Remediation of soils contaminated with oil with a biological product with immobilization of bacteria by carriers from local soils

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Abstract. The article shows the effectiveness of the biological product "SHER" developed by "Scientific Industrial Enterprise" AltaiAgroFarm Ltd (Kazakhstan) on a carrier in the form of screening out from local wastes of limestone-shell rock in the process of cleaning soils from oil with a high paraffin content. The results of NGS-sequencing of the consortium of microorganism as part of the probiotic biological product "SHER" established a microbiome of the following type: at the class level 96.15% of all representatives of the consortium were identified as Bacilli, of which 95.69% of bacteria at the order level were identified as Lactobacillales. A method has been developed for immobilizing bacteria of the biological product "SHER" on a carrier in the form of screening out from local waste of shell limestone. Based on the research results, it was established that the immobilization of bacteria of the biological product "SHER" by the carrier in the form of screening of limestone-shell rock showed a high degree of purification of oil-contaminated soil (88.63%). To study the further activity of bacteria, experiments were carried out on the secondary use of the residual stock solution of the biological product "SHER" used in the process of cleaning oil-contaminated soil. The results of experimental studies using residual mother liquor and immobilization by screenings showed the degree of soil purification (45.7%). X-ray spectral analysis determined the elemental composition of the cleaned soil, revealed the presence of a low content of metals: 1.42% Fe and 0.84% Al. It was found that on a carrier in the form of screening out from local wastes of limestone-shell rock, the effectiveness of the biological product "SHER" increases.

1. Introduction

A promising direction in soils' purification from oil is the immobilization of microorganisms on various carriers (peat, vermiculite, etc.) [1]. It is known that in most cases, a neutral medium ideal for biodegradation has pH between 6 and 9 [2]. So, for saline (slightly alkaline) soils of Kazakhstan, the introduction of a carrier in the form of limestone-shell screening improves the contact of oil-contaminated soil with microorganisms [3]. The bacterial cells of the product immobilized on the carrier are retained on the particles' surface and remain viable in hot climates.

Due to environmental pollution problems in oil-producing regions, the problem of industrial waste usage (local limestone-shell screening) for the bacteria immobilization of the biological product "SHER" used for soil purification is currently very relevant in Kazakhstan.
The use of industrial waste (local limestone-shell screening) for bacteria immobilization of the biological product "SHER" used for soil purification allows to solve the urgent task of restoring the environment in the oil-producing regions of Kazakhstan.

This article's aim was to study and select optimal conditions for bioremediation of oil-contaminated soils (OCS) with high paraffin content using the biological preparation "SHER" with bacteria immobilization by local limestone-shell waste.

Literature analysis has shown that existing methods, technologies, and means of cleaning soils saturated with paraffin oil do not allow them to be completely cleaned of paraffin [4-7].

2. Objects and methods of research
Samples of oil-contaminated soil were taken from the Uzen oil production field (Mangystau region, Kazakhstan). Oil content determination in the soil was carried out by gravimetric method.

Identification of the microorganisms' consortium was carried out by sequencing the 16S rDNA gene. Unlike classical Sanger sequencing, NGS platforms allow millions of small DNA fragments to be read in parallel from two sides, resulting in a huge data array [8].

The study of the chemical and mineralogical composition of the limestone-shell sample was carried out in the testing laboratory of Tselsim LLP in Almaty (Kazakhstan). X-ray phase analysis (XPA) was carried out on an upgraded diffractometer DRON-3M on CuKα radiation with software. Radiographs were obtained in the range of 10... 70 degrees angles.

The experiment on bioremediation in laboratory conditions was carried out using a technology consisting in mixing oil-contaminated soil with a biological product and a carrier. Containers with a capacity of 1.5 liters and a height of 20 cm were used. During the experiment, constant conditions were maintained in the laboratory for 10 days: air temperature – within 20±2 °C, indoor humidity – 60-70%. Soil moisture was established by drying the soil sample to a constant mass at a temperature of 105±5 °C [9].

Statistical processing of research materials was carried out according to the variational statistical analysis criteria with the calculation of the mean value and standard deviation (Mean±SD) with the standard mean error and the standard deviation of the sample using the Microsoft Excel 2007 software package. The statistical significance of the differences in the mean values was assessed according to generally accepted methods [10].

3. Results and discussion
On the territory of the Uzen deposit, the topsoil is characteristic of the coastal zone of the Caspian Sea and is represented by gray-brown desert soils [11]. It is known that the chemical composition of oil affects the rate of its decomposition. The lower molecular weight of the product contributes to a greater intensity of its decomposition.

Table 1 shows the properties of the studied oil with high paraffin content.

| Table 1. Some properties of the barn oil of the Uzen deposit |
|-------------------------------------------------------------|
| **Physico-chemical properties of oil** | **Unit of measurement** | **Value** |
| Density | API | 31.4 – 35.7 |
| | kg / m³ | 856.7 – 874.1 |
| Reaction of the environment | units pH | 7.3±0.5 |
| Solidification temperature | °C | + 30 - + 36 |
| Moisture | % | 13.7±2.6 |
| Soil air | mg CO₂/(dm·h) | 0.317 |
| Kjedahl nitrogen | % weight | 0.138-0.975 |
| Paraffins | % weight | 13.6-21.8 |
| Asphaltenes | % weight | 0.7-2.7 |
| Silica gel resins | % weight | 16.1-23.8 |
The table shows that according to API indicators, this oil has a chemical composition that can contribute to the rapid hydrocarbons’ decomposition over time. Thus, the properties of the Uzen deposit with high paraffin content and with a high pour point (within +30-36 °C) allow bioremediation since the density of this oil in API degrees is more than 30. The concentration of asphaltenes (c₄) in oil is much higher than the concentration of polycyclic aromatic hydrocarbons (C). A microheterogeneous system is formed in the early stages of thermal degradation because of asphaltenes’ transition into the second phase. As a result, asphaltenes are intensively converted into carbides [12].

The study of the metagenomic bacteria composition by sequencing the 16S rDNA gene is carried out without the cultivation stage; all genomic DNA is directly isolated from the analyzed sample. According to the research results, the strains included in the biological product are not pathogenic, non-toxic, and non-toxicogenic. The composition of “SHER” biological preparation is given in Table 2.

### Table 2. The composition of the SHER biopreparation

| No. | Microorganisms                  | Quantity in the consortium, % |
|-----|---------------------------------|-------------------------------|
| 1   | Saccharomycescerevisiae (unclassified) | 38.07                        |
| 2   | Lactobacillusparaffaraginis     | 3.59                          |
| 3   | Lactobacillusshulgardii         | 18.02                         |
| 4   | Lactobacillusfaeni              | 5.25                          |
| 5   | Lactobacilluscamelliae          | 15.24                         |
| 6   | Lactobacillusramnosus           | 11.66                         |
| 7   | Lactobacillusparacasei          | 3.09                          |
| 8   | Lactobacilluszeae               | 0.92                          |
| 9   | Citrobacterfrendii             | 0.66                          |
| 10  | Lactobacillusparakefiri         | 1                             |
| 11  | Lactobacillussthailandensis     | 0.49                          |
| 12  | Lactobacilluscasei              | 0.38                          |
| 13  | Otherspecies                    | 1.63                          |

Lactic acid bacteria (LAB) Lactobacillus Saccharomycescerevisiae (unclassified) were isolated by sequencing the 16S rRNA gene; these bacteria form the nucleus of this bacteria group, as well as gram-negative non-sporogenous bacteria (Citrobacterfrendii), which develop well in polluted sandy soils. Currently, strains of Lactobacillus bacteria are widely used for the development of new probiotics, which make up 82% of the preparation.

The results of NGS sequencing of microorganisms’ consortium in the probiotic composition of the biological product “SHER” established the microbiome of the following type: Firmicutes (96.42%), Proteobacteria (1.78%), unclassified 1.4%, other phyla 0.4%. The dominant species in the community are Lactobacilluscamelliae bacteria (15.24%). At the class level, 96.15% of all consortium representatives were identified as Bacilli, of which 95.69% of bacteria at the order level were identified as Lactobacillales. Classification at the family level determined the taxonomic units in Lactobacillace bacteria in 95.28% [13].

The use of the above-mentioned bacteria for cleaning oil-contaminated soil was reported in [14-16]. In our research, a consortium of lactic acid bacteria (ICD) Lactobacillus is used for the first time, their proportion in the preparation is 82%.

The experimental results on remediation of soils contaminated with paraffin oil using the biopreparation at room temperature showed that at 21 °C, oil destruction was 88.63% in 3 days (Table 3). These data are consistent with studies [17], in which it was shown that the Bacillus strain can
decompose oils at temperatures from 35 °C to 55 °C and although slightly, the decomposition rate increased with increasing temperature.

Oil degradation was assessed visually by changing the oil spot and biomass accumulation. Calcite $\text{CaCO}_3$ in limestone composition contributes to a more intense emulsion release. At the same time, no oil film was observed on the surface of the medium. That is, the oil mostly turned into a homogeneous emulsion. At the same time, small particles precipitated (no more than 6-9%).

Table 3. The degree of purification of oil-contaminated soil with the biopreparation "SHER"

| Indicator                       | Experimental research options for oil-contaminated soil (OCS) clean-up |
|---------------------------------|-----------------------------------------------------------------------|
|                                 | 1                      | 2                      | 3                      |
| Recipe                          | OCS: Screening: BP      | OCS: Screening: BP      | OCS: Screening: BP      |
| Mass ratios                     | 0.4 kg:0.6 kg:1.0 l    | 0.5 kg:0.5 kg:1.2 l    | 0.6 kg:0.4 kg:1.2 l    |
| Initial oil content in OCS, g/kg| 111.7±3.7              | 140.3±5.14             | 167.7±4.9              |
| Residual oil content in OCS after 2 days | 73.6±4.1              | 106.9±3.8              | 125.1±2.9              |
| Cleaning efficiency, %          | 34.11                   | 23.8                   | 25.4                   |
| Residual oil content in OCS after 3 days | 12.7±2.9              | 47.2±4.7              | 68.4±4.7              |
| Cleaning efficiency, %          | 88.63                   | 66.35                  | 59.21                  |

*Note: OCS – oil contaminated soil, BP – biopreparation.*

The immobilization of bacteria of the biological product "SHER" on a limestone carrier with solution mixing for 3-5 hours contributed to the stable degradation of hydrocarbons on the second day of the experiment [18]. When the injected mother solution encounters oil in the soil, the process of its destruction by microorganisms begins.

The results in Table 3 show that an increase in the biopreparation dose (up to 1.2 liters) does not contribute to the effectiveness of cleaning the soil from oil. The effective result was obtained in option No. 1 (88.63%) after 3 days with the following ratio (OCS:Screening:BP) equal to (0.4 kg:0.6 kg:1.0 l). Thus, bacteria immobilization of the biological product "SHER" by a carrier being limestone-shell screening showed a high degree of purification of oil-contaminated soil. This result of cleaning oil-contaminated soil at a temperature of 21°C was obtained in the laboratory after 72 hours. It is known that bacteria showing a high soil purification degree from oil in the laboratory are not always effective in real conditions of oil fields. The type of the initial sample of the oil-contaminated soil and the micrograph of the purified soil are shown in Figure 1.

![Figure 1](image)
To study the further activity of oil-oxidizing microorganisms, the residual mother solution (RMS) was filtered out of the emulsion and used for a second time. At the same time, the RMS was used without time delays. During the secondary RMS use with bacteria immobilization on a limestone carrier, a residual mother solution and water were added to the mixed substrate based on oil sludge and limestone-shell screening as in the previous experiment (Table 4). As a result, the mixture was divided into three phases: emulsion, RMS, mechanical admixture.

The greatest efficiency of soil purification was noted in option No. 1: after 3 days with the following ratio - OCS:Screening:RMS:N\textsubscript{2}O equal to 50 g:70 g:20 ml:80 ml, it was 45.7%.

**Table 4.** Results of recycling «SHER» mother solution

| Indicator | Experimental research options for oil contaminated soil (OCS) clean-up |
|-----------|---------------|
| Recipe    | 1             | 2             | 3             |
| OCS:       | OCS:          | OCS:          |
| Screening: | Screening:    | Screening:    |
| RUS:H\textsubscript{2}O | RUS:H\textsubscript{2}O | RUS:H\textsubscript{2}O |
| Mass ratios | 50 g:70 g:20 ml:80 ml | 50 g:60 g:50 ml:50 ml | 100 g:135 g:200 ml:20 ml |
| Initial oil content in OCS, g/kg | 13.8±3.7 | 13.8±5.14 | 27.6±4.9 |
| Residual oil content in OCS after 2 days | 9.8±4.1 | 11.4±3.8 | 21.0±2.9 |
| Cleaning efficiency, % | 28.9 | 17.4 | 23.9 |
| Residual oil content in OCS after 3 days | 7.5±2.9 | 9.1±4.7 | 19.8±4.7 |
| Cleaning efficiency, % | 45.7 | 34.1 | 28.3 |

*Note: RUS - residual mother solution.*

Laboratory studies were also carried out to determine the physico-chemical properties of the emulsion isolated using a separation funnel. The analysis results are shown in Table 5.

The emulsion mainly consists of 76.6% oil products, water saturation – 11.5%, sediment content – 14.7% by weight.

**Table 5.** Physico-chemical properties of the emulsion

| It.No. | Indicator name               | Measurement unit | Result |
|--------|------------------------------|------------------|--------|
| 1      | Water content                | % weight         | 11.5   |
| 2      | Content of petroleum products| % weight         | 75.8   |
| 3      | Sediment content             | % weight         | 12.7   |

The purified soil was examined by X-ray spectral microanalysis. The radiograph is shown in Figure 2.
Figure 2. Micrographs of the treated soil (scanning electron microscopy) left - magnification 100 µm (red circle), right - magnification area up to 400 µm.

The DX-Q map program built into the SEM gave out the distribution by area and concentration (in %) of the elements (O, C, Ca, Si, Fe, K, Al, Mg) contained in the soil. It was shown that the silicate material does not contain either sulfur S or lead Pb. Grayer levels mean CaO calcium oxide. Out of metals, Fe (1.42%) and Al (0.84%) were found. The program made it possible to determine the atomic number, atom (%) mass (%) of all the listed elements in the soil composition (Table 6).

Table 6. Chemical elements in the composition of purified soil

| Element   | At. No. | Net  | Mass[%] | Mass Norm.[%] | Atom [%] |
|-----------|---------|------|---------|--------------|----------|
| Oxygen    | 8       | 488  | 34.45   | 45,36        | 46,27    |
| Carbon    | 6       | 401  | 24.44   | 32,19        | 43,73    |
| Calcium   | 20      | 890  | 10.35   | 13,63        | 5,55     |
| Silicon   | 14      | 366  | 2.56    | 3,37         | 1,96     |
| Iron      | 26      | 103  | 1.42    | 1,87         | 0,55     |
| Potassium | 19      | 107  | 1.14    | 1,49         | 0,62     |
| Aluminium | 13      | 91   | 0.84    | 1,11         | 0,67     |
| Magnesium | 12      | 65   | 0.74    | 0,97         | 0,65     |
| Sum       |         | 75,94372 | 100   | 100          |          |

The largest mass were O (34%) and C (24.4%), respectively, and the smallest mass was Mg (0.74%).

Temperature and salinity are important environmental parameters affecting the decomposition of petroleum compounds [19]. Temperature has a huge impact on many aspects of microorganisms' life including oil-oxidizing ones. It changes the rate of chemical reactions in cells and the state of cellular macromolecules.

The climatic conditions of the oil-producing regions of Kazakhstan have long remained an elective factor in the use of oil-oxidizing microorganisms in hot climate. Bacteria with such properties can effectively decompose oil in areas with hot, arid climate [20]. The study of oil-oxidizing microorganisms' ability with separately dosed addition of microbial consortia contributes to the decomposition of hydrocarbons in oil sludge in a short time [21]. In this study, the effectiveness of the biopreparation showed high purification degree in a short time – 72 hours. For the first time, the immobilization of Lactobacillus and Saccharomyces cerevisiae (unclassified) bacteria on a limestone carrier contributed to stable degradation of hydrocarbons on the second day of the experiment.
4. Conclusion
Thus, the conducted studies have shown that paraffin oil hydrocarbons are decomposed by lactic acid bacteria Lactobacillus and Saccharomyces cerevisiae (unclassified), which form the nucleus of this bacterial group and are able to decompose oil at elevated temperatures. The effectiveness of cleaning the soil from oil was shown during 3 days of the experiment. It was found that the bacteria immobilization of the biological product on a carrier made of local material in the form of limestone-shell waste screening contributed to stable degradation of oil and showed high purification degree of oil-contaminated soil (88.63%). The secondary use of the residual mother solution of the biopreparation and immobilization by screening showed the degree of soil purification in (45.7%). Thus, the biological product "SHER" on a carrier in the form of local limestone-shell waste screening can effectively neutralize petroleum products in the soil in the process of their cleaning from oil with high paraffin content.

References
[1] Vassanasak L, Pawinee Ch, 2010 Decolorization of molasses melanoidins and palm oil mill effluent phenolic compounds by fermentative lactic acid bacteria Journal of environmental Sciences 22 (8) 1209–1217
[2] Pleshakova E V, 2011 Introduction of oil-oxidizing microorganisms into polluted soil: problems and prospects Bulletin of the Saratov University 2 102-111
[3] Das N, Chandran P, 2011 Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview Biotechnol Res. Int. 2 1–13
[4] Silva F S, Almeida D G, Rufino R, Luna J, Santos V A, Sarubbo L A, 2014 Applications of biosurfactants in the petroleum industry and the remediation of oils spills Int. J. Mol. Sci. 15 12523–12542
[5] Liu W, Luo Y, Teng Y, Zhengao L, Lena Q, 2010 Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes Environ Geochem Health 32 23–29
[6] Saha R C, Reza A, Hasan M S, 2019 A review –bioremediation of oil sludge contaminated soil International Conference on Environment Pollution and Prevention 96 6-11
[7] Hassan Gh, Hamid M, Seyed Mohammad M D, 2018 Evaluation of heavy petroleum degradation using bacterial-fungal mixed Ecotoxicology and Environmental Safety 164 434-439
[8] Rebrikov D V, 2014 NGS high-performance sequencing 228
[9] Evdokimov I V, 2018 Methods for determining the biomass of soil Russian Journal of Ecosystem Ecology 3 (3) 65-71
[10] Glants S B, 1998 Biomedical Statistics, Practice 1998 150
[11] Kenzhetaev G Zh, Suleimenova N Sh, Perymakov V N, Nurbayeva F K, 2014 Investigation into the Physico-Chemical Properties of Soils of Caspian Sea Coastal Areain Mangystau Province Oriental journal of chemistry 30 (4) 1631-1638
[12] Zubaidy E A, Abouelnasr D M, 2010 Fuel recovery from waste oily sludge using solvent extraction Process. Saf. Environ 88 318-326
[13] Vassanasak L, Pawinee Ch, 2010 Decolorization of molasses melanoidins and palm oil mill effluent phenolic compounds by fermentative lactic acid bacteria Journal of Environmental Sciences 22(8) 1209–1217
[14] Kumari S, Regar R K, Manickam N, 2018 Improved polycyclic aromatic hydrocarbon degradation in a crude oil by individual and a consortium of bacteria Bioresource Technology. 254 174-179
[15] Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao X, Li F, Li H, Yu H, 2018 Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis Frontiers in Microbiology 9 2885-2891
[16] Pleshakova Ye V, Belyakov A Yu, Deev D V, 2018 Characteristics of Hydrocarbon Degradation by Bacteria Isolated from Drill Cuttings Biology Bulletin. 45 (10) 1174–1181
[17] L. Boqun J, Meiting L, Jinpeng W, Wentao X, Xiaojing L, 2016 Isolation, identification, and crude oil degradation characteristics of a high-temperature, hydrocarbon-degrading strain *Marine Pollution Bulletin* **106** (1–2) 301-307

[18] Das N, Chandran P, 2011 Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview *Biotechnol Res Int.* **5** 1–13

[19] Ruling W F, Head I M, Larter S R, 2003 The microbiology of hydrocarbon degradation in subsurface petroleum reservoirs: perspectives and prospects *Research Microbiology* **154** (5) 321-328

[20] Abed R M M, Al-Sabahi J, Al-Maqrashi F, Al-Habsi A, Al-Hinai M, 2014 International Biodeterioration *Biodegradation* **58** 89-95

[21] Hepziba S, Shabnam M, Sriswama S, Ramani K, 2018 Enhanced biodegradation of hydrocarbons in petroleum tank bottom oilsludge and characterization of biocatalysts and biosurfactants *Journal of Environmental Management* **220** 87-95