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ORIGINAL RESEARCH PAPER

FACTORS AFFECTING ANTIBIOFILM PROPERTIES OF MICROBIAL SURFACTANTS

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Abstract: Antibiofilm activity of surface-active substances (SAS) synthesized by *Rhodococcus erythropolis*, *Acinetobacter calcoaceticus*, or *Nocardia vaccinia* was studied. The strains were cultivated using carbon ethanol, glycerol, hexadecane, sunflower oil, and carbon-containing wastes from biodiesel production as carbon sources. *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and yeasts *Candida albicans* and *Candida utilis* were used as testing cultures. The antibiofilm activity of biosurfactants depended on the source and concentration of carbon source and duration of cultivation. The addition of calcium cations to media significantly increased antibiofilm activity. Replacement of pure sources of carbon for wastes provided the opportunity to receive biosurfactants with high antibiofilm properties on inexpensive substrates. Destruction of biofilms was the same in cases when supernatant or purified SAS was used.

Keywords: *antibiofilm properties, biosurfactants, conditions of cultivation*

INTRODUCTION

Microorganisms of biofilms are characterized by high resistance to biocides [1−3]. The ability of microorganisms to form biofilms is considered a factor of their pathogenicity, so colonization of the surfaces, for example, the surfaces of implantable medical devices, can cause the spreading of infections [4]. Generally, 80 % of all infections are caused by microorganisms able to form biofilms [1 − 4].

Biofilm formation is also a serious industrial problem. Biofilms can cause biocorrosion of pipes, biofouling of technological equipment, especially membrane filters. Biofilms in the food industry can contain spoilage and pathogenic microorganisms that cause the contamination of the food products and food-borne disease outbreaks [5].

Modern technologies for the destruction of microbial biofilms include the usage of mechanical, physical, chemical, and biological methods [3 – 5]. Biological methods have such advantages as high efficiency, sustained release, and safety for humans and the environment. However, the ability of microorganisms to acquire resistance to antibiotics and other biocides, high cost of the majority of the methods for prevention and destruction of biofilms cause searching for new substances with appropriate properties. Microbial surface-active substances (SAS) are studied for the last 15 years as substances for the destruction of biofilms on different surfaces [6].

Biosurfactants reduce surface tension and interfacial tension of biofilms. Altogether SAS has an antimicrobial activity due to microbial cell cytoplasmic membrane disintegration, increasing its permeability and cell death [7, 8]. Biosurfactants have antiadhesion activity due to the change of surface-bound charge of the cell. These mechanisms of microbial SAS action ensure the high efficiency of biofilm destruction. Moreover, biosurfactants have a lot of advantages in comparison with traditional agents used for biofilms destruction: application of microbial SAS is environmentally friendly due to their biodegradability; biosurfactants are considered low or non-toxic substances; specific mechanism of action significantly reduces the risk of getting a resistant form of microorganisms [6, 8, 9].

The composition and properties of biosurfactants are depended on the substrate and conditions used for the cultivation of SAS producers. It was shown that conditions of cultivation affect antimicrobial and antiadhesion activities of SAS synthesized by *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 [10 – 12]. Application of different carbon sources affected not only yield but also the type and antifungal activity of lipopeptide biosurfactant produced by *Bacillus amyloliquefaciens* strain AR2 [13]. Antibacterial activity of sophorolipids produced by yeasts *Candida bombicola* cultivated in fermentation medium with different sugar sources varied for the tested microorganisms [14]. However, there is no information about the influence of cultivation conditions on the ability of biosurfactants to destroy microbial biofilms.

In the present research, the effects of cultivation conditions on antibiofilm abilities of microbial surfactants synthesized by *R. erythropolis* IMVAc-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 were studied.

MATERIALS AND METHODS

Microorganisms

The strains of *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 were isolated from the oil-polluted soil and were deposited in the Collection of Microorganisms of Institute of Microbiology and Virology, National Academy of Science, Ukraine [15].
The biosurfactant produced by *R. erythropolis* IMV Ac-5017 was a set of glycolipids (trehalose mono- and dimycolates), neutral lipids (cetyl alcohol, palmitic acid, methylpentadecanoic acid, and mycolic acids) and phospholipids (phosphatidyl glycerol and phosphatidylethanolamine) [16]. SAS produced by *A. calcoaceticus* IMV B-7241 contained glycolipids (trehalose mono- and dimycolates) and aminolipids [17]. The strain *N. vaccinii* IMV B-7405 synthesized a set of glycolipids such as trehalose diacelats and trehalose mikolats and such neutral lipids as mycolic and alkanoic acids, and aminolipids [18].
Bacterial strains *Escherichia coli* IEM1, *Bacillus subtilis* BT2, *Staphylococcus aureus* BM1, *Pseudomonas sp.* MI2, *Pseudomonas aeruginosa* P55, yeasts *Candida albicans* D6 and *Candida utilis* EI8 from the Collection of Microorganisms of the Department of Biotechnology and Microbiology, National University of Food Technologies, Ukraine, were used as test microbial cultures. These cultures were chosen for this study because bacteria of the genera *Escherichia*, *Bacillus*, *Staphylococcus*, *Pseudomonas,* and yeasts of the genus *Candida* can form biofilm medically implanted devices as well on the food matrixes [1, 2, 5].

Cultivation of biosurfactants producers

Bacterial strain *R. erythropolis* IMV Ac-5017 was cultivated in the liquid mineral medium with the following composition, $g \cdot L^{-1}$: $NaNO_3$, 1.3, $MgSO_4 \cdot 7H_2O$, 0.1, NaCl, 1.0, Na_2HPO_4, 0.6, KH_2PO_4, 0.14, $FeSO_4 \cdot 7H_2O$, 0.01, distilled water 1 L, *p*H 6.8–7.0 (basic medium). $CaCl_2$, 0.1 $g \cdot L^{-1}$, was added to the basic medium in some experiments (modified medium). Ethanol, 2 % ($v \cdot v^{-1}$), purified glycerol, 1.2 and 3.6 % ($v \cdot v^{-1}$), or crude glycerol, waste from biodiesel production on Komsomolskyi pant, Poltava region, Ukraine, 2.0 and 6.0 % ($v \cdot v^{-1}$) were used as the carbon sources.
Bacterial strain *A. calcoaceticus* IMV B-7241 was cultivated in liquid mineral medium with the following composition, $g \cdot L^{-1}$: $(NH_2)_2CO$, 0.35, $MgSO_4 \cdot 7H_2O$, 0.1, NaCl, 1.0, Na_2HPO_4, 0.6, KH_2PO_4, 0.14, distilled water 1 L, *p*H 6.8–7.0. Yeast autolysate, 0.5 % ($v \cdot v^{-1}$) and solution of microelements, 0.1 % ($v \cdot v^{-1}$), were added in medium. Composition of microelements solution was as follow, $g \cdot mL^{-1}$: $ZnSO_4 \cdot 7H_2O$, 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, 0.5 (basic medium). $CaCl_2$, 0.1 $g \cdot L^{-1}$, and $MgSO_4 \cdot 7H_2O$, 0.1 $g \cdot L^{-1}$, were added to basic medium (modified medium). Ethanol, 2 % ($v \cdot v^{-1}$), purified glycerol, 1.7 and 3.6 % ($v \cdot v^{-1}$), *n*-hexadecane, 1.3 % ($v \cdot v^{-1}$), refined sunflower oil, 2 % ($v \cdot v^{-1}$), spent oil, 2 % ($v \cdot v^{-1}$), crude glycerol, 6,0 % ($v \cdot v^{-1}$) were used as the sources of carbon.
Bacterial strain *N. vaccinii* IMV B-7405 was cultivated in liquid mineral medium with the following composition, $g \cdot L^{-1}$: $NaNO_3$, 0.5, $MgSO_4 \cdot 7H_2O$, 0.1, $CaCl_2 \cdot 2H_2O$, 0.1,

KH_2PO_4, 0.1, $FeSO_4 \cdot 7H_2O$, 0.01, yeast autolysate, 0.5% ($v \cdot v^{-1}$) (basic medium). $CaCl_2$, 0.4 $g \cdot L^{-1}$, was added to basic medium (modified medium). Purified glycerol, 1.2, 2.0 and 4.8 % ($v \cdot v^{-1}$), crude glycerol, 2.9, 3.3 and 8.0 % ($v \cdot v^{-1}$), refined oil, 2.0 % ($v \cdot v^{-1}$), spent oil, 2.0 % ($v \cdot v^{-1}$), were used as sources of carbon.
The inoculum was grown in a liquid medium containing 0.5 – 1.0 % ($v \cdot v^{-1}$) of the corresponding carbon source. The culture liquid with the concentration of bacterial cells of 10^4–10^5 $cells \cdot mL^{-1}$ was taken from the exponential growth phase and was added to the medium for the strain cultivation, 10 % ($v \cdot v^{-1}$). Cultivation of bacteria was conducted at 30 ºC in the flasks with a volume of 750 mL in 100 mL of the medium under shaking at 320 rpm during 120 – 168 hours.

Recovery of surface-active substances

After cultivation, the liquid was centrifuged at 5000×g for 25 minutes using laboratory centrifuge LP–8, Kiev, Ukraine. The modified method [19] was used for the extraction of surface-active substances. The supernatant, 25 mL, was placed in the funnel with volume 100 mL, 1 N solution of HCl was added to adjust *p*H to 4.0 – 4.5, the mixture was shaken for 3 min, then Folch solution (chloroform and methanol in volume ratio 2:1), 15 mL, was added, the mixture was shaken for 5 min and left for phase separation. The lower fraction (organic extract 1) was removed, and the extraction procedure was repeated twice to obtain organic extracts 2 and 3. Chloric acid was not added during the third extraction. All extracts were combined and evaporated on the rotor evaporator ER-1M2 (Russia) at 50 °C and pressure 0.4 bar to the constant mass. The dry SAS was dissolved in distilled water to initial volume and sterilized at 112 °C for 30 min. The concentration of SAS was determined by the mass method [19].

Evaluation of biofilm destruction using biosurfactants

A biofilm disruption study using SAS was done to follow the method described by Gomes and Nitschke [20]. The polystyrene microplates were used for biofilm formation. Beef-extract broth (BEB), 180 µL, or mash, 180 µL, and suspension of 1-day testing bacterial culture, 20 µL, were placed in the well of polystyrene microplate and incubated for 24 hours at a temperature optimal for this microorganism. Then, culture liquid was removed, and fresh BEB or mash, 180 µL, and suspension of testing bacterial culture were added and incubated for 48 hours. This time is enough for the formation of biofilm in the well of the microplate [20]. Culture liquid was removed, and SAS's solution with different concentrations from 10 to 640 $\mu g \cdot mL^{-1}$, 200 µL, was added to the well with formed biofilm. Sterile water, 200 µL, was added instead of the SAS solution in control. After 24 hours of biosurfactant contact with the biofilm, the wells were washed three times, with 200 µL of tap water. The quantity of adhered cells was determined with the spectrophotometric method.
The wells were treated with 200 µL of methanol (99 %) for 15 min for fixation of adherent cells, and then the cells were stained with Crystal violet (1% solution) for 15 min. The wells were washed with tap water, and 33 % of acetic acid, 200 µL, was added. After 15 min liquid was removed from the well, and its extinction was determined at 630 nm using spectrophotometer KFK-3 (Russia). Biofilm destruction was determined as a ratio of extinction of liquid from an experimental well with SAS to

the extinction of liquid from a control well with water and expressed as a percentage.

Statistics

Results were expressed as the mean of at least three independent replicates, and the results are expressed as mean ± standard deviation. The difference of the means was considered reliable at significance value $p<0.05$.

RESULTS AND DISCUSSION

Destruction of biofilms by biosurfactants synthesized using conventional carbon sources

Addition of SAS synthesized by *A. calcoaceticus* IMV B-7241 in the media with such sources of carbon as ethanol, 2 % ($v \cdot v^{-1}$), purified glycerol, 1.7 % ($v \cdot v^{-1}$), or hexadecane, 1.3 % ($v \cdot v^{-1}$), with equimolar concentrations of carbon showed that level of biofilm destruction depended on SAS concentration, type of test culture and source of carbon used for cultivation of biosurfactant producer (Table 1).

Table 1. *Destruction of biofilms by biosurfactants synthesized by A. calcoaceticus IMV B-7241 using traditional carbon sources*

Carbon source	SAS concentration [$\mu g \cdot mL^{-1}$]	Destruction of biofilm [%]			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Candida utilis***
Ethanol	40	54.1 ± 0.4	35.1 ± 0.3	33.3 ± 0.3	0
	80	69.1 ± 0.5	46.1 ± 0.2	37.1 ± 0.3	0
	160	70.8 ± 0.4	53.8 ± 0.5	38.9 ± 0.2	0
	320	77.2 ± 0.6	55.9 ± 0.6	39.1 ± 0.4	0
	640	85.8 ± 0.7	59.1 ± 0.4	47.2 ± 0.3	0
Purified glycerol	40	55.3 ± 0.4	42.0 ± 0.2	30.7 ± 0.2	8.1 ± 0.1
	80	57.7 ± 0.5	49.8 ± 0.4	33.1 ± 0.2	8.2 ± 0.0
	160	61.1 ± 0.5	54.1 ± 0.4	35.3 ± 0.2	13.1 ± 0.1
	320	63.2 ± 0.4	55.7 ± 0.3	35.1 ± 0.3	17.8 ± 0.2
	640	86.7 ± 0.4	58.0 ± 0.2	37.4 ± 0.1	23.0 ± 0.1
Hexadecane	40	15.1 ± 0.1	64.6 ± 0.5	39.0 ± 0.4	13.1 ± 0.0
	80	22.9 ± 0.1	67.1 ± 0.7	43.1 ± 0.3	13.4 ± 0.1
	160	31.3 ± 0.2	69.0 ± 0.4	42.9 ± 0.1	21.9 ± 0.1
	320	40.1 ± 0.3	72.7 ± 0.6	45.2 ± 0.1	32.1 ± 0.2

Increase of SAS concentration from 40 to 640 $\mu g \cdot mL^{-1}$ increased destruction of biofilm of *B. subtilis* in 1.6 – 2.9 and biofilm of *S. aureus* in 1.4 – 1.7 times, destruction of biofilm of *E. coli* remained on the same level. SAS synthesized on ethanol did not influence the destruction of biofilm of *C. utilis*.

It was shown that destruction of biofilms was the same in cases when supernatant of purified SAS produced by *R. erythropolis* IMV Ac-5017, grown in the basic medium with ethanol, 2 %, and *N. vaccinii* IMV B-7405, grown in basic medium with glycerol,

2 %, was used (Table 2).

Table 2. *Destruction of biofilms by supernatants and solutions of biosurfactants synthesized by R. erythropolis IMV Ac-5017 and N. vaccinii IMV B-7405*

Producer of SAS	Preparation of SAS	SAS [μg·mL^{-1}]	Destruction of biofilm [%]		
			E. coli	*B. subtilis*	*C. albicans*
R. erythropolis IMV Ac-5017	Supernatant	20	39.8 ± 0.2	32.8 ± 0.2	50.0 ± 0.0
	SAS solution	20	35.7 ± 0.3	42.8 ± 0.2	53.8 ± 0.3
	Supernatant	40	44.1 ± 0.3	38.1 ± 0.3	53.1 ± 0.4
	SAS solution	40	37.0 ± 0.2	47.0 ± 0.4	57.2 ± 0.3
N. vaccinii IMV B-7405	Supernatant	20	15.8 ± 0.0	62.1 ± 0.5	78.2 ± 0.5
	SAS solution	20	19.9 ± 0.1	57.7 ± 0.3	75.1 ± 0.6
	Supernatant	40	18.1 ± 0.0	65.8 ± 0.3	81.1 ± 0.6
	SAS solution	40	20.9 ± 0.1	60.0 ± 0.5	78.8 ± 0.4

The addition of SAS produced by these bacterial cultures resulted in high-level destruction (50-81 %) of yeast biofilm *C. albicans*.

Destruction of biofilms by biosurfactants synthesized using different wastes

The level of biofilm destruction by SAS depended not only on the test culture and SAS concentration but also on the carbon source and its concentration in the medium used to cultivate SAS producer (Tables 3 and 4).

Table 3. *Destruction of biofilms by biosurfactants synthesized by N. vaccinii IMV B-7405 using different wastes*

Carbon source [v·v^{-1}]	SAS [μg·mL^{-1}]	Destruction of biofilm [%]		
		E. coli	*B. subtilis*	*C. albicans*
Refiend sunflower oil, 2.0	10	30.2 ± 0.2	70.3 ± 0.5	12.0 ± 0 0
	20	30.8 ± 0.3	72.0 ± 0.5	13.2 ± 0.1
	40	29.3 ± 0.1	65.8 ± 0.6	28.3 ± 0.2
	80	24.0 ± 0.1	66.1 ± 0.3	33.2 ± 0.2
Spent sunflower oil after meat roasting, 2.0	10	26.2 ± 0.2	30.3 ± 0.3	15.1 ± 0.1
	20	27.1 ± 0.3	29.9 ± 0.2	15.2 ± 0.1
	40	27.7 ± 0.3	31.2 ± 0.2	21.3 ± 0.1
	80	27.0 ± 0.1	20.4 ± 0.1	25.7 ± 0.2
Spent sunflower oil after potatoes roasting, 2.0	10	52.4 ± 0.4	35.8 ± 0.2	27.9 ± 0.1
	20	53.9 ± 0.4	37.1 ± 0.2	22.8 ± 0.2
	40	47.2 ± 0.2	40.0 ± 0.2	34.8 ± 0.3
	80	44.9 ± 0.4	45.8 ± 0.3	40.4 ± 0.2
Purified glycerol, 2.0	10	18.0 ± 0	67.2 ± 0.2	71.2 ± 0.3
	20	18.0 ± 0.2	66.0 ± 0.4	72.2 ± 0.2
	40	20.1 ± 0.1	59.8 ± 0.3	69.3 ± 0.3
	80	21.3 ± 0.1	57.0 ± 0.5	60.3 ± 0.3
Crude glycerol, 3.3	10	15.3 ± 0.2	36.2 ± 0.1	55.4 ± 0.3
	20	15.0 ± 0.1	37.1 ± 0.2	56.1 ± 0.3
	40	17.7 ± 0.1	46.2 ± 0.3	50.0 ±0.1
	80	17.9 ± 0.1	53.2 ± 0.2	50.0 ± 0.3

Even the source of spent oil, used by *N. vaccinii* IMB B-7405 for SAS production, affected the level of biofilm destruction (Table 3). The level of biofilms destruction by SAS synthesized using spent oil from roasted potatoes was in 1.2 – 2 times higher than SAS synthesized using spent oil from roasted meat (Table 3). The destruction levels of biofilms of *E. coli* and *C. albicans* were the same and even higher when spent oil was used instead of refiened oil. However, the destruction levels of biofilms of *B. subtilis* and *C. albicans* with SAS synthesized using crude glycerol (wastes from biodiesel production) were lower than SAS synthesized using purified glycerol (Table 3).
However, another trend was observed when SAS, synthesized by *R. erythropolis* IMB Ac-5017 in a medium with purified glycerol or wastes from biodiesel production, was used for biofilms destruction (Table 4).

Table 4. *Destruction of biofilms by biosurfactants synthesized by R. erythropolis IMV Ac-5017 using purified glycerol or crude glycerol*

Carbon source, % [v·v^{-1}]	**SAS [µg· mL^{-1}]**	**Destruction of biofilm [%]**			
		Pseudomonas sp.	***E. coli***	***B. subtilis***	***C. albicans***
Crude glycerol, 2.0	15	58.1 ± 0.3	25.0 ± 0.2	61.2 ± 0.3	35.7 ± 0.2
	30	72.1 ± 0.4	26.0 ± 0.1	63.0 ± 0.3	46.8 ± 0.4
	60	78.9 ± 0.3	28.3 ± 0.2	77.7 ± 0.5	48.3 ± 0.2
Crude glycerol, 6.0	15	44.1 ± 0.4	14.2 ± 0.1	58.2 ±0.5	55.0 ± 0.4
	30	46.0 ± 0.3	30.3 ± 0.2	56.3 ± 0.4	60.3 ± 0.4
	60	47.7 ± 0.4	49.2 ± 0.3	56.1 ± 0.3	61.1 ± 0.3
Purified glycerol, 1.2	15	33.2 ± 0.2	42.7 ± 0.3	39.2 ± 0.1	50.3 ± 0.2
	30	49.1 ± 0.4	31.3 ± 0.1	44.6 ±0.1	71.8 ± 0.6
	60	69.8 ± 0.4	26.0 ± 0.1	71.0 ± 0.3	71.9 ± 0.2
Purified glycerol, 3.6	15	65.1 ± 0.5	42.8 ± 0.2	51.2 ± 0.1	54.0 ± 0.2
	30	60.2 ± 0.3	32.8 ± 0.3	39.6 ± 0.2	49.3 ± 0.1
	60	39.4 ± 0.2	16.1 ± 0.1	25.1 ± 0.1	24.2 ± 0.1

Replacement of the purified glycerol with equimolar by carbon concentration crude glycerol for the cultivation of *R. erythropolis* IMV Ac-5017 increased the ability of synthesized SAS to destruct biofilms of *B. subtilis* and *Pseudomonas* sp. (Table 4).
The level of biofilm destruction depended not only on carbon sources but also on its concentration to cultivate biosurfactant producers. An increase of crude glycerol concentration from 2 to 6 % (v·v^{-1}) resulted in SAS synthesis with a lower destruction level of bacterial biofilms; meanwhile, the destruction level of biofilm of *C. albicans* was higher (Table 4).

Destruction of biofilms by biosurfactants synthesized at different producer growth time

It was shown for *N. vaccinii* IMV B-7405 that time of cultivation had an influence on antibiofilm activity of synthesized SAS that was used in a concentration of 20 µg·mL^{-1} (Table 5).
Antibiofilm activity of SAS received by long-term cultivation of the strain *N. vaccinii* IMV B-7405 in basic medium with purified or spent sunflower oil from roasted meat or with purified glycerol, 1.2 % (v·v^{-1}) was decreased in comparison with SAS produced

after short-term cultivation (Table 5).

Table 5. *Destruction of biofilms by biosurfactants synthesized by N. vaccinii IMV B-7405 at different growth time*

Carbon source, % [v·v^{-1}]	**Stationary growth phase**	**Desctruction of biofilm [%]**		
		Ps. aeruginosa	***B. subtilis***	***C.utilis***
Sunflower oil, purified, 2.0	early	30.2 ± 0.2	71.9 ± 0.5	54.1 ± 0.3
	late	12.1 ± 0.0	37.6 ± 0.2	38.0 ± 0.3
Spent sunflower oil after meat roasting, 2.0	early	10.2 ± 0.1	19.8 ± 0.1	20.3 ± 0.2
	late	3.1 ± 0.0	7.3 ± 0.0	12.2 ± 0.1
Spent sunflower oil after potatoes roasting, 2.0	early	39.8 ± 0.4	37.3 ± 0.3	35.3 ± 0.3
	late	43.0 ± 0.3	38.2 ± 0.3	47.9 ± 0.4
Purified glyzerin, 1.2	early	72.9 ± 0.6	66.2 ± 0.4	79.0 ± 0.6
	late	17.1 ± 0.1	49.2 ± 0.3	30.1 ± 0.2
Purified glyzerin, 4,8	early	57.1 ± 0.4	40.3 ± 0.2	25.0 ± 0.1
	late	57.1 ±0.2	43.7 ± 0.3	11.8 ± 0.1
Waste from biodisel production, 2,0	early	30.8 ± 0.2	37.2 ± 0.2	27.1 ± 0.3
	late	50.1 ± 0.4	49.1 ± 0.1	56.2 ± 0.4
Waste from biodisel production, 8,0	early	57.2± 0.5	42.6 ± 0.2	21.7 ± 0.1
	late	56.8 ± 0.6	42.2 ± 0.3	22.7 ± 0.2

Meanwhile, SAS from the late stationary growth phase of strain *N. vaccinii* IMV B-7405 cultivated using waste materials from biodiesel production had higher antibiofilm activity than SAS from the early stationary growth phase. Antibiofilm activity of SAS produced in the medium with spent oil from roasted potatoes did not depend on the time of biosurfactant producer growth.

Destruction of biofilms by biosurfactants synthesized in basic and modified media

It was shown that the composition of medium for cultivation of SAS producer had a strong influence on SAS antibiofilm activity (Table 6).
Basic and modified media differed in calcium content were used in this experiment. Cations of calcium are activators of NADP-dependent glutamate dehydrogenase, the key enzyme of biosynthesis of surface-active aminolipids [12, 21, 22]. SAS synthesized by all studied strains on modified medium had higher antibiofilm activity than ones received on basic medium (Table 6).
So, even low concentrations (from 8 μg·mL^{-1}) of surface-active substances synthesized by *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405 possess a high level of destruction of bacterial or yeasts biofilms (Table 6). These SAS can be synthesized using a wide spectrum of carbon sources, including toxic industrial wastes. Thus, the SAS concentration in a medium with crude glycerol can reach up to 10 g·L^{-1}, and the yield of SAS per unit mass of carbon-containing substrate consumed varies from 40 to 50 % [18]. Spent oil can also be successfully used for SAS production (Tables 3 - 5).
The ability to destruct biofilms is shown not only for solutions of SAS synthesized by *R. erythropolis* IMB Ac-5017 and *N. vaccinii* IMB B-7405 but also for corresponding supernatants (Table 2). Application of SAS-containing supernatants for biofilms

destruction gives the possibility to exclude from the technological process stage of SAS extraction.

Table 6. *Destruction of biofilms by biosurfactants synthesized by A. calcoaceticus IMB B-7241, N. vaccinii IMB B-7405 and R. erythropolis IMB Ac-5017 in basic and modified media*

SAS producer	Test culture	Medium	Destruction of biofilm [%] after addition of SAS [μg·μL^{-1}]			
			8	16	32	64
R. erythropolis grown on ethanol, 2% (v·v^{-1})	*B. subtilis*	basic	20.2 ± 0.1	34.3 ± 02	42.7 ± 0.3	69.1 ± 0.5
		modified	39.7 ± 0.4	56.6 ± 0.4	67.2 ± 0.6	73.0 ± 0.4
	E. coli	basic	46.0 ± 0.2	44.2 ± 0.2	35.7 ± 0.2	28.1 ± 0.2
		modified	53.1 ± 0.2	49.6 ± 0.3	43.2 ± 0.3	34.0 ± 0.2
	C. albicans	basic	48.9 ± 0.2	53.1 ± 0.4	57.1 ± 0.4	59.1 ± 0.4
		modified	55.0 ± 0.4	62.8 ± 0.4	64.2 ± 0.5	64.8 ± 0.5
A. calcoaceticus grown on ethanol, 2% (v·v^{-1})	*B. subtilis*	basic	27.9 ± 0.2	29.2 ± 0.1	50.8 ± 0.4	63.2 ± 0.4
		modified	43.3 ± 0.3	44.8 ± 0.4	69.2 ± 0.3	75.1 ± 0.6
	S. aureus	basic	3.0 ± 0	14.2 ± 0.1	27.1 ± 0.2	38.0 ± 0.3
		modified	17.2 ± 0.1	29.9 ± 0.3	47.8 ± 0.3	49.8 ± 0.4
N. vaccinii grown on purified glycerol, 2% (v·v^{-1})	*B. subtilis*	basic	31.1 ± 0.3	53.4 ± 0.3	58.8 ± 0.4	63.2 ± 0.4
		modified	39.7 ± 0.2	63.2 ± 0.2	76.3 ± 0.5	80.8 ± 0.7
	E. coli	basic	44.4 ± 0.2	29.1 ± 0.2	26.6 ± 0.2	20.1 ± 0.1
		modified	67.2 ± 0.4	53.8 ± 0.5	52.0 ± 0.3	50.0 ± 0.4
	C. utilis	basic	17.0 ± 0.1	24.1 ± 0.2	24.0 ± 0.2	25.2 ± 0.1
		modified	19.2 ± 0.1	35.0 ± 0.1	37.2 ± 0.1	38.8 ± 0.3

CONCLUSIONS

Antibiofilm properties of surface-active substances synthesized by *Rhodococcus erythropolis* IMB Ac-5017, *Acinetobacter calcoaceticus* IMB B-7241, or *Nocardia vaccinii* IMB B-7405 cultivated using different carbon sources including pure substances such as ethanol, glycerol, hexadecane, refined sunflower oil and wastes such as spent sunflower oil and crude glycerol (wastes from biodiesel production) was shown. Replacement of pure substances for waste products will diminish the cost of SAS production with high antibiofilm activity. The level of bacterial and yeast biofilms destruction by SAS was affected by the conditions for the cultivation of SAS producers such as source, concentration, and quality of carbon source and time of biosurfactant producer growth. In addition to media for SAS producers, cations of calcium resulted in a significant increase in their ability to destroy bacterial and yeast biofilms.

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