TECHNOLOGY FOR OPTIMIZATION OF PROCESSES OF BIODEGRADATION OF NATURAL AND WASTEWATER BY VARIOUS SORBENTS CONTAMINATED WITH USED MINERAL, SEMI-AND SYNTHETIC LUBRICANT OILS

ABSTRACT

Aims: Study of processes of degradation of spent mineral, semisynthetic and synthetic motor oils by selected hydrocarbon-oxidizing microorganisms and selection of sorbents contributing to optimization of processes of decontamination of natural and waste water from these types of pollutants.

Study Design: The studies were carried out on controlled biodegradation with mineral, semi-synthetic and synthetic lubricating oils with a consortium of nine types of hydrocarbon-oxidizing microorganisms (HOM), in concentrations with initial contamination of 90 ± 6, 400 ± 6 and 1000 ± 6 mg/dm^3 per 400 cm^3 of effluent under statistical conditions at a temperature of + 16 to + 29 °C. The growth of microorganisms was within the range of 304•106 - 306•106 cells/cm^3 (with a continuous purification mode - over 14 days). Also, in the experiment on biodegradation of lubricating oils, HOM sorbent materials were used: river sand, coal powder, polyethylene chips, crushed Tetra paket, sawdust and peat (natural sizes), husks (buckwheat, barley, wheat and oats) with a particle size of 0.017, 0.034, 0.07 and 0.1 mm. Their concentration was, depending on the series of tests, from the trace, 10, 20, 30, 40, 50 and up to 100 mg/dm^3.

Methodology: To biodegrade natural and wastewater with a variety of sorbents contaminated with spent mineral, semi-synthetic and synthetic lubricating oils, nine main bacterial strains isolated from various industrial sewers were used. Microorganisms from environmental objects were isolated by direct inoculation and by cumulative culture. For comparison, different cultures of *Alcaligenes* (1 species), *Micrococcus* (1 species), *Brevibacterium* (2 species), *Bacillus* (1 species), *Flavobacterium* (1 species), *Clostridium* (1 species) and *Pseudomonas* (2 species), which is part of the oil and oil-destroying microbiological consortium. Microorganisms are combined into consortia on morphological-physiological and biochemical properties and are represented by both gram-positive and gram-negative, motile and motionless aerobic and facultatively anaerobic forms. The physiological activity of the strains is variable, their enzymatic activity is high.

The degree of biotransformation of wastewater was assessed by the dynamics of chemical oxygen consumption (COC), biological oxygen consumption (BOC_5)

and dissolved oxygen (O_2) in the process of biodegradation of wastewater pollution - by chemical methods.
Results: In the presence of sorbents, the husks of grain crops with microorganisms involved in purifying water from transformer oil receive an addiction process in the interval from 1 to 3 days of contact and diesel oil, respectively, 3 days. At the same time, the increase in the amount of transformer oil reaches 10-12 times in the version with buckwheat and oat husks and up to 18 times in the presence of barley husks in water, and the increase in the amount of diesel oil reaches 2-3 times in the versions with wheat and barley husks.
According to the dynamics, the increase in the number of HOM in semi-synthetic oil in the presence of husks of grain crops, its reduction in concentration pollution reaches 55.0-63.7 % by 6-9 days. The share of mineral oils subject to biodegradation of HOM, within the same time frame, exceeds more than 30 %. The total cleansing effect in versions with oat husks reaches 90.6 %, barley 92.2 %, and buckwheat and wheat 93.7-93.8 %. Whereas the efficiency of water purification from used lubricating oils in the presence of river sand, polyethylene chips, grass dust, coal powder and peat remained at the level of indicators or lower, observed under the influence of HOM.
Conclusion: When biodegrading natural and waste water from oil contamination, it can be used through optimization of sorbents with hydrocarbon-oxidizing microorganisms, which leads to the achievement of the maximum maximum permissible norm for water purification from used lubricating oils. Thus, we recommend the controlled use of sorbents of grain crops, such as husks of oats, wheat, barley and buckwheat (particle size 0.017-0.034 mm with a concentration of 50 mg/dm^3) together with hydrocarbon-oxidizing microorganisms (up to 350•10^6 cells/cm^3).

Keywords: biodegradation, hydrocarbon-oxidizing microorganisms, used lubricating oils, sorbents, natural and wastewater, oil contamination.

1. INTRODUCTION

Among the numerous pollutants continuously entering the environment from industrial enterprises, agricultural facilities and others, used lubricating oils are still the most global [1, 2].

With annual global production of up to 50 million tons. [3] the share of used oils exceeds 30% [4]. Of these, 20% is used for production needs [5], part is burned [6], and the bulk is diverted to open water bodies [7, 8].

Many years of experience in protecting natural water bodies from these types of pollution show that the most effective way to neutralize them is to use biosorption technologies. Especially in the first phase of an emergency or other inflow of oil, petroleum products and their derivatives, accompanied by pollution of significant volumes of water and water areas.

In the subsequent period, there is an urgent need for biodegradation of residual amounts of petroleum products, including lubricating oils (film, emulsified and

dissolved) in water. It is economically inexpedient to solve this problem globally by mechanical adsorption using organic and inorganic porous materials and substances.

A promising area is the use of controlled biotechnology, which combines sorption and effective biodegradation of compounds with heterotrophic microflora purposefully used for this purpose in one material. We are talking about the joint use of selected HOM with a wide oxidation spectrum of various classes of hydrocarbons and oils of various nature, structure and sorbing substances included in the general cell metabolisms as additional easily oxidizable organic matter.

This area of work is at an early stage of development and is not well understood. Based on this, the purpose of this study was to study the processes of degradation of spent mineral, semi-synthetic and synthetic motor oils by selected HOMs and the selection of sorbents that contribute to the optimization of the processes of neutralization of natural and wastewater from these types of pollutants.

Based on the set goal, the tasks are determined:

1. Determination of the degree and optimization of decontamination of natural and wastewater from used lubricating oils of different composition and properties by a consortium of HOM.

2. Study of the effect of organic and inorganic sorbents on the biodegradation of lubricating oils with determination of their sizes, concentrations, as well as types that ensure the optimization of water biotreatment processes.

2. MATERIAL AND METHODS

The object of research was mineral oils: transformer (Lukoil VG, Russia), transmission (Mobil ATF 220 Premium Automatic, USA), diesel (Shell Helix Diesel 10W-40, UK, Netherlands), solar (Tatneft, Russia), as well as semi-synthetic (Mobil Super 2000 10W-40, USA) and synthetic oils (Castrol Magnatec A3/B4 5W-40, UK). Their content in experiments was: 90 ± 6, 400 ± 6 and 1000 ± 6 mg/dm^3 per 400 cm^3 of runoff [9-13].

HOM united in a consortium, including nine species belonging to the genera: *Alcaligenes* (1 species), *Micrococcus* (1 species), *Brevibacterium* (2 species), *Bacillus* (1 species), *Flavobacterium* (1 species), *Clostridium* (1 species) and *Pseudomonas* (2 species) [14].

A HOM suspension culture for water challenge was prepared from pure isolates stored in the laboratory on liquid Münz (composition (g/l): $(NH_4)_2HPO_4^{-2}$; K_2HPO_4 – 1.0; KNO_3 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.2; NaCl – 0.2; $FeCl_2$ - traces; tap water - 0.1 dm^3; distilled water - 0.9 dm^3; pH - 7.2) medium supplemented with vaseline oil. At the initial stage, each strain was inoculated on an oblique meat-peptone agar

(MPA), grown in a thermostat for 2 days at a temperature of 28 °C. After verifying the purity of the grown cultures on MPA, they were then washed with saline (0.44% NaCl solution), mixed into a single association on fresh medium with petroleum jelly oil (0.5% by volume). Passaging was carried out at 25 °C for 7-10 days. Having received the number of HOM in the range of 340•106-360•106 cells/dm^3, the experimental variants of effluents were further infected [15, 16].

Sorbent materials were used: river sand, coal powder, polyethylene shavings, crushed Tetra paket, sawdust and peat (natural sizes), buckwheat husks, barley, wheat and oats with particle sizes of 0.017, 0.034, 0.07 and 0.1 mm. Their concentration was, depending on the series of tests, from the trace, 10, 20, 30, 40, 50 and up to 100 mg/dm^3.

Water purification from used lubricating oils in laboratory model and field experiments with HOM, sorbents of various nature and without them was evaluated by chemical (O_2, COC (chemical oxygen consumption), oil content) and biological (BOC_5 (biological oxygen consumption), total number of microorganisms, including hydrocarbon-oxidizing bacteria) unified methods [17-19].

3. RESULTS AND DISCUSSION

At the stage of studies, transformer, transmission, diesel (solar) oils were subjected to biodegradation. Against the background of the mineral medium (Münz medium) and tap water with a concentration of initial pollution of 90 ± 6 and 1000 ± 6 mg/dm^3.

Experiments showed that with an initial number of HOM in the range of 24-75 thousand cells/cm^3 on the 2-3rd day, water with lubricating oils began to darken, increasing by 5-6 days. At the same time, the oil film formed on the surface began to lose color, turning from light gray to gray (transformer, transmission), and diesel into light pink. The observed destruction of the oil film into separate pieces, which immediately began to settle and the water was released sharply from the initial contamination. In general, water release from the film occurred within 8-14 days, depending on the nature of the oils, and reached up to 80% of the surface area.

Analysis of residual amounts of lubricating oils in the water column by this date revealed that their content decreased from 56.6 to 72.1 %. The difference between the variants in the number of unoxidized oils was 15-17 %. In the control without infection with HOM in the same period there were no more than 7 %.

In parallel, with the preservation of the same experimental parameters, on natural (river) water with the introduction of sorbents - river sand, peat, coal powder, film chips, sawdust and grain husks, it was shown that the process of releasing water from suspended, emulsified and dissolved oils (mineral, semi- and synthetic) depends on the activities involved in the oxidation of HOM and the nature of the materials with which they contact. There is a direct connection with the

degradation of the above mineral oils from their properties to intensive biooxidation. So, for example, in the presence of sorbents - husks of grain cultures, microorganisms involved in the purification of water from transformer oil obtain a «doping effect» in the interval from 1 to 3 days of contact. This is reflected in their rapid growth in an oil environment. The increase in numbers reaches 10-12 times in the version with buckwheat and oat husks and up to 18 times in the presence of barley husks in the waterand the increase in the amount of diesel oil at the same time reaches 2-3 times in versions with wheat and barley husks (Fig. 1) [20].

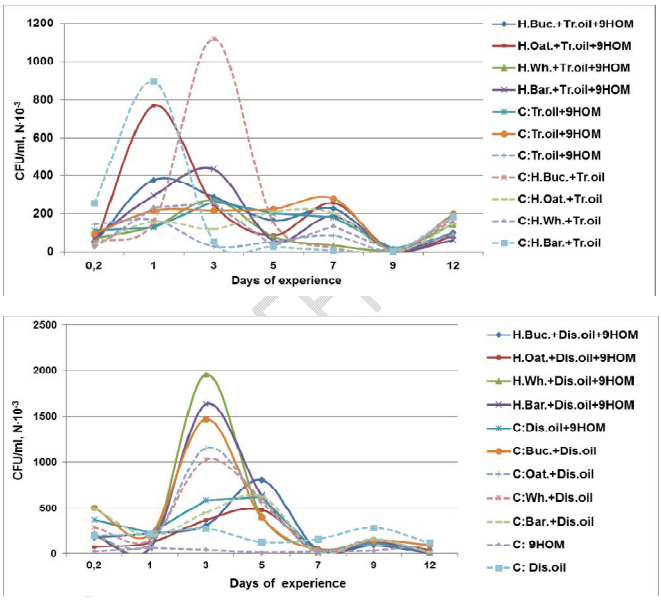

Fig. 1. Dynamics of HOM change in the process of water purification from transformer oil under the influence of substrates and without them
Tr.oil. - transformer oil; 9HOM - 9 hydrocarbon oxidizing microorganisms; H.Buc. - buckwheat husks; H.Oat - oats husk; H.Wh. - wheat husks; H.Bar. - barley husks; C - control.

This period coincides with a significant release of the water surface from the oil film and a reduction in suspended and dissolved products in the water column. The number of them varies within 65.0-87.0 %, respectively.

With semisynthetic oil from Mobil Super 2000 10W-40 in the dynamics of HOM in the process of cleaning water from initial pollution, a different picture was obtained (Fig. 2) [21].

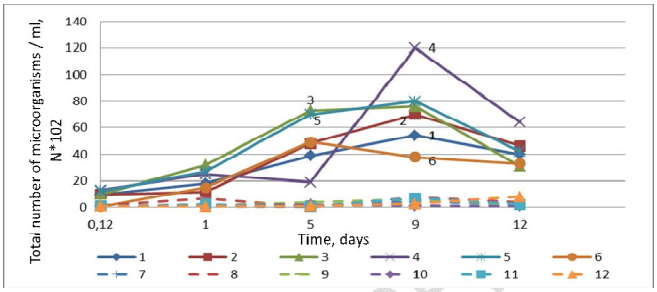

Fig. 2. Dynamics of the growth of the number of HOM (semisynthetic oil of Mobil Super 2000 10W-40):
1 – Coal powder; 2 – Peat; 3 – River sand; 4 – Polyethylene shaving; 5 – Sawdust; 6 – Grass dust; 7 and 8 – Control

Hydrocarbon oxidizing microorganisms introduced into the water slowly adapting to the initial pollution give the largest increase by 6-9 days. By this period, the total reduction of lubricating oil on the surface, as well as in the water column under the influence of mineral sorbents, remains at the level of 44-50 %. In the presence of cereal husks with the same contamination, its reduction in concentration reaches 55.0-63.7 %.

When considering in detail the change in the main purification indicators (dissolved oxygen content (O_2), BOC_5 and COC) of water, it was found that their dynamics in versions with buckwheat husks, oats, wheat, barley fully confirm the intensively current process of moderate bioreduction of water quality under the influence of the latter, both from mineral and semisynthetic oils. The proportion of mineral oils subject to biodegradation of HOM, in the same period, exceeds more than 30 %. The total cleaning effect in versions with oat husks reaches 90.6 %, barley 92.2 %, and buckwheat and wheat 93.7-93.8 % (Table. 1) [20].

Table 1. Biocleaning of water from spent mineral lubricating oils with and without sorbing materials (average of 5 definitions)

Experiment options	Sizes and concentration of sorbents		% water purification from oils
	Dimensions, mm	Concentration, mg/dm^3	

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River sand + HOM [1)]	River fine-grained	up to 50.0	44.9
Coal powder + HOM	-	- // -	40.6
Peat + HOM	Natural	- // -	53.2
Polyethylene shavings + HOM	Various - from shavings to small particles	- // -	67.3
Grass dust + HOM	0.1	- // -	45.0
Husk buckwheat + HOM	0.017-0.034	- // -	93.7
Husk oat + HOM	0.017-0.034	- // -	90.6
Husk wheat + HOM	0.017-0.034	- // -	93.8
Husk barley + HOM	0.017-0.034	- // -	92.2
Water with HOM without sorbents	-	- // -	56.6
Water without HOM and without sorbents	-	- // -	9.0
Inert materials (sand, coal powder, polyethylene shavings, peat, herb dust)	Natural	up to 50.0	11.0
Husks of cereals	0.017-0.034	- // -	22.6

Note: [1)] Hydrocarbon oxidizing microorganisms from 24 to 75 cells / cm^3.

Further studies confirmed this fact. In addition, we have established that the intensity of purification of water from spent lubricating oils is mainly determined by the time of their contact, which is involved in the process of degradation of HOM and the influence of sorbents.

Some sorbent materials and microorganisms have been identified as having a direct link during biodegradation. This is characteristic of the husk of grain crops, in particular buckwheat, barley, oats and wheat. Most likely, the observed is due to the presence of proteins, carbohydrates, amino acids, organic acids and other substances that stimulate the activity of HOM and have an inducing effect on the oxidation of certain hydrocarbons, including lubricating oils. Similar effect of the latter on processes of biodegradation of oil and oil products is proved experimentally [22, 23].

Whereas in the presence of river sand, polyethylene chips, rubbish, coal powder and even peat, rich in many organic (humus, apocrenic, acids) and inorganic compounds (inorganic nitrogen, phosphorus, potassium, etc.). The efficiency of water purification from spent lubricating oils remained at or below the level observed under the influence of HOM (Table 1, Fig. 3) [21].

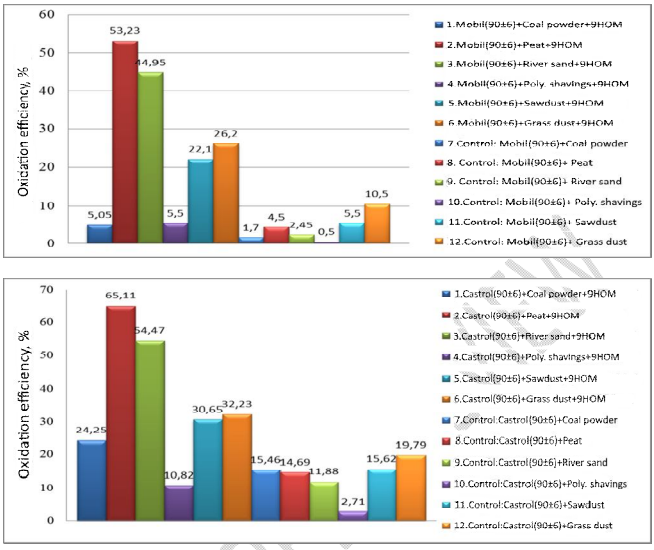

Fig. 3. Efficiency of biooxidation of semi-synthetic Mobil Super 2000 10W-40 oil (A) and synthetic Castrol Magnatec A3/B4 5W-40 oil (B) with use of sorbents and without them:

1-Coal powder; 2-Peat; 3-River sand; 4-Poly. shavings – Polyethylene shavings; 5-Sawdust; 6-Grass dust; 7, 8, 9, 10, 11, 12-Control; 9HOM – 9-th hydrocarbon oxidizing microorganisms

Like it is noted also at oxidation of synthetic Castrol Magnatec A3/B4 5W-40 oil. Under the influence of HOM, the maximum reduction in pollution occurred only on the 12-14th day of contact and ranged from 24.2 (coal powder) to 65.1 % (peat). In the control without sorbents and without HOM, the change in the content of Castrol oil ranged from 10.1-11.9 (river sand, coal powder) to 19.4 % (peat).

4. CONCLUSION

So, from the results of detailed studies, it follows that inert sorbents of any nature in the fight against oil pollution, including spent lubricating oils supplied to reservoirs by emergency or other, must be considered as a preliminary stage of natural and wastewater treatment.

Increasing the neutralization of water from such contaminants by these sorbents can be achieved by intensifying them together with HOM for maximum purification of water from oil products, used lubricating oils and natural or contaminants (open ponds, production effluents) it is recommended to use controlled sorbents of grain crops - husks of oats, wheat, barley and buckwheat (particle size 0.017-0.034 mm with concentration from 50 mg/dm^3) together with HOM (up to 350•106 kl/cm^3). The formed complex – sorbents and microorganisms, being included in oxidative processes, together provide water recovery from these types of contaminants to natural qualities.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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