SOLUTION PROPERTIES AND EFFECTIVENESS OF NEW BIOLOGICAL SURFACE-ACTIVE SUBSTANCES

R. Makhkamov,
F. Saidkulov,
M. Nurmanova,
I. Makhkamova,
Sh. Samandarov
Institute of General and Inorganic Chemistry,
Academy of Sciences of the Republic of Uzbekistan,
Email: ravshanmak@gmail.com

Abstract
The solution properties and effectiveness of new biological surface active-substances obtained from microbial cultural liquids and medical plants have been investigated. 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 lipophilic surfaces have been investigated. It has been shown that the hydrophilisation of lipophilic surfaces by biosurfactant water solutions at low biosurfactant concentrations. At high biosurfactant concentrations the contact angles increased due to the orientation of biosurfactant molecules by their nonpolar groups towards the lipophilic surfaces. It has been established that the increase in the cosine of the contact angle at higher surfactant concentrations was associated with the lyophilization of the surfaces due to the formation of micelles on the surfaces. Field tests of new antimicrobial biological surfactants for plant seed growth on saline soils have been carried out. The obtained results showed, that the use of new biological surfactants ensures a good germination and growth of carrot seeds. The obtained positive results were associated with the antimicrobial and surface activity of the new biological surfactants. The positive effects of biological surfactants also connected with the optimization of the micronutrients and antistress elements intake into germinating seeds and increasing plant resistance. The use of new biosurfactants has been increased the plant resistance to various diseases, stimulates seeds germination in saline soils and unfavorable conditions.

Keywords: biosurfactants, wettability, contact angles, surface modification, surface activity, 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-9T at different temperatures and concentrations. In order to obtain statistically significant results, each measurement was repeated 5 times. Testing 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. Determination of 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. Using 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. Obtaining 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. Determination of 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.

Results and Discussions

The foam forming ability of new biological surfactants in water solutions have been investigated. The obtained results of a study of the foam forming ability of aqueous solutions of new biologic surface-active substances are shown in Table 1 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 1), 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 1. 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%.					
		0,1	0,5	0,62	1,25	2,5	5,0
BIS-1	293	175	212	219	265	282	329
	313	188	233	245	285	319	347
	333	207	244	259	303	329	349
BIS-2	293	179	217	225	268	289	338
	313	193	238	248	290	329	355
	333	205	249	265	307	337	359
BIS-3	293	183	219	229	275	295	344
	313	197	243	255	297	334	359
	333	209	255	268	315	345	365
BIS-4	293	188	225	233	283	298	348
	313	205	246	258	304	337	365
	333	214	259	275	325	348	367
BIS-5	293	189	226	235	285	299	349
	313	204	248	259	306	338	367
	333	215	258	275	327	349	369

Analysis of the Table 1 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 hydrophobic surfaces such as polyethylene, plexiglass, paraffin, parylene and polytetrafluoroethylene (PTFE) were investigated. The contact angles of new biosurfactant water solutions on hydrophobic surfaces were determined depending on the biosurfactant concentration in solutions. The contact angles of new biosurfactant water solutions on polyethylene, plexiglass, paraffin, parylene and PTFE surfaces depending on the biosurfactant concentrations are shown in Table 2. The analysis of the results presented in Table 2 showed, that for hydrophobic surfaces (polyethylene, plexiglass, paraffin, parylene and PTFE 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 of biosurfactants.

Table 2. The cosine of the contact angles of biosurfactant water solutions on different hydrophobic surfaces depending on the biosurfactant concentrations.

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
BIS-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	0.26	0.28	0.33	0.39	0.48	0.55	0.61	0.68	0.75	0.76
	Parylene	0.19	0.31	0.45	0.47	0.49	0.56	0.59	0.63	0.65	0.66
	PTFE	0.12	0.19	0.25	0.29	0.33	0.41	0.46	0.49	0.51	0.51

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.

The field tests of new composite preparations based on antimicrobial biological surfactants for plant seed growth on saline soils have been carried out. For field tests, aqueous solutions of composite preparations with new biological surfactants have been 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. The seeds treated in this way with aqueous solutions of composite preparations were dried. Then the 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 3 below.

Table 3. Results of field tests of composite preparations based on new biological surfactants for carrot seed growth.

№	Name of 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	10	14
1	BSF-1	5,00	100,0	12,4	75,6	86,7
		5,00	200,0	16,5	86,4	95,2
		5,00	300,0	24,3	90,5	100,0
2	BSF-5	5,00	100,0	18,6	78,7	88,5
		5,00	200,0	19,6	89,5	97,4
		5,00	300,0	27,8	93,4	100,0
3	Control	0,00	-	0,00	46,3	81,5

The data obtained from the field trials showed, that using composite preparations BSF-1 and BSF-5 based on biological surfactants increased carrot seeds growth on saline soils. As can be seen from the data in Table 3, the use of new composite preparations BSF-1 and BSF-5 based on biological surfactants ensured a good germination and growth of seeds. Field trials showed that when using 5.0% aqueous solutions of the composite preparation BSF-1, based on biosurfactant, 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 3, when using a 5.0% aqueous solution of the composite preparation BSF-5 based on biosurfactant 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 BSF-1 and BSF-5, the germination and growth of seeds were 46.3% within 10 days and 81.3% within 14 days. The results of testing new composite preparations BSF-1 and BSF-5 based on biological surfactants showed, that they accelerate the germination and growth of seeds on saline soils compared to the control (water treatment). The positive result obtained was associated with the antimicrobial activity and surface activity of the new preparations. The positive effects of BSF-1 and BSF-5 preparations also connected with the optimization of the micronutrients and anti-stress elements intake into germinating plant seeds and increasing plant resistance. The use of new biosurfactants ensures increased plant resistance to various diseases, stimulates seed germination in saline soils and unfavorable conditions.

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

Acknowledgement
This research was supported and funded by the Ministry of Innovative Development of the Republic of Uzbekistan (grant IL-4821091658 for the Institute of General and Inorganic Chemistry, Academy of Sciences of Uzbekistan).

References
1. Mulligan, Catherine N. (2005). "Environmental applications for biosurfactants". Environmental Pollution. 133 (2): 183–198. doi: 10.1016/j.envpol. 2004.06.009. PMID 15519450.
2. Ron, Eliora Z.; Rosenberg, Eugene (2001). "Natural roles of biosurfactants. Minireview". Environmental Microbiology. 3 (4): 229–236. doi:10.1046/j.1462-2920.2001.00190. x. PMID 11359508.
3. Zahed MA, Matinvafa MA, Azari A, Mohajeri L (April 2022). "Biosurfactant, a green and effective solution for bioremediation of petroleum hydrocarbons in the aquatic environment". Discover Water. 2 (1): 1–20. doi:10.1007/s43832-022-00013-x.
4. Rosenberg E, Ron EZ (August 1999). "High- and low-molecular-mass microbial surfactants". Appl. Microbiol. Biotechnol. 52 (2): 154–162. doi:10.1007/s002530051502. PMID 10499255. S2CID 23857287.
5. Chattopadhyay A, Mittal KL (1996). Surfactants in Solution, CRC Press
6. Islam N, Kato T (2005). Effect of temperature on the surface phase behaviour and micelle formation of a mixed system of nonionic/anionic surfactants J. of Colloid and Interface Science 282(1)142-148
7. Holmberg K, Jansson B, Kronberg B, Lindman B (2002) Surfactants and Polymers in Aqueous Solution. Second edition.
8. Mixed surfactant systems. Surfactant Science series. (2004) ed. Masahiko Abe, rev. and expanded second ed.)
9. Karsa DR (1998). New Products and Applications in Surfactant Technology, 245 p.
10. Bhadani A, Hokyun J, Kafle A et al. (2020) Synthesis and properties of renewable citronellol based biodegradable anionic surfactant. Colloid and Polymer Science 298:(11)1543-1550
11. Kunieda H, Matsuzawa K, Makhkamov R, Horii M et al. (2003) Effect of amino-acid-based polar oils on the Krafft temperature and solubilization in ionic and nonionic surfactant solutions. Journal of Dispersion Science and Technology 24:(6) 767-772
12. Makhkamov R, Kim V, Aminov S, Sirazhiddinova D (1992) Correlation between the structure of a carbohydrate substituent and the adsorption ability in a series of the derivatives of hexylene-succinic acid. Kolloid Journal 54:(4)121-124

13. Makhkamov R, Kim V, Aminov S, Sirazhiddinova D (1992) Correlation between the aggregative stability of emulsions and the hydration of an emulsifier cation. Kolloid Journal 54:(4)118-120
14. Gemma C. Shearmana, Stephane Ugaziob, Laurent Soubiranb et al (2009) The lyotropic phase behaviour of ester quaternary surfactants. Journal of Colloid and Interface Science 331:(2)463-469.
15. Desai, Jitendra D.; Banat, Ibrahim M. (1997). "Microbial production of surfactants and their commercial potential". Microbiology and Molecular Biology Reviews. 61 (1):47–64. doi:10.1128/mmbr.61.1.47-64.1997.PMC 232600. PMID 9106364.
16. Zhaowei Hou, Xiaolin Wu, Guopeng Wu, Erlong Yang et. al. (2020) Self-Assembled Vesicles Formed by Positional Isomers of Sodium Dodecyl Benzene Sulfonate-Based Pseudogemini Surfactants. Langmuir, 36 (26),7593-7601.
17. Fang Fu, Yulin Fan, Langqiu Chen et al. (2020) Water Solubility and Surface Activity of Alkoxyethyl β-d-Maltosides. Journal of Agricultural and Food Chemistry 68:(31)8330-8340.