






Article

Degradation of Oil and Petroleum Products in Water by Bioorganic Compositions Based on Humic Acids

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Abstract: The use of nature-like technologies, leading to acceleration of the processes of natural destruction of pollutants, is one of the promising directions for oil and petroleum product degradation in the environment. The joint use of oil-degrading bacteria and natural polymers with the properties of surfactants and humic acids (HAs) in bioorganic compositions is effective. In this study, humic acids from reed peat, which have a critical micelle concentration (CMC) of 0.6 g/L, were used as an organic component. Oil-degrading bacteria of the genera *Rhodococcus* and *Pseudomonas*, which have an increased biodegrading ability in relation to crude oil and waste engine oil, were used as a bacterial component. Mono- and polybacterial bioorganic compositions based on these components are proposed. The emulsification index is maximum (94% ± 2%) using a bioorganic composition based on the association of strains of three bacteria. Analysis of films of model petroleum products in vitro showed a high degree of their destruction after 7 days using monobacterial bioorganic compositions (up to 80%) and after 2 days using a bioorganic composition based on 3 strains (almost 90%). A high ability to stabilize emulsions of humic acids and develop bioorganic compositions has been established, which indicates a significant potential for their use for cleaning the environment from oil pollution. The use of a combination of humic acids and oil-degrading bacteria (all strains) makes it possible to achieve the maximum stabilizing effect of emulsions: the transmission coefficients of emulsions are 37–75% lower relative to control (oil-polluted water) and 50% lower in relation to humic acids.

Keywords: nature-like technologies; sustainable development; humic acids; bacteria; oil; petroleum products; emulsification; biodegradation



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1. Introduction

Environmental pollution from oil and petroleum products represents one of the most pressing ecological challenges [1–3]. Leaks and accidental spills regularly occur during the exploration, production, transportation, processing, and storage of petroleum products. Substances contained in oil enter aquatic and terrestrial ecosystems and have a toxic effect on living organisms [4–6]. The ingress of oil hydrocarbons and oil products into the environment is the most widespread pollution of natural waters and soils [7–9]. At the same time, the existing methods for eliminating the consequences of oil pollution, including

the use of standard cleaning methods such as mechanical, physical, physicochemical, and biological treatment, do not always meet the requirements of environmental safety [10–12]. They can cause a threat of secondary pollution, are limited by the area of the source of pollution, and are often unprofitable from an economic point of view [13–16].

Now, bioremediation is becoming a promising way to clean up oil-contaminated ecosystems [16–19]. The process of bioremediation, which is understood as the use of microorganisms for detoxification or removal of organic toxicants due to the various metabolic capabilities of the bacteria themselves [20], is a developing and promising method for the biodegradation of oil hydrocarbons in environmentally sound components. Studies have shown that in oil environments, such as oil spill areas and oil reservoirs, there is a wide variety of bacterial species that can decompose hydrocarbons [21]. Their choice is determined by the composition of petroleum hydrocarbons and by environmental factors [22]. However, single strains of microorganisms cannot decompose all oil fractions due to the lack of specific enzyme systems [23]. Therefore, the elimination of hydrocarbon contamination requires the combined action of many functional bacteria or the use of additional agents, including chemical ones. The combined use of chemical and microbiological methods to eliminate oil pollution is promising, and it relies upon a synergy between dispersants and oil-oxidizing bacteria that can both decompose and assimilate oil hydrocarbons. Humic acids, being widespread and readily available organic components of various natural media (soils, natural waters, coal, and peat), can be advantageously used as dispersants. The reactivity of humic acids with respect to petroleum hydrocarbons is provided by the presence of hydrophobic fragments in their structure [24,25]. Eljack and Hussam [24] argue that the interactions of humic acids with organic pollutants lead to their dissolution, thus increasing their mobility and bioavailability in the environment [25]. The dependence of these interactions on the physicochemical characteristics of humic acids, including the content of aromatic and aliphatic structural fragments, has been amply investigated [26]. The successful use of humic acids for washing oil-contaminated substrates under real conditions was first demonstrated when cleaning a sandy aquifer from diesel fuel [27].

A number of studies aiming at understanding the solubilization of hydrocarbons from samples of commercial fuels and crude oil in an aqueous environment containing various amounts of soluble humic acids have been carried out [28,29]. They showed that humic acids induce the dissolution of naphthalene hydrocarbons by a factor of 5–10 [29]. Robles-Mora et al. [30] described the efficient removal of aromatic hydrocarbons by humic acids from contaminated aquifers. At the same time, the solubilization of oil with humic acids and the subsequent formation of non-toxic, stable complexes called “HAs-hydrocarbons” cannot be considered the final stage in the remediation of ecosystems. At the same time, the addition of oil-degrading microorganisms capable of decomposing both the products of oil interaction with HAs and oil products that have not reacted with humic acids leads to a more complete and rapid remediation of ecosystems. However, there are difficulties in choosing the correct ratios of reagents (humic acids and bacteria) as well as the concentrations of humic acids, which are a nutrient substrate for bacteria. In our previous works [31,32], we studied humic acids isolated from peats of various origins in the Tula region and oil-degrading bacteria of the genus *Rhodococcus* as agents for accelerating water purification from oil products. It is known that bacteria of the genus *Rhodococcus* effectively degrade oil fractions-n-alkanes with different carbon chain lengths [33]; *Pseudomonas*, on the other hand, consume hydrocarbons that are part of both the benzene and benzene–alcohol fractions, including mono- and polyaromatic hydrocarbons [34]. These features of these genera must be taken into account in the process of selecting destructor microorganisms for bioorganic compositions. Therefore, our data indicate that for effective cleaning of oil pollution, it is advisable to use a combination of various oil-degrading strains belonging to different genera of microorganisms that have a wide range of enzyme systems.

Therefore, in this study, we considered the effect of previously developed compositions based on bacteria of the genus *Rhodococcus* with the addition of an additional

oil-degrading strain from the genus *Pseudomonas* and humic acids isolated from cane peat on the emulsification and degradation of crude oil and used engine oil (UEO).

2. Materials and Methods

2.1. Reagents and Materials

Humic acids isolated from the reed peat of the Ryazan region by alkaline extraction [19] were used. Humic acids were used individually and as part of compositions with oil-degrading microorganisms of the genus *Rhodococcus* (*Rhodococcus erythropolis* S67, *Rhodococcus erythropolis* X5) and the genus *Pseudomonas* (*Pseudomonas fluorescens* 142 NF). The bacteria were provided by the Laboratory of Plasmid Biology of the Institute of Biochemistry and Physiology of Microorganisms, named after G.K. Skryabin of the Russian Academy of Sciences [35], and the All-Russian Collection of Microorganisms. Working solutions of humic acids at a concentration of 50 mg/L were prepared in accordance with the previously described procedures [36,37]. Deighed portions of humic acids have been dissolved in 0.1 M NaOH; a neutral pH value was achieved by adding 0.05 M HNO₃ or 0.05 M NaOH, and the solutions were adjusted to the mark with a supporting electrolyte solution, 0.1 M NaNO₃ [19]. As model toxicants, crude oil from the refinery of JSC GAZPROMNEFT-MNPZ and used engine oil of the Shell HELIX ULTRA 5W40 brand were used. The experiment was carried out in distilled water at a temperature of 22 °C.

2.2. Determination of Surface-Active Properties of Humic Acids

The standard method using stalagmometry, operating on the principle of a falling drop, made it possible to deduce a correlation between the surface tension and the concentration of humic acids [38]. The reference solution was 0.1 N sodium hydroxide. Working solutions with concentrations of humic acids of 0.0625, 0.125, 0.25, 0.5, 1, 1.5, 2, and 2.5 g/L were prepared by dissolving the sample humic acids in a 0.1 N sodium hydroxide solution. Humic acid solutions in sodium hydroxide were thermostated at a temperature of 20 °C [38]. Surface tension was calculated by Formula (1):

$$\sigma = \frac{\sigma_0 \cdot \rho \cdot n_0}{\rho_0 \cdot n} \quad (1)$$

where σ is the surface tension of the investigated liquid, J/m²; σ_0 —surface tension of water, J/m²; ρ is the density of the investigated liquid, g/cm³; ρ_0 is the density of water, g/cm³; n is the number of drops of the investigated liquid; and n_0 is the number of NaOH drops.

2.3. Cultivation of Microorganisms

Microorganisms were cultured in Lysogeny broth (LB) and Evans mediums. To prepare 1 L of LB medium, 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl were added to a 1000-mL beaker, then these substances were dissolved in 800 mL of distilled water and brought to a volume of 1 L. Media were autoclaved in a VK-75-01 steam sterilizer (TZMOI, Moscow, Russia) at 121 °C for 20 min [37]. To prepare 1 L of Evans medium, the following were added to a 1000 mL beaker: 8.71 g K₂HPO₄, 1 mL 5 M NH₄Cl solution, 1 mL 0.1 M Na₂SO₄ solution, 1 mL 62 mM MgCl₂ solution, 1 mL 1 mM CaCl₂ solution, and 1 mL 0.005 mm solution (NH₄)₆Mo₇O₂₄. The solution of microelements was 1 mL. Composition: in 1% HCl solution, (g/L): ZnO–0.41; FeCl₂–2.9; MnCl₂–1.28; CuCl₂–0.13; CoCl₂–0.26; H₃BO₃–0.06. HCl conc.—up to pH = 7.0 [37].

Sowing and cultivation of strains of microorganisms in liquid nutrient media were carried out in shake flasks. 100 mL of liquid nutrient media was added to the flask and autoclaved in a steam sterilizer for 1 h at $t = 150\text{--}160$ °C and a pressure of 1 atmosphere. Then, in a laminar box, the selected microorganisms were inoculated with a microbiological loop. Microorganisms *Rhodococcus erythropolis* S67, *Rhodococcus erythropolis* X5, and 2% n-hexadecane were added to the flask containing LB medium; in Evans medium—*Pseudomonas fluorescens* 142 NF and 2% naphthalene. Microorganisms were cultivated in an Excella E25 orbital shaker (Eppendorf, Enfield, CT, USA) at 180 rpm at 37 °C for 1–6 days.

2.4. Preparation of Bioorganic Compositions

Bioorganic compositions were prepared by inducing a suspension of oil-degrading bacteria and solutions of humic acids. Mono- and polybacterial biocompositions were used in the work (Figure 1). The ratios of the components were used in accordance with previous experiments [33,34].

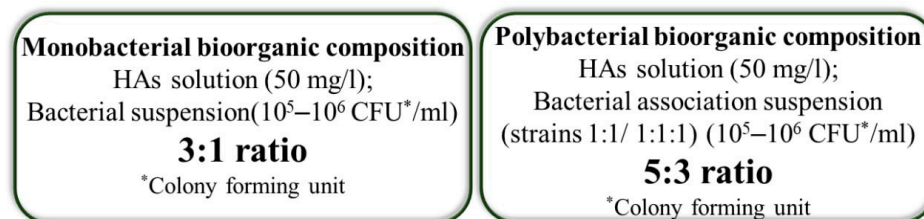


Figure 1. The developed compositions used in the work.

2.5. Investigation of the Influence of Humic Acids and Oil-Degrading Microorganisms on the Solubilization of Oil Hydrocarbons

2.5.1. Determination of the Emulsifying Ability of Humic Acids and Bioorganic Compositions In Vitro

During the experiments, 30 mL of sterile water were filled into the control Petri dishes (d = 15 cm) previously sterilized in a VK-75-01 autoclave for 1 h at t = 150–160 °C and a pressure of 1 atmosphere. Afterwards, 5% vol. oil product (crude oil or used engine oil) was carefully dropped on the water surface.

Separately, a model pollutant (5% vol.) and humic acids (50 mg/L) (3% vol.) were added to 30 mL of sterile distilled water. In the third variant of the experiment, humic acids (50 mg/L) and microorganisms *Rhodococcus erythropolis* S67/*Rhodococcus erythropolis* X5/*Pseudomonas fluorescens* 142 NF (10⁵–10⁶ CFU/mL) were added to Petri dishes. The cups were closed and left on a flat horizontal surface in diffused light at room temperature. The assessment of the state of drops of hydrophobic compounds was carried out after 2, 24, 48, 72, and 168 h. The experiment was carried out in distilled water at room temperature.

2.5.2. Determination of the Emulsion Index of Humic Acids and Bioorganic Compositions

The emulsion index test determines whether a surfactant has emulsifying properties or not by calculating the ratio of the height of the stable emulsion layer to the total height of the liquid formed after shaking and holding for 24 h. To determine the emulsification index, a hydrophobic substrate was added to HAs separately or with bacteria (3:1): crude oil/used engine oil (4.0 mL).

The mixture was shaken vigorously for 2 min. After 24 h, the height of the stable emulsion layer was measured.

$$E = \frac{\text{height of the emulsion layer}}{\text{total height}} \cdot 100\% \quad (2)$$

The emulsion index after 24 h (E₂₄) is the height of the emulsion layer divided by the total height multiplied by 100 [39].

2.5.3. Scanning Electron Microscopy (SEM)

Before measurements, the samples of humic acids and bioorganic compositions were mounted on a 25 mm aluminum specimen stub and fixed with graphite adhesive tape. Metal coating with a thin film (10 nm) of gold/palladium alloy (60/40) was performed using the magnetron sputtering method as described earlier [40]. The observations were carried out using a Hitachi SU8000 (Hitachi, Tokyo, Japan) field-emission scanning electron microscope (FE-SEM). Images were acquired in secondary electron mode at a 5 kV accelerating voltage and at a working distance of 8–10 mm. The morphology of the samples was studied, taking into account the possible influence of a metal coating on the surface [41].

2.5.4. Investigation of the Stabilization of Oil Hydrocarbon Emulsions in the Presence of Humic Acids and Oil Degrading Microorganisms

A solution of HAs with a concentration of 50 mg/L was added in order to study the stabilization of crude oil and used engine oil emulsions in the presence of humic acids. During the experiments, sterile water with model toxicants served as a control. In variants of the experiment with microorganisms, a suspension of microorganisms (10^5 – 10^6 CFU/mL) was added to solutions of humic acids (50 mg/L) and a supposed pollutant (5% by volume). The analyzed solutions were placed in an ultrasonic bath for 2 min and kept for 24 h. Samples were taken at a depth of 4 cm, and the optical density was measured at a wavelength of 535 nm on an SF-104 spectrophotometer (Akvilon, Podolsk, Russia). The obtained values of optical densities were recalculated to the transmittance (T , %), whose values served as a criterion for the stability of the oil-in-water emulsion according to Formula (3) [42,43]:

$$T = 10^{-D} \cdot 100\% \quad (3)$$

where D is the optical density.

2.5.5. Determination of the Physicochemical Properties of Model Toxicants

Determination of the physicochemical characteristics of model toxicants (kinematic viscosity, density) was carried out according to the standard methods of all-Union State Standard 33-2016 “Oil and oil products” [44]. The data are presented in Table 1.

Table 1. Physicochemical properties of model toxicants.

Toxicant	Parameter	Density at 15 °C, kg/m ³	Kinematic Viscosity, mm ² /c
Crude oil		880.2	41.4
Waste engine oil		890.4	54.2

3. Results and Discussion

3.1. Determination of the Critical Concentration of Micelle Formation of Humic Acids

The structure of humic acids includes various hydrophilic and hydrophobic fragments that provide the amphiphilic nature of HAs [45–47]. Due to this, humic acids can form micelles at a concentration above the critical micelle formation concentration (CMC). A further increase in the concentration of HAs leads to the formation of supramolecular aggregates of HAs due to nonvalent interactions [48]. The surface tension isotherm of the analyzed humic acids was obtained using the stalagmometry method (Figure 2).

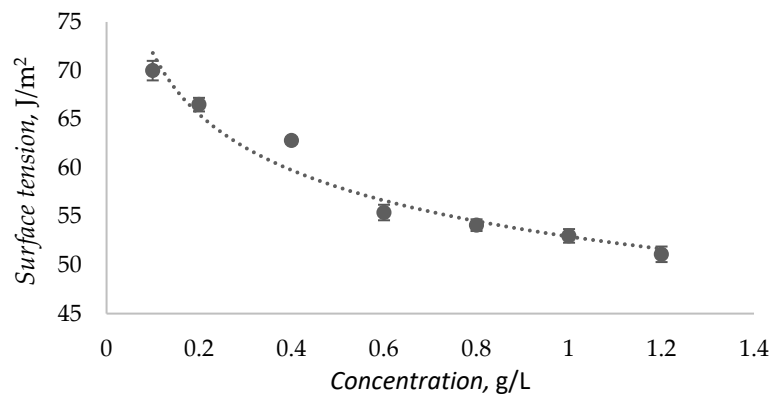


Figure 2. Surface tension isotherm of humic acids.

The HAs isotherm $\sigma = f(C)$ consists of two sections and has a characteristic form for surfactants. At a concentration lower than the CMC, HAs are in the form of separate molecules of surface active substances, and at concentrations above the CMC, the molecules

of humic acids are collected in micelles. For the analyzed humic acids, the CMC was 0.6 g/L. The obtained data on the value of CMC HAs are consistent with the data from other works: 0.6–0.8 g/L [49], 0.8 g/L [50]. The low CMC value determines the high ability of surfactants and humic acids to reduce the surface tension of the system [51,52].

3.2. Study of the Effect of Humic Acids and Bioorganic Compositions on the State of Oil Hydrocarbon Films

After applying crude oil or used engine oil (5% vol.) in model distilled water (100 mL) in the control sample, an equivalent spreading of toxicants over almost the entire surface area of the water was observed. After 7 days of exposure, no significant differences were noted in the pattern of distribution of all tested objects (Figure 3a). In the variant of adding humic acids to crude oil, after 7 days of the experiment, the oil slick was stratified into 2 parts with torn edges (Figure 3b). Subsequently, the destruction of the oil emulsion into separate components with torn edges occurred. For used engine oil, a different picture was observed: an oil film changed color and thinned by 90% (Figure 3b).

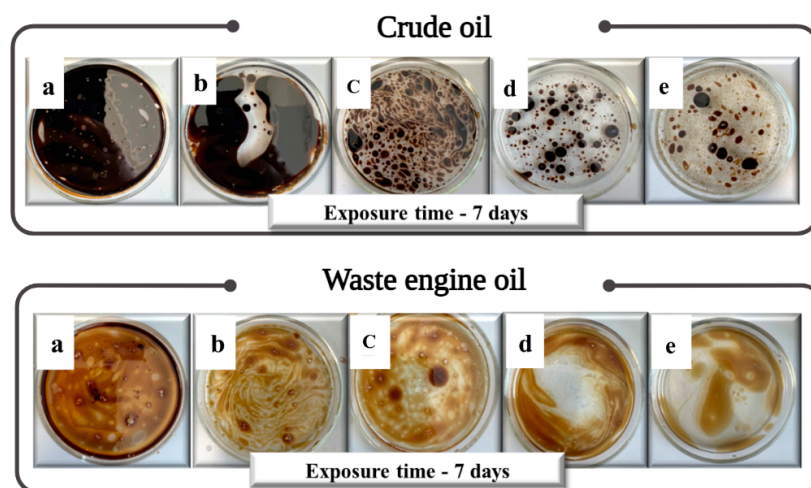


Figure 3. Distribution of crude oil and used engine oil on the surface of humic acids and bioorganic compositions: (a)—control; (b)—HAs; (c)—HAs + R.X5; (d)—HAs + R.S67; (e)—HAs + Ps.142 NF.

The use of bioorganic compositions based on HAs and bacteria significantly affects the emulsification of crude oil; after 7 days of exposure, intensive crushing and tearing of the film edges is observed. There was the maximum increase in the number of drops due to the emulsifying effect of HAs and bacteria (Figure 3c); in Figure 3d,e, the number of drops decreased due to consumption by bacteria. Moreover, the visual degrading effect of bioorganic compositions decreased in the series: “HAs + *Pseudomonas* 142 NF” > “HAs + *Rhodococcus* S67”, “HAs + *Rhodococcus* X5”. The distribution of oil emulsions under the action of the compositions was different: unlike oil emulsions, used engine oil films did not break into separate drops, but they were depleted, and a thinner, especially in the peripheral part, inhomogeneous film with long ragged edges was formed (Figure 3b–d).

The difference in the effect of the biodegrading action of bioorganic compositions and HAs may be associated with toxicants physicochemical properties (Table 1), especially viscosity. Viscous oil products are prone to the removal of dense fragments on the water surface due to their low density and dispersion. Moreover, used engine oil may have a different trend owing to the presence of many other compounds and additives like antifoam, dispersants for metal particles, metal surface detergents, phosphorus-containing additives as antiwear, etc. So, for used engine oil, a different mechanism of biodegradation is possible in comparison with crude oil.

The results of the experiment with the degradation of crude oil and used engine oil films in the presence of a bioorganic composition based on HAs and associations of used bacteria (polybacterial compositions) at a ratio of HAs to a suspension of microorganisms

of 5:3 showed that the degradation of crude oil and used engine oil occurred much faster and reached about 90% after 48 h (Figure 4). Figure 4 shows the results of the influence of the composition on the state of crude oil films and used engine oil depending on the time of the experiment (2 h; 48 h).

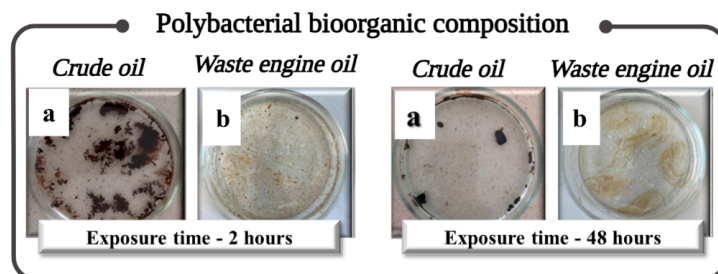


Figure 4. Influence of bioorganic composition based on humic acids and association of oil-degrading bacteria (*Rhodococcus* S67, *Rhodococcus* X5, and *Pseudomonas* 142 NF) on the state of films of model toxicants.

After 2 h, there was significant destruction of the crude oil film and almost complete destruction of the used engine oil film. After 48 h, the extent of degradation of petroleum products was almost 90%. A thin biofilm formed by bacteria on humic acids was detectable on the water surface [53]. The developed bioorganic compositions were studied by scanning electron microscopy (Figure 5). In the photographs, the initial structure of humic acids (Figure 5a) is observable, resulting from an accumulation of plates and crystals 3–15 μm in size. Upon receipt of bioorganic compositions, the surface of humic acids is covered with microorganisms (Figure 5b), which, after 48 h of the experiment, turn into a fairly dense biofilm (Figure 5c,d).

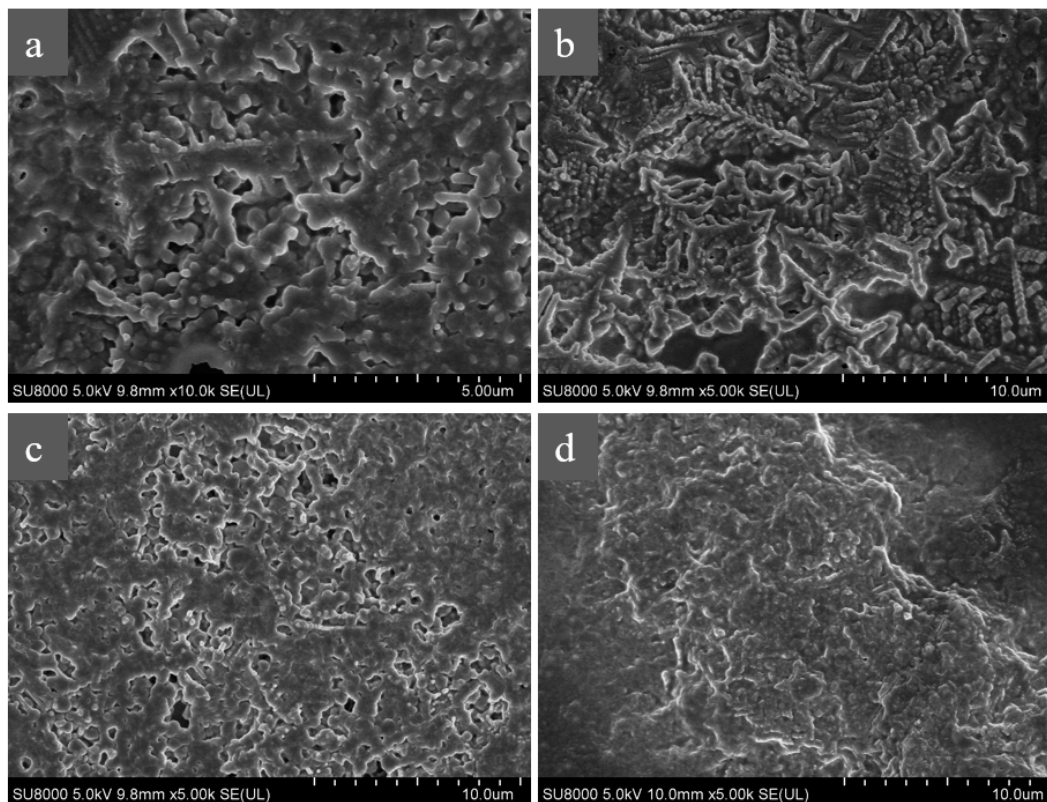


Figure 5. Overview SEM images of the prepartate based on the commercial preparation of humic acids and the association of oil-degrading bacteria (*Rhodococcus* S67, *Rhodococcus* X5, and *Pseudomonas* 142 NF) after 2 h of the experiment (a); high-magnification SEM image of the corresponding surface (b); and after 48 h of exposure (c,d).

The mechanism of degradation of oil pollution by bioorganic compositions based on humic acids and oil-degrading microorganisms can be explained as follows: Humic acids emulsify (disperse) oil and form smaller, more stable aggregates (complexes, drops). These small aggregates become more accessible for contact with bacteria due to a decrease in the energy barrier (the DLVO theory [54,55]) (Figure 6).

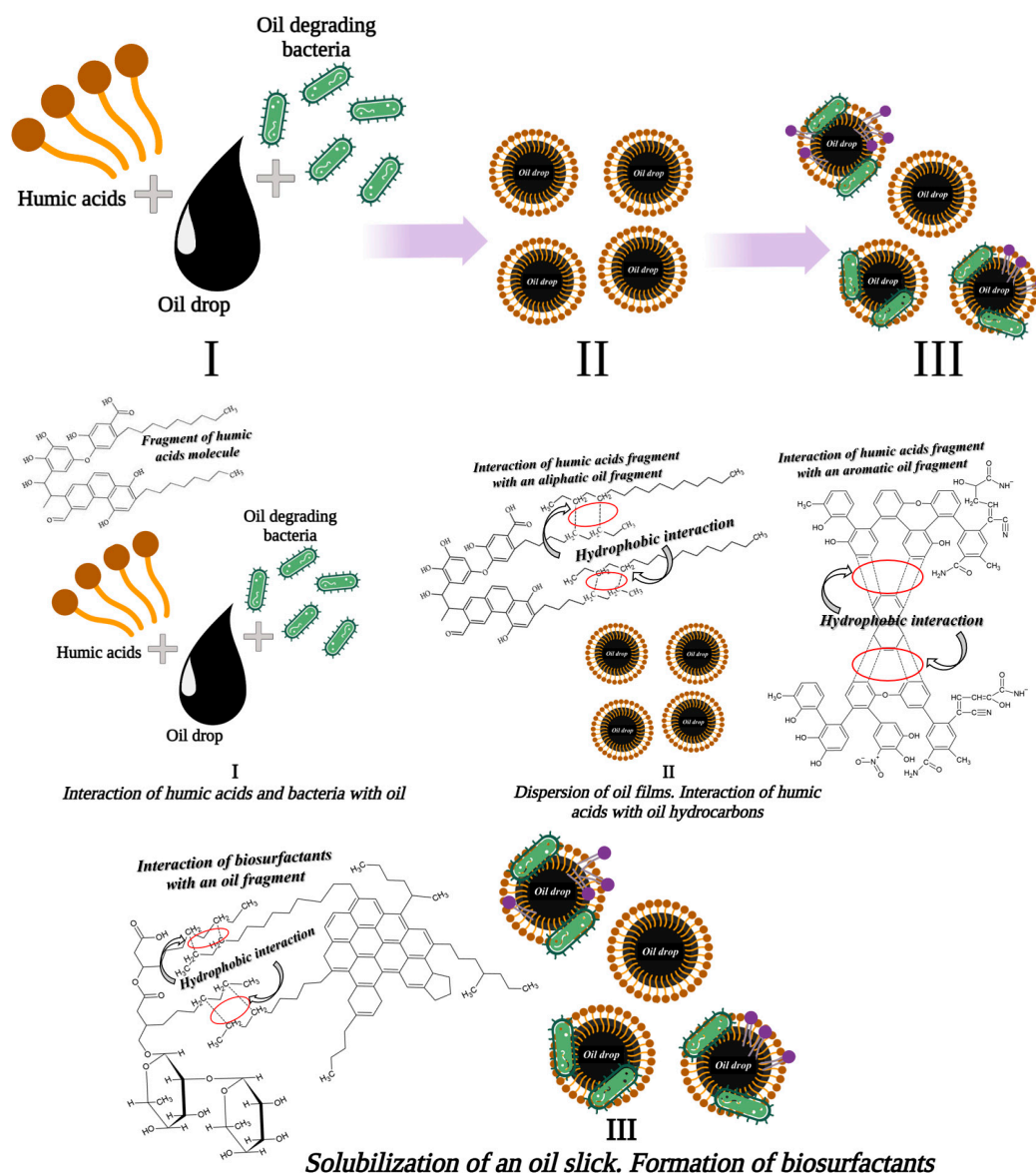


Figure 6. Stages of the mechanism of bioorganic compositions action in relation to oil hydrocarbons.

Also, complexes of “humic acids–oil” can be attractive food for bacteria due to the presence, on their surface, of functional groups and fragments belonging to HAs. Individual HAs molecules can also act as “primers” for bacterial reproduction [56]. When oil hydrocarbons are consumed, bacteria produce biologically active surfactants, or biosurfactants, which additionally degrade oil hydrocarbons [57].

The interaction of bioorganic compositions with used engine oil obviously proceeds according to a slightly different mechanism, so there is no visual fragmentation of the used engine oil film when exposed to the composition; only its thinning occurs. In this case, the emulsifying ability of HAs is clearly not enough to disperse a more viscous pollutant. However, due to the interaction with HAs, a looser film structure can form, making the oil molecules more accessible to bacteria.

The production of surfactants by bacteria obviously increases using bioorganic compositions based on humic acids and associations of bacteria of the genera *Rhodococcus* and *Pseudomonas*, and a distinct dispersion of used engine oil also occurs; its degradation begins to resemble the degradation of crude oil.

3.3. Determination of the Emulsification Index of Humic Acids and Bioorganic Compositions

The secondary evaluation of the emulsifying ability of humic acids and compositions based on them was estimated by the emulsification index [39]. This indicator is based on the property of surfactants to form an emulsion during shaking the culture liquid with oil hydrocarbons. The results of the experiments are presented in Table 2.

Table 2. Emulsification index of humic acids and compositions based on them.

Variant	Crude Oil	Engine Motor Oil
HAs	37 ± 1	41 ± 1
<i>R.erythropolys</i> X5	75 ± 1	64 ± 2
<i>R.erythropolys</i> S67	78 ± 2	69 ± 1
<i>Pseudomonas fluorescens</i> 142 NF	50 ± 1	50 ± 2
«HAs + <i>R.erythropolys</i> X5»	81 ± 2	80 ± 3
«HAs + <i>R.erythropolys</i> S67»	83 ± 2	85 ± 3
«HAs + <i>R.erythropolys</i> X5 + <i>R.erythropolys</i> S67»	87 ± 2	87 ± 1
«HAs + <i>Ps. fluorescens</i> 142 NF»	71 ± 2	73 ± 2
«HAs + <i>R.erythropolys</i> X5 + <i>R.erythropolys</i> S67 + <i>Ps. fluorescens</i> 142 NF»	94 ± 2	79 ± 1

It has been established that suspensions of all analyzed objects are able to emulsify oil hydrocarbons (Table 2). The values of the emulsification indices were 31–94%, depending on the variant of the experiment. For HAs separately, the emulsification index was 31–41%, depending on the type of oil product. For bioorganic compositions, the emulsification index increased with the simultaneous use of all analyzed strains (using crude oil as a hydrophobic substrate). The maximum emulsification index in the case of used engine oil was 87 ± 1 (using the composition “HAs + *R.erythropolys* X5 + *R.erythropolys* S67”).

3.4. Investigation of the Stabilization of Oil Hydrocarbon Emulsions in the Presence of Bioorganic Compositions Based on Humic Acids and Oil-Degrading Microorganisms

A comparison of the ability of HAs and bioorganic compositions to stabilize the emulsion was carried out four hours after mixing the components. Data on the stabilization of aqueous emulsions of pollutants (crude oil and used engine oil) with humic acids at room temperature for the analyzed samples are presented in Figure 7.

It was established that humic acids not only have an emulsifying ability but also stabilize emulsions of oil and petroleum products in water [43]. The values of the transmittance of aqueous emulsions of crude oil and used engine oil in the presence of HAs are 8–10% less than in the control sample (Figure 7). The durability of emulsion systems is associated with the formation of adsorption layers on the oil-water interface [58]. These systems may consist of asphaltenes, paraffins, mineral salts, resinous compounds, or mechanical sulfur impurities. These substances form a strong mechanical barrier on small water droplets distributed in the oil phase, which prevents the separation of water. The addition of surfactants, such as humic acids, has a great influence on the formation and stabilization of oils and oil emulsions. The use of a bioorganic composition based on HAs and the association of bacteria makes it possible to achieve the maximum stabilizing effect of emulsions: for the composition “HAs + *R.X5* + *R.S67* + *Ps.142 NF*”, the transmission coefficients of emulsions are 37–75% lower relative to control and 50% in relation to HAs.

The effectiveness of the use of these bioorganic compositions for the detoxification of oil-contaminated waters is realized due to the action of HAs as both emulsifiers and stabilizers of the emulsion of oil and petroleum products. In addition, humic acids can act

as a nutrient substrate (a source of available carbohydrates) for microorganisms involved in the decomposition of oil and petroleum products. Released by microorganisms, especially their associations, biosurfactants contribute to the even greater dispersion of petroleum hydrocarbons, increasing their bioavailability.

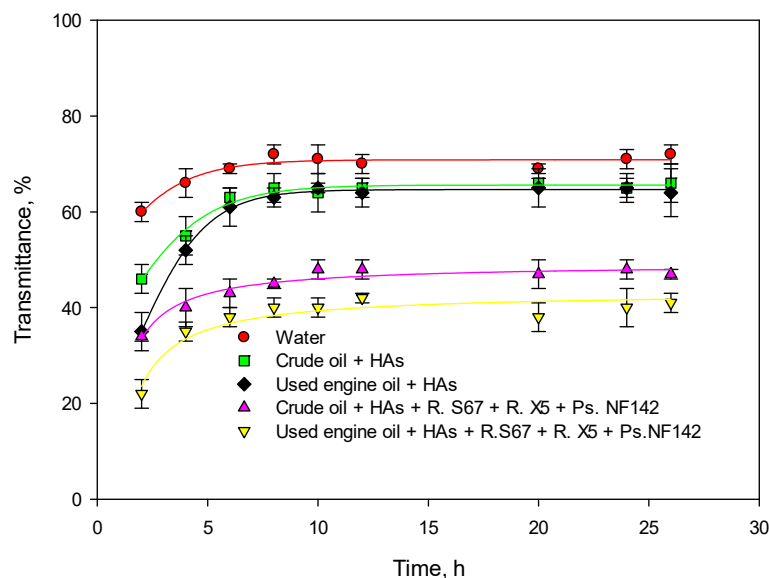


Figure 7. Stabilization of petroleum product emulsions by humic acids and bioorganic compositions.

4. Conclusions

The emulsifying ability of humic acids and bioorganic compositions in relation to crude oil and used engine oil was evaluated. It was found that in the case of the combined use of HAs and bacteria, the maximum emulsification of crude oil and used engine oil was achieved using a bioorganic composition based on HAs and the association of microorganisms *R. S67*, *R. X5*, *Ps. 142 NF*: the emulsion index exceeds the indicators for bacteria and HAs separately from each other and is $94 \pm 2\%$. An *in vitro* analysis of crude oil and used engine oil films confirms the high degree of emulsification of petroleum products using both humic acids and compositions based on them. A high degree of destruction of crude oil and used engine oil films by the analyzed objects was shown for 7 days using monobacterial compositions (up to 80%) and for 2 days using compositions based on the association of three bacterial strains (almost 90%). The mechanisms of destruction in the case of crude oil and used engine oil for 7 days were different; for 2 days they were the same. Stabilization of crude oil and used engine oil emulsions with humic acids and bioorganic compositions does not depend on the strain of bacteria used and is determined by the surface-active properties of HAs.

Author Contributions: Conceptualization, V.P.M., A.P.R. and T.B.C.; formal analysis, methodology L.V.P. and M.M.G.; formal analysis, Y.M.A.; methodology, validation, writing—original draft preparation, writing—review and editing, M.M.G.; writing—review and editing, funding acquisition, V.A.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article.

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