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Humate effect on oil-oxidizing activity of hydrocarbon-oxidizing microorganisms

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ABSTRACT
The effect of humic substances on the activity of hydrocarbon-oxidizing microorganisms is studied. It is shown that sodium humate, aminogumic and sulfogumic acids did not have a negative impact on the growth of oil-oxidizing microorganisms. Introduction of sodium humate in the culture medium stimulated the destructive activity of oil-oxidizing microorganisms. At its addition the degree of oil degradation was 72.5-84.5%, and atits absence – 70.7-78.3%.
Keywords: bioremediation, degradation, humates, oil-oxidizing activity, oil-oxidizing microorganisms, oil pollution

I. Introduction

Contamination by oil and oil products is one of the most difficult types of technological impact on the environment. The oil that arrives in water reservoirs can be carried by currents for hundreds and thousands of kilometers from the discharge point, penetrate into the sea water column, accumulate in the bottom sediments, exerting in this wayinfluence on all groups of organisms[1, 2].

In the Kazakhstan sector of the Caspian Sea, an active development of oil fields is taking place. In connection with this, a problem of creating effective, affordable agents to remove hazardous oil pollutions on the water becomes very relevant.

The leading place in the complex of processesfor purification of natural ecosystems belongs to biological factors, namely hydrocarbon-oxidizing microorganisms. The process for bioremediation of ecosystems polluted by oil and oil products is based on the ability of microorganisms to degrade complex organic oil compounds [3, 4, 5].

Sharing chemical and microbiological methods for eliminating oil pollutions is promising using dispersants and oil-oxidizing microorganisms capable of assimilating petroleum hydrocarbons. As dispersants, available and nontoxic humates and their modifications obtained from raw materials and coal mining wastes are suggested. Humic substances are able to reduce the toxicity of emulsions made of oil and oil products as well as individual oil hydrocarbons. Inaddition, theyactassurface-active agents [6, 7, 8, 9, 10].

II. Materials and Methods

The active strains of oil-oxidizing bacteria isolated from oil-polluted water and bottom sediments of the Caspian Sea belonging to the genera*Arthrobacter* and *Dietzi*aserved as objects of research.

Additionally,the humic substances obtained from the Shubarkol coal deposit (Central Kazakhstan region) were the objects of the study: sodium humate, sulfogumic acid (SGA) aminogumic acid (AGA).

Sodium humates were obtained by coal extraction with alkali solutions. Sodium sulfohumatewas obtained by sulphurizingoxidized coals with concentrated sulfuric acid followed by treatment with alkali.

Effect of humates on oil-oxidizing microorganisms was studied using the well method and the direct impact on the colonies.

To study the oil degradation with the combined introduction of oil-oxidizing microorganisms and humates, the mineral medium of the following composition was used, g/l:NH_4NO_3 – 1.0, K_2HPO_4 – 1.0, KH_2PO_4 – 1.0, $MgSO_4$ – 0.2, $CaCl_2{\times}6H_2O$ – 0.02, $FeCl_3$ – trace amount, pH =7.0-7.2. As a sole carbon source, oil(3%) was added to the medium. Culturing of microorganisms was carried out in Erlenmeyer flasks containing 100 ml of medium on an orbital shaker (180 rpm) at 28 °C for 14 days. The total content of petroleum hydrocarbons in the medium was determined by gas chromatography.

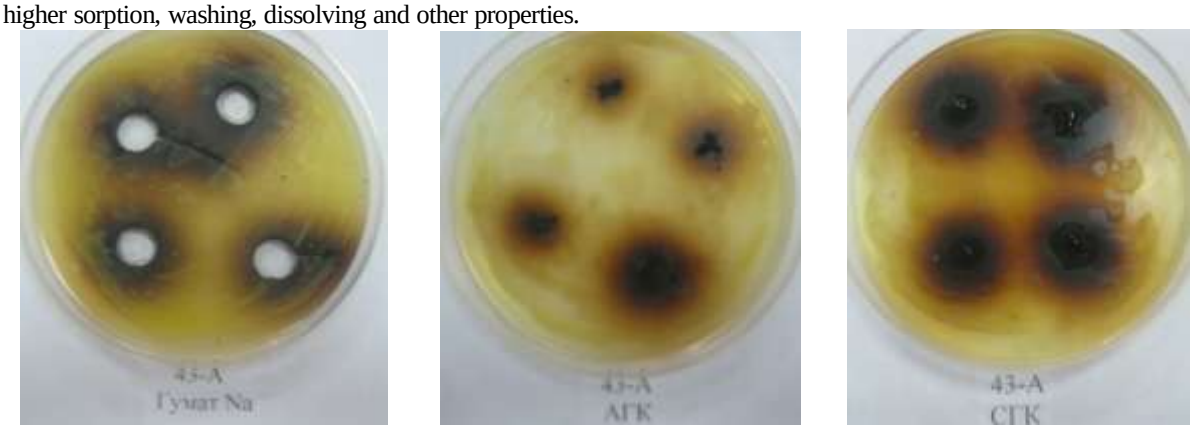
III. Results and Discussion

Humic acid (HA) according to the present-dayconcept relate to the self-organizing anionic multifunctional polyelectrolytes. Polyfunctionalityand

hydrophilic-hydrophobic balance of humic acids determines their ability to enter intothe processes of ion exchange, electron transport, complexation and intermacromolecular interactions with natural and synthetic polymers.

Investigated humic substances are medium-active surfactants. At values below the critical micelle formation concentration, they acquire a micellar structure defining a higher sorption, washing, dissolving and other properties. Furthermore, they are dispersants, and as is well known, the dispersed oil is easierexposed to biodegradation.

The effect of sodium humate, aminohumic (AHA) and sulfohumic (SHA) acids on the growth of oil-oxidizing microorganisms was studied. The results showed that these compounds had no toxic effects. A growth of cultures was not suppressed, and in some cases was even stimulated (Fig. 1).



A b c

Figure 1.Effectofsodiumhumate (a), aminohumic (b) andsulfohumic (c) acidsonthegrowthofoil-oxidizingmicroorganisms

The oil degradation has been studied under the combined introduction of oil-oxidizing microorganisms isolated from the Caspian Sea, and humic compounds. The degradation of oil mixture from the Caspian Sea regionwith the addition of humatesat doses of 500 mg/L and 1000 mg/Lwas examined (Table 1).

Table 1.Oil degradationwith the combinedaddition of oil-oxidizing microorganisms and sodium humate

Strain	The degree of oil degradation, %		
	without sodium humate	with sodium humate	
		500 mg/l	1000 mg/l
12 T	71,2	72,5	75,0
15 T	72,4	73,9	77,0
84 T	78,3	82,1	84,5
43-A	76,6	78,2	81,8
23III	72,8	75,7	77,5
25III	70,7	74,8	78,5
Note – The destruction of oil in the control (medium + oil) amounted to 25.0%			

The study results showed that the addition of sodium humateto the culture medium stimulated the destructive activity of oil-oxidizing microorganisms. If the pure oil-oxidizing cultures utilized oil to 70.7-78.3%, then the degree of oil degradation after the addition of sodium humateincreased to 72.5-84.5%.

With simultaneous application of oil-oxidizing microorganisms and sodiumhumate in an amount of 500 mg/L, thelevel of oil degradation reached 72.5-82.1%. Increase in the dose of humateenhanced utilization of oil by 2.0-3.8%. Maximal oil degradation was observed in strains 84T and 43-A.

Adding aminohumic andsulfohumic acids did not have a stimulating effect on the oil-oxidizing activity of the examined strains. Underthecombined incubation of microorganisms with AGA and SGA, degradation of oil reached 69.7-77.6% and 69.5-76.3%, respectively.

Figures 2, 3 and 4 representpetroleum chromatograms after exposure to strains *Dietziamaris* 84 T and *Arthrobacterluteus* 43-A in the presence of sodium humate.

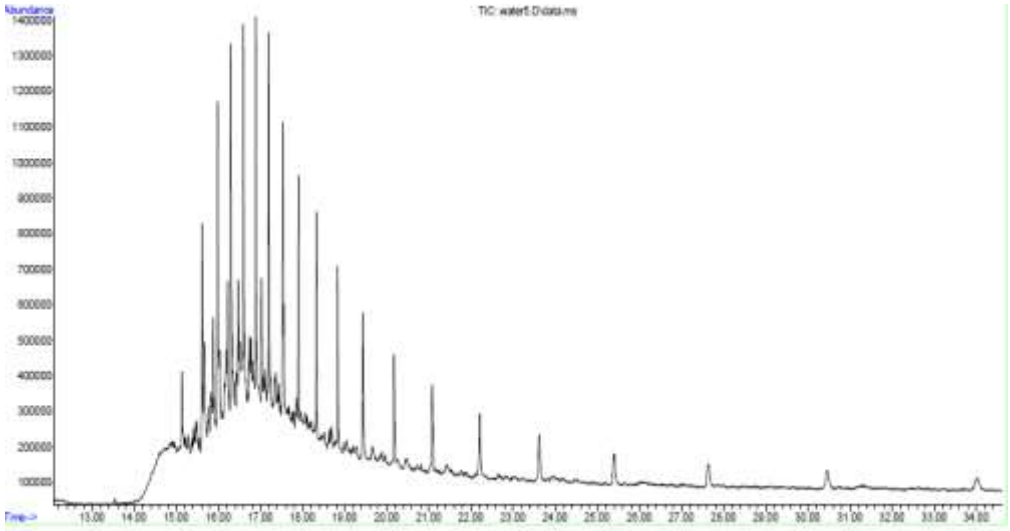


Figure 2. Chromatogram of the total content of petroleum hydrocarbons in the control

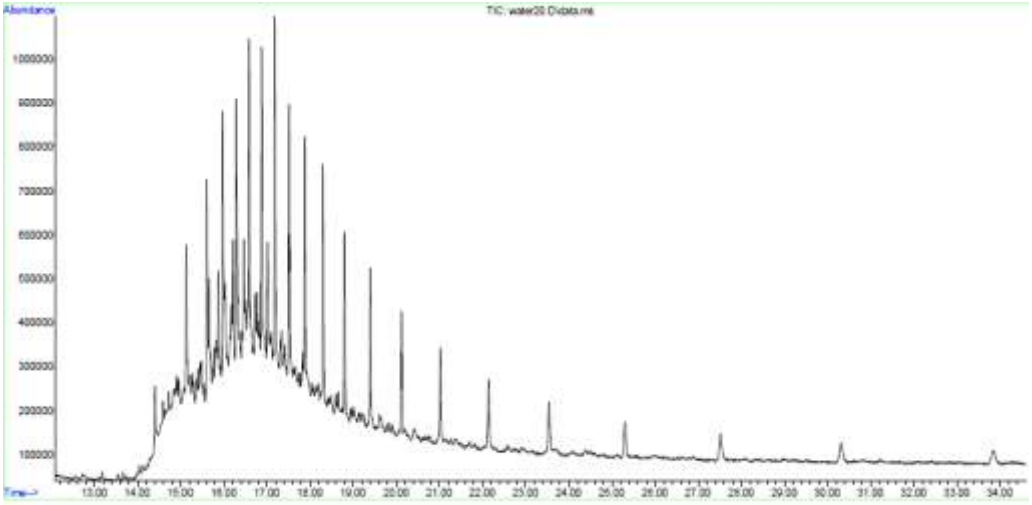


Figure 3.Chromatogram of the total content of petroleum hydrocarbon after culturing the strain *Dietziamaris* 84 in the presence of sodium humate

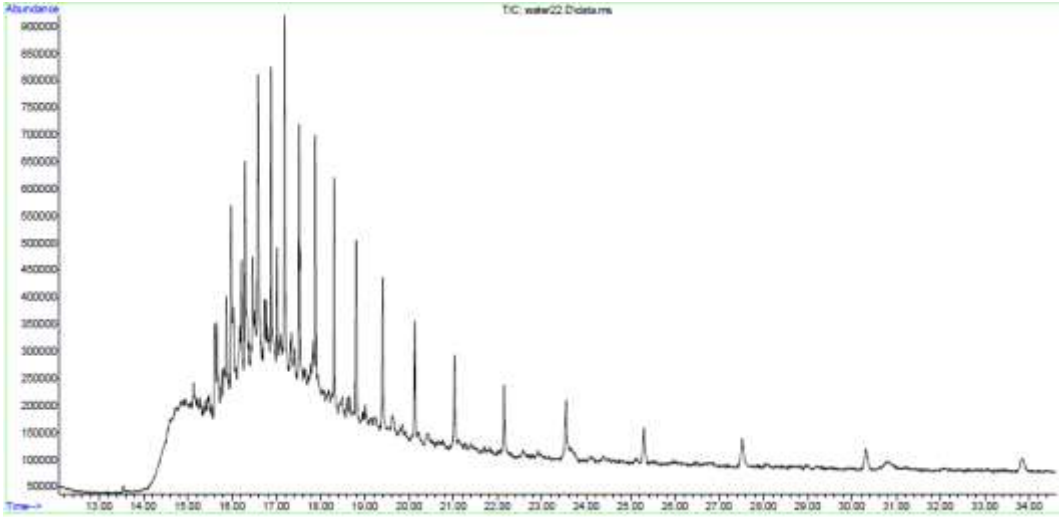


Figure 4.Chromatogram of the total content of petroleum hydrocarbon after culturing the strain *Arthrobacterluteus*43-Ain the presence of sodium humate

According to the obtained data, the content of n-alkanes in the test samples increased due to the destruction of condensed cycloparaffins with 2 and 3 rings. When culturing the strain A-43, the amount of the fluorene significantly reduced, whilein the presence of the strain 84-T –the amount of nitrobenzene.

Thereby, it was found that sodium humate has a stimulating effect on the destructive activity of oil-oxidizing microorganisms. At that, the maximal oil degradation occurred under the combined introduction of the examined strains and soluble sodium humate in an amount of 1 g/L. AHA and SHA had no effect on the activity of microorganisms.

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