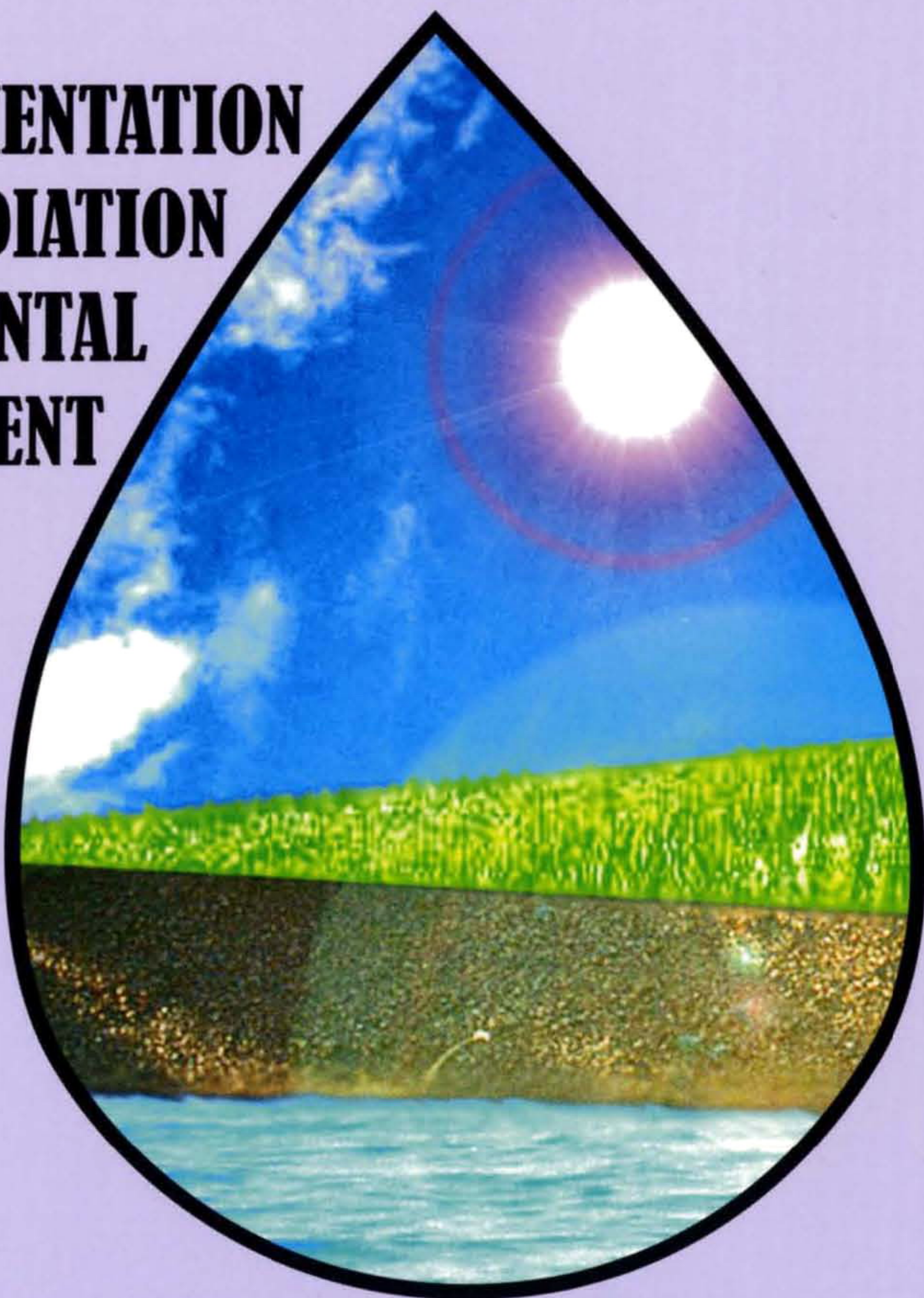


**SCIENTIFIC GATHERING
WITH INTERNATIONAL PARTICIPATION**

**IMPLEMENTATION
OF REMEDIATION
IN ENVIRONMENTAL
QUALITY IMPROVEMENT**



**BELGRADE
27 November 2006**

**SERBIAN CHAMBER OF COMMERCE
BOARD OF ENVIRONMENTAL PROTECTION
AND SUSTAINABLE DEVELOPMENT**

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ISOLATION, SELECTION AND ADAPTATION OF ZYMOGENOUS MICROORGANISMS: A BASIS OF SUCCESSFUL BIOREMEDIATION

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Abstract

Bioremediation of soil contaminated with petroleum and petroleum derivatives is a modern and economical method which satisfies all the conditions of «green technology». The open biotechnological process uses the natural capability of the soil microorganisms, mainly bacteria and fungi, to decompose petroleum hydrocarbons and transform them into a simple and non-toxic organic compounds, respectively at the worst to mineralize them. Various bioremediation techniques provide the optimal conditions for growth of microbial population which uses hydrocarbons as a substrate and thereby accelerate decomposition of contaminants. The final goal is to return the contaminated environment in the state of pre-anthropogenous influences.

The paper presents herein the results of ex situ bioremediation of soil on the pilot heap (halde) of approximately 150 m³ within the grounds of Petroleum Refinery Pančevo (Serbia). With biostimulation, bioventilation and reinoculation of autochthonous microbial consortium for about five months, decomposition of total petroleum hydrocarbons is achieved from the initial almost 30 to about 3 g/kg of dry soil respectively the efficiency of bioremediation is approximately 90 %.

Capability of naturally present microorganisms for petroleum degradation was tested in laboratory conditions in Erlenmeyer flasks on the shaker. As test substrates were used: complex mixtures of hydrocarbons of various classes (diesel fuel D-2, paraffin, extracted total petroleum hydrocarbons from the treated soil), n- and cyclo-alkanes (hexane, heptane, decane, hexadecane and cyclohexane), aromatic hydrocarbons (benzene, toluene and isomer xylene mixture), polycyclic aromatic hydrocarbons (phenanthrene and pyrene) and chloroform as chlorinated hydrocarbon derivative. Upon obtaining of satisfactory results in relation to decomposition of hydrocarbons and their derivatives (bioremediation potential), the microbial consortium was isolated and therefrom performed selection and adaptation of zymogenous microorganisms which decompose petroleum hydrocarbons. The biomass of high density of activated microorganisms was applicated once a week on the experimental heap.

Key words: soil microorganisms, consortium, petroleum, petroleum derivatives, soil, ex situ bioremediation

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INTRODUCTION

Bioremediation is a modern method in which the natural activity of microorganisms is used for reduction of concentration and/or toxicity of various chemical substances such as petroleum derivatives, aliphatic and aromatic hydrocarbons (including polycyclic aromatic hydrocarbons and polychlorinated biphenyls), industrial solvents and base organic raw materials (phenols, benzene, acetone), pesticides, metals and metalloids (mercury, chromium, arsenic, selenium,...) [1].

Biodegradation is a natural process which develops in the ground or in water under the effects of microorganisms (bacteria, fungi, algae). Some microorganisms can decompose or transform the chemical substances present in petroleum and petroleum derivatives. The bioremediation method optimizes the conditions for the growth of microorganisms present in the ecosystem in order to increase the velocity of biological decomposition of hydrocarbons [2, 9, 11, 12].

Crude oil consists of a great number of different hydrocarbons and similar components which can be classified on a saturated and aromatic fraction. The saturated fraction contains alkanes of normal and bifurcated series and cycloalkanes (naphthenes), while aromatic fraction contains mono and polycyclic hydrocarbons which can have also alkyl-lateral series. Besides hydrocarbons naphtha can contain also nitrogen, sulphur, oxygen compounds (NSO-compounds). In petroleum are also present in small quantities asphaltene, high molecular aromatic compounds or compound mixture with higher number of condensed aromatic rings and higher number of heteroatoms [4, 7].

Contamination of ground and water with petroleum and petroleum derivatives occurs at accidental effluents in exploitation, transport, processing, storage and utilization. Only 10% of pollution originates from large accidental effluents which cause contamination of sea shores, lakes or water currents, and attract significant media attention [4].

Compounds which come in petroleum ingredients are substrates for microorganisms, so that when comes to petroleum discharging in the environment, in ecosystem is already present a small population of microorganisms which shall decompose hydrocarbons. At the same time, the increase of microorganism population occurs in the soil which can use hydrocarbons as the sole source of hydrocarbons. In non-contaminated soil, 1-10% of the present microorganisms use hydrocarbons, and in contaminated soil this population is dominant [5, 14].

Some microorganisms degrade the appropriate number of petroleum components, but the mixed population, similar as in the natural conditions, the most often enable a higher degree of degradation. Some of the components can be decomposed only cometabolically-by joint action of more microorganisms. Besides microorganisms which primarily decompose petroleum, in the soil, there are also others which use for nutrition the components obtained by primary decomposition. Removal of decomposition products stimulates further degradation of petroleum.

Some of microorganisms produce also biosurfactants—natural emulsifiers (“contactors”), which facilitate dissolving of hardly soluble components and increase accessibility of those components to microorganisms [3, 8].

Growth of population of microorganisms which decompose hydrocarbons, and therefore also the velocity of microbiological degradation of petroleum and petroleum derivatives depends on a series of factors. The most important physical factors are temperature, available oxygen, saltiness, pH as well as the grade of ecosystem openness. The influence of chemical factors includes the presence and concentration of nutrients, sources of nitro-

gen, phosphorus and other elements, nature of hydrocarbon substrate and its effective concentration, as well as possible presence of toxic substances either in petroleum derivatives or in the medium itself. Biodegradability of petroleum components drops in the following series: *n*-alkanes, branched alkanes, branched alkenes, *n*-alkylaromates of small molecular mass, monoaromates, cyclic alkanes, polycyclic aromates, asphaltenes [1, 3, 15].

A low solubility in water of majority of components present in petroleum restricts capacities of microorganisms for decomposition because the process is developed in aqueous medium. Some microorganisms which decompose petroleum also produce a large number of biosurfactants of various chemical structure. Biosurfactants of low molecular mass (glycolipids, lipopeptides) are more effective in reduction of surface tension while the high molecular biosurfactants (amphipathic polysaccharides, proteins, lypopolysaccharides and lipoproteins) are effective stabilizers in water-oil emulsions [3, 4, 8, 13].

Rate and degree of biodegradation depend on the type of soil in which the process is being developed. The organic substance and small particles are mainly responsible for adsorption of pollutants. Hydrocarbons are strongly bound to humic substances and clay minerals. In the sandy terrain the accessibility of the substrate for microorganisms depends on porosity. In soil, distribution of contaminants is mainly inhomogeneous which brings to differences in the quantity of active biomass and the degree of decomposition [1, 3, 15].

Variable and suboptimal conditions in nature bring to a prolonged degradation time. Degradation rate drops with the decrease of the substrate concentration, gradually reducing also the viable microbial biomass, while a shortage of the cosubstrate or accumulation of toxic intermediate products can also stop the process [8, 13].

Bioremediation techniques stimulate the growth of microorganisms and optimize the process of degradation by correcting defects occurred in the natural process. With the presence of microorganisms in the system, in the simplest case for petroleum hydrocarbon decomposition process, the limiting factors are lack of individual nutritious substances, particularly phosphorus and nitrogen, absence of the sufficient quantity of oxygen and inhomogeneous medium [8, 13].

The method can be applied *in situ* and *ex situ* subject to concentration of the contaminant, its biodegradability and soil characteristics. The contaminated soil is treated by *in situ* bioremediation on the locality itself, which is cheaper, but also a longer procedure. *Ex situ* procedures require digging up of contaminated earth or water pumping out. The contaminated material is then treated in a bioreactor which is the quickest and the most efficient but also the most expensive procedure. The procedure in solid phase is often applied, the most often in the form of halde (designed heap)—overground treatment of contaminated material in the system with collector which prevents outlet of toxic substances and where moisture, temperature, aeration and addition of fertilizer—the source of nitrogen and phosphorus, can be controlled [3, 4, 15].

With biostimulation are added nutritious substances lacking in the soil, the most often nitrogen and/or phosphorus compounds in the form of inorganic salts or organic fertilizer. Bioventilation and injecting of hydrogen-peroxide provide oxygen. By bioaugmentation are introduced in the system the commercially available microorganisms or microorganism consortiums, which are especially prepared for every concrete situation, in the case that there are no in soil microorganisms decomposers or that the existing ones cannot decompose all the contaminant components [3, 8, 13, 15].

The paper presents our results of bioremediation of the contaminated soil for the period of about five months (162 days) on the pilot halde 150 m³ within the circle of Petroleum Refinery Pančevo.

MATERIAL AND METHODS

Pilot heap [15]: In the circle of the Petroleum Refinery Pančevo watertight base of soil of different level of contamination by petroleum and petroleum derivatives, a bioremediation halde („open bioreactor“) 150 m³ in volume is made. The halde geometry is a three-sided parallelpiped which dimensions are such that one linear meter contains 6 m³ of treated material. The natural aeration is stimulated by the system of perforated pipes. Humidity is maintained by technological water from the tank of 1 m³ (used also for biomass adding), by manual sprinkling with a pump up to 100% retention water capacity. Filtering solutions are drained in another tank with automatic level control which is coupled by the pump with water tank, respectively the system is fully closed for environmental influences. Homogenization and additional aeration of the halde content is ensured by manual stirring of the total quantity every two weeks. The halde is protected from direct external influences by a “green house”.

Bioremediation potential of the soil: Defined in Erlenmeyer with shaking (solid phase: mineral base = 1:9 m/V), for duration of 1 month at 26 °C, which is described in more details in the part Results and Discussion.

Production of biomass: Biomass is prepared in the laboratory bioreactor with mechanical stirrer, in the quantity of 10 L every week during the bioremediation process, which is described in more details in the part Results and Discussion.

Determination of the number of microorganisms: The number of microorganisms is determined by the method of a serial dilution on agar plates incubated on 26 °C. For bacteria a nutrient agar was used, for yeasts and molds Sabouraud glucose agar with chloramphenicol, and for microorganisms which decompose hydrocarbons, the mineral base medium [10] with 2000 ppm diesel fuel [6].

Chemical methods^{**}: The content of total petroleum hydrocarbons (TPH) in the soil is extracted as per method ISO 16703:2004 and gravimetrically determined in accordance with DIN EN 14345:2004. The gas chromatography as per stated ISO standard is used for the quality analysis and comparison of results. It was performed on the instrument Agilent 4890D with FID detector. Column HP-1MS 30 m x 0.25 mm. Gas-hydrogen carrier, injector temperature 250 °C, initial temperature 40 °C, isothermal at 285 °C during 12 minutes. Polycyclic aromatic hydrocarbons (PAH) are determined by the gas chromatography with mass spectrometric detection (GC-MS) as per standard ISO 18287:2006. GC-MS is performed on the mass spectrometer Finnigan Mat model 8230, with Ion Trap Detector (ITD) model ITD-705 of the same manufacturer. The gas chromatograph connected to the mass spectrometer, as well as the operating conditions were the same as for GC, with disconnected FID. Production column used was Supelco (USA) type PTE-5 of the same dimensions as the column for GC. The column output is via interphase connected directly to ITD at the temperature of 240 °C. The results were controlled by the certified reference materials ERM^{®****}. The group composition of the organic substance of contaminated earth was determined by the extraction and chromatographic separation on the adsorbent column [7]. All the results related to concentration of contaminants and other components in the ground are calculated to dry substance.

*) The common analytical procedures used for substrate analysis and procedure monitoring are not stated!

**) European Reference Materials.

RESULTS AND DISCUSSION

Exploring the possibility of bioremediation application for the certain contaminated area includes a series of analyses and experiments. The most important analysis are determination of TPH contents and the presence of microorganisms in soil which decompose hydrocarbons. Important parameters are obtained also on the basis of testing the contaminated soil by determination of: group composition of hydrocarbons, PAH contents, concentration of organic carbons, mineralogical-petrographic composition, content of nitrogen and phosphorus and of some metals (potassium, magnesium, calcium, iron, vanadium and nickel).

If the contaminated ground contains microorganisms which decompose hydrocarbons, the common procedure is to optimize the biodegradation procedure in the laboratory conditions with a small quantity of specimens. While testing in Erlenmeyer with shaking the increase of the number of microorganisms is observed and the decomposition speed determined on the basis of the difference in the initial and final contents of hydrocarbons as well as the appearance of TPH chromatogram of the tested soil specimen. With such a test in the laboratory conditions where the mixing and aeration are intensive, we can estimate the efficiency of bioremedial procedure, accessibility of hydrocarbons for microorganisms, effects of biostimulation, as well as test the effects of synthetic surfactants on the same microorganisms and on biodegradation velocity.

Soil contaminated with petroleum and petroleum derivatives, being the subject of this paper, initially contained 29.99 ± 1.88 g TPH/kg and 9.3×10^5 microorganisms in 1 g of substrate which decompose hydrocarbons. The content of PAH is 5.5 mg/kg, the content of organic extractible substance is 67.13 g/kg. On the basis of x-ray diffraction analysis of the native, incinerated and glycerin saturated preparation, the soil is dominantly clayed sand with low content of carbonate.

During the laboratory experiment in Erlenmeyer on the shaker lasting one month, there has been achieved reduction of the content of TPH up to 10.51 g/kg. The appearance of chromatogram of TPH soil at the beginning and at the end of the experiment is shown in Fig.1. Besides the reduction of the TPH, it is noticeable that the largest decomposition was achieved in the range up to C₃₀. It was established that the consumption of phosphate in the base can be correlated with the increase of biomass, which indicates that the biostimulation could be successfully applied.

In the laboratory conditions, three commercial synthetic biodegradable surfactants – “contactors” were tested. Their influence on population of microorganisms and velocity of TPH degradation were also tested. On the basis of the obtained results, “contactor” of average properties was selected and added as the solution into sprinkling water in concentration of about 0.2 kg dry substance/m³ of soil.

On the basis of the results obtained it was decided to carry out the pilot bioremediation *ex situ* with wetting, by adding a source of nitrogen and phosphorus so that the ratio C:N:P is obtained approximately 100:10:1, with the natural aeration by the system of perforated pipes and periodical mixing–overturning of the whole content.

During the experiment for 15-days were monitored changes of TPH content, number of microorganisms and a series of other indicators, the above mentioned, which are of importance for bioremediation and its procedure.

The basic aim was to increase the number of microorganisms in soil, and particularly participation of those which decompose petroleum hydrocarbons.

Fig. 2 shows changes of TPH concentration in the function of time.

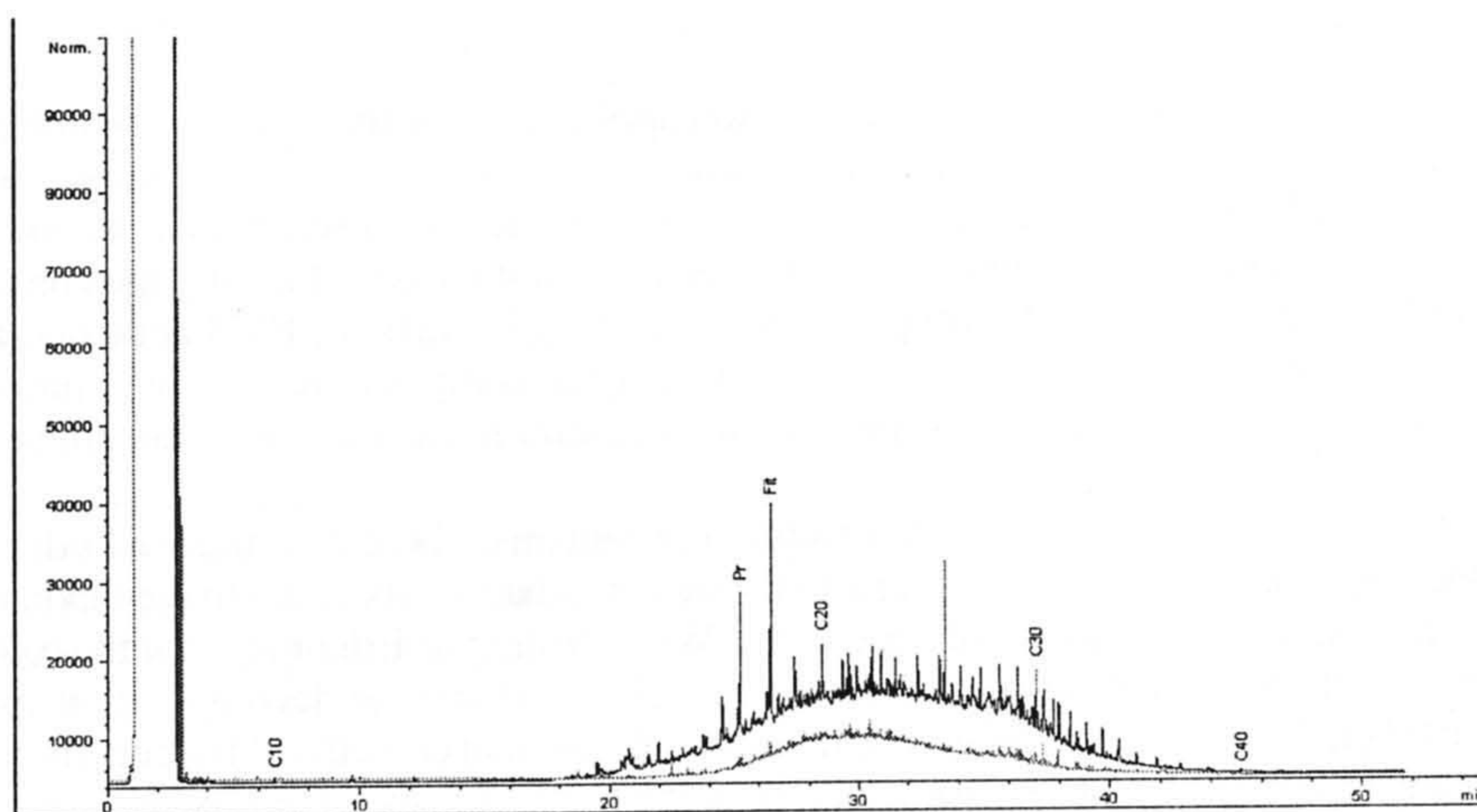


Fig. 1. Comparable presentation of gas chromatograms TPH at the beginning (upper line) and at the end of the experiment in Erlenmeyer with shaking

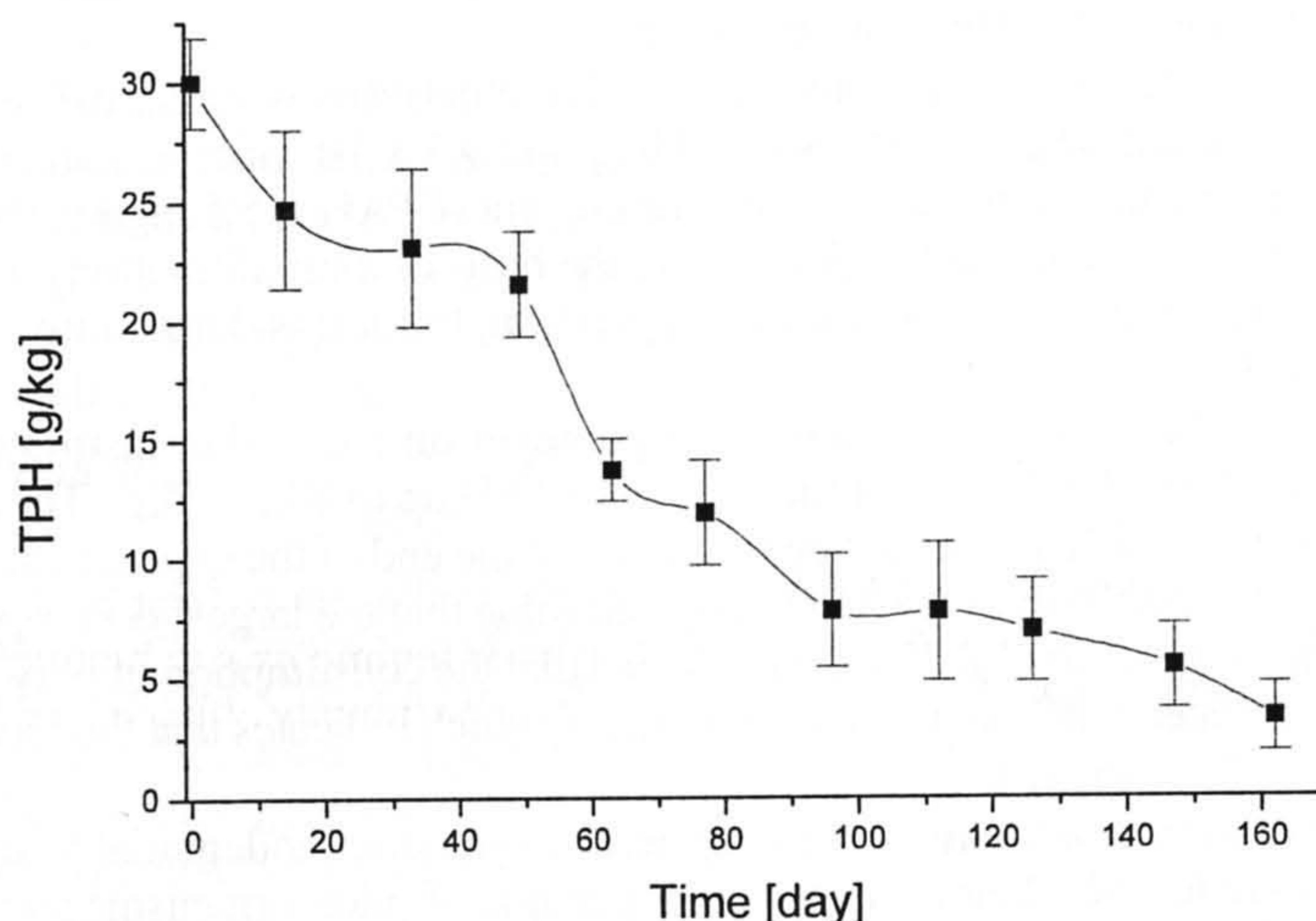


Fig. 2. Graphical presentation of bioremediation dynamics in the function of time

It is noticed that during the starting period the velocity of degradation was low, what was expected, because it corresponds to the lag-phase of microbial population growth.

The growth of the number of microorganisms and intensification of biodegradation process in addition to biostimulation have been also achieved by reinoculation of zymogenous microorganisms. In the laboratory, a mixed population of microorganisms which decomposed hydrocarbons has been singled-out from the soil specimen. Certain strains were tested for growth on hydrocarbons of various structure as the only sources of carbon in the mineral base. As the test substrates were used: composite mixtures of hydrocarbons of different classes (diesel fuel D-2, paraffin, extracted total petroleum hydrocarbons from

the treated soil), *n*- and *cyclo*-alkanes (hexane, heptane, decane, hexadecane and cyclohexane), aromatic hydrocarbons (benzene, toluene and xylene isomer mixture), PAHs (phenantrene, and pyrene) and chloroform as chlorinated hydrocarbon derivative. Out of strains which showed the widest range of growth, consortium was prepared multiplied in the laboratory fermentor. Biomass of high density (up to 10^{13} CFU/mL) of activated microorganisms was deposited on the experimental halde once a week.

Comparable presentation of gas chromatograms TPH at the beginning and upon expiry of about 5 months of the bioremediation process, when was decomposed near 90 % at the beginning present hydrocarbons originating from petroleum, the most illustratively speaks in favor of successful biodegradation, which is shown on Fig. 3.

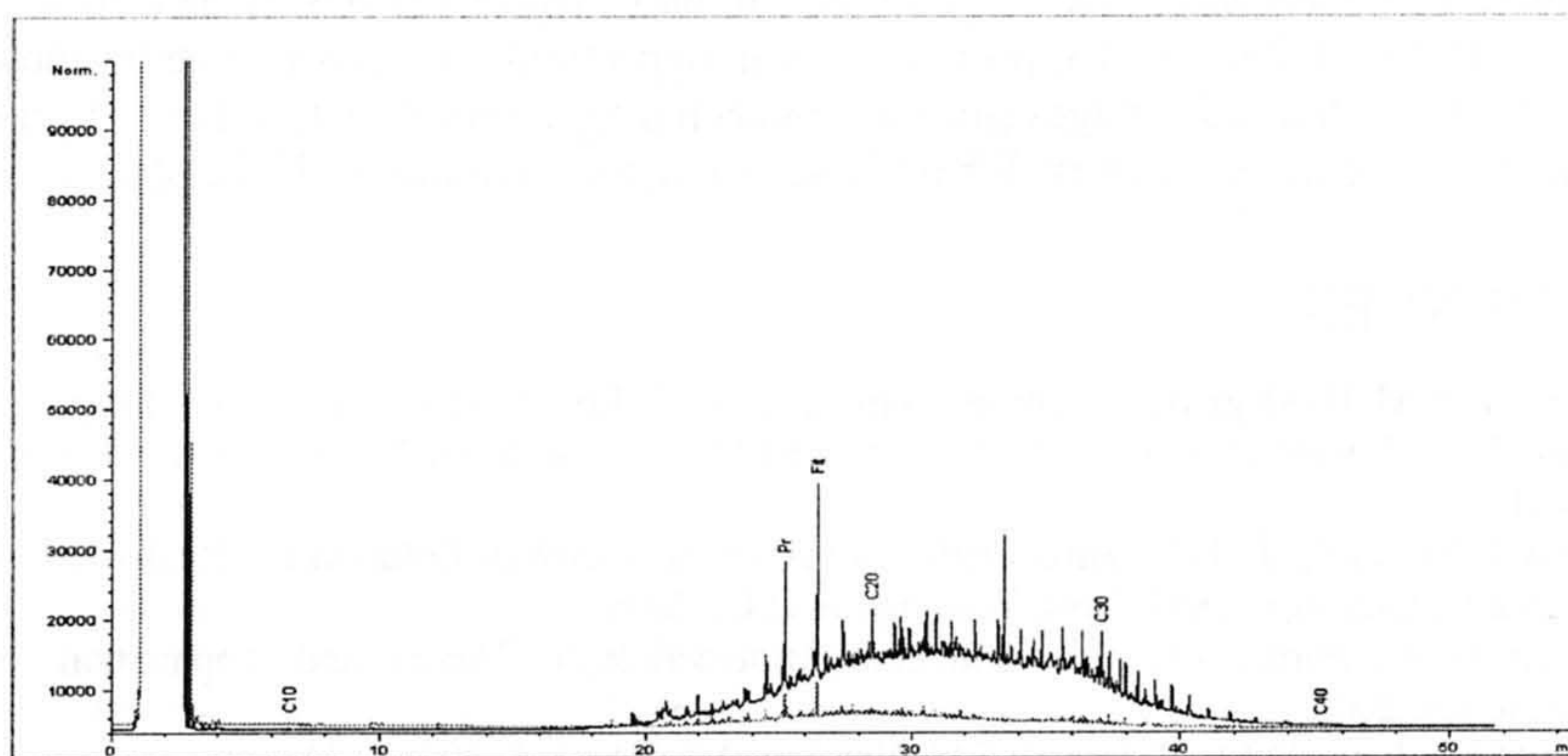


Fig. 3. Comparative gas chromatograms TPH at the beginning (upper line) and at the end of bioremediation on the pilot halde-installation

Participation of microorganisms which decompose hydrocarbons in their total number, at the beginning was somewhat higher than 9%, so that at the end of the process the hydrocarbon biodegradators were dominant with a share of more than 80% (range 10^7 CFU/g), confirming that the selected procedure and the regime *ex situ* bioremediation of soil from the Refinery of a high contamination degree is extremely efficient, thanks, first of all, to the corresponding selection of microorganisms which made the applied consortium of biomass reinoculated on the treated soil. This conclusion is also confirmed by PAH concentration at the end of bioremediation, which is 5.2 mg/kg, which means that from the initial concentration more than 5 % was biodegraded.

CONCLUSION

On the basis of all stated and discussed results, it can be concluded that:

1. The procedure *ex situ* of bioremediation of soil contaminated with high concentrations of petroleum and petroleum derivatives in the industrial-real scale is possible to efficiently realize on the basis of the laboratory tests.
2. For a successful procedure, the key task is to make the right choice of consortium of active zymogenous microorganisms, which was proved once again in the described pilot procedure of bioremediation by the work with own strains of microorganisms isolated, selected and adapted from microbiogeocenosis, which was the subject of biodegradation of contaminants of anthropogenic origin.

3. Biostimulation and bioventilation, which in the described case were not quite adequate, are indispensable and compatible elements of the successful bioremediation.

4. Our results also confirm that bioremediation is a bio(geo)technological procedure, by means of which recycled soil is obtained. This means, that no harmless waste which can be put aside on the waste depot is obtained, but the soil which has value in use.

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REFERENCES

1. Alexander M. Biodegradation and Bioremediation, 2nd Ed., Academic Press, San Diego, 1999
2. Antić M.P., Jovančević B.S., Ilić M., Vrvic M.M., Schwarzbauer J., Environ. Sci. Pollut. Res. **13** (2006) 320
3. Atlas R.M., Philp J., Eds., Bioremediation: Applied Microbial Solution for Real-World Environmental Cleanup, ASM Press, Washington D.C., 2005.
4. Evans G.M., Furlog J.C., Environmental Biotechnology: Theory and Application, Wiley, Chichester, 2003
5. Head I.M., Jones D.M., Roling W.F.M, Nat. Rev. Microbiol. **4** (2006) 173
6. Hurst C.J., Crawford R.L., Knudsen G.R., McInerney M.J., Stetzenbach L.D., Eds., Manual of Environmental Microbiology, 2nd Ed., ASM Press, Washington, 2002
7. Jovančević S.B., Practical Organic Geochemistry with Fuel Chemistry, Faculty of Chemistry, Belgrade, 1999, p. 13 (in Serbian)
8. Jördening H-J., Winter J., Eds., Environmental Biotechnology: Concepts and Application, Wiley-VCH, Weinheim, 2005
9. Korda A., Santas P., Tenente A., Santas R., Appl. Microbial. Biotechnol. **48** (1997) 677
10. Loser C., Seidel H., Zehnsdorf A., Stottmeister U., Appl. Microbial. Biotechnol. **49** (1998) 631
11. Milčić-Terzić J., Lopez-Vidal, Y., Vrvic M.M., Saval S., Water Sci. Technol. **42** (2000) 403
12. Milcic-Terzic J., Lopez-Vidal Y., Vrvic M.M., Saval S., Bioresource Technol. **78** (2001) 47
13. Rittmann B.E., McCarty P.L., Environmental Biotechnology: Principles and Applications, McGraw-Hill, New York, 2001.
14. Van Hamme J.D., Singh A., Ward O.P., Microbiol. Mol. Biol. Rev. **67** (2003) 503
15. Wise D.L., Trantolo D.J., Cichon E.J., Inyang H.I., Stottmeister U., Eds., Remediation Engineering of Contaminated Soils, Marcel Dekker, New York, 2000