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**SURFACE ACTIVITY AND COLLOID-CHEMICAL PROPERTIES OF NEW BIOLOGICAL SURFACE-ACTIVE SUBSTANCES**

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**Abstract**

The purpose of this work was obtaining of new biological surface-active substances and establishing correlations between their surface activity and colloid-chemical properties. The well-known methods for separation and determining the surface activity and colloid-chemical properties of biosurfactants were used in the work. The new biological surfactants were isolated by acid precipitation and purified by solvent extraction. The surface activity and foaming ability of the obtained new biosurfactants in aqueous solutions were studied depending on concentration and temperature of the system. The obtained results showed that with an increase of the concentration of surfactants in water solutions, the surface activity of new biosurfactants increases. The analysis of results showed a good correlation between the foam forming ability and the surface activity of the studied biosurfactants. Based on the studies it was established that the stability of foams is significantly determined by the interaction of surfactant molecules in the monolayer.

**Keywords:** Biosurfactants, separation, colloid-chemical property, surface activity, foam-forming ability.

**Introduction**

Biosurfactants are surface-active substances that are produced at the microbial cell surface or excreted, and can reduce surface and interfacial tension. Biosurfactants have several advantages over synthetic surfactants, such as low toxicity, high biodegradability, can stay active at extreme pH and salinity. Main applications of biosurfactants include herbicide and pesticide formulations, environment friendly detergents, pharmaceutical products, medicine and cosmetics, pulp and paper production, ceramic processing, textiles and food industries [1-3]. By covering the surface of various solids and liquids with the thinnest monomolecular layers, surfactants make it possible to regulate technological processes. Due to this, surfactants have found wide application to improve the quality of various materials [4-6]. Many processes cannot proceed without the participation of surfactants, such as regulation of colloid-chemical properties of disperse systems, stabilization of

suspensions, emulsions and foams, modification and protection of various surfaces [7-9]. Surfactants have a high adsorption capacity with respect to interfaces of phases of various nature, and are able to associate in the liquid phase with the formation of micellar particles [10-12]. In this regard, it is very relevant and interesting to study the colloid-chemical properties and effect of new surfactants on stabilization of disperse systems. [13-14]. In this regard, newly obtained biological surface-active substances deserve attention due to their practicality in both aqueous and non-aqueous solutions. The biosurfactants have a long hydrocarbon chain and their length are different. It is known that the chemical structure and length of hydrocarbon chains can affect the properties of surfactant solutions [15-16]. The biological surface-active substances have well-balanced hydrophilic-lipophilic properties and can form a micellar structure in both aqueous and oily media, depending on the concentration and temperature of the system [17-20]. However, few studies have been carried out on new biosurfactants obtained from *Saccharomyces cerevisiae*. In this regard, in this work, obtaining of new biological surface-active substances and establishing correlations between their surface activity and colloid-chemical properties were studied. The article presents the materials and methods used in the work, the results of the study and their discussion, conclusion based on the analysis of the results and a list of references.

### Methods and Materials

The purpose of this work was obtaining of new biological surface-active substances from *Saccharomyces cerevisiae* depending on temperature of the system and establishing correlations between their surface activity and colloid-chemical properties. To achieve the goal, the following tasks were set: obtaining of new biological surface-active substances from biomass by separation methods and purification by extraction, distillation and recrystallization methods; study surface activity and foam-forming abilities of new biosurfactants; and establishing correlations between their surface activity and colloid-chemical properties.

**Determination of 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.

**Foam forming ability.** 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 aqueous solutions of surfactants. 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 surfactant 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.

Extraction and purification of biological surfactants. Biological surfactants were isolated by acid precipitation and purified by solvent extraction. The *Saccharomyces cerevisiae* were planted in medium with nutrients and stored in a thermostat at a constant temperature. Each experiment was conducted at different temperatures in a thermostat. After incubation at a constant temperature for 168 hours in a thermostat, the cells were removed from the cultural liquid by centrifugation for 20 minutes at a constant temperature. The cell-free supernatant thus obtained was acidified with a 10% aqueous solution of acetic acid and the resulting mixture was stored for 18 hours at a constant temperature in an incubator to enhance the precipitation of biological surfactants. The precipitate formed as a result of storage in a thermostat was separated by centrifugation for 20 minutes. The thus isolated precipitate was extracted several times with ethyl alcohol at room temperature. The resulting extract was filtered and then the ethanol solvent in the extract was distilled under reduced pressure. The residue in the flask after distillation was dissolved in acetone and reprecipitated with n-hexane. The biological surfactants isolated after reprecipitation were dried in a thermostat under reduced pressure. Biological surfactants obtained in this way were reddish-brown, viscous substances with a peculiar odor. Depending on the temperature of the medium with nutrients during the experiments obtained biosurfactants have names BIS-1, BIS-2, BIS-3, BIS-4 and BIS-5.

### Results and Discussions

Biosurfactants are environment-friendly, less toxic, biodegradable in nature, have higher foaming ability, and possess lower critical micelle-forming concentration (CMC) values than the synthetic surfactants and so can be used to replace synthetic surfactants. Among the numerous surface-active substances that are widely used in practice, biological surfactants are poorly studied. In this regard, the work carried out a study of the surface activity, the colloidal-chemical properties, the effect of concentration and temperature on the surface-active and adsorption capacity of new biosurfactants. The new 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. The surface activity of new biological surfactants in water solutions were investigated. The results of a study of the surface tension of aqueous solutions of the studied new biologic surface-active substances are shown in Table 1 below.

Table 1. The surface tension of water solutions of the studied biosurfactants.

Surfactant	T, K	Surface tension $\sigma$ (mH/M) of biosurfactant water solutions at different concentrations ( $C \cdot 10^2$ ) mol/L)								
		0,02	0,04	0,08	0,16	0,31	0,62	1,25	2,5	5
BIS-1	293	71,8	70,9	68,5	64,3	53,8	46,8	39,8	35,3	34,5
	303	70,8	69,7	67,5	62,6	52,3	45,6	40,4	35,8	33,9
	313	70,2	68,6	66,8	58,8	51,5	45,3	38,7	34,6	32,3
	323	69,3	67,3	63,8	57,5	50,3	43,7	37,5	33,9	31,3
	333	67,8	65,8	61,8	55,3	49,4	42,2	35,7	33,3	30,6
BIS-2	293	71,7	70,8	67,8	62,9	52,8	45,9	39,6	34,9	33,4
	303	70,8	69,7	66,6	61,4	51,7	44,6	38,5	34,2	32,8
	313	69,8	68,8	65,4	57,6	50,6	43,7	36,6	33,5	31,9
	323	68,9	66,7	63,3	56,1	49,4	42,9	35,5	32,4	30,6
	333	67,6	65,9	60,9	52,2	48,8	41,5	34,7	31,8	30,4
BIS-3	293	68,8	66,8	62,8	56,9	52,7	40,7	33,7	30,7	29,3
	303	67,6	64,6	61,5	56,3	47,8	39,8	31,8	29,6	28,5
	313	66,8	63,6	58,7	53,5	44,9	38,9	31,6	27,8	27,9
	323	66,3	61,7	57,5	51,7	43,4	37,7	29,9	26,5	26,2
	333	60,8	59,8	56,3	49,8	42,8	36,9	29,5	25,8	25,8
BIS-4	293	68,6	66,4	62,4	57,8	52,6	40,8	33,9	30,8	30,5
	303	66,7	64,9	61,9	56,7	47,8	39,9	32,6	29,9	28,6
	313	65,8	63,5	58,5	53,9	44,6	38,8	31,4	28,4	27,8
	323	64,9	61,9	57,3	51,5	43,7	37,9	29,8	27,7	26,9
	333	61,4	59,4	55,8	49,6	41,9	37,4	28,9	26,3	25,7
BIS-5	293	67,7	64,9	60,7	54,7	50,9	39,8	31,7	29,8	28,9
	303	65,5	62,7	59,8	53,4	45,9	38,9	29,8	28,4	26,6
	313	63,8	61,8	56,7	51,5	44,8	37,9	29,4	25,9	25,9
	323	61,3	60,9	55,8	49,9	41,5	36,8	28,9	24,7	24,7
	333	58,9	57,8	54,9	47,8	40,9	35,7	27,5	24,2	23,9

Analysis of the experimental data in Table 1 showed that, in terms of surface activity, the studied biosurfactants were ranked in the following order: BIS-5>BIS-4>BIS-3>BIS-2>BIS-1. The analysis of obtained results 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.

Surfactants are of great interest as stabilizers and regulators of the properties of the corresponding dispersed systems. The effectiveness of surfactants in many cases depends on the structure and composition of their molecules. In this regard, the study of the stabilizing properties of new biosurfactants is of great scientific and practical importance.

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. Typically, the foaming capacity increased with an increase in the surfactant concentration and the volume of the foam increased by about 15 percent with an increase in the surfactant concentration by one percent.

Table 2. Foam forming ability and stability of foams in aqueous solutions of new biosurfactants.

Surfactant	T, K	Foam forming ability (V, ml)/ Stability of foams (S) at different biosurfactant concentrations, g/L.					
		0,1	0,5	0,62	1,25	2,5	5,0
BIS-1	293	173/80	210/82	218/85	263/86	280/87	328/87
	313	186/77	231/78	244/78	284/79	318/80	345/80
	333	200/41	242/45	258/47	301/48	328/49	348/50
BIS-2	293	177/83	214/86	223/90	269/90	288/91	337/91
	313	191/78	236/82	249/82	291/83	327/85	353/86
	333	203/42	248/49	263/51	308/52	338/54	357/54
BIS-3	293	181/85	218/87	227/92	273/92	292/93	341/93
	313	195/82	240/84	253/84	295/85	331/87	357/88
	333	207/46	252/51	267/53	312/54	342/56	361/56
BIS-4	293	186/85	223/89	231/93	280/92	296/93	346/94
	313	200/83	245/84	256/85	300/85	335/88	362/90
	333	211/49	258/53	272/54	320/55	346/58	365/57
BIS-5	293	192/88	230/91	236/92	284/91	301/94	351/93
	313	205/85	248/85	262/86	305/87	340/90	365/92
	333	216/56	262/55	275/56	321/56	351/60	370/60

As can be seen from the table 2, BIS-5 has the highest foaming capacity. 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 (table 2), 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 is probably due to an increase in the drainage of liquid from the foam films, and, accordingly, an increase in the rate of foam destruction. Analysis of the data presented in table 2 indicates that at a temperature of 333K slowly falling foam turns into rapidly falling.

### Conclusion

The new biologic surface-active substances were obtained and their surface activity and colloid chemical properties investigated. The new biological surfactants were isolated by acid precipitation and purified by solvent extraction. The surface activity and foam forming ability of the obtained new biosurfactants in aqueous solutions were studied. It has been shown that with an increase of surfactant concentration in water solutions, the surface activity of biosurfactants increase. It has been established that there is a good correlation between the foam forming ability and the surface activity of the studied biosurfactants. It has been shown that the stability of foams is significantly determined by the interaction of surfactant molecules in the monolayers. The results of the presented study also confirm the results of a number of studies. In the next studies, the effect of new biosurfactants on regulating of rheological properties of disperse systems will be investigated.

### Conflict of interests:

The authors declare no conflict of interests.

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### 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, Elicora 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] Fang Fu, Yulin Fan, Langqiu Chen et al. (2020) Water Solubility and Surface Activity of Alkoxyethyl  $\beta$ -d-Maltosides. *Journal of Agricultural and Food Chemistry* 68:(31)8330-8340
- [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] Organised Solutions: Surfactants in Science and Technology (1992), ed. S. Friberg, B. Lindman, New York:Marcel Deccer Inc., 383p.
- [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] Surfactants in Solution (1996) ed. A.K.Chattopadhyay, K.L.Mittal, 440 p.
- [18] Adsorption and aggregation of Surfactants in Solution (2019) ed. K.L.Mittal, Dinesh O. Shah, 724 p.
- [19] 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.
- [20] 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.