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INFLUENCE OF DYNAMIC ACTION ON BIODEGRADATION OF HYDROCARBONS IN SOIL

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Abstract. Petroleum and petroleum products, due to high adsorbing ability of soil, long time remain in it, changing its physical, chemical and biological properties. Restoration of soil fertility after mineral oil influence under natural conditions lasts tens years.

We had been developed the laboratory bioreactor for studying of process of biodegradation of the petropolluted ground in dynamic conditions. The bioreactor represents the cylindrical device on the mobile platform equipped with a dynamometer for measurement of a jet twisting moment. The device is equipped by the mechanical mixing device and an adjustable drive, and as the compressor and rotameter for measurement of the expense of air.

As a test-culture of microorganisms Candida maltosa strain №569 was used which are a part of a biological product "Oleovorin" (firm "Bigor"). For researches was it is taken "Universal" neutral soil.

As the previous experiences have shown that petrooxidizing microorganisms brought by us enter difficult interactions with autochthonic microflora, for cleanliness of experiment soil sterilized twice within an hour at temperature 121ºC. To exclude influence of structure of oil on biodegradation parameters, the further experiences were spent on paraffin. The soil was polluted by paraffin C_{14}-C_{17}.

Soil mixed with the help propeller mixer with various speed of rotation. Control experiments were carried out by placing of identical quantity of a ground in the capacity equal to diameter of the bioreactor. The control capacity was in the same temperature conditions, as the bioreactor.

The greatest difference between speeds of consumption in a static and dynamic mode is observed till 20 o'clock, speed in a dynamic mode exceeded speed of consumption of hydrocarbons in a static mode approximately in several times.

By means of hashing of the soil polluted by hydrocarbons it is possible to lower sharply concentration of hydrocarbons within the first 20 hours, and further dynamic process provides much lower maintenance of hydrocarbons in soil.

Keywords: petropolluted soils, hydrocarbons, petrooxidizing microorganisms, petroleum, petroleum products, Candida maltosa.

1. Introduction

The soil first of all throe from pollution by oil and oil products. Resins and asphaltine components sorb in humus layer, cementing it. Therefore decreases soil pore space, soil particles stick together. As a result of the soil disturbance surface erosion of soils amplifies. In areas of oil extracting soil pollution by oil is accompanied salinification because of contact to the mineralized sewage. As little oil as 2 g in 1 kg of soil does its impracticable for habitability of plants and soil microflora. Pollution of soil by oil products results in disruption of biocenoses and of their species diversity.

Negative action of oil is connected with direct toxic action, and also with change of physical properties of soil (oxygen and moisture access worsens) owing to what fertility of soil decreases (Кузнецов and Градова 2006).

Restorign of soil fertility after influence of oil products under natural conditions lasts tens years, and deep pollution can remain hundreds years (for example, landfilled oil sludge) (Кобзев *et al.* 2001). Studying and working out of environmentally sound receipt of acceleration of oil products degradation in soil is an important problem in a solution of a problem recultivation of technogenic faulted soils. The modeling of concrete ecological situations specific to processes of pollution of environment by oil is of interest.

The biological way of restoration of the polluted soils is perspective. There are natural microorganisms which consume oil products as a carbon source, oxidize toxic connections, and also transform them to a humus.

Soils polluted by oil products is possible to accelerate biorestoration process repeatedly at the expense of use of specially prepared biological products and technologies of their application.

By means of biological products two processes become more active: aerobic (in the presence of molecular oxygen) and anaerobic (at its absence). The first stage the leading role is played by aerobic process in which course hydrocarbons are oxidized to nontoxical substances, carbonic gas and water, a biomass considerable quantity also is formed. After that oxidation products, other components of oil products and a biomass will be transformed to a humus at participation anaerobic microorganisms (Мурзаков 2005).

Now the closed bioreactors of isothermal type are developed. Such reactors can all-the-year-round work at the expense of maintenance of the optimum temperature conditions necessary for reproduction of microorganisms and biodecomposition of oil products (Vestnik VNIIZhT… 2005).

Structurally bioreactors represent the chamber where the ground polluted by oil products moves and in which hashing and the periodic dosed out water delivery, fertilizers and microflora is carried out. Before loading in the bioreactor the ground is crushed by means of a cultivator as the the size of particles of a ground, the above diffusion ecotoxicant to microorganisms is less.

The technology of bioneutralization consists of following stages:

- A cutting of the polluted ground;
- Delivery of the polluted ground to a place of placing of the bioreactor;
- An unloading of the polluted ground from a motor vehicle body in the reception bunker;
- The dosed out giving of a ground in a cultivator;
- Separation of large stones and snow blocks;
- Crushing of a ground by a cultivator;
- Warming up of the crushed ground before giving in the reactor in a cold season;
- Giving of the crushed ground in the bioreactor;
- Process of biodecomposition of oil products by microorganisms;
- A periodic irrigation of a ground in the bioreactor a solution of fertilizers and microflorae in water;
- Periodic hashing of a ground in the bioreactor;
- Carrying out of analyses of tests of a ground on the maintenance of oil products;
- An unloading of the polluted ground from a motor vehicle body in the reception bunker;
- Loading of a body of the car by the cleared ground by means of the conveyor (the volume of the unloaded cleared ground shouldn't exceed 75 % of volume of the ground which is in the bioreactor).

However, conditions of hashing of a ground and communication of these conditions with kinetics the process of biodegradation practically aren't studied.

2. Methodology

We had developed the laboratory bioreactor for studying of process of biodegradation of the petropolluted ground in dynamic conditions. The bioreactor represents the cylindrical device on the mobile platform equipped with a dynamometer for measurement of a jet twisting moment. The device is equipped by the mechanical mixing device and an adjustable drive, and as the compressor and rotameter for measurement of the air flow rate.

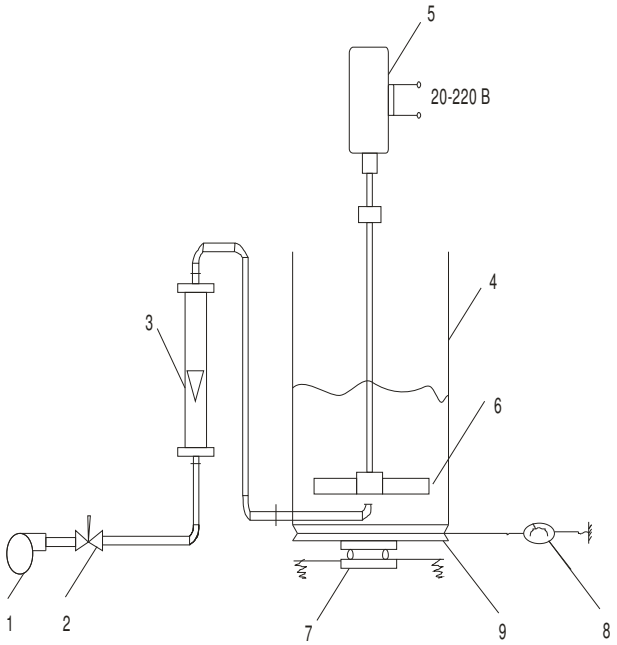

Fig 1. The scheme of experimental mechanism (1 - compressor, 2 - control valve, 3 - rotameter, 4 - reactor, 5 - variable drive, 6 - mixing machine, 7 – axial bearing, 8 – electronic dynamometer, 9 - sheave groove)

Mechanism works as follows. The reactor with a soil lot is positioned on the mobile platform equipped with the persistent bearing and a pulley. The thread connected with a dynamometer is located in a sheave groove in such a manner that at rotation of a mixing machine the forcing connected with hashing was measured by a dynamometer, and further is recalculated in a twisting moment and capacity of mechanical hashing. The variable drive allows to vary speed of rotation of a mixing machine, and also to carry out faltering modes of mixing. Soil samples were collected through certain intervals of time during the day (8 hours), the forcing of mixer rotation was measured and speed of mixer rotation. The temperature and humidity of soil in the bioreactor and in control was measured. Soil samples were dried up to air-dry condition for the further measurement of concentration of hydrocarbons. The forcing was recalculated in capacity of mechanical hashing.

In quality the culture test the culture of microorganisms Candida maltosa strain 569 which are a part of a biological product "Oleovorin" (firm "Bigor") was used. For researches was it is taken "Universal" neutral peat

and soil (manufactures of Open Company Torfozavod AGROPEAT, Russia, Leningrad region, Tosnensky area, settlement Smooth).

Cultivation of yeast Candida maltosa strain 569 was spent on thermostatted shaker with speed of rotation 200 – 220 rpm in Ehrlenmeyer's flask in volume of 750 ml containing 100 ml of a nutrient medium at temperature of 30-32 ^{0}C. Nutrient medium structure No 10 following (Морщакова *et al.* 1991):

$(NH_4)_2SO_4$	3 g/l
KH_2PO_4	6,5 g/l
$MgSO_4 \cdot 7H_2O$	0,7 g/l
$FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$	12, 5 mg/l
$CuSO_4 \cdot 5H_2O$	3 mg/l
pH 6,8-7,0	

As the previous experiences have shown that petrooxidizing microorganisms brought by us enter difficult interactions with autochthonic microflora, for cleanliness of experiment soil sterilized twice within an hour at temperature 121ºC. The maintenance of microorganisms in soil before sterilization is resulted in table 1. To exclude influence of structure of oil on biodegradation parameters, the further experiences were spent on paraffin. The soil was artificial is polluted by paraffin C_{14}-C_{17}.

Table 1. Quantity CFU of microorganisms in the unsterile and thermoprocessed soil

	Media	Soil before steriliza-tion	Soil after sterilization
Bacteria	Meat-and-peptone agar	$3*10^7$	$2*10^1$
Yeast	Wort agar	0	0
Actinomy-cete	Amylum-ammoniac	$5*10^4$	$1*10^1$
Fungi	Sabouraud's medium	$1*10^4$	0

The maintenance of hydrocarbons defined by gravimetric method with extraction hydrocarbons of oil chloroform. The essence of a method consists in chloroform extraction of oil products from samples the polluted oil of soil and gravimetric definition of degree of pollution. Definition course:

For the analysis select tests of soils. The soil hinge plate makes 2 the Sample of soil bring in a glass flask, add 20 ml of chloroform, carefully mix, allow to settle. The extract through the paper filter is transferred to round-bottomed flask preliminary weighed to within 1 mg in volume by 100 ml.

Soil chloroform extraction repeat 3 times, collecting extracts in the same flask. Then make chloroform evaporation on rotary evaporator at temperature of a water bath 68 ºC.

A flask weigh, then in 5 minutes of a purge weigh repeatedly. Weight consider as a constant if the difference between 2 last weighings makes no more than 0,004 g. Further define a mass fraction extracting substances.

The quantity of microorganisms in the soil polluted by hydrocarbons was made by seeding on the Petri dishes (Звягинцев *et al.* 1980).

3. Results

Soil mixed with the help of three mixers (propeller mixer, mixer in the form of a comb with fingers up, mixer in the form of a comb with fingers down) with various speed of rotation. Check experiments were carried out by placing of identical quantity of a ground in the capacity equal to diameter of the bioreactor. The capacity was in the same temperature conditions, as the bioreactor.

Experiment was carrying out during a week. Soil was mixed during 8 hours a day; samples took three times a day. Speed of rotation was supported at level of 60 rpm.

On Fig 2 is shown dynamics of growth of microorganisms Candida maltosa strain 569 at mixinging with a propeller mixer and in a static mode.

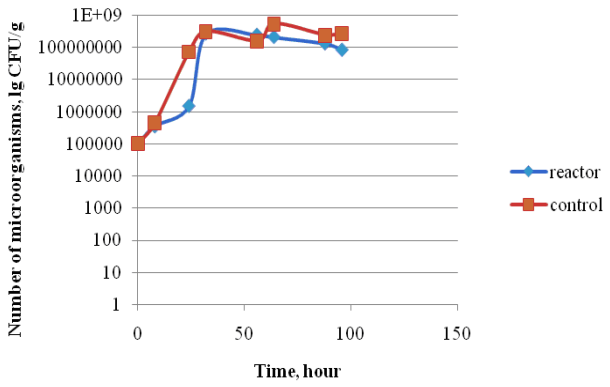

Fig 2. Dynamics of growth of microorganisms Candida maltosa strain 569 on the petropolluted soil at mixinging and in a static mode

There were not much differ between amount of microorganisms in static and dinamic modes (Fig 2). But paraffin consumption rate was remarkable greater in dinamic mode than in static (Fig 3).

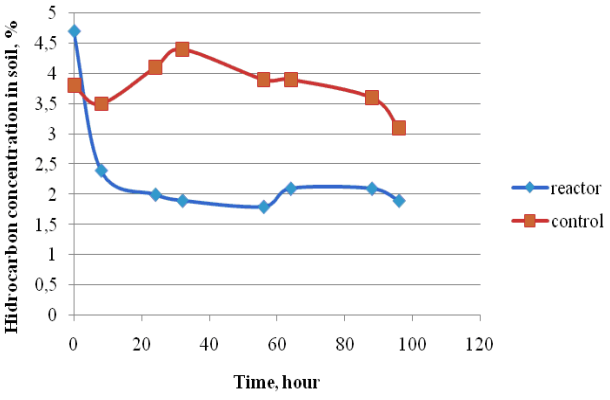

Fig 3. Dynamics of consumption of paraffin in soil culture of microorganisms Candida maltose strain 569 at mixing and in a static mode (propeller mixer)

Scale of the mean power spent for hashing of petropolluted soil was ~ 1, 7 W.

Because the greatest difference between speeds of consumption in a static and dynamic mode is observed till 20 o'clock following experiments were carrying out during 2 days.

We use also a mixer in the form of a comb for our experiments.

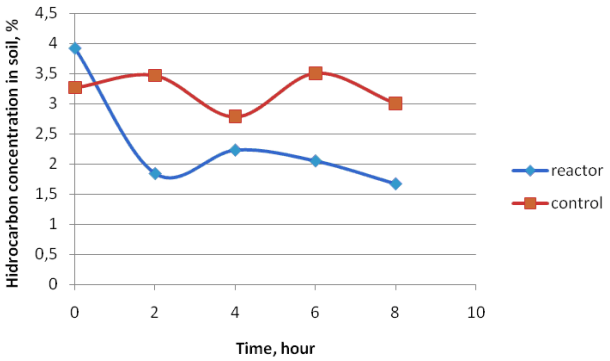

Fig 4. Dynamics of consumption of paraffin in soil culture of microorganisms Candida maltose strain 569 at mixing and in a static mode (mixer in the form of a comb with fingers up)

Scale of the mean power spent for hashing of petropolluted soil was ~ 2, 3 W.

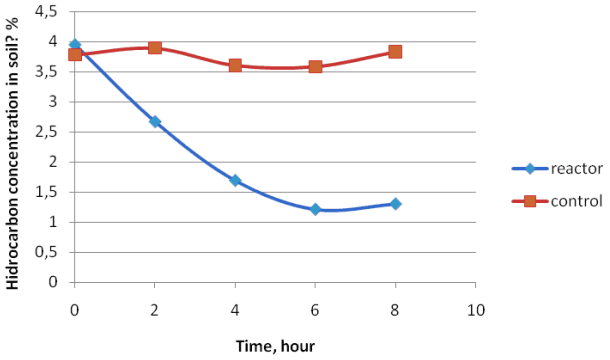

Fig 5. Dynamics of consumption of paraffin in soil culture of microorganisms Candida maltose strain 569 at mixing and in a static mode (mixer in the form of a comb with fingers down)

Scale of the mean power spent for hashing of petropolluted soil was ~ 1, 4 W.

On the Fig 1, 2, 3 is shown that all of mixers we used positively influence process of consumption of paraffin by microorganisms.

4. Conclusions

1. The greatest difference between speeds of consumption in a static and dynamic mode is observed till 20 hours, speed in a dynamic mode exceeded speed of consumption of hydrocarbons in a static mode approximately several times.
2. By means of mixing of the soil polluted by hydrocarbons it is possible to lower sharply concentration of hydrocarbons within the first 20 hours, and further dynamic process provides much lower maintenance of hydrocarbons in soil as it follows from the considered schedule.
3. There is no necessity to mix soil during all process of bioremediation, it is more favorable to mix within 1-2 days, and then to continue process in static mode.

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