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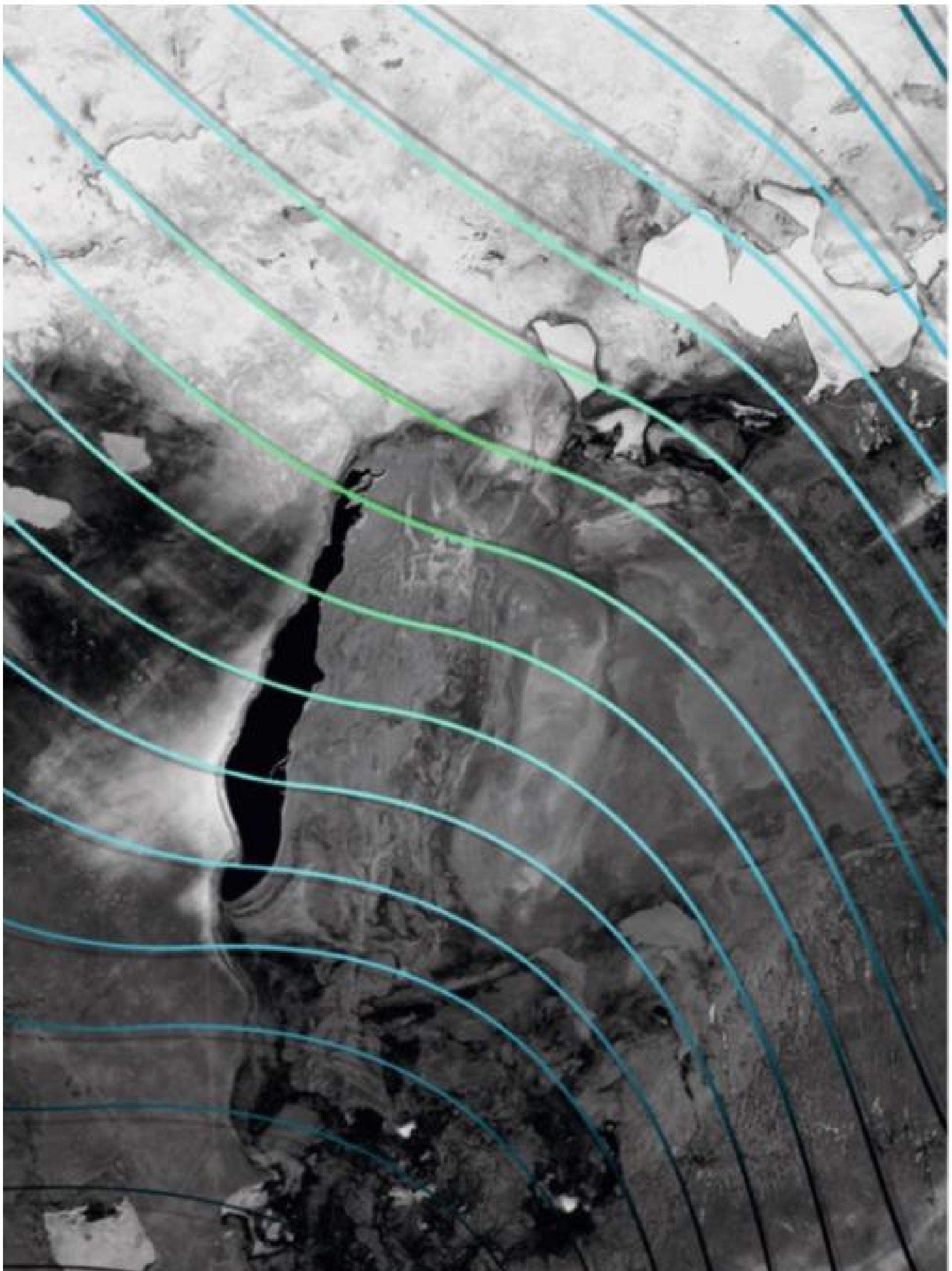
STOCKHOLM JUNIOR WATER PRIZE



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Water saving in oil bioremediation techniques

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SUMMARY

Kazakhstan is the ninth-largest country in the world covering a land area of 2,724,900 square kilometres. Country has the potential for providing agricultural products to 1,5B people worldwide. At the same time, the Republic of Kazakhstan belongs to the countries that may face the problem of water shortage in the next decade [1].

Intensive land use for agriculture could involve depleted industrial fields contaminated by oil and other pollutants. Also, land development itself involves intensive use of different types of machinery fueled by petrol. This could lead to soil and surface water contamination by oil spills.

Bioremediation for oil-contaminated soil and wastewater is known as the most natural and environmentally friendly technique. This biological method involves water consumption as well. Therefore, the objectives of involving previously unused lands in agricultural circulation in Kazakhstan are associated not only with their pollution but also with the need to conserve water.

Method of biological treatment in special installations - ex-situ, consists of the next stages [2]:

1. cultivation of oil destructing microorganisms [2];
2. soil and water treatment in the presence of a destructor [2].

This method will be effective due to the cultivation of the most active biological oil destructor and will allow more rational use of a given amount of water in the ex-situ method [3]. In the study microbial isolates were cultivated and investigated. The most active microorganism for the destruction of oil was identified.

The opportunities for saving and reusing water while using an ex-situ method discussed and proposed for future large -scale investigation.

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1 PURPOSE AND OBJECTIVES

1.1 Purpose: investigate the possibilities of saving and reusing water while using an ex-situ method to clean the soil and water from oil products and return these resources for use in agriculture.

1.2 Objectives:

1. To select a bacteria that have a higher cleaning efficiency at the same water consumption

1.1 To cultivate microorganisms capable of consuming petroleum hydrocarbons.

1.2 To detect strains of microorganisms-destructors with the highest consumption of oil hydrocarbons.

1.3 To determine the taxonomic affiliation and physiological and biochemical characteristics of oil-destructing microorganisms.

2. To check the efficiency of using destructors while adding water to the soil.

2 METHODS

In this work, the ex-situ technique was used, which consists of 4 stages:

- thermal desorption,
- biological treatment,
- biological remediation,
- land farming.

To obtain enrichment cultures of hydrocarbon-oxidizing microorganisms, hydrocarbon-oxidizing microflora of

- reddish-brown soil,
- high heat compost,
- oil,
- oil-contaminated soil and sand

were studied.

2.1 Thermal desorption

To provide a nutrient medium for microorganisms, beef extract agar was prepared and served as a source of nutrients (fig. 1). Each researched substrate (oil-contaminated soil, sand, and compost) in a weight of 1 g was introduced into flasks with 99 ml of sterile water. As a result we got 100 ml of liquid with sand. The mixture was shaken and 1 ml of it was transferred to a flask. 5% sterile oil was added to the medium as the carbon source.

A smear of the prepared suspension was applied to agar and placed in a thermostat (fig. 2). The studied cultures were grown for 3 days at a temperature of 28C. Further, using light and scanning microscopy, the selected cultures from agar were determined by the Gram stain method.

fig. 1

fig. 2

2.2 Biological treatment

To check the purity of the experiment, the obtained strains were added to the contaminated soil with oil, kerosene, and machine oil. To do this, we took samples of soil and sand with a mass of 200 g and added oil in a mass of 500 mg / kg of the weight of the soil into them (fig. 3). The soil (or sand) was thoroughly mixed and kept at a temperature of 18C for 3 days.

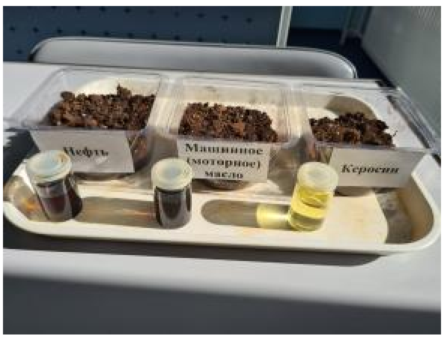

fig. 3

2.3 Biological remediation

After that, 100 ml of water per 200 g of soil was added every day to maintain the activity of microorganisms and provide conditions that may occur during intensive land use, particularly, the irrigation process or natural phenomena (rain). According to the study [3], water promotes the growth of bacteria and their reproduction since it serves as a source of energy and removes unwanted products.

After adding the water, the oil floats to the surface forming oil slicks. After the introduction of microorganisms, they help to cleanse the soil and remove oil spills within 10 days, thereby purifying the soil and water.

The ability of isolates of microorganisms to utilize oil hydrocarbons was studied using chromatography.

It can be noted that in the process of biodegradation, hydrocarbons are destroyed at first. They crept over the chromatographic background in the form of clear peaks, while hydrocarbons at the background are oxidized later [4]. Studies have shown that Rhodococcus microorganisms actively degrade oil hydrocarbons.

2.4 Land farming

Land farming is a bioremediation treatment process performed ex situ in biotreatment cells. Contaminated soils, sediments, or sludges are incorporated into the soil surface and periodically turned over to aerate the mixture [2].

After purification of soil and water from oil products by means of bioremediation, the purified water resource can be reused for other purposes.

3 RESULTS

3.1 Composition of extracted microbial populations

As a result of the experiment, the following crops, which contribute to soil cleansing, were identified.

1 – Arthrobacter

Cultures with dark blue pigmentation, oily, gram-positive. The cells are coccoid, pleomorphic. The developmental cycle of coccus - stick - coccus is clearly expressed. Old cultures consisted of cocci. Based on these characters, they were assigned to the genus Arthrobacter. [fig.4]

2 – Bacillus

Cultures with violet pigmentation, rod-shaped cells (0.3-2.2 x 1.2-7.0 μm). They are mobile, connected in chains and formed cylindrical spores located terminal and subterminal. The sporangia did not swell. According to these characteristics, the cultures are attributed to the genus Bacillus. [fig.5]

3 – Micrococcus

Cultures are purple and smooth. The cells are spherical, 0.5-2 microns in diameter, connected in pairs or in the form of clusters of irregular shape but not in chains. Gram-positive, non-spore-forming. Obligate aerobes. Catalase-positive, halotolerant (grow at 5% NaCl). Based on these characters, the species was assigned to the genus Micrococcus. [fig.6]

4 – Rhodococcus

Cultures with pink or cream pigmentation, gram-positive, aerobes, with rod-shaped or filamentous branching cells (8-10-12 microns), folding into characteristic V-shapes. Aerial mycelium is absent. Cell division was carried out mainly by the snapping type; the development cycle is clearly expressed, old cultures consisted of cocci and short rods. Based on these properties, the cultures were assigned to the genus Rhodococcus. [fig. 7]

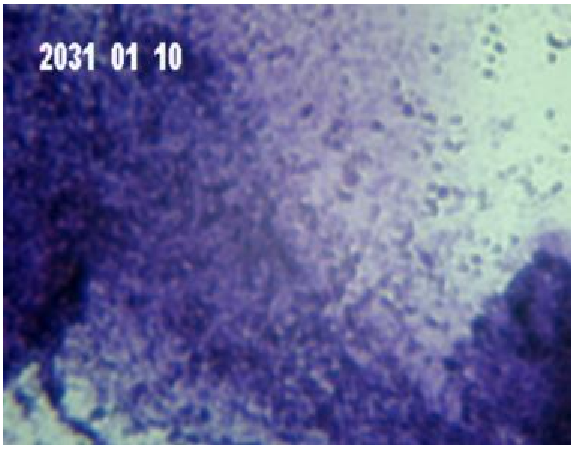

Fig.4

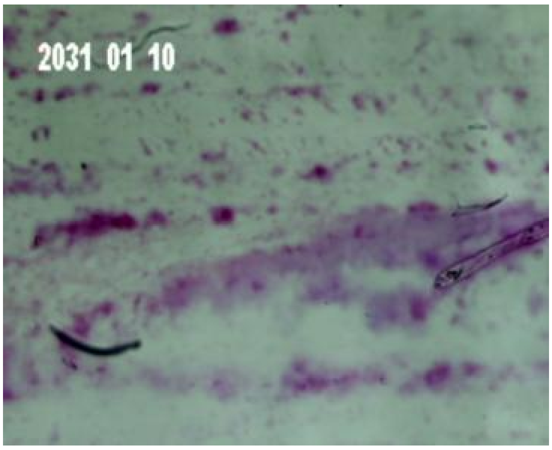

Fig.5

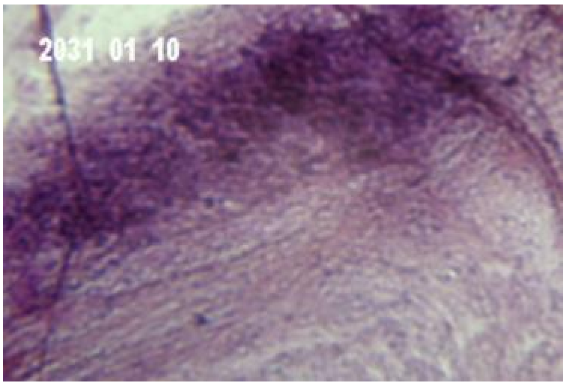

Fig.6

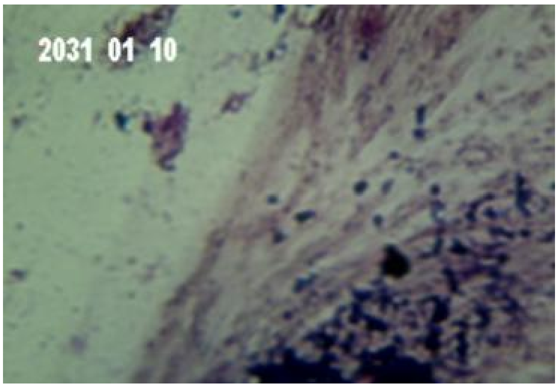

Fig.7

3.2 Microbial isolates potential to breakdown oil hydrocarbons for soil and wastewater confirmed

The ability of isolates of microorganisms to oxidize oil hydrocarbons was studied. Each strain of microorganisms was cultivated in a liquid medium in which oil was used as the only source of carbon. After 10 days of incubation, the oil contained in the medium was extracted with hexane and subjected to liquid chromatography. The change in the composition of saturated fractions of oil hydrocarbons in the process of microbial degradation was analyzed.

As a result of in vitro studies, it was determined that isolates of microorganisms, which are assigned to the genus Rhodococcus, actively degrade oil hydrocarbons. This conclusion is based on two factors:

1) The ability to accumulate microbial mass in a substrate with a single source of carbohydrates in the form of oil hydrocarbons;
2) Changes in the chromatogram of oil, which was used as a nutrient substrate. There was a sharp decrease in the amount of heavy hydrocarbon chains.

The selection criteria for the most active isolates were such indicators as a decrease in the coefficient of hard-to-reach compounds in the saturated fraction of oil. The results of the analysis showed that these isolates of microorganisms are characterized as active destructors of the saturated fraction of hydrocarbons.

3.3 Water saving opportunities for ex-situ oil remediation

Our laboratory research provides an opportunity to use outcomes as a model for ex-situ bioremediation technique. , which allows the use of a certain amount of water for clean-up and provides water recirculation options. The obtained water can be used in agricultural purposes, such as irrigation, pesticide and fertilizer application, and sustaining livestock.

After being polluted it undergoes a cleaning cycle and goes back to use. Further, the research needs to be continued on a large scale. [fig. 8]

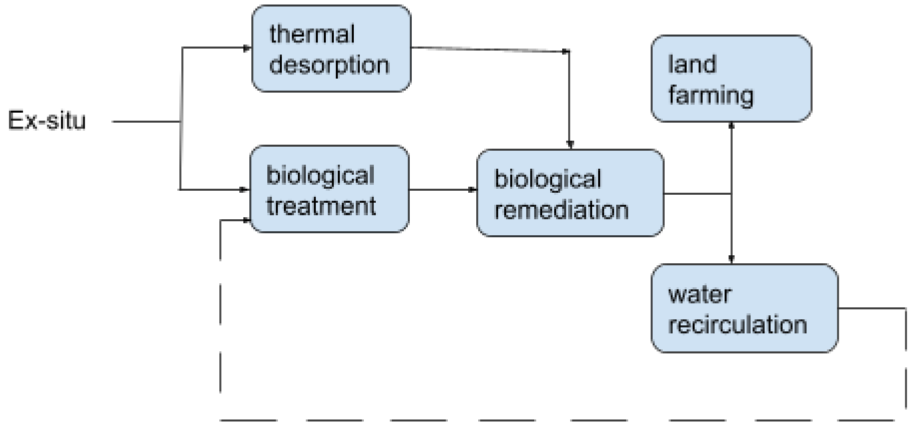

fig.8

4 CONCLUSION

For countries with wide territory and lack of water resources it is important to apply the most environmentally friendly clean up technologies when planning extensive agricultural development. Also, the potential of oil spills bioremediation is not so obvious from a water saving point of view. Our study includes laboratory research on cultivation of oil destructing microorganisms as well as soil and water treatment in the presence of a destructor. The outcomes can be used as a model for ex-situ bioremediation technique, which allows the use of a certain amount of water for clean-up and provides for water recirculation options. This requires further research and larger scale bioreactors for testing. We have a strong aspiration to continue our study and attract attention to water saving and to promoting environmentally friendly clean up technologies.

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