Solution Properties, Surface Activities And Antimicrobial Effectiveness Of New Biological Surfactants

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ABSTRACT

The solution properties, surface activities and antimicrobial effectiveness of new biological surface active-substances obtained from microbial cultural liquids and medical plant cells have been investigated. A very good correlation between the foam forming ability and the surface activity of new biological surfactants has been determined. It has been shown, that the stability of surfactant foams significantly determined by the interaction of surfactant molecules in the monolayer. The contact angles of biosurfactant water solutions on hydrophilic and lipophilic surfaces have been investigated. The obtained results with biosurfactant water solutions on the hydrophilic surfaces showed, that lipophilization of hydrophilic surfaces occurred at low biosurfactant concentrations. At higher biosurfactant concentrations the contact angles decreased due to the orientation of biosurfactant molecules by the polar groups on the surfaces. It has been established, that the increase in the cosine of the contact angles at higher surfactant concentrations was associated with the hydrophilization of the surfaces due to the formation of micelles on the surfaces. It has been established that the effect of the biosurfactant structure on the adsorptive modification of surfaces occurs only at low biosurfactant concentrations. The antimicrobial activityes of new biological surfactants water solutions have been investigated. The obtained results showed, that the new biological surfactants water solutions were effective against studied microorganisms. Field tests of new composite preparations based on antimicrobial biological surfactants for plant seeds growth on saline soils were carried out. For field tests, aqueous solutions of composite preparations with new biological surfactants were used. The obtained results showed, that the use of new composite preparations with biological surfactants ensured a good germination and growth of seeds.

Keywords: biosurfactants, wettability, contact angles, surface modification, surface activity, foam-forming ability, antimicrobial effectiveness.

Introduction

Biological surfactants are amphiphilic surface-active substances of biological origin, produced by microorganisms and plant cells. The microorganisms including bacteria, fungi and algae are the most efficient microorganisms at producing biosurfactants. The biosurfactants offer many advantages compared to synthetic surfactants: they are environment friendly, have bioavailability, biodegradability, activity under extreme conditions, low toxicity and structural diversity. The biosurfactants have well-balanced functional groups and hydrophilic-lipophilic properties. However, few studies have been carried out on new biological surface active-substances obtained from the microbial cultural liquids and from plants. In this regard the establishing the physical and colloid-chemical properties of biological surface active-substances, investigating their antimicrobial activities are very important and useful. In this regard, we have investigated the solution properties, surface activities and antimicrobial effectiveness of new biological surface active-substances obtained from microbial cultural liquids and medical plants.

Methods and Materials

Determination of biosurfactant water solutions surface tension. The surface tension of surfactant solutions was determined using tensiometer DCAT-15 at different temperatures and concentrations. In order to obtain statistically significant results, each measurement was repeated 5 times.

The wettability of surfaces with biosurfactant solutions. The wettability of solid surfaces with surfactants water solutions was characterized by the values of the contact angles on different hydrophilic and lipophilic surfaces. The measurements were carried out using Theta Flow optical tensiometer by projecting the deposited drop onto the screen. The drop was applied using a micro-syringe in a strictly defined amount.

Foam forming ability of biosurfactant water solutions. The foaming ability was determined at a temperature of 293K, while 100 ml of a freshly prepared surfactant solution with a certain concentration was shaken in a graduated container for 60 s. Then the height of the foam column at the initial moment in the graduated container was measured.

Determination of antimicrobial activities. Antimicrobial activityes of new biological surface-active substances were tested and investigated against gram-positive and gram-negative bacterias, and a pathogenic strain of microscopic fungi. The pathogenic strains were diluted in sterilized distilled water and the density adjusted to the McFarland standard. Then a suspension of pathogenic strains was transferred to Muller Hinton agar medium. After preparing agar medium water solutions of new biological surface-active substances were poured into open pits on the surface of pathogenic strains grown on agar medium. The plates with agar medium, suspension of pathogenic strains and surfactant solutions were incubated at 37 ± 2ºC for 24 hours and the zone of inhibition was measured. The experiment was performed in triplicate.

Thin layer chromatography. Thin layer chromatography (TLC) was carried out at room temperature in two different systems. For the study, ascending TLC was used in chambers preliminarily saturated with solvent vapors forming the mobile phase. Chromatography was carried out on plates with a polar stationary phase on aluminum and polymer matrices.

Refractive indices of biosurfactant water solutions. An Easy plus refractometer was used to determine the refractive index of aqueous solutions of the obtained new surfactants. The refractive index of aqueous solutions was measured at a temperature of 293 K.

Density of biosurfactant samples. To determine the density of new surfactants a density meter Easy plus was used. The density of the obtained surfactants was measured at a temperature of 293 K.

Biological surfactants were isolated by acid precipitation and extraction methods, then purified by recrystallisation from the solvents. The isolated and purified biological surfactants were weighed and aqueous solutions with different concentrations were prepared from them.

Results and Discussions

The surface activity of new biological surface-active substances in water solutions were investigated. The obtained results of a study of the surface tension of aqueous solutions of new biologic surface-active substances are shown in Table 1 below.

Table 1. The surface tension of new biosurfactant water solutions.

Surfactant	T, K	Surface tension σ (мН/м) of biosurfactant water solutions at different concentrations (C, %)								
		0,02	0,04	0,08	0,16	0,31	0,62	1,23	2,45	4,95
BIDS-1	293	71,8	70,9	68,6	64,5	53,8	46,9	39,7	35,4	34,6
	303	70,8	69,7	67,7	62,7	52,3	45,7	40,5	35,9	33,8
	313	70,2	68,6	66,9	58,9	51,5	45,4	38,6	34,7	32,5
	323	69,3	67,3	63,7	57,6	50,3	43,8	37,7	33,8	31,4
	333	67,8	65,8	61,9	55,4	49,4	42,4	35,8	33,1	30,7
BIDS-2	293	71,9	70,7	67,9	62,8	52,9	45,8	39,8	34,8	33,5
	303	70,7	69,8	66,7	61,5	51,8	44,7	38,6	34,4	32,9
	313	69,9	68,9	65,5	57,7	50,7	43,5	36,7	33,6	31,7
	323	68,7	66,6	63,4	56,3	49,5	42,6	35,4	32,5	30,8
	333	67,8	65,8	60,8	52,4	48,6	41,4	34,0	31,9	30,5
BIDS-3	293	68,9	66,9	62,9	56,8	52,8	40,8	33,9	30,8	29,6
	303	67,7	64,7	61,6	56,5	47,9	39,9	31,7	29,7	28,7
	313	66,9	63,8	58,8	53,6	44,8	38,7	31,8	27,9	27,8
	323	66,5	61,9	57,7	51,9	43,5	37,8	29,7	26,6	26,5
	333	60,7	59,7	56,5	49,7	42,9	36,6	29,6	25,5	25,9
BIDS-4	293	68,7	66,7	62,6	57,8	52,8	40,9	33,8	30,9	30,7
	303	66,9	64,8	61,8	56,9	47,7	39,8	32,7	29,8	28,8
	313	65,8	63,6	58,6	53,9	44,5	38,7	31,5	28,5	27,9
	323	64,7	61,8	57,4	51,7	43,8	37,8	29,9	27,8	26,8
	333	61,5	59,6	55,9	49,8	41,7	37,6	28,8	26,5	25,7
BIDS-5	293	68,4	66,4	62,4	57,7	52,7	40,5	33,9	30,8	30,4
	303	66,5	64,9	61,9	56,5	47,8	39,8	32,5	29,9	28,5
	313	65,8	63,5	58,5	53,7	44,5	38,7	31,6	28,5	27,7
	323	64,9	61,7	57,4	51,6	43,6	37,5	29,7	27,7	26,9
	333	61,6	59,4	55,6	49,7	41,9	37,4	28,6	26,3	25,6

Analysis of the experimental data in Table 1 showed that, with the increase of surfactant concentrations in water solutions the surface activity of new biosurfactants increased. This result is connected with increase of adsorption capacity of new biosurfactants depending on the increase of biosurfactant concentration in water solutions. The analysis of experimental results in Table 1 also showed that with the increase of the temperature of the disperse system the surface tension of water solutions of new biosurfactants decreased. This result is connected with increase of adsorption new biosurfactants molecules on the solution surface with the increase of the temperature of the disperse system. The stabilizing abilities of new biosurfactants dispersions have been investigated. The results of a study of the foam forming abilities and stability of foams in aqueous solutions of the studied new biologic surface-active substances are presented in Table 2 below. It was also important to quantify the influence of a number of factors, such as the temperature of the system, the concentration of surfactants, the presence of mineral salts or organic substances on the foaming ability of new surfactants. The research results showed that with an increase in the surfactant concentration in water solutions and the temperature of the disperse systems (Table 2), foam forming ability of new biosurfactants increased. It should be noted that there is a good correlation between the foaming ability and surface activity of the studied biosurfactants.

Table 2. The foam forming ability of new biosurfactants in aqueous solutions.

Surfactant	T, K	Foam forming ability (V, ml) of new biosurfactants at different biosurfactant concentrations, C%.					
		0,155	0,317	0,635	1,270	2,540	5,080
BIDS-1	293	175,5	212,4	219,6	265,3	282,5	329,4
	313	188,0	233,7	245,9	285,5	319,8	347,6
	333	207,3	244,9	259,3	303,8	329,4	349,8
BIDS-2	293	179,2	217,2	225,6	268,2	289,7	338,7
	313	193,6	238,5	248,8	290,6	329,6	355,4
	333	205,8	249,8	265,4	307,8	337,8	359,3
BIDS-3	293	183,3	219,4	229,6	275,5	295,6	344,6
	313	197,5	243,7	255,8	297,8	334,8	359,9
	333	209,7	255,8	268,4	315,9	345,9	365,3
BIDS-4	293	188,2	225,8	233,8	283,4	298,6	348,8
	313	205,4	246,4	258,4	304,6	337,9	365,5
	333	214,9	259,6	275,6	325,8	348,3	367,6
BIDS-5	293	189,5	226,5	235,3	285,4	299,6	349,4
	313	204,7	248,2	259,5	306,7	338,3	367,5
	333	215,8	258,8	275,8	327,9	349,9	369,8

Analysis of the table 2 showed, that at low temperatures (293-313K) the foam stability is very high and equal to 0.8-0.9. Apparently, this is due to the formation of a highly viscous structured film of surfactant molecules at the solution-air interface. As the temperature rises, the foaming capacity increases sharply. It can be assumed that this is due to a change in the kinetic parameters of adsorption of molecules, and, accordingly, in the parameters of the dielectric layer at the interface. However, it should be noted that an increase in the volume of the formed foam is accompanied by a decrease in its stability. This result is due to an increase in the drainage of liquid from the foam films, and, accordingly, an increase in the rate of foam destruction. It should be noted that there is a good correlation between the foaming ability and surface activity of the studied biosurfactants.

The contact angles of new biosurfactants water solutions on different hydrophilic and lipophilic surfaces have been investigated too. To obtain the information on the orientation of biosurfactant molecules on the outer surface of the layer facing the solution we have studied new biosurfactants by determining the contact angles of biosurfactant water solutions on different solid surfaces. Such investigation will also provide information on the orientation of the adsorbed biosurfactant molecules on the surfaces. For this purpose, the contact angles of biosurfactant water solutions on different hydrophilic and hydrophobic surfaces such as aluminum, copper, steel, glass, polyethylene, plexiglass and paraffin were investigated. The contact angles of new biosurfactant water solutions on aluminum, copper, steel and glass surfaces depending on the biosurfactant concentrations are shown in Table 3. Analysis of the data presented in Table 3 showed that the contact angles of surfactant water solutions on the hydrophilic surfaces significantly depends on the composition and structure of surfactant molecules. Obtained results showed, that lower concentration of biosurfactant in the water solution, higher the contact angle. It is interesting to note that at low biosurfactant concentrations, the hydrophobization of aluminum, copper, glass and steel surfaces takes place. Obtained results in Table 3 also showed, that the treatment of hydrophobic surfaces with the diluted biosurfactant water solutions increased the contact angles on such surfaces.

Table 3. The cosine of the contact angles of biosurfactant water solutions on different hydrophile surfaces depending on the concentration of biosurfactants

Biosurfactant	Surface	Biosurfactant concentration in water solution (C %) / The cosine of the contact angles (CosΘ).										
		0,013	0,025	0.05	0,15	0,25	0,35	0,45	0,55	0,65	0.85	1.00
BIDS-3	Steel	0,44	0,39	0.37	0,45	0.53	0.64	0.68	0.73	0.75	0.76	0.76
	Glass	0.87	0.80	0.83	0.88	0.89	0.95	0.97	0.98	0.98	0.98	0.99
	Aluminum	0,68	0,58	0.59	0,61	0.68	0.76	0.83	0.86	0.87	0.87	0.87
	Copper	0,58	0,57	0.46	0,51	0.66	0.75	0.78	0.85	0.86	0.87	0.88

Obtained results in Table 3 showed that at high biosurfactant concentrations, the contact angles were sharply decreased. Obviously, these results were due to the fact that during adsorption, the biosurfactant molecules were oriented by their polar groups to the hydrophobic surfaces. At a certain biosurfactant concentration in water solution, at which the maximum filling of the monolayer took place, the values of the contact angles reach minimum values. The values of the contact angles were decreased with the increase in the hydrophobic nature of the biosurfactant. Lower critical micelle-forming concentration values of studied new biosurfactants the validity of this assumption. It is interesting to note that the concentration dependence of the contact angle passes through a minimum only for hydrophilic surfaces (aluminum, copper, glass, steel surfaces). This can be explained by the fact that a monolayer of biosurfactant molecules is formed on the hydrophilic surface with orientation by polar groups to the solid surface, and by hydrophobic chains to water. During the adsorption of the second surfactant layer with the orientation of polar groups to the water, the surface is again hydrophilized, the contact angle decreases, and a minimum is observed on the concentration dependence of the contact angle. It should be noted that the concentration corresponding to the maximum contact angle is in the concentration range between the critical micelle-forming concentration and the concentration at which the maximum build-up of the adsorption layer of biosurfactant molecules occurs at the solution-air interface. The increase in the cosine of the contact angle at higher surfactant concentrations is associated with the hydrophilization of the surface due to the formation of surface micelles. The new biosurfactant water solutions were also investigated on polyethylene, plexiglass and paraffin surfaces depending on the biosurfactant concentration and contact angles were determined. The contact angles of new biosurfactant water solutions on polyethylene, plexiglass and paraffin surfaces depending on the concentration are presented in Table 4. Analysis of the results presented in Table 4 showed, that for hydrophobic surfaces (polyethylene, plexiglass and paraffin surfaces), the cosine of the contact angle has been increased monotonically with the increase in the biosurfactant concentrations. It should also be noted, that the isotherms of the dependence of the contact angle on the biosurfactant concentration for hydrophobic surfaces are similar to the surface tension isotherm

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Table 4. The cosine of the contact angles of biosurfactant water solutions on different hydrophobic surfaces depending on the biosurfactants concentration.

Biosurfactant	Surface	Biosurfactant concentration in water solution (C,%) / The cosine of the contact angles (CosΘ).									
		0,03	0,05	0,15	0,25	0,35	0,45	0,55	0,65	0.85	1.00
BIDS-3	Polyethylene	0.26	0.39	0.47	0.48	0.55	0.66	0.69	0.75	0.76	0.76
	Plexiglass	0.28	0.45	0.48	0.56	0.59	0.67	0.75	0.79	0.80	0.80
	Paraffine	0.19	0.31	0.45	0.47	0.49	0.56	0.59	0.63	0.65	0.66

These results can be explained by the fact that only monolayers of biosurfactant molecules can be formed on hydrophobic surfaces, oriented by hydrophobic groups to a solid surface, and hydrophilic groups to the water. The second layer cannot form, as the energy of intermolecular interaction of polar groups with water is greater than with each other. The absence of minima on the dependence of the cosine of the contact angle of paraffin on the biosurfactant concentration confirms the correctness of the formation of a monolayer of surfactant molecules on such surfaces. Thus, the dependence of the contact angle on the biosurfactant concentration for hydrophobic surfaces is similar to the surface tension isotherm, and for hydrophilic surfaces it has the form of a curve with a maximum. The obtained results on the contact angles of the different surfaces with new biosurfactant solutions indicate that the effect of the biosurfactant structure on the adsorptive modification of surfaces occurs only at low surfactant concentrations, under conditions of specific adsorption and formation of a monolayer. At high concentrations above the critical micellar concentrations, the adsorptive modification of surfaces is mainly determined by the structure of the surfactant hydrocarbon chain.

Antimicrobial activityes of new biological surface-active substances were tested and investigated against gram-positive bacteria Staphylococcus aureus, Bacillus subtilis, gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, pathogenic strains of microscopic fungi Candida albicans. Pathogenic strains were diluted in sterilized distilled water and the density adjusted to the McFarland standard. Then a suspension of pathogenic strains was transferred to Muller Hinton agar medium. After preparing agar medium water solutions of new biological surface-active substances were poured into open pits on the surface of pathogenic strains grown on Mueller Hinton agar medium. The plates with agar medium, suspension of pathogenic strains and surfactant solutions were incubated at 37 ± 2ºC for 24 hours and the zone of inhibition was measured. The experiment was performed in triplicate. For the preparation of inoculants of the test cultures, cultures grown on Mueller Hinton agar medium were transferred to sterilized 5 ml flasks containing distilled water. The resulting suspension was adjusted to a turbidity equivalent of 0.5 McFarland standard to bring it to 1.5×108 CFU/ml. After that, it was inoculated on the surface of pre-prepared Mueller Hinton agar medium using a sterilized L-shaped glass rod in a lawn mold. Cultures were then kept at 37°C for 15 minutes. After that, pits of 6 mm size were carved on the surface of the medium where the test cultures were planted. 10 µl of biosurfactants water solutions were poured into these engraved wells. At the next stage, test cultures were incubated at 37°C for 18-24 hours. After the incubation period, the zone of inhibition around the wells into which the biosurfactants water solutions were poured was measured. Experiments were repeated three times to confirm the reliability of the obtained results.

Antimicrobial activityes of new biological surface-active substances were tested and investigated against different gram-positive bacterias and gram-negative bacterias. The obtained results of antimicrobial activityes of new biological surface-active substances are presented in Table 5 below.

Table 5. Antimicrobial activityes of new biological surfactants

Test number	Biosurfactants names	S. aureus, mm	B. subtilis, mm	P. aureginosa, mm	E. coli, mm	C. albicans, mm
1.	BIDS-1	11	18	16	15	12
2.	BIDS-2	14	15	16	14	13
3.	BIDS-3	12	15	14	16	14
4.	BIDS-4	13	15	13	15	14
5.	BIDS-5	15	13	14	12	15

The obtained results in table 5 showed the effectiveness of new biological surface-active substances against the tested gram-positive and gram-negative bacterias. The new biological surface-active substances were activ and effective against gram-positive bacteria Staphylococcus aureus, Bacillus subtilis, gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, pathogenic strains of microscopic fungi Candida albicans. On the bases of the obtained results the new biosurfactants were recommended for applications as the antimicrobial surface-active agents in different compositions in protection and stimulating plant grows in different soils and climatic conditions.

Field tests of new composite preparations based on antimicrobial biological surfactants for plant seed growth on saline soils were carried out. For field tests, aqueous solutions of new biological surfactants and composite preparations based on them were used. In our field tests, solutions of the compositions were used in doses of 100, 200 and 300 ml of aqueous solution per 1 kg of seeds.

To establish the effectiveness of new composite preparations based on biological surfactants, carrot seeds were pre-treated with aqueous solutions of composite preparations in various concentrations in accordance with the consumption rates established in our laboratory studies. For this, 1 kg of seeds were weighed in plastic containers and treated with 100, 200 and 300 ml of 5% aqueous solutions of composite preparations based on biological surfactants for 2 hours. Then the seeds treated in this way with aqueous solutions of composite preparations were dried. Then the treated seeds were placed in paper cartridges and soaked for 4 hours in a 1% aqueous solution of calcium nitrate. After treatment, the seeds were planted in selected areas of the soil and the growth and outgrowth of the planted seeds were examined. The results of field tests of new composite preparations based on biological surfactants for carrot seed growth on saline soils are presented in Table 6 below.

Table 6. Results of field tests of composite preparations with new biological surfactants in carrot seeds growth.

№	Name of the biosurfactant in the composition	Concentration of aqueous solution of composition, C%	Consumption of aqueous solution of composition, ml/kg	Dependence of seed growth in percentage (%) on the time after sowing seeds, in days.		
				6 days	10 days	14 days
1	BIDS-2	5,00	100,0	12,4	75,6	86,7

2	BIDS-2	5,00	200,0	16,5	86,4	95,2
3	BIDS-2	5,00	300,0	24,3	90,5	100,0
4	BIDS-4	5,00	100,0	18,6	78,7	88,5
5	BIDS-4	5,00	200,0	19,6	89,5	97,4
6	BIDS-4	5,00	300,0	27,8	93,4	100,0
7	Control (river water)	0,00	-	0,00	46,3	81,5

The data obtained from field trials indicated, that very good results in carrot seed growth on saline soils were obtained using composite preparations based on biological surfactants BIDS-2 and BIDS-4.

As can be seen from the data in Table 6, the use of new composite preparations with biological surfactants BIDS-2 and BIDS-4 ensured a good germination and growth of seeds. Field trials showed, that when using a 5.0% aqueous solution of a composite preparation based on new biosurfactant BIDS-2 from 100 to 300 ml per 1 kg of seeds, the germination and growth of seeds ranged from 75.6 to 90.5% within 10 days and from 86.7 to 100% within 14 days. As can be seen from the test results presented in Table 6, when using a 5.0% aqueous solution of the composite preparation with new biosurfactant BIDS-4 from 100 to 300 ml per 1 kg of seeds, the germination and growth of seeds ranged from 78.7 to 93.4% within 10 days and from 88.5 to 100% within 14 days.

The obtained test results showed, that in the control experiments using water without adding new composite preparations with biological surfactants BIDS-2 and BIDS-4, the germination and growth of seeds were 46.3% within 10 days and 81.3% within 14 days. The results of testing of new composite preparations based on biological surfactants BIDS-2 and BIDS-4 showed, that they accelerated the germination and growth of seeds on saline soils compared to the control (water treatment). The positive result obtained is associated with the antimicrobial activity and surface activity of the new preparations. The positive effects of new preparations with biosurfactants also connected with the optimizing the intake of micronutrients and anti-stress elements into germinating plant seeds and increasing plant resistance. The use of new biosurfactants increased the plant seeds resistance to various diseases and stimulated seed germination in saline soils and unfavorable conditions.

Conflict of interests:

The authors declare no conflict of interests.

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