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BIODEGRADATION OF OIL PRODUCTS IN POLLUTED SOIL BY USING THE MIXTURE OF THE POLYMER COMPLEX [CHITOSAN – LACTOSERUM PROTEIN]–ACTIVE SILT

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Abstract. Biodegradation processes of oil products in the polluted soil were studied by using the mixture of the polymer complex [chitosan – lactoserum protein]–active silt. It is shown that all these processes take place due to the synergism of the components which form part of the mixture; the use of the developed biomixture ensures the effective biodegradation of compound oil hydrocarbons, a high degree of cleaning soil from pollutions, except the possible secondary pollution of environment. As a result of biocomposting the cleaned ground containing biogenic substances fits in natural cycle and can be used for realization agricultural work.

Key words: biodegradation, petroleum products, chitosan-lactoserum protein polymer complex, active silt, biomixture.

1. Introduction

During oil outflows the vital functions of biota are repressed to a considerable degree [1, 2]. That is why the problem of recultivation and biodegradation of oil polluted soils becomes the urgent one [3, 4]. Unfortunately, the visible practical success of oil polluted soils biodegradation has not been achieved yet.

Today the most progressive technology in the oil polluted soils cleaning is considered to be the usage of introductive microorganisms [5, 6], strains of fungus, actinomycetes [7-9], bacteria [10-12]. At the same time the microorganisms are able to destroy only the upper layers (15–20 cm) of oil pollutions and have a selective effect on the definite fractions of oil products; the strains do not solve the problems of recultivation, the possibilities of their transformation have also their limits.

To a considerable degree the difficulty of biodegradation is caused by the high concentration of oil products in soil that results in obstacles in the biochemical processes of oxidation and biodestruction. Sorbing oil products on sorbents particles and macromolecules allows decreasing the concentration in the local systems. It is important that a sorbent should degradate by itself in the course of time. Biopolymers are very perspective for sorbing of oil products.

Chitosan is a biopolymer of the future; the interest to it is connected with unique physiological and ecological properties such as biocompatibility, biodegradability, non-toxic physiological activity, and easy access to resources needed for its production. The polymer complexes based on chitosan in combination with other synthetic and biodegradable polymers are of particular interest.

However, the use of the mixture of bio- and synthetic polymers and microorganisms in biodegradation processes of oil products in the polluted soil has not been sufficiently studied.

The aim of this work is the study of biodegradation processes by using the mixture of the polymer complex [chitosan–lactoserum protein (ChS-LP)]–active silt.

2. Experimental

The soil, used in this work, contains oil products left after oil outflows; it was taken in the industrial area of the Volzhsky Chemical Complex.

The mixture, which consists of ChS-LP polymer complex and active silt, was used as an agent accelerating biodegradation processes.

The advantages of the polymer complex are: accessibility of raw materials, non-toxicity, biodegradability and ecological safety [13, 14].

The active silt from Volzhsky waste disposal plant was used as the test-culture. It consists of the filiform bacteria, *Bodo ovatus, Oikomonos mutabilis, Euglena visidis, Amotba limax, Arcella centropyxis, Arcella diskodes, Eoglipha leavis, Vorticella convallaria, Vorticella mikrostoma, Epistulis plikatilis, Carchesium*

polipinum, Opercularia coarktata, Fhabdostyla ovum, Aspidiska costata, Lionotus lamella, Amphileptus carchesi, Colpidium colpoda, Chilodonella uond, Lakrumaria pupula, Stulonicha mvtilis, Oxitricha pelionella, Euplotec patella, Prorodon teres, Tokohrya mollis, Podophrya fixa, Rabdofrya, Monostyla, Cathyrnia, Notommata ansata, Rotatria, Philodina roseola, Colurella, Nematoda, Aelosoma. The bacteria and microorganisms that form part of active silt are able to use different materials of natural and synthetic origin for their nutrition. Moreover, the active silt is the source of biodegradants, nitrogen and the organic component of the soil.

In the spring-summer period the oil polluted soil was cultivated on planed and balanced plot by the initial mixture of the polymer complex [chitosan–lactoserum protein]–active silt. After that the soil was loosened to 15–20 cm for the improvement of moisture and aeration. The optimum moisture of the soil essential for ensuring the favorable conditions of microorganisms vital functions must be 50–60 %.

Biodegradation processes of oil products have been studied for 90 days at the temperature of 283 K, the optimum temperature range was 298–318 K. A cover material was used for maintaining the optimum temperature. Every month the loosening was made to a depth of 50–55 cm, and the soil was watered whenever its humidity achieved 50 %. Besides, the periodic soil loosening prevented the moisture evaporation. The decreasing of oil saturation (oil destruction) index indicated to the efficiency of cultivation of the worked soil. To provide a complete soil recultivation in autumn it was exposed to the phytoremediation – the soil sowing by annual and biennial seeds.

The optimum ratio of the mixture "oil polluted soil/ sorbent/active silt" was found by the formula describing the dependence of the degree of oil product biodegradation on the content of sorbent in the mixture, received by data handling, using the following dependence [15]:

$$S(n) = k \cdot (1 - e^{-n/T}) \quad (1)$$

S – degree of oil product biodegradation, %; n – ratio of soil quantity (volume unit) to the sorbent quantity (volume unit); k – scale coefficient, used for calculation of oil product biodegradation with introduced sorbent depending on coordinate scale; T – time constant of oil product biodegradation with introduced sorbent; $e \approx 2.718$, for all that, the optimum components ratio was chosen in the point of intersection function graph $S(n)$ with horizontal line, which is smaller than a steady-state value to 3–5 %.

The value of the optimum doze of active silt was determined according to the formula describing the dependence of the degree of oil product biodegradation on doze of active silt, received by data handling, using the following dependence [15]:

$$S(d) = k_1 \cdot (1 - e^{-d/Q}) \quad (2)$$

d – doze of active silt, mg/kg soil; k_1 – scale coefficient, used for calculation of oil product biodegradation with introduced active silt depending on coordinate scale; Q - time constant of oil product biodegradation with introduced active silt, for all that, the optimum doze of active silt was chosen in the point of intersection of function graph $S(d)$ with horizontal line, which is smaller then steady-state value to 3–5 %.

In this work 50 g of the developed biomixture was used for every 1 m^2 of the soil for biodegradation of oil polluted soil; the optimum polymer complex: active silt ratio is 1:4.

The residual index of oil products in the soil was used for valuation of decomposition intensity. It was determined using spectra-photometrical method (photo-electro-calorimeter "KFK-2") and weight method [16].

The number of the main groups of microorganisms, which take part in biodegradation of oil products, was defined by sewing the soil suspension, using the dilution technique in the dense nutrient medium [17].

The measurement of mass concentrations of the oil products in waste waters samples was carried out using the fluorescent method by means of "Fluorat-02" liquid analyzer [18].

The quantitative and structural-group composition of residual oil products was determined by methods of the quantitative NMR-spectroscopy [19] and liquid chromatography [20], using "Mercury-300" and "Lumachrom" apparatuses, respectively.

3. Results and Discussion

The proposed method of oil product biodegradation is based on the introduction of biological active mixture of ChS-LP polymer complex-active silt into the soil. The given mixture is used for decreasing or full degradation of different structured oil hydrocarbons, which take place in processes of oxidative destruction and mineralization during the vital functions of hydrocarbons oxidizing microorganisms.

The use of of biomixture ChS-LP polymer complex-active silt in biodegradation processes is very perspective.

ChS-LP polymer complex (Fig. 1) represents the microsubstances with cavities of nanosizes, fixed between each other with hydrogen links, in which the sorbing processes take place. The complex has good sorbing, protective and reconstruction properties [21]. Due to different nature of its functionally-active groups, chitosan withholds the lactoserum protein in the complex, preventing its washing-out under the action of different natural factors.

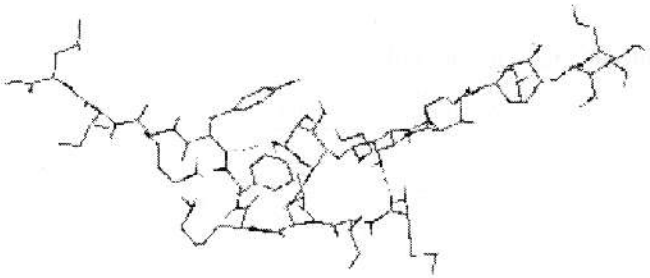

Fig. 1. The structure of the fragment of ChS-LP polymer complex

Biodegradation processes of oil products in the polluted soil take place due to the synergism of the components which form part of the mixture: ChS–LP polymer complex and active silt. The biodegradation processes proceed with of CO_2 and water release, biomass and partially oxidized biologically inert by-products formation. At that time the transformation processes of organic substances are accompanied by the humidification processes. The completeness of oil hydrocarbons biodegradation with increased resistance and with the low content (less 3 wt %) of oil products in the polluted soil is provided by the use of ChS-LP polymer complex-active silt mixture in the proposed method.

Chitosan is the base of the polymer complex and the sorbent that accelerates biodegradation processes of oil products; lactoserum protein is the active nutrient medium for bacteria and microorganisms of active silt. The structure model of ChS-LP polymer complex with sorbed oil products (OP) in its cavities and with active silt is represented in Fig. 2. Moreover, due to the most activity not only of the polymer complex, but also of the active silt, the whole mixture has an increased activity towards the oil products biodegradation. The introduction of such a mixture in the polluted soil affects the habitat of natural microflora, causing the consistent continuity of the microorganisms complex in the soil.

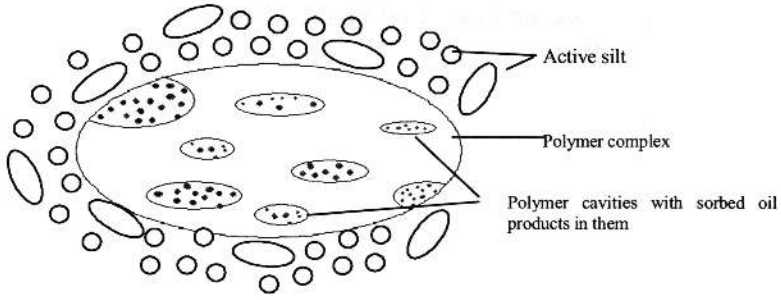

Fig. 2. The structure model of ChS-LP polymer complex with sorbed oil products in its cavities and with active silt

The dynamics of changing the number of microorganisms in soil is represented in Table 1. As it is shown in the table, the number of microorganisms in the polluted soil increases in 12 times, at the same time the number of microorganisms in the processed by the developed mixture soil increases more than in 1500 times. The considerable augmentation of the number of microorganisms is explained by the high activity of added mixture of the polymer complex-active silt. The mixture activates the soil microbiological activity; the biomass of active silt plays the role of an effective fertilizer, which stimulates the development of microbiological biota in oil polluted soil.

Table 1

The dynamics of changing microorganisms number in soil

Compositions	The dynamics of changing microorganisms number in soil (in 1 g), 10^{-6}				
	before processing	after 2 weeks	after 1 month	after 2 months	after 3 months
Soil before processing	6.1	6.5	7.3	7.9	8.4
Soil with OP (control)	0.005	0.008	0.01	0.03	0.06
Soil with OP and with of ChS-LP polymer complex-active silt mixture	0.005	0.09	2.1	5.5	8.1

Table 2

The dynamics of OP content changing in soil

Compositions	The degree of OP biodegradation in soil, %				
	before processing	after 2 weeks	after 1 month	after 2 months	after 3 months
Soil before processing	–	–	–	–	–
Soil with OP (control)	0.02	8.9	18.6	47.8	71.4
Soil with OP and with ChS-LP polymer complex-active silt mixture	0.02	24.8	65.2	81.3	98.9

The combination of chitosan and lactoserum protein in the polymer complex ensures a comparatively larger efficiency of a degree of the oil biodegradation in soil, which can be explained by the presence of a great number of active complex-forming centers due to the chelatogenic protein group, which are able to hold the sorbed particles of oil products firmly and fixedly and to block their repeated return into soil.

The data in Table 2 shows, that the use of ChS-LP polymer complex-active silt mixture in biodegradation process of oil products allows to decrease the content of oil hydrocarbons in 98.9 %.

As it is shown in Table 2, nature is not able to ensure a rather complete cleaning of oil polluted soils. The time of biodegradation is prolonging too much and the degree of biodegradation achieves only 71.4 %, while using the developed mixture the degree of biodegradation achieves 98.9 %.

The process of changing in time of a relative concentration of oil products in soil has been studied in this work (Fig. 3). It is shown that under the action of the developed mixture the considerable changes of concentration of oil products occur for a short period of time.

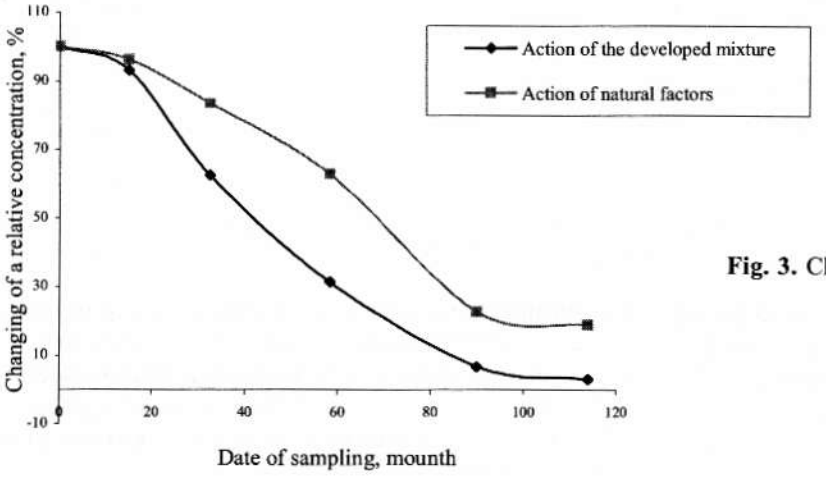

Fig. 3. Changing in time of a relative concentration of oil products in soil

Table 3

The quantitative and structural-group composition of residual OP

Compositions	Biodegradation time, days	Gross content of residual OP, wt %	The quantitative and structural-group composition of residual OP, wt %		
			paraffin	resins	asphaltenes
Soil before processing	–	12.8	80.1	15.2	6.3
Soil with OP (control)	30	11.3	78.1	13.6	5.9
	60	9.8	74.5	13.0	5.2
	90	7.7	71.2	12.8	5.0
Soil with OP and ChS-LP polymer complex-active silt mixture	30	6.8	70.6	12.5	5.2
	60	3.0	55.8	8.2	4.1
	90	0.6	49.2	5.9	3.6

A character of the developed mixture of ChS-LP polymer complex-active silt used for biodegradation processes of oil products in the polluted soil is the high degree of decomposition of compound oil hydrocarbons: resinous and asphalt fractions (Table 3). Due to its functional activity the polymer complex "prepares" asphaltenes for entering the humus layer of soil and allows achieving the high cleaning degree of the soil from pollutions.

The experiments show that the use of the developed biomixture allows achieving a fuller decomposition of resinous and asphalting oils fractions, which can be explained by the presence of microorganisms and enzymes growth substratum in the mixture; all these alive organic beings take part in oxidation of compound polycyclic oil hydrocarbons.

So, bringing of the polymer complex-active silt mixture into the polluted soil extends the sphere of microorganisms are drawn into the process of hydrocarbons decomposition, which helps to use wider potential possibilities of microcenosis. All these processes allow to reduce the time of full biodegradation in the polluted soil with different degree of pollution and achieving a higher degree of oil hydrocarbons transformation into the harmless substances.

As a result of biocomposting the cleaned ground containing biogenic substances fits well in natural cycle and can be used for realization agricultural work. Besides, the used mixture allows not only to clean the soil from pollution, but also to receive the soil material which contains valuable organic substances, ensures the nutrition for wide spectrum of plants and microorganisms.

The use of chitosan and lactoserum protein in the mixture allows to obtain biologically active material enriched with nitrogen and to decrease the amount of mineral nitric fertilizers, which are necessary for composting oil polluted soil, and to exclude the possible secondary pollution of environment.

4. Conclusions

1. Biodegradation of oil products in the polluted soil is carried out by using the biological active mixture, which consists of ChS-LP polymer complex and active silt. The advantages of this biomixture are: accessibility of raw materials, non-toxicity, biodegradability, and ecological safety.

2. Biodegradation of oil products in the polluted soil takes place due to the synergism of the components which form part of the mixture – ChS–LP polymer complex and active silt. As a result of it, expenses on the production of oil-oxidizing bacteria and microorganisms decrease, and expenses on the utilization of active silt disappear.

3. The use of the biomixture polymer complex-active silt ensures an effective biodegradation of compound oil hydrocarbons and allows to achieve a high degree of cleaning soil from pollutions. The given mixture allows obtaining the final biologically active soil material which contains valuable organic substances and nitrogen, ensures the nutrition for wide spectrum of plants and microorganisms and allows to decrease the amount of mineral nitric fertilizers, which are necessary for composting of oil polluted soil, and to exclude the possible secondary pollution of environment.

4. As a result of biocomposting the cleaned ground containing biogenic substances fits well in natural cycle and can be used for realization agricultural works.

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БІОДЕСТРУКЦІЯ НАФТОВИХ ПРОДУКТІВ У ЗАБРУДНЕНОМУ ҐРУНТІ З ВИКОРИСТАННЯМ СУМІШІ ПОЛІМЕРНОГО КОМПЛЕКСУ [ХІТОЗАН–БІЛОК МОЛОЧНОЇ СИРОВАТКИ]–АКТИВНИЙ МУЛ

Анотація. *Вивчено біодеструктивні процеси нафтових продуктів у забрудненому ґрунті з використанням суміші полімерного комплексу [хітозан–білок молочної сироватки]–активний мул. Показано, що всі процеси відбуваються завдяки синергізму компонентів, які утворюють суміш; використання розробленої біосуміші забезпечує ефективну біодеструкцію складних нафтових вуглеводнів, високий ступінь очищення ґрунту від забруднень, за винятком можливого вторинного забруднення довкілля. В результаті біокомпостування очищені ґрунти, що містять біогенні речовини, беруть участь у природному циклі і можуть використовуватись для сільськогосподарських робіт.*

Ключові слова: *біодеструкція, нафтові продукти, полімерний комплекс хітозан-білок молочної сироватки, активний мул, біосуміш.*