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COLLOID-CHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF NEW BIOLOGICAL SURFACTANTS

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Abstract
The colloid-chemical properties and antimicrobial activities of new biological surfactants obtained from microbial cultural liquids and medical plants have been investigated. Very good correlations between the surface activities and the foam forming abilities of new biological surface-active substances have been determined. It has been shown, that the stability of biosurfactant foams in water solutions significantly determined by the interactions of surfactant molecules in the monolayers. The contact angles of biosurfactant water solutions on hydrophilic surfaces have been investigated. A lipophilisation of hydrophilic surfaces at low biosurfactant concentrations in the biosurfactant water solutions has been determined by the laboratory experimental research work. At higher biosurfactant concentrations in water solutions the contact angles on of hydrophilic surfaces decreased. Such results were due to the orientation of biosurfactant molecules by the polar groups towards the hydrophilic surfaces. It has been established that the increase in the cosine of the contact angles at high 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 the effectiveness of new biological surfactants against several microorganisms.

Keywords: biosurfactants, wettability, contact angles, surface modification, surface activity, antimicrobial effectiveness.

Introduction

The biological surfactants are amphiphilic surface-active compounds of biological origin, produced by microorganisms and plant cells. The microorganisms including bacteria and fungi are the most efficient microorganisms at producing biosurfactant molecules. The biosurfactants offer many advantages compared to synthetic surfactants, they are environment friendly, have got very good biodegradability, activity under extreme conditions, low toxicity and structural diversity. The biosurfactants have

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well-balanced functional groups and hydrophilic-lipophilic properties. However, few investigations and publications have been carried out on new biological surface active-substances obtained from the microbial cultural liquids and plant cells. In this regard the establishing the colloid-chemical properties of biological surface active-substances, investigating their antimicrobial activities are very important and useful. In this regard, the colloid-chemical properties and antimicrobial activities of new biological surface active-substances obtained from microbial cultural liquids and medical plants have been investigate in this research work.

Methods and Materials

Determination of biosurfactant water solutions surface tension. The surface tension of surfactant solutions was determined using tensiometer DCAT-9T 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.

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 (n_D^{20}) 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 (d_4^{20}) 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 purified by solvent extraction. The isolated and purified biological surfactants were weighed and aqueous solutions with different concentrations were prepared from them.

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Results and Discussions

The surface activity of new biological surfactants 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
BS-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
BS-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
BS-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
BS-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
BS-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.

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The contact angles of new biosurfactants water solutions on different hydrophilic surfaces have been investigated. 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 surfaces such as aluminum, copper, steel and glass 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 2. Analysis of the data presented in Table 2 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 2 also showed that the treatment of hydrophobic surfaces with the diluted biosurfactant water solutions increased the contact angles on such surfaces.

Table 2. The cosine of the contact angles of biosurfactant water solutions on different hydrophilic surfaces depending on the biosurfactants concentration.

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
BS-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 2 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

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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 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 ± 2°C 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 Tab. 3 below.

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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.	BS-1	11	18	16	15	12
2.	BS-2	14	15	16	14	13
3.	BS-3	12	15	14	16	14
4.	BS-4	13	15	13	15	14
5.	BS-5	15	13	14	12	15

The obtained results in table 3 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.

Conflict of interests:
The authors declare no conflict of interests.

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