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Using Microorganisms for Cleaning Oil-Contaminated Concrete

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Abstract
Revealed that the distribution of spots on the surface of the concrete depends on its density and structure, and the penetration of oil into the concrete occurs through micro- and nano-pores and cracks of the further spread of spots along the pathways. It was found that the use of hydrocarbon-oxidizing microorganisms strains as Micrococcus luteus IS16, M. varians IS41, micromycetes: Aspergillus sp., Penicillium sp., Alternaria sp., thiobacteria Acidithiobacillus ferrooxidans Ach1, A. ferrooxidans Ach2 promising for biological treatment of oil-contaminated concrete. Create optimal conditions for maintaining the activity of microorganisms: 55,0 ± 15,0% moisture, nutrients, temperature 38,0 ± 2,0^{0}C, trace elements, optimize the biodegradation of petroleum hydrocarbons.
Keywords*:* oil-contaminated concrete, bioremediation, hydrocarbon-oxidizing microorganisms, thiobacteria.

1. Introduction

The problem of environmental pollution by oil and oil products takes, recently, one of the leaders in the environment. There are studies conducted in different countries and associated with biological treatment of soil and water from oil and oil products using a variety of biological objects (Admon *et al*. 2001, Hutchinson*et al.* 2004, Ainon *et al*. 2006, Nejada *et al*. 2011). Particular attention is given to hydrocarbon-oxidizing microorganisms (HOM) as the main consumer of petroleum hydrocarbons (Chaineau *et al*. 2003, 2005, Azlinah *et al*. 2008, Obuekwe *et al*. 2008, Milic *et al*. 2009, Obayori *et al*. 2009. However, information on the biological treatment of oil-contaminated surfaces concreted scarce (Beklemishev &Kozliak 2003, Kogbara 2014), mostly using different detergents. Most likely, this is due to the porosity of concrete and asphalt, which complicates the process of deep cleaning of the substrate from organic pollutants.

The purpose of work- study the possibility of biological treatment of oil-contaminated concrete using microorganisms based on a study of the migration of oil.

2. Material and methods of researches

Collection of Samples: Samples of concrete were selected in test laboratory of construction materials. As material of research blocks of various brands of concrete of 10,0±0,5 x 10,0±0,5 cm in size served.
Oil. In researches oil of the Kumkol field with the following characteristics was used: temperature of hardening 100C, contents silikagel of pitches of 19,2%; karbeno-karboides of 5,82%; asfalten of 5,4%; paraffin of 7,5%; sulfurs of 0,064%. At a temperature of 200C has density – 0,850 g/cm3.
Physic-Chemical Properties of Contaminated Concrete: The concrete physic-chemical parameters were analyzed. The temperature was determined using a mercury thermometer. Nature of penetration and distribution of an oil slick in concrete blocks was investigated visually and with the helpelectronic and raster microscope "Jeol JSM-6490 LV" (Japan).
Isolation and Enumeration of Bacteria: Isolation and enumeration of bacteria were performed by soil dilution plate technique using mineral salts agar media. One gram of dried soil was dissolved in 9ml of distilled water and agitated vigorously. Different aqueous dilutions, 10, 10, 10 of the suspension were applied on to plates and 20ml of melted medium at around 50°C was added to it. After gently rotating, the plates were incubated at 37°C for 24 hours. Enumeration of different isolates was carried out. Selected colonies of bacteria were transferred from mixed culture of the plates on to respective agar plates and incubated at 37°C for 24 hours. Plates containing pure cultures were stored at 4°C until for the examinations.
Isolation and screening cultures. *HOM***:** a. Mineral salts medium (MSM) (g/L): K2HPO4 (2g), KH2PO4 (0.5g), NaCl (0.5g), NH4Cl (0.5g), MgSO4 (0.2g), CaCl2 (10mg), pH 7.2, containing different concentration of diesel and kumkol oil. b. Enrichment medium (EM): meat extract, peptone culture medium and glucose medium. c. Preservation medium (PM): meat extract, peptone, agar medium (MPA).
Micromycetes: The fungi were extracted by direct print method and dry needle method (Methods of experimental mycology, 1982). The cultures were grown using humid camera method and on the Czapek culture media. Samples were incubated in Petri dishes at a temperature of 25^0 C. Starting on the 7th day, the dishes were examined and micromycetes colonies were isolated into pure culture. Incubation period lasted for 21 days. Upon pure cultures isolation, Czapek agar was used for their identification and further storage.
Thiobacteria: For the growth of bacterial strains, iron Liquid medium (9kFe2+) (by Sugio *et al.* 2008).

Characterization of Isolates: Each isolate was examined many times for its size, shape, margin, consistency opacity, elevation, pigmentation, Gram reaction and cell morphology as described by Holt et al (1994). Diagnostic properties used include motility, production of catalase, indole, urease, oxidative fermentation of sugars, methyl red test, vogues proskauer test and citrate utilization test.

3. Results of researches

After putting oil on a surface of concrete blocks features of their distribution in horizontal and vertical vectors were studied **(Figure 1).**

Besides, for studying of possibility of cleaning of concrete blocks of oil them the microanalysis for definition of element and weight structure was carried out previously (**Table 1**).

It was revealed that the smallest depth of penetration of oil in samples 1, 2 and 8, but diameter of an oil slick on a block surface the greatest.. Electronic and microscopic inspection showed that the structure of these blocks dense without jointing, as explains spreading of a spot on a block surface. In blocks 3, 5, 6 and 9 oil gets deep into micro and nanocracks. In blocks 2, 4, 7 and 10 oil there passes through micropores. Thus it is visible that oil drops located in ways of penetration, pollute to 10-12% of nearby concrete (**Figure 2**). It is visible by this fact and difficulties in cleaning of a surface of the petropolluted concrete speak.

For cleaning of the petropolluted concrete blocks as options synthetic detergents in the form of powder and gel, HOM composition consisting of strains of Micrococcus luteus IS16, M. varians IS41 with a caption of 10^8 C/ml, composition of thiobacteria (Tb) of Acidithiobacillus ferrooxidans Ach1, A. ferrooxidans Ach2, composition of micromycetes which were applied in 1,0 ml on a surface of an oil slick were used. It was established that use as HOM, micromycetes and thiobacteria is more effective than chemical means **(Figure 3).**

Thus it was revealed that creation of optimum conditions for maintenance of activity of microorganisms somehow: 55,0± 15,0% humidity, biogenous elements, temperature 38,0±2,0^{0}C **(Figure 4)** , optimize processes of biodegradation of hydrocarbons of oil.

4. Conclusion

On the basis of the conducted researches it is established that nature of oil pollution of concrete depends on their structure. Penetration of oil in concrete happens by means of micro and a nanotime and cracks to further distribution of a spot along ways of penetration. Most likely, difficulties in purification of the petropolluted concrete and asfalt are also connected with it. Oil slicks remain even after carrying out actions for physical and chemical cleaning that is confirmed by visual inspections of the petropolluted concreted sites at a number of the enterprises of oil and gas branch. Besides, the used cleaners promote secondary environmental pollution. Results of these researches show prospects of use of compositions of microorganisms which biodegradations of hydrocarbons of oil promote. Creating favorable conditions for their activity: 55,0± 15,0% humidity, biogenous elements, temperature 38,0±2,0^{0}C, microcells, optimize processes optimizes processes of biological purification of the petropolluted concrete.

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Andrey S. Babenko, ScD, Professor of Ecology, Tomsk State University PhD thesis "Rove beetles (Coleoptera, Staphylinidae) of the south part of West Siberia" (1983), ScD thesis "Ecology of Staphylinidae of forest and disturbed territories of South Siberia" (1999). Sphere of scientific interests include ecology of soil invertebrates, organic waste decomposition. The main awards: Fulbright Scholarship (2001-2002), Rockefeller Foundation (2003), Green Found Grant (2010).
Aigul M. Sattarova, doctoral PHD. Fields of research - the study of the processes of bioremediation of oil-contaminated substrates.
Asel Ye.Tleukeeva, Master of Biology, Research Associate. Fields of research - the study of the reaction of microorganisms on a variety of factors.
Yerzhan B.Issayev, PhD, associate professor, has been studying the effect of xenobiotics on biological objects. PhD thesis "Flora of Southern Altai" (1994).

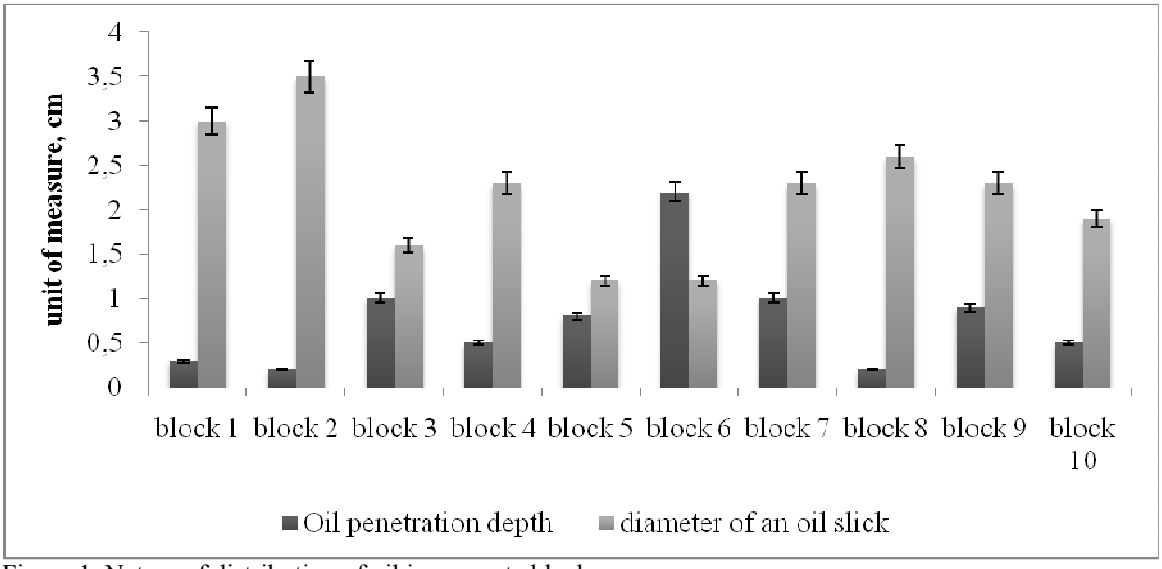

Figure 1. Nature of distribution of oil in concrete blocks

Table 1. Element and weight structure of concrete blocks

Blocks	Na	Mg	Al	Si	S	Cl	K	Ca	Fe
1	0.97±0.80	3.03±0.29	5.07±0.48	7.74±0.65	1.96±0.15	0.42±0.02	1.13±0.09	38.54±3.25	5.39±0.50
2	5.96±0.48	*	0.60±0.05	2.17±0.18	17.51±1.52	*	33.35±2.87	4.70±0.41	3.51±0.21
3	*	0.59±0.04	0.63±0.05	3.88±0.35	0.60±0.03	*	0.52±0.04	22.76±2.14	*
4	1.17±0.11	2.26±0.19	2.17±0.20	8.58±0.64	2.18±0.20	*	1.02±0.01	44.68±4.42	2.62±0.11
5	*	0.19±0.01	1.61±0.15	7.69±0.59	*	*	*	5.39±0.45	*
6	0.28±0.02	2.26±0.21	2.17±0.19	8.58±0.72	1.53±0.11	*	1.02±0.01	44.68±4.23	2.62±0.18
7	1.09±0.10	2.55±0.23	2.32±0.20	9.05±0.82	2.39±0.18	1.12±0.10	0.25±0.01	42.61±3.92	3.05±0.21
8	*	0.38±0.02	0.41±0.03	2.05±0.15	*	*	*	10.37±1.01	*
9	2.40±0.19	*	2.51±0.19	0.34±0.02	2.35±0.18	1.36±0.10	9.54±0.81	7.46±0.52	2.59±0.19
10	2.40±0.21	*	2.78±0.21	0.34±0.03	2.39±0.19	1.36±0.09	9.57±0.85	8.1±0.74	3.01±0.24

Note: * - it isn't revealed

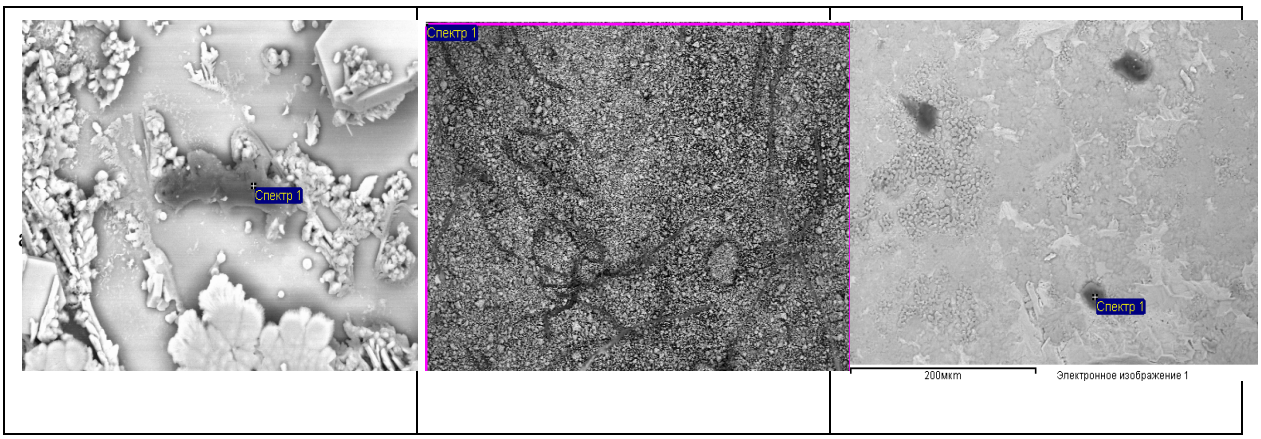

Figure 2. Electronic and raster pictures of cuts of the petropolluted blocks 2 (a) ,6 (b) and 7(c)(h1000)

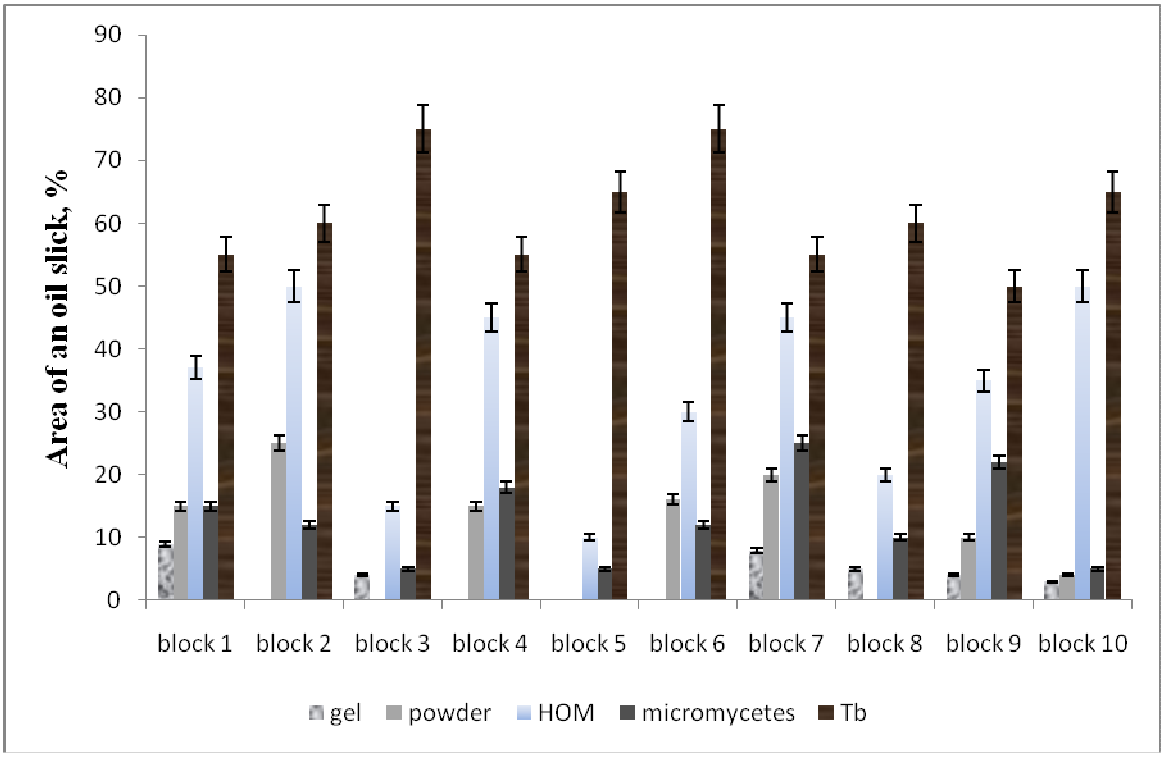

Figure 3. Extent of cleaning of concrete blocks of oil with detergents: gel and powder, microorganisms: HOM-hydrocarbon-oxidizing microorganisms, micromycetes, Tb- thiobacteria

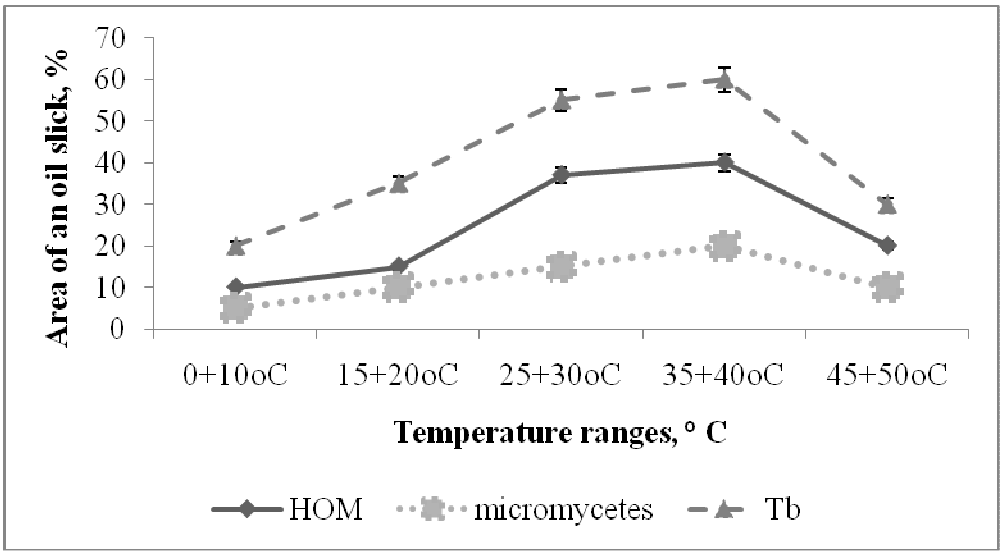

Figure 4. Influence of temperature on process of bioremediation of the petropolluted concrete

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