Installation for testing the ability of oil-oxidizing microorganisms

O. Hamroyev[1*], *J.* Togaev[1], and *R. N.* Keldiyorov[2]

[1]Karshi Engineering-Economics Institute, 225, Mustakillik str., 180100, Karshi, Uzbekistan
[2]Karshi Institute of Irrigation and Agro Technologies of the National Research University Tashkent Institute of Irrigation and Agricultural Mechanization Engineers, 180119, Karshi, Uzbekistan

Abstract. The paper proposes an experimental setup simulating a storage tank for petroleum products under agricultural conditions. The research was carried out with periodic cultivation of microorganisms. The growth activity and degree of utilization of hydrocarbon-containing compounds of several strains of microorganisms were checked. The results of quantitative determination of hydrocarbons show that the examined contaminants contain hydrocarbons with a chain length from C8 to C27. The latter contain on average 68.7 % n-alkanes, 29.2 % isoalkanes and a relatively small amount of aromatic hydrocarbons. The results of studies to determine the dependence of substrate consumption and biomass growth on the duration of the process are presented.

1 Introduction

Data on the above ability of microorganisms are usually obtained in small laboratory facilities. This makes it possible to select an active strain of microorganism, study its physiological properties and further apply under experimental conditions on the fermentation modes worked out for it, which are optimal for a given microorganism.

The purpose of this work is to justify the possibility of biological treatment of technical objects from contaminants of oil origin.

The task of the work is to find out the ability of previously selected active microorganisms to assimilate the residues of petroleum products in the experimental setup. The issues of efficient use of fuel and lubricants were dealt with by S. Komiljonov [1], T Razzaqov [2], B. Tulaganov [3, 6], B Shaymardanov [4], I. Temirov [5], R. Khudayqulov [6], M. Amonov [7], B. Mirzaev [8, 9, 11, 12, 20], F. Mamatov [21-25], and others.

2 Materials and methods

Based on these considerations, we have made an experimental installation on the type of tank for storage of petroleum products. Let us describe the main part of the installation. It is a tank, which is a horizontal cylinder of 10 liters' capacity, made of organic glass. The

* Corresponding author: r.nargiza@mail.ru

following units are mounted in the tank: a barboter (air supply system); a heat exchanger, communicated by silicone hoses with a thermostat and serves to maintain a certain temperature; electrodes to measure pH of the medium; resistance thermometer; pump-dosing unit for feeding titrating liquids into the tank; a valve for sampling (Figure 1).

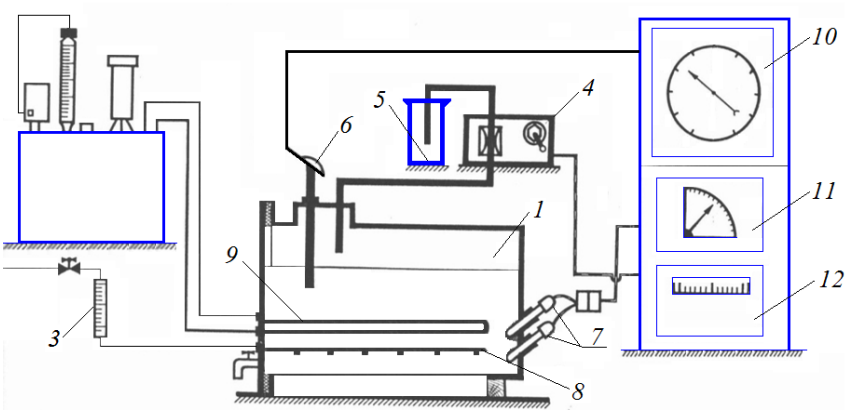

Fig. 1. Scheme for checking the ability of microorganisms to assimilate oil pollution: 1 – tank; 2 – thermostat; 3 – rotometer; 4 – pump-dosing unit; 5 – vessel for liquids; 6 – resistance thermometer; 7 –electrodes for measuring pH-media; 8 – barbometer; 9 – heat exchanger; 10 – temperature-register; 11 – pH-meter; 12 – pH-media register.

Seed was obtained in flasks on mineral medium according to prescription.

As a source of carbon, diesel fuel residues in the concentration of 5 % of the volume were used, the initial composition of which had been previously studied (see below).

Tests were conducted at a temperature optimal for each microorganism, pH of the medium for yeast was 4.5-5.0, and for bacteria, 6,8-7.0. Experiments were performed with and without the addition of a surface-active substance (surfactant) technical sulfoureide at a concentration of 0.05%.

A 20% aqueous solution of ammonium sulfate (4 ml per liter of culture medium) and 6% aqueous ammonia solution applied for titration were used as a source of nitrogen. The sources of phosphorus and other nutrients were a set of concentrated salts according to prescription No. 8 in the amount of 20 ml per liter of culture medium [11-14].

During investigations, the working volume of the unit tank was five liters.

It is known that the residues of petroleum products during storage are deposited on the inner walls of the tanks, especially their bottom part. In order to determine suitability of bio preparations (microorganisms) for removal of contamination residues from the surface of objects we used samples of steel in the form of a truncated cylinder. The outer diameter of the sample corresponds to the inner diameter of the installation tank. Then on the prepared samples (on the inner part) a layer of sediment of oil products was applied and put them into the tank of the installation.

3 Result and discussion

Microorganisms for their growth use hydrocarbons from the composition of oil pollution in the process of biological treatment.

The growth of microorganisms on oil contaminants depends on the group hydrocarbon composition of the contaminants. Therefore, it was necessary to determine the group hydrocarbon composition of the studied contaminants. In accordance with the adopted

methodology, the composition of the samples was determined by chromatographic method. The results of the quantitative determination of hydrocarbons show that the examined contaminants contain hydrocarbons with a chain length from C8 to C27. The latter contain on average 68.7 % n-alkanes, 29.2 % is alkanes and a relatively small amount of aromatic hydrocarbons.

Thus, the analysis of the composition of the samples studied allows us to conclude that it is expedient to use biological method for cleaning technical objects from the residues of petroleum products. Microorganisms oxidizing a wide range of n-alkanes (C8-C33) and other classes of compounds such as is compounds and aromatic hydrocarbons are the most promising for biological treatment of various objects from oil products.

The results obtained allowed us to apply modular (standard) hydrocarbons of similar composition to study the ability of microorganisms to utilize petroleum products.

Microorganisms are divided into three groups in relation to temperature: psychrophilic or cold-loving, mesophiles and thermophiles.

The growth of each species of microorganism can only occur within a certain temperature range, limited to the minimum and maximum values for the species, below and above which growth ceases.

The temperature optimum of the cultures under study was determined by comparing the biomass values obtained during cultivation in different temperature regimes. Biomass concentration was determined by weight method in a 48-hour culture. Diesel fuel was used as a source of carbon; its concentration to the volume of the medium was 10

Oil-oxidizing microorganisms are mainly mesophiles. These microorganisms develop in the temperature range of 22-34°C.

For most yeast strains of the genus Candida optimum is in the temperature range of 30-34° C, some species of Candida tropicalize actively grow at 36-38 ° C.

Unlike yeast, bacteria have a wider range of temperature interval of cultivation (20-45° C), some thermophilic cultures can withstand the temperature of 55-60 ° C.

The results of studies to determine the temperature optimum of the selected cultures are shown in Table 1.

Table 1. Temperature optimum of selected active strains oxidizing various petroleum products.

Mesophilic crops		**Thermotolerant crops**	
26-30°C	32-36°C	36-38°C	38-40°C
Bacteria	Yeast	Yeast	Bacteria
1. VSB-160	1. VSB-569, VSB-638	1. VSB-935,	1. SKF-3
2. 9-1, 9-2	2. VSB-637	VSB-928	2. VSB-568,
3. VSB-570	3. VSB-777	-	VSB-574, VSD-5
-	4. VSB-779	-	3. VSB-567 (42°C)
-	5. VSB-906, VSB-908	-	-
-	6. M-2I, M-25, M-425	-	-

Table 1 shows that yeast and bacterial strains that oxidize various petroleum products are divided into two large groups: mesophilic (26-36°C) and thermotolerant (36-42°C) cultures.

When studying the effect of temperature on the growth of selected cultures, it was found that there are microorganisms capable of growing on media with oil products in a wide temperature range (26-42° C), which implies that the biological treatment of objects can be carried out in regions with different climatic conditions.

Earlier [1, 2, 3], we selected active cultures of oil-oxidizing microorganisms. The oil-oxidizing ability of some active cultures was tested on an experimental setup simulating an oil product storage tank at the oil complexes of the agro industrial complex.

The installation reduced the cleaning time (compared with the results obtained in flasks) from 48 hours to 24-27 hours. This is explained by the fact that the intensity of aeration of the culture liquid in the experimental unit is much higher than in the experimental flasks. As a result of the study, it was found that the optimum amount of air fed into the tank of the installation is 3 l/l min (3 liters per liter of contamination per minute); the amount of air fed below the optimum leads to a decrease in culture activity, and more is inexpedient because air, not having time to dissolve in the culture liquid, escapes into the atmosphere.

Studies to determine the degree of utilization of hydrocarbons after the cultivation of selected cultures in the experimental setup are also given. Experiments were carried out with the surface-active substance (surfactant)-technical sulfoureide at a concentration of 0.05 % and without it. The results of the studies are given in Table 2.

Table 2 Hydrocarbon utilization by microorganisms in the experimental (feedstock - diesel fuel; hydrocarbon concentration - 38,8 g/l).

N	Strain designation	Disposed of, in %	
		n - alkanes	aromatic hydrocarbons
1	VSB-638	85.5	86.4
2	VSB -638 + 0,05 % PAV	91.7	91.9
3	VSB- 935	95.6	93.7
4	VSB – 935+ 0,05 % PAV	97.7	92.1
5	VSB -160	91.4	90.05
6	VSB -160 + 0,05 % PAV	91.4	84.7

Table 2 shows that with the addition of surfactant to the culture medium, the degree of hydrocarbon utilization slightly increases with strain VSB-638 by 6.2 %, with strain VSB-935 - by 2.1 %, and with strain VSB-160 does not change. This can be explained by the fact that under intensive aeration of the culture liquid the dispersing ability of surfactants is insignificant, which means that the purification process can be carried out without adding surfactants.

It should be noted that in all experiments it was possible to drain the culture liquid without oil emulsion. There were no traces of petroleum products on the surface of the metal samples, except for a small portion of the biomass, which was easily removed by a stream of water.

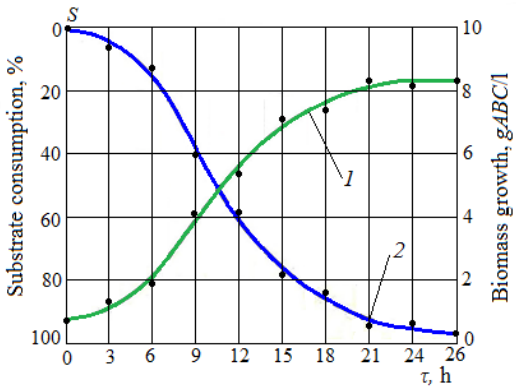

Fig. 2. Curves of biomass growth (1) of VSB-935 strain and substrate consumption (2) over time (10 g/l diesel fuel).

From obtained microphotographs of cells of yeast genus Candida tropicalis VSB-935 in the first hours and at the end of the fermentation process (magnification 1*1350 in a light microscope MBI-15) showed that the particles of diesel fuel are completely oxidized by microorganisms.

Results of examinations to determine dependence of substrate consumption and biomass growth on process duration are presented in Figure 2 (under the action of WCB-935 strain).

The growth of microbial culture in time follows a certain regularity, which is usually established as follows: a certain amount of culture of microorganisms is added to the nutrient medium and the growth of cells is determined at regular intervals. During the experiment nutrients are not added to the medium and the metabolic products of the cells are not removed. Figure 3 shows a classic growth curve of a batch culture.

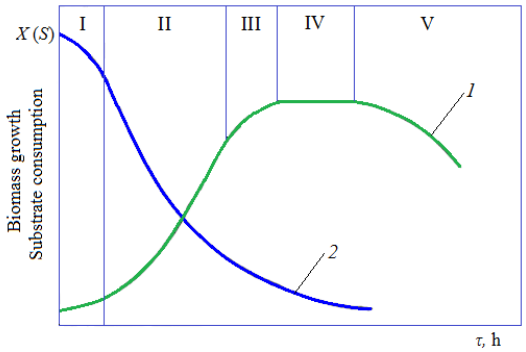

Fig. 3. Periodic culture growth curves: 1 – culture growth in time; 2 – substrate consumption curve. I – initial or lag phase; II – logarithmic growth phase; III – growth retardation phase; IV – stationary growth phase; V – cell death phase.

Curve 1 describes the growth of the culture in time and consists of several sections (development phases).

Curve 2 characterizes the process of cell consumption of substrate *S*.

Comparison of Fig. 2 and Fig. 3 shows that the experimental data agree quite satisfactorily with the results of theoretical studies.

4 Conclusion

According to the classification adopted in biotechnology, the microorganisms selected during experimental studies are divided into two groups: mesophilic with an optimal temperature of their active life of 26-36 ° C; thermotolerant cultures with an optimal temperature of their active life activity of 36-42 ° C.

It was found that mesophilic yeast strains of the genus candida ensure 90-95 % utilization of n-alkanes and isoalkanes, and mesophilic and thermotolerant bacterial strains - 63-96 %. Aromatic hydrocarbons are utilized by these microorganisms by 43-90 %.

Thus, the obtained results of the research serve as the basis for the creation of technology for biological treatment of technical objects from oil pollution.

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