Ministry of Education and Science of Ukraine

ODESSA NATIONAL ACADEMY OF FOOD TECHNOLOGIES

International Competition of
Student Scientific Works

BLACK SEA SCIENCE 2021

PROCEEDINGS

ODESSA, ONAFT 2021

Ministry of Education and Science of Ukraine

Odessa National Academy of Food Technologies

International Competition of Student Scientific Works

BLACK SEA SCIENCE 2021

Proceedings

Odessa, ONAFT 2021

UDC 001.8(063)

Recommended for print by the Academic Council of
Odessa National Academy of Food Technologies
on April 6, 2021, Protocol No. 13

Black Sea Science 2021: Proceedings of the International Competition of Student Scientific Works / Odessa National Academy of Food Technologies; B. Iegorov, M. Mardar (editors-in-chief.) [*et al.*]. – Odessa: ONAFT, 2021. – 731 p.

Proceedings of International Competition of Student Scientific Works «Black Sea Science 2021» contain the works of winners of the competition.

The author of the work is responsible for the accuracy of the information.

Organizing committee:

Prof. Bogdan Iegorov, D.Sc., Rector of Odessa National Academy of Food Technologies, Head of the Committee

Prof. Maryna Mardar, D.Sc., Vice-Rector for Scientific and Pedagogical Work and International Relations of Odessa National Academy of Food Technologies, Deputy Head of the Committee

Prof. Stefan Dragoev, D.Sc., Vice-Rector for Scientific Work and Business Partnerships of University of Food Technologies (Bulgaria)

Prof. Baurzhan Nurakhmetov, D.Sc., First Vice-Rector of Almaty Technological University (Kazakhstan)

Prof. Mircea Bernic, Dr. habil., Vice-Rector for Scientific Work of Technical University of Moldova (Moldova)

Prof. Jacek Wrobel, Dr. habil., Rector of West Pomeranian University of Technology (Poland)

Prof. Michael Zinigrad, D.Sc., Rector of Ariel University (Israel)

Dr. Mei Lehe, Ph.D., Vice-President of Ningbo Institute of Technology, Zhejiang University (China)

Prof. Plamen Kangalov, Ph.D., Vice-Rector for Academic Affairs of "Angel Kanchev" University of Ruse (Bulgaria)

Dr. Alexander Sychev, Ph.D., Assoc. Professor of Sukhoi State Technical University of Gomel (Belarus)

Dr. Hanna Lilishentseva, Ph.D., Assoc. Professor, Head of the Department of Merchandise of Foodstuff of Belarus State Economic University (Belarus)

Prof. Heinz Leuenberger, Ph.D., Professor of the Institute of Ecopreneurship of University of Applied Sciences and Arts (Switzerland)

Prof. Edward Pospiech, Dr. habil., Professor of the Institute of Meat Technology of Poznan University of Life Sciences (Poland)

Prof. Lali Elanidze, Ph.D., Professor of the Faculty of Agrarian Sciences of Iakob Gogebashvili Telavi State University (Georgia)

Dr. V. Kozhevnikova, Ph.D., Senior Lecturer of the Department of Hotel and Catering Business of Odessa National Academy of Food Technologies, Secretary of the Committee

5. ECOLOGY AND ENVIRONMENTAL PROTECTION

BIOREMEDIATION OF PETROLEUM - CONTAMINATED SOIL USING MICROORGANISMS

Author(-s): Tamila Shiukashvili, Mariam Ichkiti, Tornike Khutuashvili

Advisor(-s): Prof. Magda Davitashvili
Iakob Gogebashvili Telavi State University, Georgia

Abstract. *Biosurfactants are surface - active molecules that can reduce surface tension. Microorganisms are able to produce biosurfactants and bio emulgators, that are characterized with structural and functional diversity. There are 39 bacterial strains that are produced by petroleum - contaminated soil biot in Georgia. There are 11 Pseudomonas, 17 Bacillus and 4 Rhodococcus strains identified here. With the help of screening, seven bacterial strains are chosen that are produced by biosurfactants. Producing biosurfactants by the genus Bacillus reaches its maximum in 24 hours, but by Pseudomonas and Rhodococcus strains - in 72 hours. Surface tension tensiometer method has discovered three strains that can synth extracellular biosurfactants. In order to increase the biosurfactant yield, there are carefully selected feeding areas and good conditions for cultivating. Among the strains that are well known with its extracellular biosurfactant yield, Pseudomonas sp. GV19 stands out with its 30.1 milinuton/meter strain.*

Key Words: *biosurfactants, bioemulgators, strain, desorption, emulsify, solubilization, extracellular, bioremediation.*

I. INTRODUCTION

One of the most important ways in the oxidation of petroleum hydrophilic hydrocarbons is producing biosurfactants by the microorganisms that can degrade petroleum. This process causes desorption, emulsification and solubilization of petroleum hydrocarbons. On the other hand, this process makes it easy for them to enter in the microbial cell. Nowadays, the interest toward biogenic surfactants has raised, because they are environmentally friendly and economically effective. When we are comparing synthetic surfactants to biosurfactants, biosurfactants are in advantage because of their low rate of toxicallity, high definition of biodegradation, sustainable activity during the process of reaction, etc. [5, 8]

Nowadays, the synthesis of biosurfactants with the help of microorganisms is a well known process [7, 10, 11, 12, 38]. But our understanding of the method of discovering biosurfactant producers remains limited. Their functional evaluation criterias are not defined as well as their phisical, chemical and biological characteristics. Their phisiology and producing of surface active metabolites is partially studied.

The goal of our work is to screen surface active substances - biosurfactant producers that were produced from the microbiota of petroleum contaminated soil in Kakheti district (Sighnaghi, Dedoplistskaro, Sartichala, Sagarejo). To reach these goals, we had some tasks:

1. Taking different species of bacteria from petroleum contaminated soil in Kakheti district and changing them to pure culture of bacteria.
2. Screening of bacteria according to surface active substances - biosurfactants synthesis.
3. Selection of optimal conditions for biosurfactants synthesis.

For the first time in Kakheti district, we managed to allocate surface active substances - biosurfactants that produce microorganisms aboriginal strains from petroleum contaminated oil.

As a result of our studies we discovered biosurfactants that produce bacterial strains, that were from the genes of *Rhodococcus*, *Bacillus* and *Pseudomonas*. Selected strains may be used to bioremediate petroleum contaminated soil.

In the work, we chose to do the research with modern methods that included spectrophotometry and thensometry. With the help of these methods, we could evaluate the activity of extracellular biosurfactants. Also, in the research we used laboratory methods such as taking soil samples, separating bacterias [22, 36], selecting feeding areas and cultivating conditions for bacteria [32].

The work is done on the technical - material basis of the biological laboratory of Iakob Gogebashvili Telavi state university, faculty of exact and natural sciences.

II. LITERATURE ANALYSIS

2.1 Review of the Literature with Analysis

2.1.1 The Spread of Surface Active Substances - Biosurfactants in the Microbial World

Nowadays the interest in biological originated surface active substances has dramatically increased. The reason is that they are ecologically safe and economically effective agents. People use them for different purposes such as emulsification, solubilisation, antithesis and detergent processes [9].

As a consequence of anthropogenic influence, ruining natural ecosystems faced mankind to a giant problem. One of the most important factors of environmental pollution is the oil refining industry. Millions of tons of solid and liquid remains are being collected during the process of oil extraction, recycling, transportation and consuming. These hydrocarbons are characterised with low levels of degeneration and are highly toxic. Self cleaning of a polluted territory is a very long lasting process. It is also dangerous to natural ecosystems. Microorganisms have the ability to transform petroleum hydrocarbons and degrade them. This affords us to use them for bioremediation of contaminated areas [1].

Microorganisms, as they are biologically active substances producers, are used in the industry for creating no-waste technology. In the means of ecology, it is a very important process. They are fast - reproducing organisms and are not dependent on different seasons. They are not toxic and can assimilate to any raw material or waste. Microorganisms are the most important biocatalysts. They can produce biosurfactants and emulgators that are characterized with structural and functional diversity.

Aside from synthetic surfactants, biosurfactants have advantages in lower rates of toxicity, high definition of biodegradation, sustainability in extreme conditions [5, 8]. Ability of strong foaming, effectiveness in extreme temperature, ph or under conditions of salt concentrations [30]. Biosurfactants that are the products of microbial synthesis are the most effective in biochemical production. The reason is that in order to cultivate their producers microorganisms we need more simple mineral areas. Also, if we manage the process of fermentation it will increase its productivity with the less waste [15, 34].

Surfactants, as they are physiologically active substances, are not used only to emulsify and solubilizate hydrophobic substances, but their role in medicine, pharmaceutical technology and agriculture is huge [5, 13, 26]. The Growth of microorganisms and the ability of producing biosurfactants on renewal substrates is very important. This is the reason why the potential user of biosurfactants turns out to be petroleum production [2].

The restriction of protecting the environment has led us to the growing interest in biogenic substances. This process will eventually ban hightoxical chemical substances. It is well known that chemical surfactants are produced from the organic synthesis of petroleum [9]. This is why, obeying biosafety rules is crucial. This fact is a prerequisite for developing the field of biotechnology that learns biological agents and products of biosynthesis [21, 24, 31, 39].

Producing biosurfactants in situ by microorganisms is playing a vital role in biotechnology of protecting the environment. For example, its vital in remediation of soil and water that are polluted by hard metals and organic pollutants [6]. In the last few years, the traditional field of using biosurfactants has been rising. They are used in medicine, as they are physically active substances.

Biosurfactants producers are discovered in the living world. The role of microorganisms is bigger than anything. They are produced from different sources such as soil, sea and freshwater and etc. According to Maier [17] biosurfactants that were synthesized by microorganisms with different phylogenetic affiliation are functionally different. This fact indicates the vital role of these compounds in producers. Genetic and phenotypic links of biosurfactants refer to their independent evolutionary development [4]. Usually biosurfactants that were synthesized by the same gene but different species are structurally and functionally same [18, 19]. That's why it is difficult to find new producers and identify its belongings to any of the taxonomic groups. In spite of the fact that nowadays all of the genetic determinants of biosurfactants synthesis are defined [28], their molecular marks for in situ detection is not created yet. Nowadays functional approach has no alternative in the search of biosurfactants producers. It takes into consideration screening of pure cultures according to the surface active substances.

2.1.2 Physical and Chemical Characteristics of the Microbial Biosurfactants and their Classification

Surfactants are superficially active substances, which have the ability to reduce stretch ot the fluid surface. They are adsorbed on the surface of the liquid, create transition layer at the border of the air and fluid, which reduse stretch of the liquid surface. Emulsifiers are superficially active substances too. They can make emulsion etween two liquids, which don’t mix with each other. Surfactants and Emulsifiers are amphiphilic molecules, which has two functional parts: polar-hydropilic head and nonpolar-hyprophobic tail. In amphiphilic molecules arrangement and size of the hydrophilic and hydropobic functional groups causes properties and purpose of the surfactants.

Biosurfactants are characterized by high structural diversity. Their structure varies from glyco- and phospholipids to high molecular weight polysaccharide, lipid and protein biopolymer nature, which determines their wide range of functional properties [25]. In terms of practical application, glycolipid surfactants, which are complexes with complex ether bonds of fatty acids and mono- and disaccharides, are intensively studied [16, 18, 20, 27]. Biosurfactants are characterized by the same physicochemical properties as synthetic surfactants. They are characterized by a critical concentration of surface and inter-phase stretch micelle formation, hydrophilic-lipophilic balance, etc. [35].

The amphiphilic nature of biosurfactants is determined by the presence of hydrophobic amino acid or peptide anions and cations and the presence of hydrophobic unsaturated or saturated fatty acids.

Five classes of biosurfactants are known today:

- ❖ Glycolipids
- ❖ Lipopeptides
- ❖ Fatty acids and neutral fats
- ❖ Lipopolysaccharides
- ❖ Polysaccharide - lipid complexes

Different classes of biosurfactants are synthesized by different groups of microorganisms:

1. Glycolipids (ramnolipids) - *Pseudomonas aeruginosa*; Tregalosolipids - *Rhodococcus erythropolis, Nocardia rhodochrous, N. erythropolis, Mycobacterium phlei;* Sophosorolipids - *Torulopsis bombicola, T. ampicola, T. petrophilum*.
2. Lipoproteins and lipopeptides: Lichenazine-- *Bacillus licheniformis*; Subtilizine - *B. subtilis*; Circulocin - *B. circularis*; Polymyxin - *B. subtilis*; Viscosin - *Pseudomonas fluorescens*; Emulsans - *Phormidium* sp.; Liposan - *Candida lypolytica*.
3. Polysaccharides: Emulsifiers - *Arthrobacter* sp., *A. calcoaceticus*; *Phormidium* sp.; Xanthan - *Xanthomonas campestris*.
4. Fatty acids - *Candida* spp., *C. lepus*.
5. Phospholipids - *Tiobacillus thiooxidans*; *Corynebacterium* sp.; *Candida* sp.

Heteropolysaccharide xanthan in pure form is a soft powdery mass that dissolves easily even in small amounts of water and forms a gel. This substance is thermostable. Resistant to the action of electrolytes, maintains viscosity in saline solutions. Does not adsorb into large clumps [33]. Used in the food industry and cosmetics as an emulsifier.

There is a great deal of interest in emulsions due to the formation of emulsions in the "fat-water", which is associated with low energy expenditure. An emulsion forms an emulsion as an aliphatic or aromatic hydrocarbon. It also forms a 2 nm thick apk around the fat droplet, with hydrophilic groups that preclude mixing with water and forming an emulsion. Stabilization of the emulsion is observed. This bioemulsifier is stable because the emulsion does not disintegrate by centrifugation [35]. Emulsion maintains activity even at high salt concentrations and is resistant to other extreme conditions. Emulsifiers are particularly effective emulsifiers that are specific to crude oil and are synthesized in an ethanol-enriched area. Emulsifiers that do not contain proteins are resistant to high temperatures. Maintains stability and emulsifying ability in neutral and alkaline areas at 100°C for 2 hours. These types of biosurfactants have the ability to remove oil from the soil surface. At the same time, the viscosity of the oil decreases and, consequently, the surface tension.

The maximum activity of liposan depends on the ratio of hexane to lipizan 50:1, which reduces the emulsifying activity. Its maximum emulsifying activity was observed in the acidic environment in the pH range of 2.0-5.0, liposan exhibits thermostability at 70°C and loses 60% of its activity when heated to 100°C for 1 hour. The ability to emulsify water-insoluble compounds by liposan depends on the chain length of the substrate.

Lipopeptide biosurfactant - inturin, isolated from bacterial culture *B. amyloliguefacies* reveals stability in the laboratory. Demonstrates surface and antibiotic properties at 20°C for 2 months and maintains 30 minutes at 100°C

incubation temperature. It should be noted that at 121°C autoclaves 20 minutes, only 40% of the activity is lost. Even during sunlight and ultraviolet radiation, its surface and antibiotic activity does not change.

With rhamnolipid synthesized by *Pseudomonas aeruginosa*, a significant amount of oil spilled on Alaska was removed. The ability to remove hydrocarbons from 25-70% of contaminated sludge and 40-80% of clay has also been studied by this biosurfactant [2]. In addition, the use of biosurfactants is effective - in heavy metals (cadmium, uranium, lead)], phenanthrene and polychlorinated biphenyls - in soil bioremediation [29].

An experiment in Venezuela showed that the viscosity of crude, heavy oil treated with biosurfactant emulsion was reduced from 200000 to 100, allowing heavy oil to be pumped through a 26000 - mile-long industrial pipeline, which is impossible with oil chemical surfactants. In Kuwait, crude oil treated with surfactant has been pumped into oil storage.

In the United States, "Petrogen Inc" has managed to remove 90% of the oil in its wastewater sediment using a microbial biosurfactant [3].

Biosurfactants are also used in the food industry as a food additive. Lecithin and its derivatives - glycerol-containing fatty acid esters, sorbitol or ethylene glycol and ethoxylated derivatives of monoglycerides are widely used as emulsifiers. Biosurfactants are also used in cosmetics. Company "Kao Co. Ltd "uses a biosurfactant - sophorolipid, in "Sofina" skin and hair conditioners and lip balms [14]. Some biosurfactants are promising for use in medicine and veterinary medicine: Succinyl-trehalose lipid produced by Rhodococcus erythropolis has been shown to inhibit lethal doses of herpes and influenza viruses (10-30 μg / ml). Biosurfactant - Surfactin is used to dehydrate peat, in the paper, coal, textile industries, and to extract uranium ores [8].

By microorganisms of the genus *Rhodococcus*, the amount of biosurfactants synthesized on hydrophobic substrates varies in the range of 0.5-30 g/l, this variation depends on the carbon source, the nature of the carbon and nitrogen source and their ratio, pH value and many other factors. [37]. In-depth cultivation of rhodococci, when the content of hexadecane or kerosene in the liquid feed area is 2%, 1-3 g/l biosurfactant is synthesized.

Thus, today microorganisms, as producers of biologically active substances, are used in the production of waste-free technologies in production and are very important from an ecological point of view as well. The biosurfactants produced by them cause the desorption, emulsification and solubilization of petroleum hydrocarbons, which in turn facilitates their penetration into the microbial cell and is extremely important in the bioremediation process.

III. OBJECT, SUBJECT, AND METHODS OF RESEARCH

3.1 Isolation of Bacteria from Oil Contaminated Soils, Nutritional Areas of Microorganisms and Cultivation Conditions

The object of the study was microorganisms isolated from oil-contaminated soils of the Kakheti region (Sighnaghi, Dedoplistskaro, Sartichala, Sagarejo). We took

averaged soil samples e.g. By the “envelope” method. [22] Soak 10 grams of test soil in 80 ml of sterile rodin for 5 min. With gradual addition of sterile tap water. The resulting suspension was stirred for 30 min. The suspension was then transferred to a test tube with a 1 ml sterile pipette containing 9 ml of sterile tap water. We had one drop of each dilution with a 1 ml sterile pipette. We sowed 3-5 petals in each dilution. To obtain separate colonies of bacteria, we sowed the 4th - 6th dilutions. We put the petri dishes in a thermostat at - 30°C for 6-10 days.

To separate the bacteria from the soil suspension, we used the following areas:
1. Chapek area for bacteria of different genus [g/l]: Glucose - 20; $NaNO_3$ - 2; K_2HPO_4 - 1; $MgSO_4 \times 7H_2O$ - 0.5; KCl - 0.5; $FeSO_4 \times 7H_2O$ - 0.01; Agar - 20. pH 7.
2. Meat-peptone agar
3. King-B
4. Synthetic area for nocardia - like bacteria (g/l): urea - 1.5; Na_2HPO_4 - 4; KH_2PO_4 - 3; $MgSO_4$ –1; Glucose - 23; Sucrose - 10; $FeCl_3$ - 8 mg / L; B1 - 1 mg / l; Agar -20. pH 6.8-7.2.

We took different colonies from each petri dish and moved them in a loop to the appropriate areas to obtain pure crops. The cultures were stored on agar in a microbiological test tube. In order to detect microbial biosurfactant producers and oil-degrading strains, we grew the crops on petroleum junctions in crude oil-containing areas, adding oil to a sterile, solidified aerated feed area from above and then sterile loops were transferred to clean crops in areas where the only source of carbon was crude oil. Transplanted material was prepared in the appropriate liquid food areas without oil 50 ml of food is contained in 750 ml flasks, circular stirring (180 rpm) at 28-30ºC for 5-7 days. The material transferred to the liquid area was a 10% bacterial suspension in the exponential growth phase.

The liquid feed area for the sowing material was the chapek area without agar.

Intensity of crop growth on solid food areas was assessed visually by a four-point system [+ - very weak growth, 2+ - growth, 3+ - medium growth, 4+ - good growth].

3.2.Determination of surface tension

To test the ability of biosurfactants to synthesize, we planted the selected strains in the crude oil field in the Chapek liquid food area. We measured the surface tension of the culture fluid in dynamics on days 1, 2, 3, 4, 5 of the tensiometric method of cultivation. The cultural fluid was centrifuged at 8000 rpm and the surface tension of the supernatant was measured with a tensiometer.

3.3 Separation of biosurfactant from the reaction zone

Biomass was precipitated or centrifuged to extract biosurfactants from the culture fluid. After removing the biomass, we acidified the culture fluid to pH-2.0 with hydrochloric acid.

We stayed 24 hours. We centrifuged on the K-23 at a speed of 11000 rpm. Dissolve the centrifuge in methanol. The precipitated mass is a biosurfactant.

IV. RESULTS

From the soil samples studied (Sighnaghi, Dedoplitskaro, Sartichala, Sagarejo) 39 bacterial strains were isolated, they were identified using selective food areas and microscopy of crops. Strains of 11 *Pseudomonas*, 17 *Bacillus* and 4 *Rhodococcus* were identified, the remaining strains could not be identified.

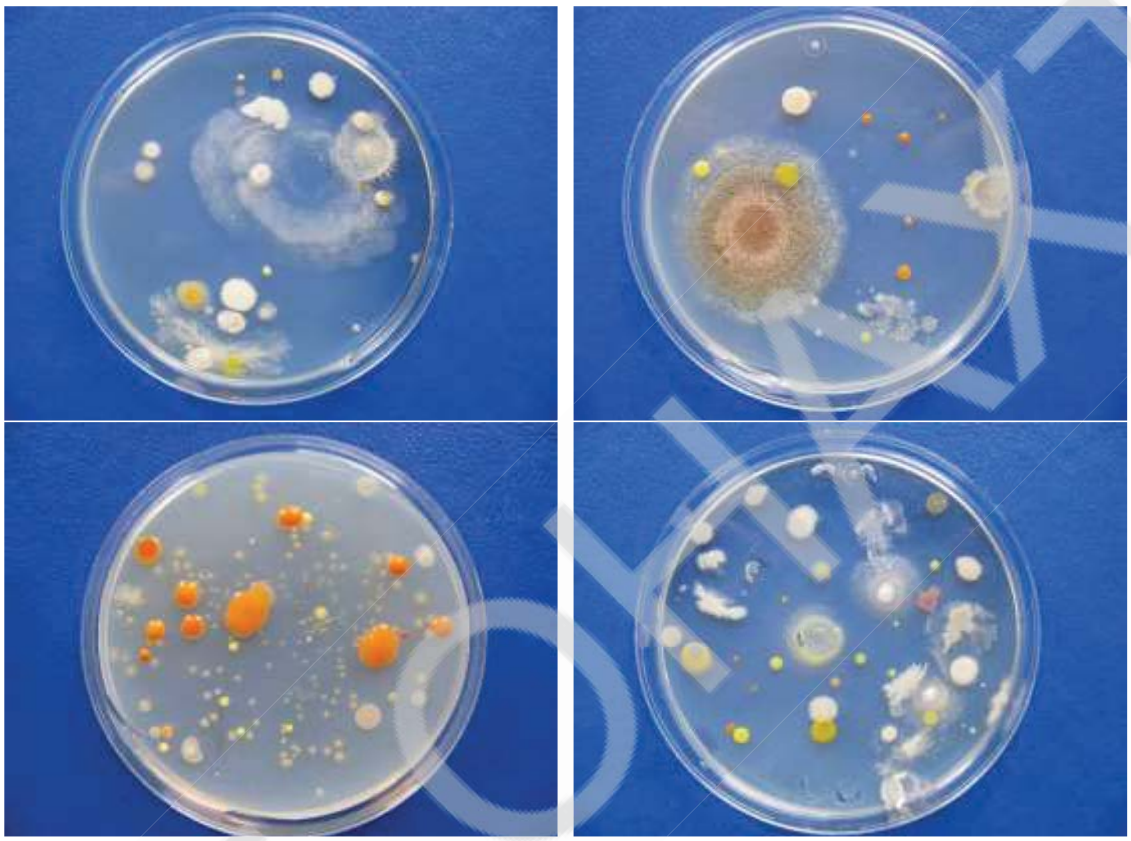
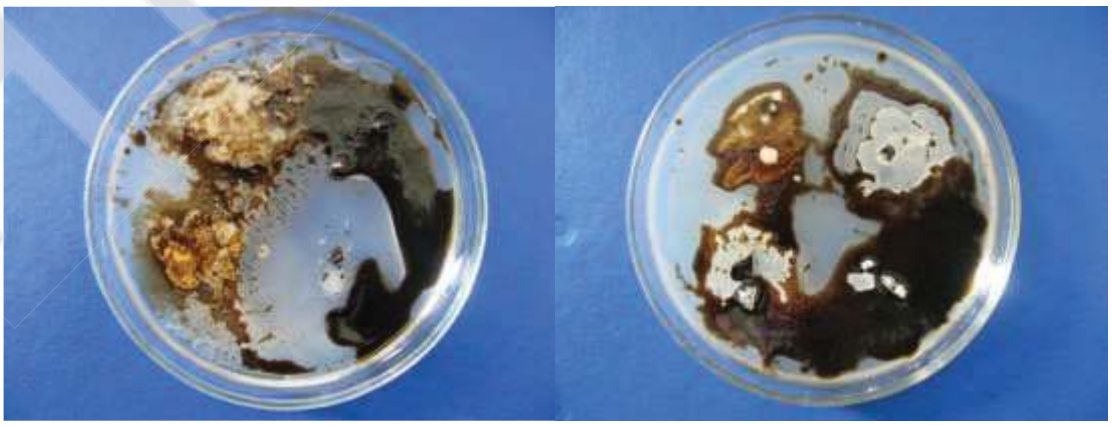


Fig.1. Microorganisms isolated from soil in different selective areas

For the primary screening of biosurfactant-producing microorganisms, the selected strains were sown in an oil-laden area. As a result of cultivation, strains of the genus Rhodococcus, Pseudomonas and Bacillus growing in crude oil were selected (28 crops in total), around which a clear zone appeared in the oil-rich area (Fig. 2).



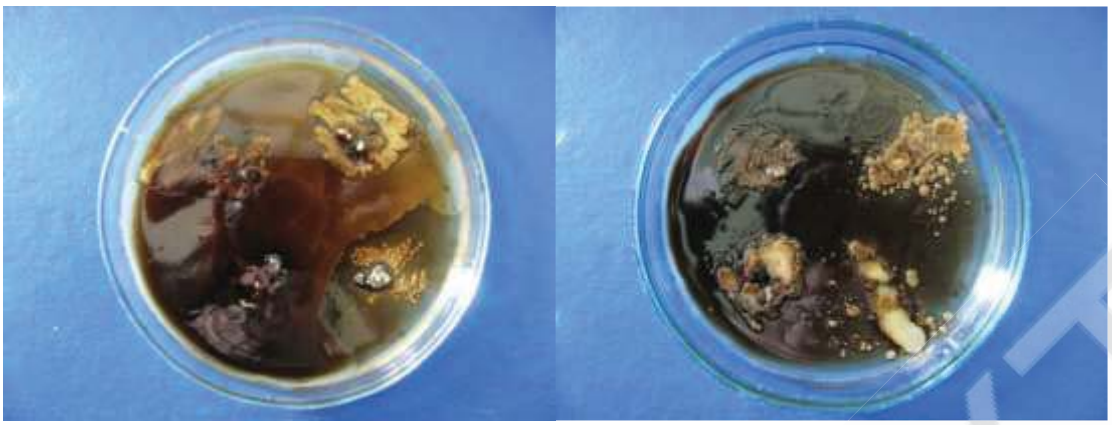


Fig.2. Growth of Bacterial Strains On Oily Nutrient Soils
Bacillus sp. GV34, *Pseudomonas* sp. GV19, *Rhodococcus* sp. GV13.

We selected 7 strains according to the intensity of culture growth in the agitated oil area and the formation of a clear zone around (Fig. 3).

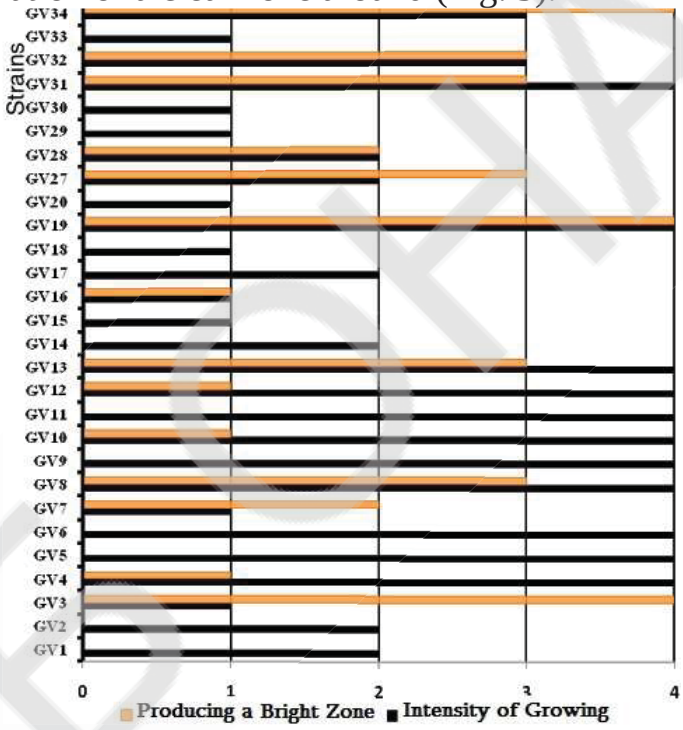


Fig. 3 . Intensity of crop growth in the oil field

Selected crops were grown in the liquid nutrient area under deep cultivation conditions. When using hexadecane as the sole source of carbon, all strains of the genus *Rhodococcus*, *Pseudomonas* and *Bacillus* produce exo-biosurfactants, which according to the literature belong to extracellular glycolipids. So instead of glucose in the chapek area we had hexadecane with 2% content.

The results of the study on the dynamics of biosurfactant release by strains of the genus *Rhodococcus*, *Pseudomonas* and *Bacillus* are presented in figures 4, 5, 6. As shown in Picture 4, strains of the genus Bacillus start synthesizing biosurfactants from the first day and reach the maximum number in 24 hours. And strains of Pseudomonas and Rhodococcus within 72 hours.

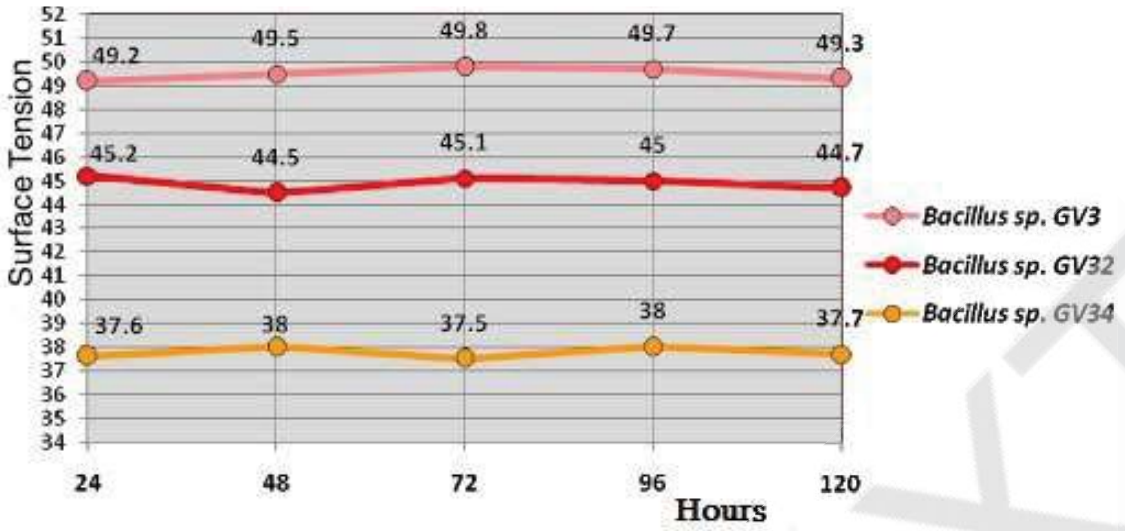


Fig. 4 . Biosurfactive activity of strains of the genus Bacillus

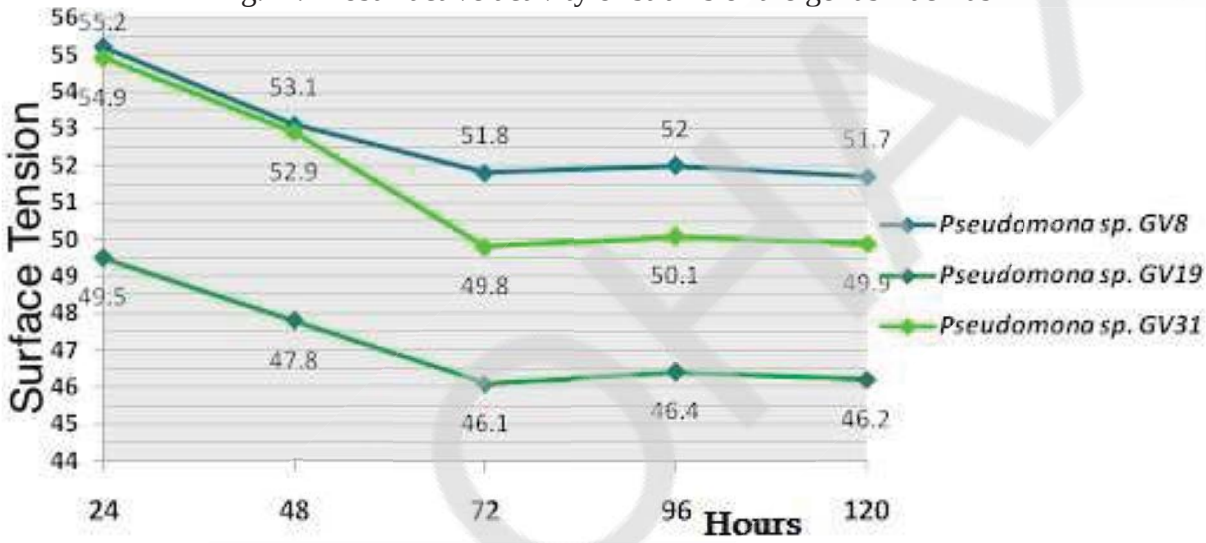


Fig. 5 . Biosurfactive activity of Pseudomonas g svari strains

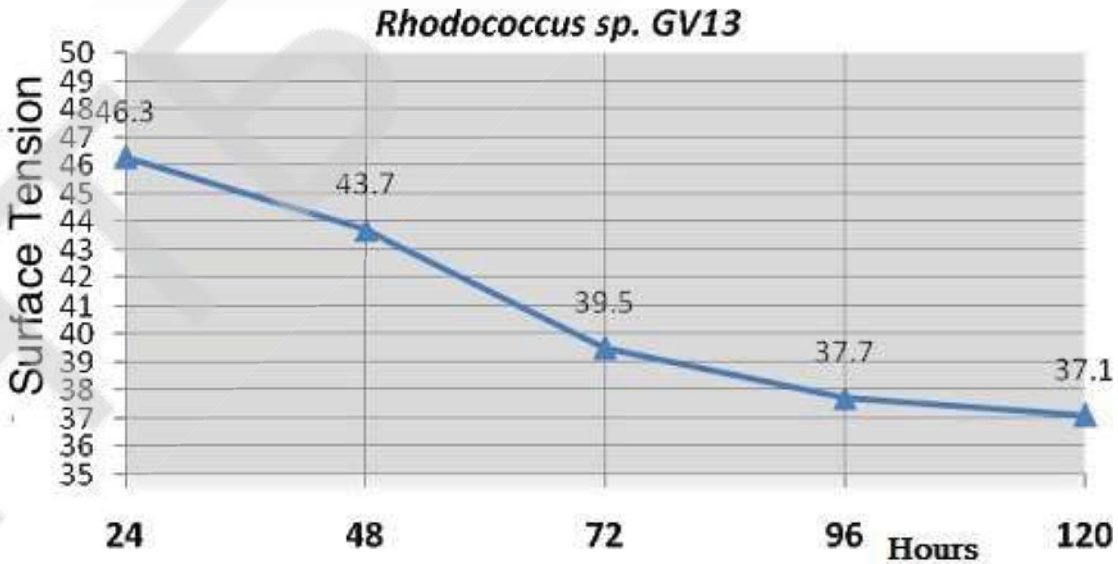


Fig. 6 . Biosurfactive activity of a strain of the genus Rhodococcus

It turned out that only *Rhodococcus* sp. GV13 and *Bacillus* sp. GV34 synthesizes extracellular biosurfactants. However, as can be seen from figure 4, Pseudomonas

sp.GV19 degraded the oil best in the agitated crude oil field and also produced a clear zone. Presumably this strain is an active producer of biosurfactants.

The next phase of the work aimed to cultivate the strains in liquid food areas of different compositions in order to increase the yield of biosurfactants (the obtained results are presented in Table №1). We used the following food areas for the experiment:

1. Chapek area with glucose [g/l]: glucose - 20; $NaNO_3$ - 2; K_2HPO_4 - 1; $MgSO_4 \times 7H_2O$ - 0.5; KCl - 0.5; $FeSO_4 \times 7H_2O$ - 0.01; pH 7.2;
2. Chapek area with hexadecane [g/l]: hexadecane - 20 ml; $NaNO_3$ - 2; K_2HPO_4 - 1; $MgSO_4 \times 7H_2O$ - 0.5; KCl - 0.5; $FeSO_4 \times 7H_2O$ - 0.01; pH 7.2;
3. Meat-peptone broth .
4. Meat-peptone broth + 2% glycerin;
5. Meat-peptone broth + 2% molasses.

Table 1. Food is the effect of composition on surface tension

#	Name of the strain	Surface tension (Mill Newton / Meter)				
		Chapek area + hexadecane	**Chapek area + glucose**	**MPB**	**MPB + 2% glycerin**	**MPB+ 2% molasses**
1	*Bacillus* sp. GV3	49.7	49.2	49.1	48.1	49.0
2	*Pseudomonas* sp. GV8	51.2	55.0	50.8	47	46.7
3	*Rhodococcus* sp. GV13	37.1	51.7	48.1	43.5	44.1
4	*Pseudomonas* sp. GV19	46.1	52.0	47.4	34.8	30.1
5	*Pseudomonas* sp. GV31	49.3	53.9	52.7	48.8	48.5
6	*Bacillus* sp. GV32	45.2	44.5	45.1	44.4	44.0
7	*Bacillus* sp. GV34	37.7	32.0	32.5	31.7	31.5

As we see from the table, 3 active producers of extracellular surfactant were identified: *Bacillus* sp. GV34, *Pseudomonas* sp. GV19 and *Rhodococcus* sp. GV13.

Food is the effect of the composition of *Rhodococcus* sp. GV13, *Pseudomonas* sp. GV19 and *Bacillus* sp. The synthesis of biosurfactants by GV34 cultures and their reactivity is the ability to reduce elongation shown in figure 7.

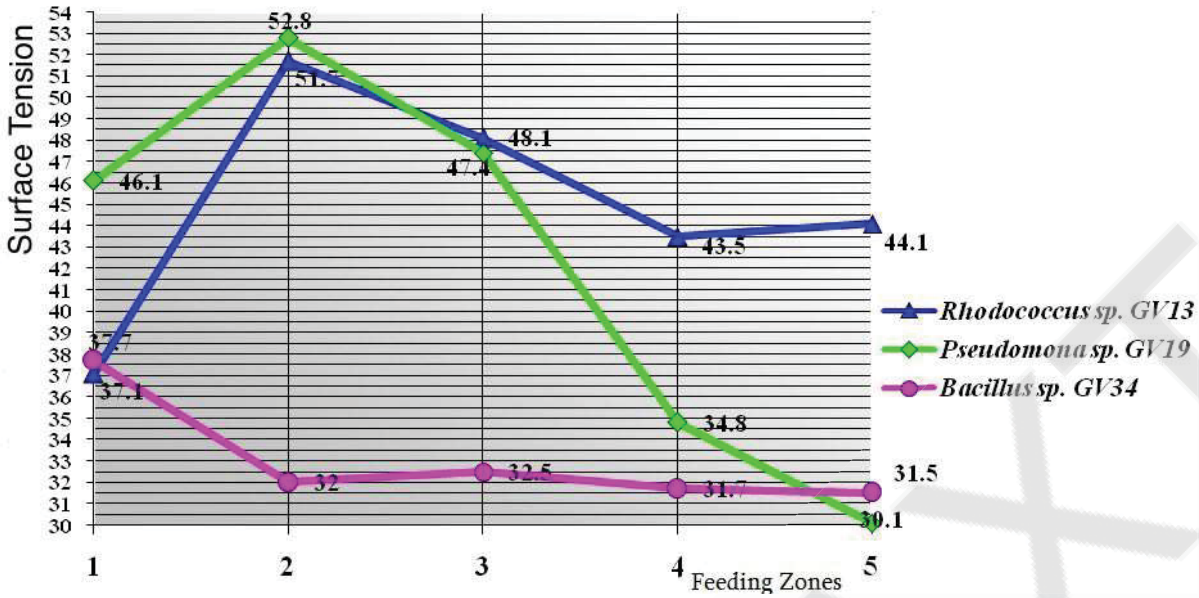


Fig. 7 The influence of the nutrient area on the surface tension of strains

1.1 Chapek area with glucose . 2.Chapek area with hexadecane. 3. Meat-peptone broth. 4. Meat-peptone broth + 2% glycerin . 5 Meat-peptone broth + 2% molasses.

As we see from figures 7, *Bacillus* sp. GV34 synthesizes biosurfactants equally well in almost every area we select. For this crop in the Chapek mineral area, the best source of carbon is glucose. *Rhodococcus* sp. the best for GV13 was the Chapek area with hexadecane content. *Pseudomonas* sp. for GV19 optimal was meat-peptone broth, where molasses was added as a carbon source in the amount of 2%, in this case we obtained the highest rate of biosurfactant yield - 30.1 mN/m [18, 23, 33, 37, 38]. We also got good results in the glycerin area (34.8 mn/m). Presumably, extracellular surfactants are adsorbed on the membranes and glycerin aids in desorption, while molasses enhances the metabolic process.

Most likely, the other 5 crops that grow well in a solid area where oil is the only source of carbon are the producers of intracellular surfactants or are isolated only by bioemulsifiers. Food is further optimized to increase the yield of biosurfactants both in these studied crops and in other strains identified during primary sowing.

V. CONCLUSIONS

1. 39 bacterial strains have been isolated from different soils of Kakheti oil contaminated soils. Strains of 11 *Pseudomonas*, 17 *Bacillus* and 4 *Rhodococcus* have been identified.
2. 7 strains of the genus *Rhodococcus*, *Pseudomonas* and *Bacillus* are selected by screening.
3. In strains of the genus *Bacillus*, the synthesis of the biosurfactant reaches a maximum in 24 h, and in the strains of *Pseudomonas* and *Rhodococcus* in 72 h.
4. The use of hexadecane, glucose, glycerin and molasses as a source of carbon in the diet has different effects on the synthesis of biosurfactants.

5. When using hexadecane as a carbon source, strains of the genus *Rhodococcus*, *Pseudomonas* and *Bacillus* produce an exo-biosurfactant.
6. The best rate of superficial stretching from the studied strains was observed in *Pseudomonas* sp. in GV19 and is 30.1 mn/m.

VI. REFERENCES

1. Amiranashvili L., Gagelidze N., Tinikashvili L., Varsimashvili Kh., Chrikishvili D, Gelashvili N., Graves D., Kirtadze E., Ghoghoberidze M. and Kvesitadze G. Selection of Soil Microorganisms Capable of Degrading Mineral Oil. Journal of biological physics and chemistry. 2005. Vol. 5. No 2/3. P.84-88
2. Banat I.M. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. // Bioresurce Technol., 1995. V. 51. P. 1-12.
3. Benerjee S. Biosurfactant for desludging crude/fuel oil storage tank. // Chem. Ind. Dig., 1998. V. 4. P. 75-78.
4. Bodour A. A. Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils / A. A. Bodour K. P. Drees, R. M. Maier // Appl. Environ. Microbiol. 2003. - V. 69. -P. 3280-3287.
5. Cameotra S. S. Recent applications of biosurfactants as biological and immunological molecules / S. S. Cameotra, R. S. Makkar // Curr. Opin. Microbiol. 2004. - V. 7. - P. 262-266.
6. Christofi N. Microbial surfactants and their use in field studies of soil remediation / N. Christofi, I. B. Ivshina // J. Appl. Microbiol. 2002. -V. 93.-P. 915-929
7. Denger K. New halo- and thermotolerant fermenting bacteria producing surface-active compounds / K. Denger, B. Schink // Appl. Microbiol. Biotechnol. 1995. - V. 44. - P. 161-166. 127.
8. Desai J.D., Banat I.M. Microbial production of surfactants and their commercial potencial. // Microbiol. Molecular Biol. Rev., 1997. V. 61. P. 47-64.
9. Design for the Environment Program. Cleaner Technologies Substitutes Assessment: Professional Fabricare Processes. U. S. Environmental Protection Agency. EPA 744-B-98-001, 1998.
10. Deziel E. Biosurfactant production by a soil Pseudomonas strain growing on polysyclic aromatic hydrocarbons / E. Deziel, G. Paquette, R. Villemur, F. Lepine, J. Bisaillon // Appl. Environ. Microbiol. 1996. -V. 62.-P. 1908-1912.
11. Gunther N. W. Production of rhamnolipids by Pseudomonas chlororaphis, a nonpathogenic bacterium / N. W. Gunther, A. Nunez, W. Fett, D. K. Y. Solaiman // Appl. Environ. Microbiol. 2005. - V. 71.-P. 2288-2293.
12. HauBler S. Purification and characterization of a cytotoxic exolipid of Burkholderia pseudomallei / S. HauBler, M. Nimtz, T. Domke, V. Wray, I. Steinmetz // Infect. Immun. 1998. - V. 66. - P. 1588-1593.
13. Kitamoto D. Functions and potential applications of glycolipid biosurfactants from energy-saving materials to gene delivery carriers / D. Kitamoto, H. Isoda, T. Nakahara // J. Biosc. Bioengin. - 2002. -V. 94.-P. 187-201.
14. Klekner V., Kosaric N. Biosurfactants for cosmetics. In: Biosurfactants production, properties, applications (N. Kosaric, ed.). N.Y.: Marcel Dekker, 1993. P. 3нты: продуценты, свойства и практическое использование
15. Kosaric N. Biosurfactants in industry / N. Kosaric // Pure Appl. Chem. 1992. - V. 64. - P. 1731-1737.
16. Lang S. Rhamnose lipids biosynthesis, microbial production and application potential / S. Lang, D. Wullbrandt // Appl. Microbiol. Biotechnol. - 1999. - V. 51. - P. 22-32.
17. Maier R. M. Biosurfactants: evolution and diversity in bacteria / R. M Maier// Adv. Appl. Microbiol. 2003. - V. 52. - P. 101-121.
18. Maier R. M. Pseudomonas aeruginosa rhamnolipids: biosynthesis and potential applications / R. M. Maier, G. Soberon-Chavez // Appl. Microbiol. Biotechnol. 2000. - V. 54. - P. 625-633.

19. Neilson J. W. Characterization of lead removal from contaminated soils by nontoxic soil-washing agents / J. W. Neilson, J. F. Artiola, R. M. Maier//J. Environ. Qual. 2003. - V. 32. - P. 899-908.
20. Nunez A. LC/MS analysis and lipase modification of the sophorolipids produced by Rhodotorula bogoriensis / A. Nunez, R. Ashby, T. A. Fogilia, D. K. Solaiman // Biotechnol. Lett. 2004. -V. 26.-P. 1087-1093.
21. Parales R. E. Biodegradation, biotransformation, and biocatalysis [B3] / R. E. Parales, N. C. Bruce, A. Schmid, L. P. Wackett // Appl. Environ. Microbiol. 2002. - V. 68. - P. 4699-4709.
22. Pramer D., Bartha R. 1972. Preparation and processing of soil samples for biodegradation studies. Environ. Letters, 2(4), p.217-224.
23. Rawls, W.J., Ahuja, L.R., Brakensiek, D.L., and Shirmohammadi, A. 1993. Infiltration and soil water movement, in Maidment, D.R., Ed., Handbook of hydrology, New York, NY, USA, McGraw-Hill, p. 5.1–5.51.
24. Renault P. Genetically modified lactic acid bacteria: applications to food or health and risk assessment / P. Renault // Biochimie. 2002. -V. 84.-P. 1073-1087.
25. Rosenberg E., Ron E. Z. // Appl. Microbiol. Biotechnol. 1999. -V. 52.-P. 154-162.
26. Ryll R. Immunological properties of trehalose dimycolate (cord factor) and other mycolic acid-containing glycolipids a review / R. Ryll, Y. Kumazawa, I. Yano // Microbiol. Immunol. - 2006. - V. 45. -P. 801-811.
27. Spoeckner S. Glycolipids of the smut fungus Ustilago maydis from cultivation on renewable resources / S. Spoeckner, V.Wray, M. Nimtz, S. Lang // Appl. Microbiol. Biotechnol. 1999. - V. 51. - P. 33-39.
28. Sullivan E. R. Molecular genetics of biosurfactant production / E. R. Sullivan // Curr. Opin. Biotechnol. 1998. - V. 9. - P. 263-269.
29. Van Dyke M.I., Gulley S.L., Lee H., Trevors J.T. Evaluation of microbial surfactants for recovery of hydrophobic pollutants from soil. // J. Jnd. Microbiol., 1993. V. 11. P. 163-170.
30. Velikonja J., Kosaric N. Biosurfactant in food applications. In: Biosurfactant production, properties, applications (N. Kosaric, ed.). N.Y.: Marcel Dekker, 1993. P. 419-446.
31. Walsh U. F. Pseudomonas for biocontrol of phytopathogens: from functional genomics to commercial exploitation / U. F. Walsh, J. P. 2001
32. Герхарт и др., 1983. Методы общей бактериологий, Москва, Мир.
33. Гоготов И.Н. Полисахариды: свойства, получение и практическое использование. В: Материалы Межд. научно-практич. конф. «Перспективы и проблемы развития биотехнологии в рамках единого экономического пространства стран содружества», Минск-Нароч: РИВШ, 2005. С. 54-55.
34. Елисеев С. А. Поверхностно-активные вещества и биотехнология / С. А. Елисеев, Р. В. Кучер. Киев: Наук, думка, 1991. - 116 с.
35. Елисеев С.А., Кучер Р.В. Поверхностно-активные вещества и биотехнология. Киев: Наукова думка, 2001. 60 с.
36. Звягинцев Д.Г. Микроорганизмы и охрана почв. 1989, М.: Изд-во МГУ, 206.
37. Пирог Т.П., Шевчук Т.А., Волошина И.Н., Карпенко Е.В. Образование поверхностно-активных веществ при росте штамма Rhodococcus erythropolis ЭК-1 на гидрофильных и гидрофобных субстратах. // Прикл. биохим. и микробиол., 2004. Т. 40. С. 544-550.
38. Турковская О. В. Штамм Pseudomonas aeruginosa продуцент биоПАВ / О. В. Турковская, Т. В. Дмитриева, А. Ю. Муратова // Прикл. биохим. микробиол. -2001. - Т. 37. № 1. - С. 80-85.
39. Шевелуха В. С. Биотехнология и биобезопасность/ В. С. Шевелуха // Сельскохозяйственная биология. 2002. № С. 3-15.