ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Microbiological Removal of Engine Oils from Natural Water Using Plant-Derived Sorbents.

Nikolei Vasilievich Morozov*, and Ilnur Machmutovich Ganiev.

Kazan Federal University, Kremlevskaya St.18, 420008, Kazan, Russian Federation.

ABSTRACT

The ongoing pollution of water resources with a variety of lubricating oils, the insufficiently developed methods of purification of natural and waste water poses the problem of finding ways to restore the natural qualities of the environment. The authors see a solution to this problem in the wide use of activity of individual associations of oil-oxidizing microorganisms (OOM) in combination with the plant-derived sorbents (buckwheat, oat, wheat and barley husk), which allows deep controlled oxidation of these contaminants down to CO_2 and H_2O. It was found that the multi-species OOM communities take more active part in biodegradation of mineral, semi-synthetic and synthetic oils, than those with the limited species composition. The growth, development and activity in the oil biodegradation is determined by the nature of the contamination. The population growth maxima lie between 5 and 14 days, and decrease to 2 to 6 hours under the influence of sorbents This affects the oil consumption amount, which is 1.7-3.5 times higher under the influence of the sorbents, and 3-7.2 times higher in the control. The first by the efficiency of water cleaning from lubricating oils by the association of nine species of OOM is barley husk, then buckwheat husk, then oat and wheat husk (laboratory experiment); barley, oat, buckwheat and wheat in field experiments (close to natural water bodies) with the particle size of 0.018 mm and 0.036 and at a concentration of 50 mg/l. An important factor in the intensification of water purification from oil with OOM is the introduction of sorbents (type, combination and ratio of the substrate to the bacteria) in the water body. It was found that the maximum purifying effect (32.2-45.4%) 9-12 days prior to the contact is achieved with the introduction of sorbents and OOM in an amount of $102 \cdot 10^6$-$106 \cdot 10^6$ cells/ml, mixed together in the form of a suspension, in contaminated water. This allows achieving a uniform distribution of ingredients that positively affects the biotransformation processes of the contaminants. Upon spraying sorbents on the oil film surface we observe the formation of separate lumps, slowly decomposing and dispersing throughout the area. This negatively affects the immobilization of the OOM cells in sorbents and slows down the process of water purification.

Keywords: the mineral, semi-synthetic, and synthetic oils, consortium, hydrocarbon-oxidizing microorganisms, biodegradation, plant-derived sorbents, the controlled biodegradation of lubricating oils.

**Corresponding author*

INTRODUCTION

Lubricating oils of various nature are one of the major sources of environmental pollution. Their draining into the soil and water exceeds in volume the accidental oil discharges and losses during production, transportation and processing [1]. According to some forecasts, the global consumption of lubricating oils increases every year: by 2010 it reached 42 million tons, in 2015 it was close to 45 million tons [2], and in the future will exceed 50 million tons.

There are four types of base oil: mineral, obtained by vacuum distillation of residue followed by refining; hydrocracking (mineral oil hydrocracking) - leichtlauf, extra high performance, extra wigh performance; semisynthetic (a mixture of mineral and synthetic oils) - Synthetic, Semi-Synthetic, Synthetic Based, Synthetic Blend; and synthetic (directed synthesis) - Fully Synthetic, 100% synthetic [3].

In percentage terms, mineral oils account for up to 60% of all oil produced and consumed. At the same time, indicators for the semi-synthetic and synthetic oils range widely from 18 to 25% for developed countries, respectively [4].

A quarter of total produced oil is reused, 19-20% is disposed, and the rest is partially burnt or discharged into the water bodies, soil, sewage system, or sprayed into the atmosphere. Getting into the environment, waste oil (gear, transformer, motor, diesel, hydraulic, industrial oil and many others) pose a danger to the human health. It is known that 1 litre of waste motor oil poured into the soil makes unusable 100-1000 tons of groundwater [5].

High-quality and safe utilization requires significant investments. In this context, the development of biotechnologies, which will provide biodegradation of lubricating oils of various nature (commercial and waste) to less toxic intermediates, simple oxygenated compounds up to carbon dioxide and water, is an urgent regional- and global-scale problem.

Rare studies devoted to this problem in the domestic and foreign literature [6, 7, 8, 9] show that various groups of heterotrophic microorganisms and natural and artificial sorbents immobilizing their activity play an important role in the degradation of lubricating oils. Both of these areas of work are at the stage of primary elaborations and require in-depth and detailed research. Moreover, in practice, there are no today any targeted association of hydrocarbon oxidizing microorganisms (HOM) specially created for this purpose with a wide range of application and exploitation.

Subject to the above stated, the objective of this study was the choice of association of heterotrophic microorganisms actively oxidizing the lubricating oils of various nature, the investigation of their participation in active biodegradation of contaminants under the influence of high-performance plant-derived sorbent and the formation on their basis of oil-oxidizing microflora for the highly effective controlled purification of natural water from these pollutants.

In accordance with the objective, the following tasks were set:

- study the biodegradation of various types of lubricating oils with the selected OOM species in various combinations of strains (isolated from industrial wastewater of JSC "Kazanorgsintez", CHPP No.2, car fleets, oil depots and the sewer of the city of Kazan) in static conditions and under different loads
- identify a community of oil-oxidizing microorganisms in the selected isolates for high-quality water purification from lubricating oils
- determine the effect of plant-derived sorbents on the biodegradation of the mineral, semi-synthetic and synthetic oils, and establish their dimension and concentration, and develop methods of OOM introduction in the aquatic environment, ensuring the complete neutralization of contaminants.

OBJECTS AND RESEARCH METHODS

Study objects: 1) mineral oils – transformer (TU 38.101-1025-85; gear oil (nigrol) TU 38.101-529-18, compressor oil TU 38.101-543-78; solar oil TU 38.401-58-100-94 (by Rosneft), diesel oil (Shell Helix Diesel 10W-40, Finland), semi-synthetic (Mobil 10W-60, Holland) and synthetic (Castrol Magnatec 5W-40, Germany); and

2) the consortium of oil-oxidizing microorganisms (OOM), comprising of: a) nine isolates of the generra: Alcaligenes, Micrococcus, Brevibacterium, Pseudomonas, Flavobacterium, Bacillus и Clostridium; b) three species of the genera: Micrococcus, Rhodococcus and Pseudomonas.

An OOM suspension culture was prepared from pure cultures of the microorganisms retained in a laboratory in Muntz liquid medium supplemented with vaseline oil.

As non-traditional sorbents serving for immobilizing surface of microorganisms and affecting the biodegradation processes of the lubricating oils we selected organic vegetable substrates such as the buckwheat, oat, barley and wheat husk of different particle size and concentration. For the experiment, they were sorted, crushed and screened in the laboratory sieve mesh of 0.071, 0.036 and 0.018 mm in diameter.

Oil biodegradation was evaluated in laboratory and field experiments by the dynamics of changes in the amount of oxidizing microorganisms expressed in the accepted bacteriological, biochemical and chemical oxygen demand (BOD_5, COD), dissolved oxygen (O_2), and the oil biodegradation – by chemical and spectrophotometric methods.

RESULTS

The use of microbiological transformations in the utilization of mineral, synthetic and semi-synthetic oil is an effective and environmentally safe way to neutralize these wastes in the environment. It is based on the decomposition of the lubricating oil (commercial and waste) in the water by oil-oxidizing microorganisms (OOM) to harmless oxidation products, down to CO_2 and water.

Neutralization of these types of pollution, as in the case of oil products discharged in the water sources, should be conducted by controlled biological method. An example is a widely used method of microbiological purification of natural and waste water from oil with the use of thickets of higher aquatic vegetation (artificial universes or naturally overgrown ecosystems). In the water body, the naturally developing microorganisms of different taxonomic groups and the higher aquatic plants (macrophytes), interconnected in a single system, participate in the restoration of the quality of the polluted environment. At the same time, the first act as the main destructors and mineralizers, and the second - as inducers, eliminators and consumers of lightly, semi- and fully oxidized compounds [10, 11]. This ensures a high degree of purification of oil-contaminated water, down to sanitary and fishery standards. Moreover, this is achieved in the areas of water bodies of 1.2-1.5 km long, with 60-80% covering of water surface with macrophytes at growing density of 70 to 200 m^2/ex. and residence time of 18 to 36 hours.

Despite the "simplicity" of the technology and highly effective purification of the water surface from a variety of oil products, this natural-biological macrophyte-based method is limited in use because of the complexity to create such ecosystem everywhere, where uncontrolled flow of oil products and lubricating oils of various nature can occur.

The world experience in solving this problem shows that the most effective and very promising method of controlling the contamination of water bodies with the lubricating oils coming from various industrial facilities (storage, processing, recycling facilities, industrial waste, agricultural facilities) is the use of bio-sorptional technologies.

At the initial stage of research, we made an attempt to choose microflora able to actively oxidize lubricating oils, without causing any physical or chemical action. For this purpose, we tested two associations, including nine and three species of oil-oxidizing bacteria (see Objects and research methods).

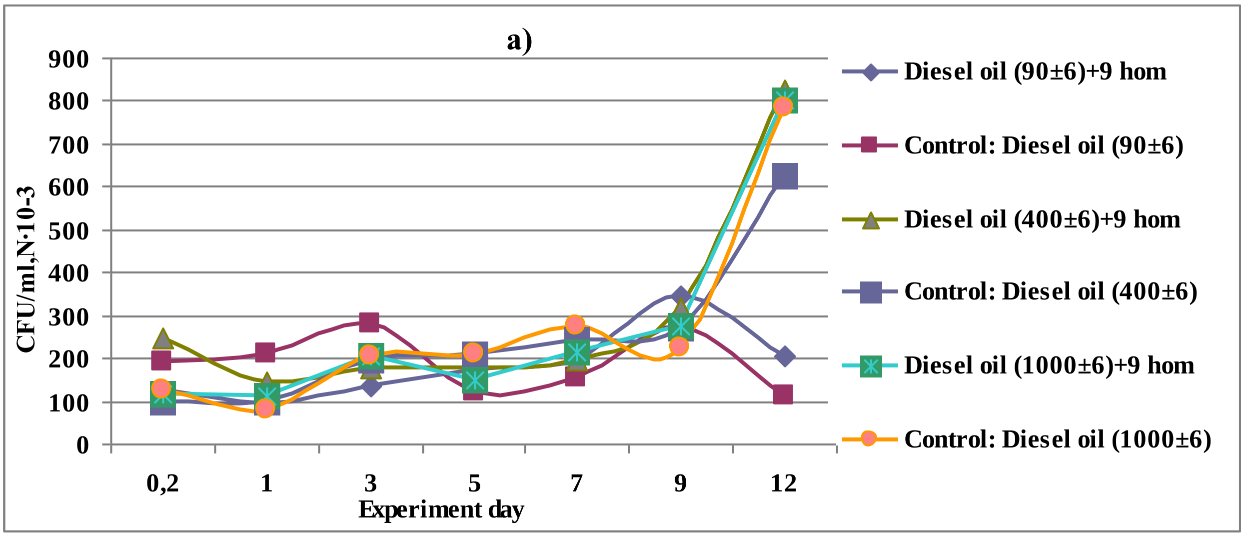
Our experiments have shown that the development of the selected microorganisms in the presence of certain lubricants has the same tendency. The decline in their number on day 1-3 of passaging, which lasted for the next 4-5 days, and then the increase on day 6-9 with the adaptation of microorganisms to the source substrate, and then a sharp reduction up to the initial level and below.

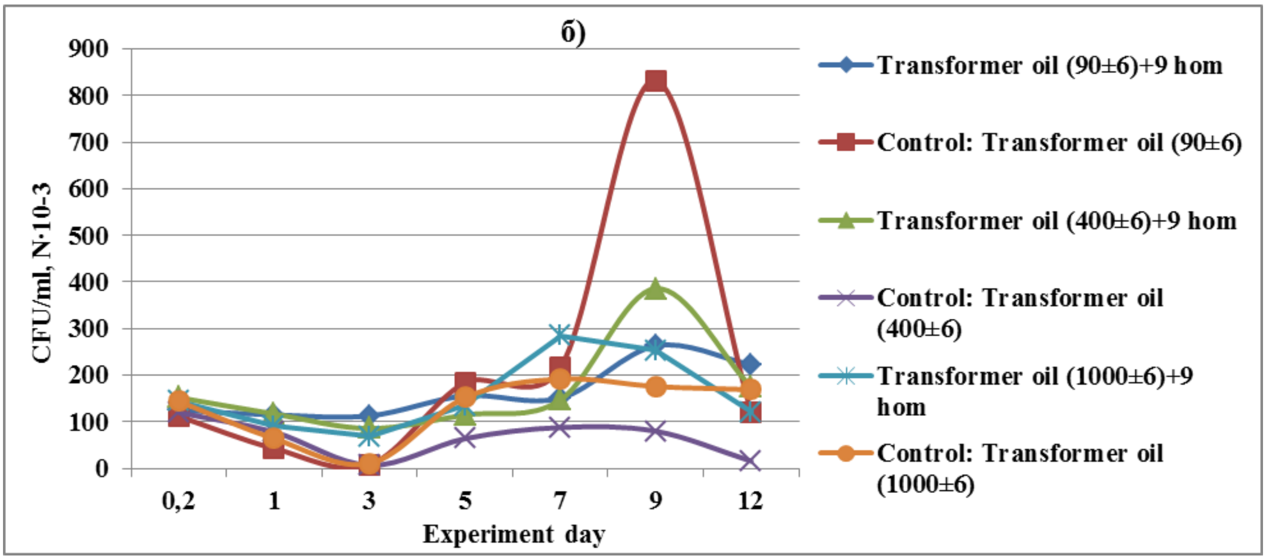
In the presence of solar, transformer, gear and diesel oil in the environment in the amount of 90±6 mg per 400 ml of Muntz medium, the number of bacterial cells by the maximum growth period was equal to

224·10³-480·10³; 320·10³ and 328·10³ cells/ml, respectively. In case of general pollution with these oils at a concentration of 400±6 mg per 400 ml, the number of microorganisms on day 12 of the experiment ranged from 25·10³ to 400·10³ cells/ml. This was mainly observed in experiments with nine strains of hydrocarbon-oxidizing bacteria in the community. In experiments with three strains (second association) in the same growing conditions and under the influence of the aforementioned lubricating oils (concentration of 90±6 and 400±6 mg per 400 ml of the medium), the growth stabilization and increase in cell number occurred later, i.e. on day 16-20. This indicates lower activity of this group of microorganisms in the metabolism of lubricating oils. We have already pointed [12] that the microorganisms of different taxonomic groups ranging from 1 to 9 species, combined into a community, are capable of exposing a variety of hydrocarbon factions to biodegradation for a short period of contact, including those with a complex structure and extremely toxic. While those consisting of few species (within 1-3 isolates) are less active in the decomposition of oil products.

A clearer "work" of the first consortium in comparison with the second one with less number of microbial species can be illustrated by the growth dynamics of HOM during decomposition of diesel and gear oil (Fig. 1).

Figure 1. Dynamics of hydrocarbon oxidizing microorganisms in the purification of water from the diesel (a) and the transformer (b) oils.





Balance calculation of the oxidation of sterile diesel oil showed by the end of the experiment that the HOM with 9 strains amount on average to 60.2%, while under the influence of three strains – not more than 11%.

Subsequent laboratory and field experiments aimed at identifying the role of the sorbents (husks of barley, buckwheat, oat and wheat) in the processes of degradation of oils were conducted only with 9 strains of hydrocarbon-oxidizing microorganisms.

We established in a series of experiments that under the influence of these sorbents the transformer, solar, gear, and diesel oil (content in water of 90±6 mg/l) began to degrade rapidly on day 3-4 of contact with microorganisms. This was indicated by a visible change of oil film color on the water surface, which in the next few days changed from dark gray to light gray in a rapidly disappearing form.

Analysis of the HOM quantity revealed that the number of microorganisms increases together with oil oxidation reaching its maximum on day 2-4, and then decreases, reaching its minimum on day 7-12. By the time of the intensive release of water surface from the oil film their number decreases by 4-6 times, and after complete decontamination - decreases almost to the original level.

In the experiments with elevated concentrations of lubricating oils 400±6 and 1000±6 m/l per 400 ml of medium the development intensity oil-oxidizing microflora was based on the type of oil. Gear oil promoted their gradual increase in number since the beginning of the experiment to day 6-8. The maximum increase during the period under statistical conditions reached 2.5-4 times. The same was observed with diesel and gear oils.

Subject to the effect on HOM development and bioremediation of mineral oil, the sorbents have been arranged in the following descending order: barley > buckwheat > oat > wheat. The average efficiency of biodegradation of contaminants in the experimental variants with sorbents ranges 1.7-3.5 times, which is beyond the control of more than 3-7.2 times.

The study of biodegradation of semi-synthetic (Mobil) and synthetic (Castrol) oil with HOM demonstrated that this process is also connected with the oil nature. Semi-synthetic oil "Mobil" is faster and more intensely exposed to biooxidation. With biooxidation, a number of HOM reaches maximum on day 3-4 of contact, and depending on the concentration of water pollution is determined as follows - $225{\cdot}10^3$ CFU/ml at 90±6 mg, $212{\cdot}10^3$ CFU/ml at 400±6 mg and $118{\cdot}10^3$ CFU/ml at 1000±6 mg. On day 7-9 their number decreases sharply. By this time, the amount of oxidized oil ranges 52.7-64.9% with involvement of sorbents, and remains at level of 17.0% without them.

The field experiments with the combined introduction of oils, oil oxidizing microorganisms and substrates in the contaminated water revealed that the development process is similar to that in the laboratory experiments. As far as OOM adapts to lubricating oils, their number starts to grow and reaches maximum on day 5-9 (almost 2 times longer than in the laboratory experiments). A decreasing concentration of the lubricating oil in the water is accompanied by rapid decrease in the number of the microflora, involved in the biodegradation, to the original level.

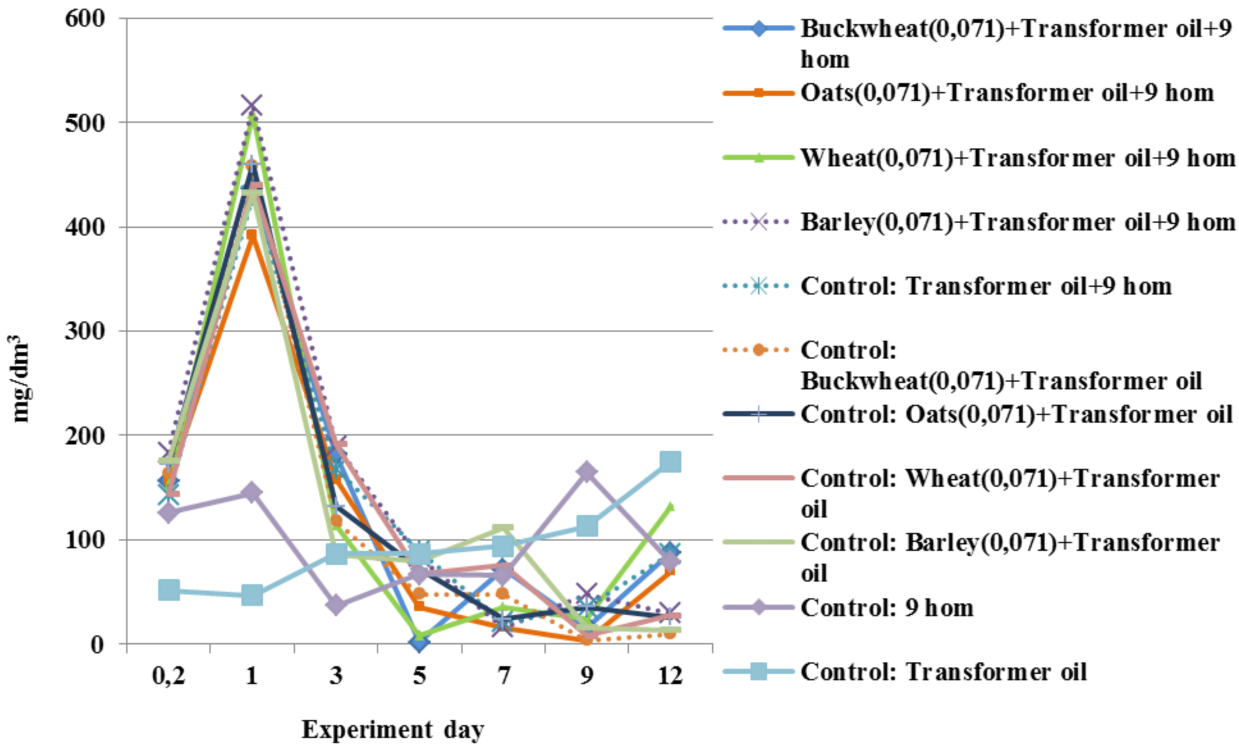
A comparison of the results of OOM development with sorbents and without them showed that an increase in the population of microorganisms by the time of maximum growth under the influence of substrates is faster and larger in number by almost 2.5-3 times. It reaches its maximum with the substrate of barley, then wheat, oat, and is minimum with buckwheat.

Based on the results of the experiments, the effect of vegetable substrates on the degree and intensity of oil oxidation depends also on the size and concentration of the sorbents. In this regard, we have studied the particle size of husks - 0.018, 0.036 and 0.071 mm, and the concentration - traces, 10, 25 and 50 mg/l (experimentally selected). The main purpose was to determine the properties of the substrates having an immobilizing and catalytic effect on OOM in the purification of water from oils of various nature. We have experimentally found that particle sizes ranging 0.018 and 0.036 are the most effective in the process of water purification. This is true for all types of husk, for example, diesel oil decontamination efficiency was: in the presence of buckwheat – 92.2-93.7%, oat – 98.1-98.7, wheat – 93.6-96.3%, and barley – 78.8 -92.2%. These values are also close to the transformer and gear oils, and as for "Mobil" and "Castrol", the highest percentage of oxidation was observed with buckwheat and barley, and less with oat and wheat. This is due to biooxidability of the presented oils.

We have found that the effect of vegetable substrates on the degree and intensity of biodegradation of all tested oils occurs in two ways. On the one hand, the substrates with particle size of 0.018 to 0.071 mm and concentration not exceeding 50 mg/l may well be used by OOM in oil biodegradation as an easily oxidisable substrate to improve oxidative processes associated with the development of the adapted enzymes, and, on the other hand, to increase the contact area of sorbent immobilized microorganisms involved in water purification. As for the impact of each substrate on the intensity of oil biodestruction with OOM, it is obvious. This is also confirmed by the dynamics of dissolved oxygen, BOD_5 and COD in the process of water purification.

Biochemical and chemical oxygen demand (BOD_5, COD) is one of the important indicators of the level of pollution by organic substances. Considering the dynamics of changes in BOD_5 in the process of water purification from transformer oil we have found that it varies in inverse proportion to the value of O_2, and as for the dynamics of OOM, it varies in direct proportion (Fig. 2).

Figure 2. The dynamics of changes in the BOD_5 during removal of transformer oil (90±6 mg/L) from water with and without the substrate



As can be seen from the figure, the transformer oil is involved in the oxidation process on day 1-3 of contact of microorganisms with the contamination. This is largely typical of the experiments with sorbents. The overall decline in O_2 by the stated term is more than 2.5 times, and BOD_5 increases by almost the same value. The latter is due, probably, to involvement of both light oil fractions and sorbents in oxidative processes. Control variants show unchanged values of O_2 and BOD_5 at the same period of time. The OOM number is at the level of 20 10^3 -50·10^{3-} cell/ml, O_2 – 3-3.5, and BOD_5 – 50-140 mgO_2l.

Analysis of the chemical oxygen demand (COD) during water purification from the oils has revealed almost the same dynamics as with the BOD_5. An increase in its value in the first two days and then decline with the reduction of water pollution concentration. An increase in COD in the beginning of the experiment was largely due to the doubling of water pollution with the combination of oils and sorbents. As far as microorganisms adapt to the original substrate, the oil metabolic processes begin to accelerate and as a result there is a decrease in COD values down to the completed purification of water from pollutants. Similarly, the changes occur in the COD content with mineral oils (solar, transformer, and diesel). This is especially evident while water stirring. Its value reduces from 540-1060 to 80-240 mg/l on day 9-12 of OOM contact with the pollution. While the COD values in the control group remain at the same level of 480-320 mg/l even on day 14. In general, the minimum COD residual value is in experiments with barley and oat husks, and maximum with wheat and buckwheat.

It was found that a crucial factor in the stimulation of oil-oxidizing activity of microflora during water purification from the lubricating oils is a form, and combinations of sorbents introduced in the contaminated water. We have tested the following variants of introduction:

1) spraying oil sorbents separately on the oil film surface after the introduction of micro-organisms;
2) mixing the culture suspension of oxidizing microorganisms (the HOM number before introduction ranges from $102 \cdot 10^6$ to $106 \cdot 10^6$ cells/ml) with sorbents with particle size of 0.018-0.036 mm and concentration of up to 50 mg/l.

Introduction of the proposed substrates (husks of barley, buckwheat, oats and wheat separately) to the oil-contaminated surface together with the selected oil-oxidizing microflora resulted in immediate interaction of a solid surface of sorbents with a liquid film phase. The film, formed by emersion of the emulsified, suspended and dissolved lubricating oils from the water column, and "attacked" by microorganisms, breaks into separate, irregular structures or parts and is pulled onto a hydrophobic surface of sorbents. Such collection is followed by immobilization of microbial cells on these formations and their enhanced development starts through the use of oil pollution. The porous husk surface accumulates moisture and dissolved oxygen in its pores, which improves the aeration of the environment and promotes oxidative processes with the involvement of microorganisms. Simultaneously, there occurs biodegradation of the sorbent serving as an easily oxidized organics, or other accompanying substances specific to this substrate. Water surface rapidly releases from oil film and generally from the pollution present in the water body.

In the case of introducing sorbents separately on the surface of the oil stains without its prior mixing with a suspension of oil-oxidizing bacteria (associations of the selected oils and HOM), the intensity of the surface purification from the oil film and waste water in general is much lower. This is due to poor contact of the introduced oil-oxidizing microorganism with the suspended, emulsified and dissolved in water oils because of lack of rapid immobilization of cells on sorbents, and therefore, the lack of adaptation of the latter to the initial pollution as the sole carbon and energy source. It also slows down the processes associated with inclusion of sorbents as an easily oxidisable organic substance in the overall metabolism of bacteria.

Thus, in the process of water purification from natural lubricating oils, the use of sorbent-absorbed oil-oxidizing microorganisms is more effective (by their mixing in advance at a certain ratio) as compared with spraying of the bacterial mass onto the surface of purified water. Bacterial cells attached to a solid support ensure high quantity of microflora involved in the purification per surface unit of the system, and generally intensify the process of water purification from the lubricating oils and other related compounds.

SUMMARY

1. The biodegradation intensity of various lubricating oils depends on the composition, and above all, the types of oil-oxidizing microorganisms (OOM) involved in this process. The absorption extent with the OOM association of nine isolates exceeds 40% for equal period of 12-14 days, while with three isolates – only 11-17%.
2. The total effect of water purification from diesel, transformer, gear, semi-synthetic (Mobil) and synthetic (Castrol) oils with oil-oxidizing microorganisms is even higher (52.7-98.7%) after adding the vegetable sorbents into the water body. Such sorbents are barley, buckwheat, wheat and oat husks with particle size of 0.018-0.036 mm and a concentration in the environment of up to 50 mg/l.
3. Subject to the effect on OOM development and bioremediation of lubricating oil, the sorbents have been arranged in the following descending order:
   - barley ≥ buckwheat ≥ oat > wheat.
4. We have formed an association that includes nine species of oil-oxidizing bacteria of genera *Alcaligenes, Bacillus, Brevibacterium, Chromobacterium*, Clostridium, Micrococcus и Pseudomonas, able to oxidize the commercial and waste lubricating oils in a changing environment (pH 4-9, temperature 16-25^{0}C etc.). To quickly remove the local and accidental spills of lubricating oils in water, the microorganisms and sorbents

are co-introduced as a suspension solution by directly mixing it with aqueous streams or spraying onto the contaminated surface.

CONCLUSION

We have developed a biotechnology for the elimination of pollution of natural water with mineral, semi-synthetic and synthetic oils of various nature on the basis of wide application of agricultural waste, namely husks of buckwheat, barley, oat and wheat in combination with the selected association of oil-oxidizing bacteria. The sorbent particle size shall not be less than 0.018-0.036 mm at concentrations of up to 50 mg/l, and the number of microorganisms shall range $102 \cdot 10^6$ - $106 \cdot 10^6$ cells/cm^3.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

[1] Litvinova A. What is the processing of waste oil? / A. Litvinova // «Time» environmental awareness website. waste recycling. – 2014. – 12 p.
[2] Mirfaizova A.A. Fate of waste oil: a view from Kazakhstan / A.A. Mirfaizova // Chemical safety problems. Chemistry and Life. Eco True of Kazakhstan, 2005. – No.517. – 7 p.
[3] Debuan T., Hansele P. Aliphatische dicarbon saurester fur Sinthetische Schi-merole / T. Debuan, P. Hansele // Erdolund Kohle-Erdgas-Petroleum.-1984,-Bd. 34, №11.-S. 511-514.
[4] Morozov N.V. Lubricating motor oil and its disposal with oil-oxidizing microorganisms / N.V. Morozov, I.M. Ganiev, Z.S. Petrov // Proceedings of VIII Moscow International Congress "Biotechnology: State and Prospects of Development" – M.: 2015. – Part 2. – Pp. 111-113.
[5] http://www.ecobrain.ru/
[6] Morozov V.N. Biotechnical System and Technology for Hydrocarbon-Containing Waste Water Purifying In a Controlled Manner / V.N. Morozov, N. V. Morozov // Research Journal of Pharmaceutical, Biological and Chemical Sciences. – 2015.-6(6)-P.1703-1710.
[7] Kulakov S., Sorokin G., Mikhaleva E. Prediction of Wear in Biodegraded Lubricants. Proc. of the Inter. Conf. on ENVIRONMENTAL ASPECTS IN PRODUCTION AND UTILIZATION OF LUBRICANTS. Sopron, Hungary, 1995, p.p.- 291-29S.
[8] Pleshakova E.V. Biodegradation of surfactants and mineral oil and its genetic nature / E.V. Pleshakova // Saratov. – 1998. – Author's abstract Cand.Sc. (Biology). 17 p.
[9] Morozov N.V. Semi-synthetic and synthetic oils and their disposal with the selected hydrocarbon-oxidizing microorganisms / N.V. Morozov, A.V. Ivanov, I.M. Ganiev, E.L. Tuimatova // Proceedings of VIII Moscow International Congress "Biotechnology: State and Prospects of Development" – M.: 2013. – Pp. 248-250.
[10] Morozov N.V. Environmental biotechnology: purification of natural and sewage water with microphytes. Kazan, TSPU, 2001. – 394 p.
[11] Morozov N.V. Environmental biotechnology in the quality regulation and management of land and water resources in terms of pollution ... Coll. "Environmental biotechnology in the XXI century: Ed. Ph.D., Professor N.V. Morozov - Kazan.: TSHPU, 2010. - Pp. 10-24.
[12] Morozov V.N. Biotechnical System and Technology for Hydrocarbon-Containing Waste Water Purifying In a Controlled Manner / V.N. Morozov, N. V. Morozov // Research Journal of Pharmaceutical, Biological and Chemical Sciences. – 2015.-6(6)-P.1703-1710.