Microbiology, Vol. 72, No. 2, 2003, pp. 173–178. Translated from Mikrobiologiya, Vol. 72, No. 2, 2003, pp. 206–211.

EXPERIMENTAL ARTICLES

Production of Oil-Releasing Compounds by Microorganisms from the Daqing Oil Field, China

T. N. Nazina*, [1], D. Sh. Sokolova*, A. A. Grigor'yan*, Y.-F. Xue, S. S. Belyaev*, and M. V. Ivanov***

**Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*
***Institute of Microbiology, Chinese Academy of Sciences, Zhong Guan Cun, P.O. Box 2714, Beijing, 100080 China*
Received April 15, 2002; in final form, July 30, 2002

Abstract—Twenty pure cultures isolated from formation waters of the Daqing oil field were studied with respect to their capacity to produce surface-active compounds in media with individual hydrocarbons, lower alcohols, and fatty acids. Aerobic saprotrophic bacteria belonging to the genera *Bacillus*, *Brevibacillus*, *Rhodococcus*, *Dietzia*, *Kocuria*, *Gordonia*, *Cellulomonas*, *Clavibacter*, *Pseudomonas*, and *Acinetobacter* decreased the surface tension of cultivation media from 55–63 to 28–44 mN/m. Strains of *Bacillus cereus*, *Rhodococcus ruber*, and *Bacillus licheniformis* produced biosurfactants most actively. Bacteria of the genera *Rhodococcus*, *Dietzia*, *Kocuria*, and *Gordonia* produced exopolysaccharides in media with hydrocarbons. Culture liquids of the strains of *R. ruber* and *B. licheniformis* exhibited an oil-releasing effect. Thus, the Daqing oil field is inhabited by aerobic bacteria capable of producing effective oil-releasing agents.

Key words: aerobic bacteria, oil fields, surface-active compounds, exopolysaccharides, MEOR.

Biogenic surface-active compounds (biosurfactants) are produced by bacteria, archaea, yeasts, microscopic algae, and some mycelial fungi. Biosurfactants are biphilic molecules consisting of a hydrophilic (polar) moiety and a hydrophobic (nonpolar) moiety; they tend to bind to each other, interact with surfaces of various polarity, adsorb at water–air or water–oil boundaries, cause wetting of hydrophobic surfaces, form structures analogous to lipid films or membranes, and reduce the surface and interfacial tension of solutions [1–3].

The functions of biosurfactants in the cell are poorly studied. Biosurfactants are known to be secondary metabolites involved in the transport of hydrophobic water-insoluble compounds into the cell, formation of biofilms, and adhesion of cells to various surfaces; they promote microorganism survival in communities; in pathogenic microorganisms, biosurfactants are an important factor of pathogenicity [1–3].

Biosurfactants can be conventionally subdivided into two groups. The first group comprises biosurfactants with a low molecular weight, such as glycolipids (rhamnolipids, trehalosolipids, sophorolipids) and lipopeptides (surfactin, strepofactin, polymyxin, gramicidin). The second group includes high-molecular-weight polymeric surfactants: polysaccharides, lipoproteins, lipopolysaccharides, and complexes of these compounds. The best studied are biosurfactants of the bacteria *Rhodococcus erythropolis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *B. brevis*, *B. polymyxa*, *B. licheniformis*, and *Acinetobacter calcoaceticus* and of the *Torulopsis* yeasts [2–4].

Like chemically synthesized surfactants, biosurfactants can be used in various fields of medicine, agriculture, and industry, including oil extraction and processing; they can also be used for ecosystem bioremediation (removal of water-insoluble pollutants), cleaning of vessels from oil fractions, acceleration of well drilling, and enhancement of oil recovery [1, 4–6]. Due to their physicochemical properties, activity at high salt concentrations, and lack of adsorption to limestones and sandstones, biosurfactants, applied in a mixture with other (e.g., nonionic) surfactants, can be efficient in the enhancement of oil recovery [1, 5, 6, 10]. Introduction of surfactant-producing microorganisms into oil reservoirs, followed by their development and surfactant production in situ, can significantly increase oil recovery.

With the use of microorganisms, surfactants with definite physicochemical properties can be obtained. In this respect, hydrocarbon-oxidizing bacteria deserve special attention, since their growth on oil is always accompanied by the formation of surfactants, which promote hydrocarbon penetration into cells [2, 3]. Some hydrocarbon-oxidizing bacteria also form, in addition to surfactants, exopolysaccharides, which can change the rheological characteristics of injected liquids, increasing their viscosity and thereby extending the compass of flooding of the oil stratum; these properties are especially pronounced in xanthans produced by bacteria of the genus *Xanthomonas* [5].

[1]Corresponding author. E-mail: nazina@inmi.host.ru

0026-2617/03/7202-0173$25.00

The effect of biosurfactants on the displacement of oil and on bioremediation of oil-contaminated soils was studied in model experiments that employed cores of oil-bearing rocks and pure cultures of *B. subtilis*, *B. licheniformis*, and *Pseudomonas fluorescens* [1, 2, 7].

Microbial production of surfactants in natural habitats has been insufficiently studied. It has been shown that surfactant producers inhabit both unpolluted soils and soils polluted with hydrocarbons; in the latter soils, the population of surfactant producers reaches 10–35% of the total population of aerobic heterotrophs [8]. A high emulsifying activity was characteristic of aerobic microorganisms (hydrocarbon oxidizers in particular) inhabiting subsurface horizons contaminated with aviation fuel [9]. However, the surfactant-producing capacity of oil field microorganisms remains unstudied.

The present work is a part of a complex investigation undertaken to determine the population density, geochemical activity, biodiversity, and metabolic potential of the microflora of the Daqing oil field (China), whose exploitation involves flooding with fresh or slightly mineralized waters. Earlier in this oil field, we found aerobic heterotrophic bacteria (including hydrocarbon-oxidizing) and anaerobic fermentative, sulfate-reducing, and methanogenic bacteria [11]. Twenty pure cultures of aerobic saprotrophic bacteria were isolated, which, according to their phenotypic and genotypic properties, were assigned to the genera *Bacillus*, *Brevibacillus*, *Rhodococcus*, *Dietzia*, *Kocuria*, *Gordonia*, *Cellulomonas*, *Clavibacter*, *Pseudomonas*, and *Acinetobacter* [12]. The aim of our present work was to study the ability of these aerobic bacteria to produce surface-active compounds and exopolysaccharides in media containing individual hydrocarbons, lower alcohols, or volatile fatty acids.

MATERIALS AND METHODS

The subjects of study. This work was concerned with the production of oil-releasing compounds by aerobic saprotrophic bacteria isolated from the Daqing oil field (China): *Bacillus subtilis* strains 32a and 32e; *B. licheniformis* 421; *B. cereus* strains 7 and 62; *Brevibacillus parabrevis* strains 13 and 14; *Gordonia* sp. 321; *Clavibacter michiganensis* 310-2-3; *Cellulomonas cellulans* 23; *Rhodococcus ruber* strains 14H, 33, and 41; *Dietzia* sp. 263; *Kocuria erythromyxa* 32f; *Kocuria* sp. 32d; *Arthrobacter oxydans* strains 32c and 1-3; *Pseudomonas putida* 1b; and *Acinetobacter* 1a. Physicochemical and microbiological characterization of the Daqing oil field, the phenotypic properties of the isolates, and the grounds for the determination of their taxonomic positions were presented earlier [11, 12].

Nutrient media and cultivation conditions. The cultures were maintained on potato agar. Production of oil-releasing compounds (surfactants and exopolysaccharides) was studied on a medium of the following composition (g/l distilled water): $NaNO_3$, 2.0; $MgSO_4 \cdot 7H_2O$, 0.25; KH_2PO_4, 1.0; $CaCl_2 \cdot 2H_2O$, 0.01; yeast extract, 1.0. The carbon sources were sucrose (40 g/l), glucose (10 g/l), acetate, propionate, butyrate (5 g/l), a mixture of liquid paraffins (C_{12}–C_{22}, 6 vol %), or *n*-hexadecane (2–6 vol %). The medium was also supplemented with trace elements according to Pfennig and Lippert [13]. The initial optical density (OD_{600}) of inoculated media was 0.05. The cultures were grown on a rotary shaker (200 rpm) at 30°C; after 5 days, biomass, surface and interfacial tension, emulsifying activity, viscosity, and other parameters were determined in the cultures. The rheological characteristics were determined in cultures that were not normalized with respect to biomass, since the aim was primary screening for producers of surfactants and exoglycans.

Analytical methods. The growth of bacteria was judged from the increase of protein content determined by the method of Lowry *et al.* [15], using the Protein Assay Kit (Sigma Diagnostics). The formation of exopolysaccharides was assessed from the amount of reducing sugars in the medium (as recalculated to glucose), which was determined in the reaction with phenol and sulfuric acid [14].

Emulsifying activity was analyzed by a method modified from [4]. Four ml of the grown culture was added to 4 ml of hexadecane, and the mixture was agitated on a Vortex for 3 min; after 24 h, the volume and the stability of the emulsion formed was recorded. Emulsifying activity was expressed as the per cent ratio of the emulsion volume to the total volume of the mixture. Kinematic viscosity of grown cultures was determined with a VPZh-4 capillary viscosimeter at 25°C.

Surface and interfacial tensions were measured in 5-ml culture samples at the medium/air and medium/hydrocarbon interfaces, respectively, at 30°C by the ring-tearing-off method on an ST-1 tensiometer (Shimadzu).

Investigation of oil-releasing properties of cultures. The effect of microbial metabolites on the release of oil from a sandy matrix [16] was studied in model experiments. One gram of washed sand was mixed with 0.1 ml of methane oil ($\rho = 0.73$ kg/m^3), and the samples were agitated in 100-ml flasks with 10 ml of 50 mM Tris–HCl buffer, pH 7.2, containing 10 mM magnesium sulfate. Varied amounts of the grown culture were added to the samples; uninoculated flasks served as controls. The samples were incubated on a shaker (200 rpm) for 60 min at 30°C and then in a stationary state for 60 min to let the sand settle. The aqueous phase was separated by decantation. The samples were washed twice with 10 ml of Tris–HCl–Mg buffer, reunited with the aqueous phases earlier separated by decantation, and treated with diethyl ether. The ether

extracts were dryed under vacuum. The amount of oil residing in the sand after the impact of microbial cultures was determined as the amount of material extracted from the sand by the ether.

RESULTS AND DISCUSSION

In this work, we studied the ability of aerobic saprotrophic bacteria from the Daqing oil field to produce oil-releasing metabolites, such as surface-active compounds and exopolysaccharides.

Production of surfactants and exopolysaccharides by aerobic bacteria during growth on various substrates. The lack of a common reactive group or of a chromophore in most of the molecules of surfactants does not allow a universal chemical or spectral method for their analysis to be elaborated. Therefore, for the estimation of surface-active properties of microorganisms, their emulsifying activity is determined based on the ability of surfactants to produce an emulsion upon agitation of a mixture of a whole culture broth or a microbial suspension with a carbohydrate or oil.

We found that several bacteria grown on simple media exhibited high emulsifying activity and produced surfactants and exopolysaccharides. On media with *n*-alkanes, the highest emulsifying activity was exhibited by the bacteria *R. ruber* (strains 41, 33, 14H), *B. licheniformis* 421, *Kocuria erythromyxa* 32f, and *Dietzia* sp. 263 (Fig. 1). The formation of surfactants depended on the carbon source and the strain (Fig. 2, Tables 1, 2). Thus, strain 263 showed the same rate of surfactant production on sugars, volatile fatty acids,

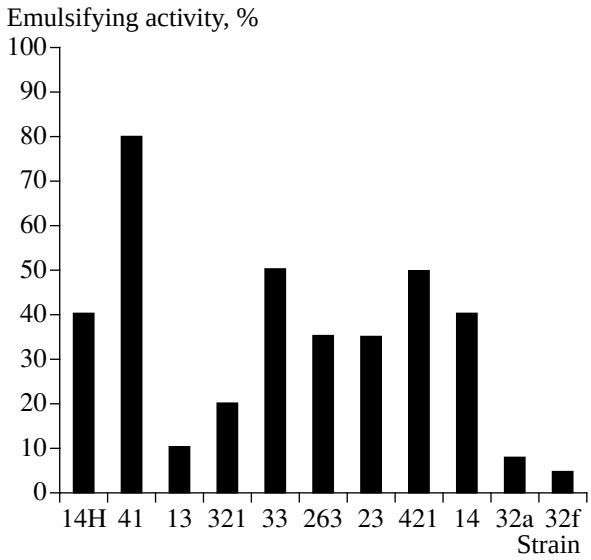

Fig. 1. Emulsifying activity of the culture broths of hydrocarbon-oxidizing bacteria grown on *n*-hexadecane: *Rhodococcus ruber* (strains 14H, 33, and 41), *Brevibacillus parabrevis* (strains 13 and 14), *Gordonia* sp. 321, *Dietzia* sp. 263, *Cellulomonas cellulans* 23, *Bacillus licheniformis* 421, *Bacillus subtilis* 32a, and *Kocuria erythromyxa* 32f.

Table 1. Emulsifying activity (EA), surface tension (ST), and interfacial tension (IT) of culture broths of aerobic bacteria grown on a mixture of liquid paraffins (1) or glucose (2)

Species and strain	Medium	Protein, mg/l	Sugar, mg/l	EA, %	ST, mN/m	IT,* mN/m	Viscosity, mPa s
Kocuria sp. 32d	1	590	820	5	37	4	ND
Dietzia sp. 263	"	820	1760	35	38	3	ND
Brevibacillus parabrevis 13	"	150	0	10	44	14	1.365
Br. parabrevis 14	"	700	640	40	45	15	2.625
Bacillus licheniformis 421	"	730	400	50	30	22**	2.38
Gordonia sp. 321	"	800	744	20	35	30**	1.12
Rhodococcus ruber 41	"	1950	1720	80	51	19	3.99
*R. rube*r 33	"	750	20	50	33	22	3.08
R. ruber 14H	"	330	140	40	40	37***	1.365
Cellulomonas cellulans 23	"	360	0	35	38	20**	1.05
Bacillus subtilis 32a	2	1070	ND	8	28	1	1.645
B. subtilis 32e	"	410	ND	5	31	1	1.645
B. cereus 62	"	330	ND	8	46	14	1.505
Clavibacter michiganensis 310-2-3	"	430	ND	10	31	3	1.54
Pseudomonas sp.27a	"	180	ND	5	28	1	1.54
Control	1	0	0	0	63	24	1.12
Control	2	0	ND	0	50	16	1.19

Note: The content of sugars was converted to the content of glucose. “ND” stands for “not determined”.

* The interfacial tension at the culture broth/decane interface.

** In 100-fold diluted culture broth.

*** In 1000-fold diluted culture broth.

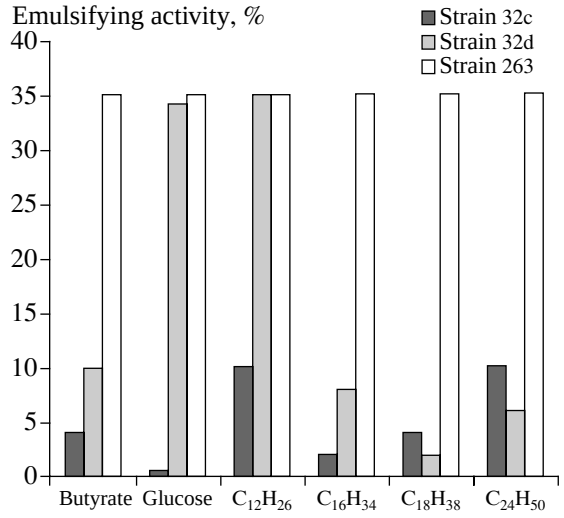

Fig. 2. Emulsifying activity of the culture broths of hydrocarbon-oxidizing bacteria *Arthrobacter* sp. 32c, *Kocuria* sp. 32d, and *Dietzia* sp. 263 grown on media with various substrates.

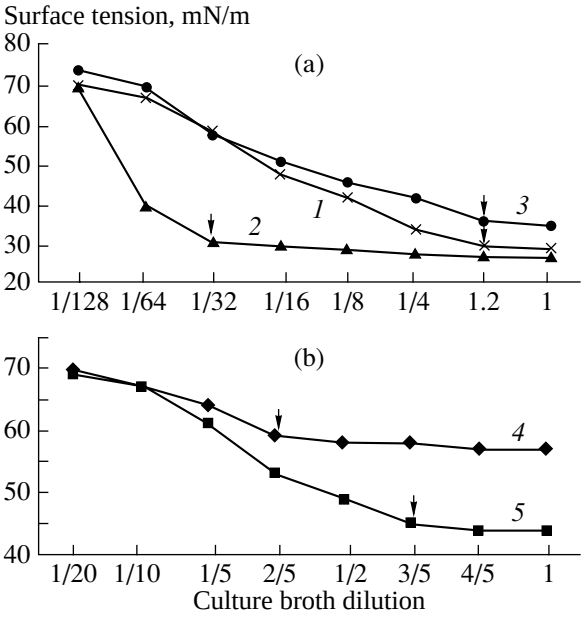

Fig. 3. Changes in the surface tension of culture broths upon their dilution. Arrows correspond to the dilution values that resulted in a surfactant concentration equal to CMC. Cultures: (*1*) *C. michiganensis* 320-2-3, (*2*) *B. subtilis* 32a, (*3*) *Kocuria* sp. 32d, (*4*) *Br. parabrevis* 13, and (*5*) *R. ruber* 41. Growth substrates: (a) sucrose and (b) a mixture of C_{12}–C_{22} *n*-alkanes.

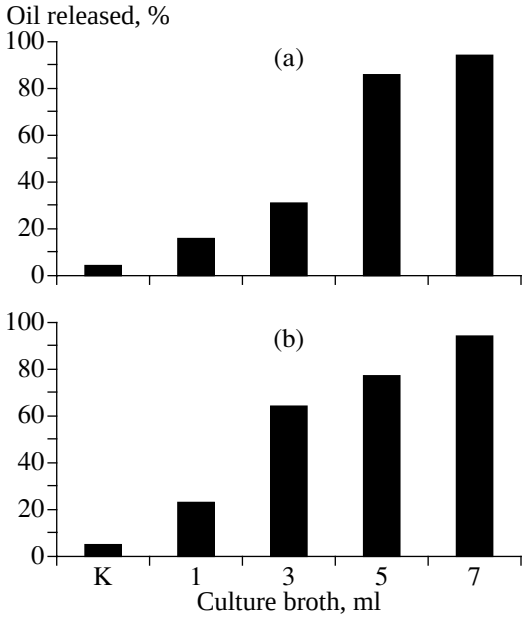

Fig. 4. Release of oil from sand under the effect of the bacteria (a) *B. licheniformis* 421 and (b) *R. ruber* 14H grown on medium with sucrose.

and some individual hydrocarbons; for strain 32f, media with glucose or *n*-decane were preferable; strains of *R. ruber* (41, 33, 14H) were still more capricious with respect to the substrates.

The ability of bacteria to produce surfactants from organic compounds of various classes is an important property under the conditions of the oil stratum, where the carbon source is represented by oil, which is a multicomponent substance.

Microorganisms that decrease surface tension of cultivation media by more than 10 mN/m are considered promising producers of surfactants [9]. Among the cultures that we studied, efficient emulsifying agents were produced by *B. licheniformis*, *Gordonia* sp., *Cellulomonas cellulans*, and *R. ruber* 14H, whose culture broths had to be diluted 100-fold and 1000-fold in order to determine the value of interfacial tension at the interface with decane (20–37 mN/m).

For indirect assessment of the critical micelle concentration (CMC), which characterizes the efficiency of a surfactant, culture broths of five strains were diluted to various extents with sterile medium and analyzed for the surface tensions (Fig. 3). The point of inflection of the curve obtained by measuring surface tensions of a series of dilutions corresponds to the surfactant concentration equal to CMC. The lower the CMC, the more industrially promising the surfactant [17]. We did not perform direct determination of the CMC values, because isolation of emulsifying agents from the culture liquids was beyond the scope of our work. The indirectly assessed CMC values corresponded to the following culture broth dilutions: 1.7-fold for *R. ruber* 41; 2.0-fold for *Clavibacter michiganensis* 310-2-3 and *Kocuria* sp. 32f; 2.5-fold for *Brevibacillus parabrevis* 13; and 32-fold for *B. subtilis* 32a. These data demonstrate the production of surfactants by the oil field microorganisms, including surfactants effective after more than 30-fold dilution of the culture broth.

The growth of several strains on liquid paraffins was accompanied by a considerable increase in the medium

Table 2. Rheological properties of culture broths after cultivation of bacteria on various substrates

Substrate	pH	ST, mN/m	IT,* mN/m	EA, %	Viscosity, mPa s
B. licheniformis 421					
Glucose	4.6	21.7	39.1	15	1.074
Sucrose	5.2	21.6	47.7	15	ND
Propionate	5.1	21.6	56.6	0	1.020
Butyrate	8.5	24.2	49.2	10	1.185
Acetate	7.8	26.3	49.1	0	1.085
R. ruber 14H					
Glucose	4.6	54.4	21.6	0	1.018
Sucrose	5.1	57.1	40.8	0	0.919
Propionate	7.2	57.7	34.9	0	0.941
Butyrate	9.6	45.5	56.5	0.2	2.446
Acetate	9.6	39.1	41.2	0	1.196
R. ruber 41					
Glucose	5.55	53.2	53.8	50	1.074
Sucrose	5.50	57.7	44.9	12.5	0.985
Propionate	6.65	53.1	55.7	3.75	1.063
Butyrate	8.55	57.9	46.2	57	1.107
Acetate	8.80	58.8	42.5	19	0.196
R. ruber 33					
Glucose	5.9	54.3	53.9	20	1.129
Sucrose	5.65	54.5	48.9	49	1.905
Propionate	6.1	67.7	60.3	0	0.985
Butyrate	9.1	52.7	48.6	48	1.539

* The interfacial tension at the culture broth/decane interface.

viscosity and in the content of polysaccharides, determined by the increase of sugar content in the medium (Table 1). The viscosity of the culture broth of *R. ruber* 41 corresponded to the viscosity of a 33% solution of sucrose at 25°C.

Microorganisms can produce extracellular polysaccharides of various composition and structure, from simple homoglycans to complex heteropolysaccharides, including those that are components of glycoproteins and glycolipids. Such biopolymers as xanthan, emulsan, scleroglucan [5] are used to increase viscosity of the water injected into oil strata; this results in an increased compass of flooding and, eventually, to enhancement of oil recovery. It can be expected that the activation of microorganisms producing surfactants and exopolysaccharides in the oil stratum will promote the release of oil.

Release of oil from a sandy matrix under the action of microorganisms. We studied the effect of culture broths of *R. ruber* 14H and *B. licheniformis* 421 on the release of oil from a porous medium represented by white sand. Bacteria were grown for 3 days on a medium with sucrose (200 rpm, 30°C), and various amounts of the cultures were introduced into oil-containing sand. Introduction of sterile medium served as the control.

We found that introduction of even a small amount (10% of the liquid phase volume) of a culture (containing biomass, surfactants, exopolysaccharides, and other metabolites) resulted in an increased release of oil as compared to the introduction of sterile medium (Fig. 4). The emulsifying properties of *R. ruber* were superior to those of *B. licheniformis.* Importantly, the bacteria could produce surfactants and polysaccharides from hydrocarbons and oil, and not only from expensive sugar-containing substances such as molasses.

The expenditure of chemically synthesized surfactants for the enhancement of oil recovery is usually 0.2–2 g/l injected water [5]. The CMC values of biosurfactants are usually 10- to 40-fold lower than those of chemically synthesized surfactants [5, 18]. The CMC values of known biosurfactants vary from 1 to 200 mg/l, and the value of the dilution of the culture broth necessary to achieve CMC varies from 8 to 500 [5]. Thus, the expenditure of biosurfactants for the enhancement of oil recovery is severalfold lower, which makes their application preferable [1, 19].

The bacteria *R. ruber* 14H and *B. licheniformis* 421 produced efficient biosurfactants, and their culture broths can be used for the enhancement of oil recovery and decontamination of oil-polluted ecosystems.

The results obtained in the present work show that the Daqing oil field is inhabited by aerobic saprotrophic (including hydrocarbon-oxidizing) bacteria that are able to produce oil-releasing metabolites (surfactants and exopolysaccharides) during growth on a wide range of substrates (hydrocarbons, lower alcohols, volatile fatty acids, sugars). It seems expedient to apply, at this oil field, the methods of microbial enhancement of oil recovery based on activation of the indigenous aerobic microflora.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project nos. 01-04-49250, 02-04-06060, and 00-15-97897) and by the Russian Ministry of Industry and Science (subproject “Biotechnologies for Extraction and Processing of Mineral Resources” of the Federal Science and Technical Program “Research and Development along Priority Lines of Science and Technology” and the International Russian–Chinese Science and Technical Project).

REFERENCES

1. Banat, I.M., Biosurfactant Production and Possible Uses in Microbial Enhanced Oil Recovery and Oil Pollution Remediation: A Review, *Bioresource Technol.*, 1995, vol. 51, pp. 1–12.

2. Lin, S.-C., Biosurfactants: Recent Advances *J. Chem. Tech. Biotechnol.*, 1996, vol. 66, pp. 109–120.
3. Ron, E.Z. and Rozenberg, E., Natural Role of Biosurfactants, *Environ. Microbiol.*, vol. 3, no. 4, pp. 229–236.
4. Zajic, J.E., Guignard, H., and Gerson, D.F., Emulsifying and Surface Active Agents from *Corynebacterium hydrocarboclastus*, *Biotechnol. Bioeng.*, 1977, vol. 19, pp. 1285–1301.
5. Rozanova, E.P., Belyaev, S.S., Ivanov, M.V., Mats, A.A., Kulik, E.S., and Mamedov, Yu.G., *Mikrobiologicheskie metody povysheniya nefteotdachi plastov* (Microbiological Methods for the Enhancement of Oil Recovery), Moscow: VNIIOENG, 1987.
6. Ganitkevich, Ya.V., Microbial Surfactants, *Biotekhnologiya*, 1988, vol. 4, no. 5, pp. 575–583.
7. Adkins, J.P., Tanner, R.S., Udegbunam, E.O., McInerney, M.J., and Knapp, R.M., Microbially Enhanced Oil Recovery from Unconsolidated Limestone Cores, *Geomicrobiol. J.*, 1992, vol. 10, pp. 77–86.
8. Jennings, E.M. and Tanner, R.S., Biosurfactant-Producing Bacteria Found in Contaminated and Uncontaminated Soils, *Proc. of the 2000 Conference on Hazardous Waste Research*, 2000, pp. 299–306.
9. Francy, D.S., Thomas, J.M., Raymond, R.L., and Ward, C.H., Emulsification of Hydrocarbons by Subsurface Bacteria, *J. Ind. Microbiol.*, 1991, vol. 8, pp. 237–246.
10. Belyaev, S.S., Laurinavichus, K.S., Obraztsova, A.Ya., Gorlatov, S.N., and Ivanov, M.V., Microbial Processes in Near-Bottom Zones of Injection Wells of Oil Fields, *Mikrobiologiya*, 1982, vol. 51, no. 6, p. 997.
11. Nazina, T.N., Xue, Y.-F., Wang, X.-Y., Grigoriyan, A.A., Ivoilov, V.S., Belyaev, S.S., and Ivanov, M.V., Diversity and Activity of Microorganisms in the Daqing Oil Field of China and Their Potential for Biotechnological Applications, *Res. Environ. Biotechnol.*, 2000, vol. 3, pp. 121–132.
12. Nazina, T.N., Grigor'yan, A.A., Yan-Fen Xue, Sokolova, D.Sh., Novikova, E.V., Tourova, T.P., Poltaraus, A.B., Belyaev, S.S., and Ivanov, M.V., Phylogenetic Diversity of Aerobic Saprotrophic Bacteria Isolated from the Daqing Oil Field, *Mikrobiologiya*, 2002, vol. 71, no. 1, pp. 103–110.
13. Pfennig, N. and Lippert, K.D., Uber das Vitamin B_{12}-Bedurfnis phototropher Schwefelbakterien, *Arch. Mikrobiol.*, 1966, vol. 55, no. 3, pp. 245–256.
14. Dubois, M., Gullis, K.A., Hamilton, J.K., *et al.*, Colorimetric Method for Determination of Sugars and Related Substances, *Anal. Chem.*, 1956, vol. 28, no. 3, pp. 350–356.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.
16. Eliseev, S.A., Vil'danova-Martsishin, R.I., Shul'ga, A.N., Shabo, Z.V., and Turovskii, A.A., Oil-Releasing Emulsifier Produced by *Bacillus* Species, *Mikrobiol. Zh.*, 1991, vol. 53, no. 6, pp. 61–66.
17. *Micelle Formation, Solubilization, and Microemulsions*, Mittel, K., Ed., Moscow: Mir, 1980, vol. 1, pp. 12–87.
18. Lang, S. and Philp, J.C., Surface-Active Lipids in Rhodococci, *Antonie van Leeuwenhoek*, 1998, vol. 74, pp. 59–70.
19. Desai, J.D. and Banat, I.M., Microbial Production of Surfactants and Their Commercial Potential, *Microbiol. Mol. Biol. Rev.*, 1997, vol. 61, no. 1, pp. 47–64.