
# Colloid-Chemical Properties And Antimicrobial Activities Of New Biological Surface Active-Substances

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**ABSTRACT**

The new biological surface active-substances were obtained from microbial cultural liquids and medical plants. The obtained surface active-substances were purified using solvents, distillation and recrystallisation methods. The physical and colloid-chemical properties, and antimicrobial activities of new biological surface active-substances have been investigated. A 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 antimicrobial activityes of new biological surfactants were investigated. The obtained results showed the very good effectiveness of new biological surfactants against microorganisms.

**Keywords:** biosurfactants, purification, properties, surface activity, antimicrobial effectiveness, foam-forming ability.

## Introduction

The biological surface active-substances (Biosurfactants) separated from the microbial cultural liquids and plants have several advantages such as low toxicity, high biodegradability, active at extreme pH and salinity of media. 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 separating and obtaining of new biological surface-active substances and establishing their physical and colloid-chemical properties, investigating their antimicrobial activities are very important and useful. The purpose of this work was separation of new biological surface-active substances from the microbial cultural liquids and medical plants and investigation of their physical and colloid-chemical properties in water solutions, and also investigating their antimicrobial activities and effectiveness. The well-known methods for separation, purification, establishing physical properties and determining the surface activity and colloid-chemical properties of new biological surface-active substances from the microbial

cultural liquids and medical plants were used in this research work. The new biological surfactants from the microbial cultural liquids and medical plants were isolated and purified using different methods including extraction, precipitation, recrystallisation and chromatography methods. The biological surface-active substances were extracted from above-ground and root parts of medical plants. The isolated and purified biological surfactants were weighed and aqueous solutions with different concentrations were prepared from them for investigation of their colloid-chemical properties. Extraction and purification of biological surfactants from microbial cultural liquids as follows. Biological surfactants were isolated by acid precipitation and purified by solvent extraction. The Saccharomyces cerevisiae was cultivated in a 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.

**Methods and Materials**

The surface tension of surfactant solutions was determined using tensiometer DCAT-9T at different temperatures and concentrations. The foam forming 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 (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. 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. 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.

**Results and Discussions**

The isolated and purified biological surfactants were weighed and aqueous solutions with different concentrations were prepared from them. Then 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. 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.

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

| Surfactant | T, K | Surface tension σ (mN/m) of biosurfactant water solutions at different concentrations (C·$10^2$) mol/L) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0,02 | 0,04 | 0,08 | 0,16 | 0,31 | 0,62 | 1,25 | 2,5 | 5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BIOS-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 |
| BIOS-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 |
| BIOS-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 |
| BIOS-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 |
| BIOS-5 | 293 | 68,1 | 66,1 | 62,1 | 57,4 | 52,5 | 40,6 | 33,7 | 30,7 | 30,3 |
| | 303 | 66,2 | 64,7 | 61,7 | 56,3 | 47,6 | 39,7 | 32,3 | 29,6 | 28,3 |
| | 313 | 65,6 | 63,3 | 58,3 | 53,6 | 44,4 | 38,5 | 31,2 | 28,2 | 27,6 |
| | 323 | 64,7 | 61,6 | 57,1 | 51,3 | 43,5 | 37,4 | 29,4 | 27,5 | 26,7 |
| | 333 | 61,2 | 59,2 | 55,5 | 49,4 | 41,6 | 37,1 | 28,5 | 26,2 | 25,5 |

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. 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 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BIOS-1 | 293<br>313<br>333 | 173/80<br>186/77<br>200/41 | 210/82<br>231/78<br>242/45 | 218/85<br>244/78<br>258/47 | 263/86<br>284/79<br>301/48 | 280/87<br>318/80<br>328/49 | 328/87<br>345/80<br>348/50 |
| BIOS-2 | 293<br>313<br>333 | 177/83<br>191/78<br>203/42 | 214/86<br>236/82<br>248/49 | 223/90<br>249/82<br>263/51 | 269/90<br>291/83<br>308/52 | 288/91<br>327/85<br>338/54 | 337/91<br>353/86<br>357/54 |
| BIOS-3 | 293<br>313<br>333 | 181/85<br>195/82<br>207/46 | 218/87<br>240/84<br>252/51 | 227/92<br>253/84<br>267/53 | 273/92<br>295/85<br>312/54 | 292/93<br>331/87<br>342/56 | 341/93<br>357/88<br>361/56 |
| BIOS-4 | 293<br>313<br>333 | 186/85<br>200/83<br>211/49 | 223/89<br>245/84<br>258/53 | 231/93<br>256/85<br>272/54 | 280/92<br>300/85<br>320/55 | 296/93<br>335/88<br>346/58 | 346/94<br>362/90<br>365/57 |
| BIOS-5 | 293<br>313<br>333 | 187/86<br>201/84<br>212/49 | 224/88<br>246/86<br>259/55 | 233/95<br>257/86<br>273/55 | 282/94<br>302/86<br>321/56 | 298/95<br>336/89<br>347/59 | 348/96<br>364/92<br>367/58 |

As can be seen from the table 2, 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 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.

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 Tab. 2 below.

Table 2. 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. | BIOS-1 | 11 | 16 | 14 | 12 | 10 |
| 2. | BIOS-2 | 10 | 12 | 15 | 11 | 12 |
| 3. | BIOS-3 | 11 | 13 | 12 | 14 | 11 |
| 4. | BIOS-4 | 10 | 14 | 11 | 13 | 12 |
| 5. | BIOS-5 | 12 | 12 | 11 | 14 | 14 |

The obtained results (Tab.2) 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.

## 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 extraction, precipitation methods and purified by recrystallisation and solvent extraction methods. 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. Antimicrobial activityes of new biological surface-active substances were tested and investigated against different gram-positive bacterias and gram-negative bacterias. The obtained results showed the effectiveness of new biological surface-active substances against the tested gram-positive and gram-negative bacterias. 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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