activity.

Thus, under the action of surfactants synthesized in such medium, the adhesion *S. aureus* BMS-1 to abiotic materials was 3−28% and was lower than in the presence of surfactants formed in a medium without calcium cations – 11−33%. In the case of adding of 0.2 g/l $CaCl_2$ into the medium with the waste of biodiesel production, the synthesis of surfactants was observed, in the presence of which the adhesion of *S. aureus* BMS-1 on tiles, steel and glass was only 7−25%, while the anti-adhesive activity of surfactants, synthesized in a medium without calcium cations, was lower: the degree of adhesion was 10−64% (Table 2).

Similar patterns were observed during the study of the anti-adhesive activity of surfactants against *B. subtilis* BT-2. The adhesion of this test culture cells on abiotic surfaces treated with surfactant solutions synthesized in a medium with calcium chloride was 26–45%, that is lower than after the treatment of surfactants obtained on a base medium (31–48%). At the same time, the number of *E. cloacae* C-8 cells attached to the surfaces decreased (compared to the control) by several percent only in the case of their treatment with surfactants synthesized in the presence of 0.2 g/l in the medium. Note that only at the lowest concentration of surfactants (0.55 μg/ml) synthesized in the presence of 0.2 g/l of calcium chloride, a decrease of 12-13% in the adhesion of *C. albicans* D-6 on tiles and steel in compared with the indicators established for surfactants obtained on the base medium. Note that such patterns were observed for surfactants synthesized on both refined glycerol and waste of biodiesel production.

The obtained data points out that the anti-adhesive activity of surfactants depends on many factors: the concentration of surfactants, the type of abiotic surface and the type of test cultures. In addition, no direct correlation was found between the antimicrobial and anti-adhesive activity of surfactants synthesized under different culture conditions of *A.*

calcoaceticus IMV B-7241. It is obvious that the anti-adhesive action of microbial surfactants is based not only on antimicrobial activity but also on other mechanisms (for example, changes in the surface charge of cells or the surface).

Table 2

Effect of *A. calcoaceticus* IMV B-7241 surfactant, synthesized in a medium with different content of calcium cations, on the adhesion of *Staphylococcus aureus* BMS-1 to different surfaces

Carbon source	Contents $CaCl_2$, g/l	Surfactant concentration, μg / ml	Adhesion, %		
			Tile	Steel	Glass
Waste of biodiesel production	0	8.87	23	11	10
		4.43	51	17	17
		2.2	55	19	21
		1.1	58	28	21
		0.55	64	33	24
	0,1	8.87	62	6	10
		4.43	68	14	24
		2.20	70	14	24
		1.10	77	22	38
		0.55	83	25	52
	0,2	8.87	17	25	10
		4.43	11	22	7
		2.20	8	14	10
		1.10	9	14	7
		0.55	9	8	7
Refined glycerol	0	8.87	19	13	11
		4.43	25	17	17
		2.20	25	23	25
		1.10	30	23	25
		0.55	33	27	30
	0,1	8.87	15	11	10
		4.43	15	15	11
		2.20	20	19	19
		1.10	23	19	25
		0.55	28	26	32
	0,2	8.87	13	8	3
		4.43	19	11	14
		2.20	23	14	17
		1.10	28	19	21
		0.55	28	25	28

Note. When determining adhesion, the error did not exceed 5%.

Despite the information on the antimicrobial activity of surfactants synthesized on glycerol, in the available literature there is much less information about their anti-adhesive action.

In (Chebbi et al., 2017) it was found that the degree of adhesion of *Bacillus licheniformis* CAN55 and *Staphylococcus capitis* SH6 was 15 and 35 %, respectively, on polystyrene after treatment with solutions of rhamnolipids *P. aeruginosa* W10 synthesized on refined glycerol at a concentration of 3125 μg/ml.

The amount of adherent *S. aureus* ATCC 29523, *B. cereus* MTCC 7190, *Salmonella typhimurium* ATCC 19430 cells to a polystyrene surface treated with solutions of *B. subtilis* VSG4 lipopeptides obtained on refined glycerol (3000 μg/ml) was in the range of 33-40% (Giri et al., 2019).

Therefore, synthesized in both base and modified medium with refined glycerol and waste of biodiesel production surfactants *A. calcoaceticus* IMV B-724 are significantly more effective anti-adhesive agents than those described in (Chebbi et al., 2017; Giri et al., 2019) because they show anti-adhesive activity in orders of magnitude lower concentrations (0.55−8.87 μg/ml).

Destruction of biofilms under the action of surfactants synthesized in an medium with different concentrations of calcium cations

Surfactants of microbial origin also have the ability to destroy biofilms in addition to antimicrobial and anti-adhesive activity. Therefore, the destruction of bacterial and yeast biofilms under the action of *A. calcoaceticus* IMV B-7241 surfactants, synthesized in medium of different compositions were studied in the next step.

The results showed that surfactants synthesized in a medium with refined glycerol and $CaCl_2$ were more effective in destroying bacterial and yeast biofilms than surfactants obtained on a similar base medium without calcium cations (Tables 3–6).

Table 3

Destruction of *Enterobacter cloacae* C-8 biofilm by the action of surfactants synthesized on glycerol

Carbon source	Concentration $CaCI_2$ (g/l)	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)		
		5.5	1.4	0.7
Waste of biodiesel production	0	38.6	32.9	21.6
	0.1	48.8	41.0	33.8
	0.2	54.5	46.3	41.0
Refined glycerol	0	68.7	71.5	75.6
	0.1	56.0	83.4	86.5
	0.2	92. 0	93.2	93.9

Note. Table. 3−6: when determining the destruction of the biofilm, the error did not exceed 5%.

Table 4

Effect of calcium cations content in the culture medium of *A. calcoaceticus* IMV B-7241 on the ability of synthesized surfactants to destroy the biofilm of *Bacillus subtilis* BT-2

Carbon source	Concentration $CaCl_2$ (g/l)	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)					
		44.3	**22.2**	**5.5**	**2.7**	**1.4**	**0.7**
Waste of biodiesel production	0	52.6	45.3	37.1	33.7	33.7	30.0
	0.1	33.7	36.8	38.2	40.3	41.4	N.d.
	0.2	38.9	41.3	42.3	45.6	48.7	49.4
Refined glycerol	0	44.2	51.6	59.7	72.3	78.5	N.d.
	0.1	45.1	56.7	64.7	79.2	85.2	N.d.
	0.2	57.4	63.1	72.4	85.2	87.3	N.d.

Note. N.d. - not determined.

Table 5

Destruction of *Staphylococcus aureus* BMS-1 biofilm under the influence of *A. calcoaceticus* IMV B-7241 surfactants, synthesized in a medium with different content of calcium cations

Carbon source	Concentration $CaCl_2$ (g/l)	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)		
		44.3	**2.7**	**1,4**
Waste of biodiesel production	0	28.8	28.8	31.7
	0.1	29.5	30.5	33.7
	0.2	38.1	40.4	43.7
Refined glycerol	0	74.5	54.7	45.6
	0.1	88.3	64.3	56.1
	0.2	94.2	72.6	68.3

Table 6

Antibiofilm activity against *Candida albicans* D-6 surfactants synthesized by *A. calcoaceticus* IMV B-7241

Carbon source	Concentration $CaCl_2$ (g/l)	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)			
		22.2	**5.5**	**1.4**	**0.7**
Waste of biodiesel production	0	71.4	60.2	53.0	52.1
	0.1	25.5	33.7	37.7	40.8
	0.2	33.7	43.9	45.1	48.2
Refined glycerol	0	79.1	78.1	76.5	72.3
	0.1	88.6	86.5	84.4	78.2
	0.2	95.8	94.0	91.8	84.7

Thus, under the action of surfactants synthesized in a medium with 0.1 and 0.2 g/l of calcium chloride, the destruction of biofilms was on average 5−10 and 12−19%, respectively, higher than in the presence of surfactants obtained on a medium without $CaCI_2$.

Note that the destruction of biofilms of all studied test cultures was 6−12% higher under the influence of surfactants synthesized in a medium with refined glycerol containing 0.2 g/l of calcium chloride than under the action of surfactants obtained on a similar medium with 0.1 g/l $CaCl_2$. In addition, a high degree of biofilms destruction (on average 70-90%) was achieved at low (0.7-5.5 μg/ml) concentrations of surfactants synthesized on refined glycerol.

Slightly different patterns were observed during the study of the biofilms destruction by surfactants obtained on the waste of biodiesel production with different calcium chloride content (see Tables 3–6). First, surfactants synthesized in the medium with calcium cations proved to be more effective destructors of bacterial biofilms compared to those obtained in the base medium only at low concentrations (0.7–5.5 μg/ml). Second, the additional introduction of calcium chloride into the medium with the waste of biodiesel production was accompanied by the formation of surfactants, which were characterized by a lower ability to destroy yeast biofilm than synthesized in the base medium: the degree of destruction *C. albicans* D-6 biofilm was 25−48 and 52−71% respectively (see table. 6). In addition, surfactants obtained under different conditions of *A. calcoaceticus* IMV B-7241 cultivation on the waste of biodiesel production have a lower ability to destroy bacterial and yeast biofilms than those synthesized on refined glycerol (destruction 21-71 and 56−96 accordingly, see Tables 3–6).

There is limited data in the literature on the ability of surfactants synthesized in a glycerol-containing medium to destroy microbial biofilms. Giri et al. (2019) found that the destruction of biofilms of *S. aureus* ATCC 29523, *E. coli* MTCC 65, *S. typhimurium* ATCC 19430 was in the range of 58−78% in the presence of lipopeptides *B. subtilis* VSG4 in a sufficiently high concentration (3000−5000 μg/ml). The destruction of the biofilm of *F. oxysporum* (strain not specified) was observed by 58% in the presence of 500 μg/ml lipopeptides of *B. thuringiensis* pak2310 (Deepak and Jayapradha, 2015). The work of Sen et al. (2020) show that the destruction of biofilms of fungi *T. rubrum* MTCC 8477 and *T. mentagrophytes* NCCPF 800049 reached 80-85% under the influence of rhamnolipids at a concentration of 2000 and 250 μg/ml, respectively. De Rienzo and Martin (2016) found that rhamnolipids of *Burkholderia thailandensis* E264 at a concentration of 400 μg/ml are able to destroy the biofilm of *B. subtilis* BBK066. In this work, the destruction of the biofilm was examined visually using confocal microscopy, and the degree of its destruction by the authors is not given.

Thus, described in the literature the rhamnolipids and lipopeptides synthesized on purified glycerol, destroy 58–85% of bacterial and fungal biofilms at sufficiently high concentrations (400–5000 μg/ml). Our results (see Table 3−6) show that the same degree of destruction of bacterial and yeast biofilms is achieved by the action of *A. calcoaceticus* IMV B-7241 surfactants in much lower (several orders of magnitude) concentrations (0.7−44, 3 μg/ml).

Conclusion

1. The results demonstrate the possibility tof regulate the biological activity of surfactants produced by *A. calcoaceticus* IMB B-7241 by changing in the composition of medium with refined glycerol and waste of biodiesel production content of calcium cations, which are activators of $NADH^+$-dependent glutamate dehydrogenase (key enzyme of surface-active aminolipids biosynthesis).

2. Surfactants synthesized under different cultivation conditions by *A. calcoaceticus* IMB B-7241 on refined glycerol and waste of biodiesel production are more effective biofilm destructors and antimicrobial and anti-dhesive agents compared to the known lipopeptides and rhamnolipids formed on glycerol.

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