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Extraction of Biogenic Rhamnolipid Surfactants

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Abstract—Extraction of biodegradable rhamnolipid surfactants from culture fluid of *Pseudomonas sp. PS-17* has been studied. Higher alcohols are the best extracting agents.

Keywords: extraction, solvent, rhamnolipid surfactant, correlation analysis

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Recently, a significant scientific interest arose toward natural surfactants and their mixtures [1]: being relatively new objects of studies of physical and colloid chemistry, such surfactants were recognized as promising for practical applications. In particular, the biogenic surfactants bear a peculiar combination of physical (reducing the liquid surface tension, emulsifying, and wetting activity) and biological (changing the cell membrane permeability, enzymatic activity, and metabolism of microorganisms) properties [2–4]. Natural surfactants are efficient over wide ranges of temperature, pH, and salts concentration; at reasonably low concentration they are non-toxic and environmentally friendly. The application of biodegradable surfactants in industry and agriculture will reduce the environment pollution.

Besides screening of microorganisms producing the surfactants and optimization of biosynthesis of the latter, development of efficient methods for isolation of the produced natural surfactants is a topical issue of modern biotechnology.

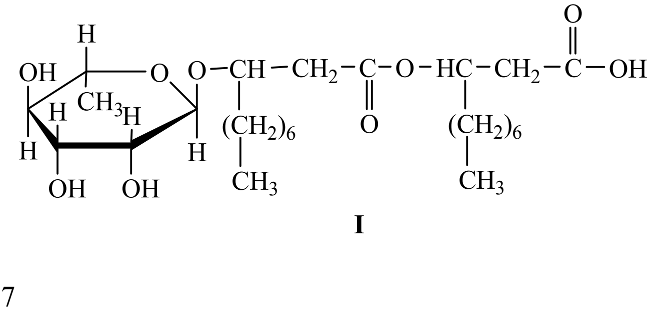
Previously, a variety of products of microbial synthesis by *Pseudomonas, Gordonia,* and *Rhodococcus* bacteria (rhamnolipid, tregalozolipid, polysaccharide lipids, and their complexes) were obtained and studied in detail [5–7]. The *Pseudomonas* microorganisms were recognized among the most promising producers of biogenic surfactants. The most important products synthesized by the *Pseudomonas sp. PS-17* species (our collection) are homologous rhamnolipids **I** consisting of rhamnose and 3-hydroxydecanoic acid (Scheme 1).

The highest surface activity was observed in the case of dirhamnolipid **II** [7] (Scheme 2).

The primary product of biosynthesis of *Pseudomonas sp. PS-17* is the cultural fluid supernatant; it does not contain any microbial cells and is in fact a solution of metabolic surfactants and other components. The major supernatant constituents determining the solution properties are rhamnolipids (7–10 g/L), exopolysaccharides (3 g/L), their complexes, and some other compounds (salts, fatty acids, etc) [5, 8].

The cultural fluid itself possesses certain surface activity, but isolation of pure components is desirable for further applications.

Scheme 1.



I

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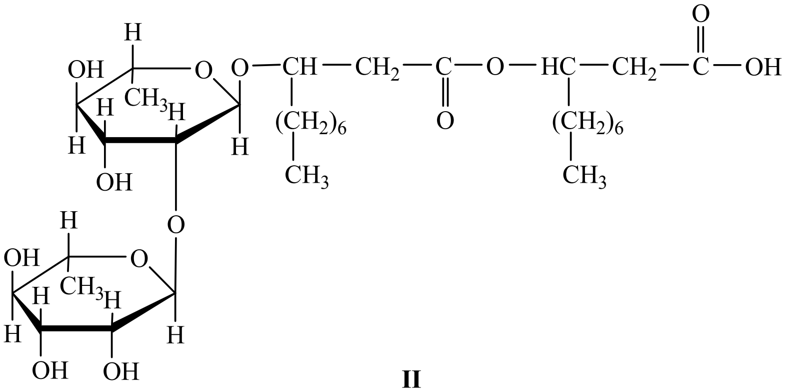
† Deceased.

Scheme 2.



II

Herein we report on studies of extraction of rhamnolipid surfactants from supernatant of *Pseudomonas sp. PS-17* culture fluid; in particular, we analyzed the degree of the target components extraction from the culture fluid by several extraction agents of different nature. The most reasonable method of rhamnolipids isolation from the supernatant is their transfer into the organic solvent phase. Due to the complex structure of the extracted compounds, so far it has been impossible to predict extractant's efficiency without direct experiment. On the other hand, it is known that the extraction efficiency (as reflected in the partition coefficient) can be correlated with certain physicochemical properties of the extracting agent using the linearity of free energy principle. The extraction process is affected by a number of the solvent properties, rather than a single (prevailing) one; therefore, multivariate calibration should be used in such analysis.

As the solvation processes accompanying the extraction are in fact intermolecular interactions, the experimental data on mass of the product extracted with a certain volume of the extracting agent were first recalculated into the amount extracted with 1 mole of the agent. The so processed data were generalized using the modified Koppel–Palm equation [9] [Eq. (1)].

$$\log G_m = a_0 + a_1 f(n^2) + a_2 f(\varepsilon) + a_3 B + a_4 E_\mathrm{T} + a_5 \delta^2 + a_6 V_\mathrm{M}. \quad (1)$$

The first two variable terms in Eq. (1) reflected the capability of the solvent towards non-specific solvation, depending on its polarity and polarizability; the next two parameters showed the solvent ability towards specific solvation, depending on the Palm basicity parameter B [10, 11] and the Reichardt electrophilicity parameter E_T [12]; the squared Hildebrand solubility parameter δ accounted for the degree of self-association (or, equivalently, for the cohesion energy) of the organic solvent of the molar volume V_M [9]. The experimental data were processed taking into account the recommendation of the International Group for Correlation Analysis in Chemistry of IUPAC [13].

Equation (1) proved to be efficient in generalization of data on extraction of organic as well as inorganic species [9]. According to the principle of linear free energies relationship, logarithm of the partition coefficient should be used rather than the substance concentration in order to describe the effect of the solvent type on the extraction efficiency. As a mixture of homolog rhamnolipids was extracted from the culture fluid supernatant, we took the mass of the substance extracted with 1 mol of the solvent as the parameter to be analyzed. The corresponding data are collected in the table.

The generalization of the data for 16 studied solvents gave the six-parametric equation with too low multiple correlation coefficient R = 0.7179. Following the IUPAC recommendation [13] we therefore successively excluded the data for single solvents with subsequent recalculation of the correlation coefficient in order to indicate the outliers.

After excluding cyclohexanone, the equation accounting for the data for 15 remaining solvents gave a much higher correlation coefficient 0.9242; exclusion of toluene and ethyl acetate resulted in acceptable correlation coefficient of 0.9736 for the six-parametric Eq. (2) (13 solvents in the model).

$$\begin{gathered}\log G_m = -0.2646 - (2.5006\pm0.5607)f(n^2) \\ - (1.0102\pm0.3069)f(\varepsilon) - (0.0006\pm0.0002)B \\ + (0.0257\pm0.0081)E_\mathrm{T} + (0.0001\pm0.0004)\delta^2 \\ + (0.0025\pm0.0003)V_\mathrm{M}, \qquad (2) \\ R = 0.9736,\ s = \pm0.0305.\end{gathered}$$

Experimental and calculated [Eq. (4)] values of log G_m

Solvent	G_m, g/L	G_m, g/mol	log G_m		
			experiment	calculation	Δ
Pentane	9.320	1.4441	0.1596	0.0943	–0.0653
Hexane	6.697	1.0957	0.0397	0.1083	0.0686
Decane	9.075	1.7688	0.2477	0.2224	–0.0253
Benzene	8.385	0.7476	–0.1263	–0.1958	–0.0695
Tetrachloromethane	8.324	0.5149	–0.0943	–0.1097	–0.0154
Chloroform	10.795	0.8048	–0.0603	–0.0136	0.0467
Chlorobenzene	6.485	0.8703	–0.1820	–0.1358	0.0462
2-Methylpropan-1-ol	12.115	0.6576	0.0491	0.0481	–0.0010
Butan-1-ol	13.324	1.1196	0.0903	0.0638	–0.0265
Pentan-2-ol	9.675	1.2310	0.0219	0.0368	0.0149
Octan-1-ol	9.695	1.0516	0.1853	0.1552	–0.0301
Pentyl acetate	6.547	1.5322	–0.0123	0.0414	0.0537
Diethyl ether	6.855	0.5991	–0.1474	–0.1447	0.0027
Ethyl acetate[a]	6.121	0.9720	–0.2225	–0.0182	0.2043
Toluene[a]	4.845	0.7122	–0.2883	–0.1639	0.1244
Cyclohexanone[a]	18.985	1.9660	0.2936	–0.1810	–0.4746

[a] Solvents excluded from the final model [Eq. (4)].

Positive value of the coefficient at the E_T term evidenced the electrophilic solvation of carbonyl oxygen atom in rhamnolipids; therefore, the extraction with alcohols revealed high G_m values. However, direct estimation of the other terms contribution into the overall extraction efficiency (log G_m) was impossible: the paired coefficients of correlation between log G_m and single terms of the equation were below 0.5, and in some cases the standard deviation of the coefficient exceeded their absolute value. In view of that, in order to elucidate the significance of the single terms in Eq. (2), we successively excluded single parameters and recalculated the correlation coefficients for the simplified equations [13]; if the R value did not decrease much upon a parameter exclusion, the parameter could be claimed non-significant. In such a way, the non-significance of the Hildebrand solubility parameter was revealed; the correlation coefficient for the resulting five-parametric Eq. (3) was of 0.9736.

$$\log G_m = -0.3026 - (2.4266\pm0.4332)f(n^2) - (1.0350\pm0.2831)f(\varepsilon) - (0.0006\pm0.0002)B + (0.0272\pm0.0033)E_T + (0.0025\pm0.0003)V_M, \quad s = \pm0.0305. \tag{3}$$

The polarity parameter was also of relatively low significance, after its exclusion the four-parametric Eq. (4) was obtained. The corresponding correlation coefficient (0.9432) was only slightly below the recommended threshold of $R \geq 0.95$.

$$\log G_m = -0.0808 - (3.1075\pm0.5686)f(n^2) - (0.0010\pm0.0002)B + (0.0175\pm0.0030)E_T + (0.0027\pm0.0004)V_M, \quad R = 0.9432,\ s = \pm0.0445. \tag{4}$$

The calculated values of log G_m are shown in the table along with their deviation from the experimental values.

To conclude, we demonstrated that the main parameter of the solvent contributing to its efficiency towards rhamnolipids extraction was electrophilicity. The extraction data could be reasonably generalized using the four-parametric equation, the Hildebrand solubility parameter and the solvent polarizability being the non-significant parameters. The best extraction efficiency was found in the cases of higher alcohols, due to electrophilic solvation of the ester groups via the hydrogen bonding and (likely) due to the possibility of the parallel orientation of the alcohol alkyl group and the 3-hydroxydecanoic fragment of rhamnolipids.

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