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Bioremediation of Grounds Contaminated With Petroleum Products by HEAP Method

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Abstract— Effective and sustainable reclamation of degraded postindustrial and military areas still requires improvements in methods and approaches. It applies especially for physical, chemical as well as biological remediation methods. In case of the latter method activity of microorganisms is influenced by the environmental conditions which can be controlled by providing the contaminated grounds and the ground waters with biogenic substances. Of no less importance, however, is aeration as well as inoculation the ground using high productive microorganisms in relation to petroleum products degradability. The paper presents a new approach to the bioremediation of areas polluted with hydrocarbons involving implementation of unique closed system of effluents circulation in ex-situ process, use of the selected, dedicated autochthonous microflora capable of hydrocarbons degrading and oxygen degradation conditions. Effectiveness of the implemented remediation method was verified in real environment on the area polluted with hydrocarbons at a former railroad transshipment station. The developed preparations and technology enabled to achieve the contaminants level compliant with the legal purity standards and safe for the environment.
Keywords— autochthonous bacteria; biodegradation process; bioremediation; environment pollution; petroleum products

I. INTRODUCTION

Development of civilization within last several decades entailed the growth of demand for crude oil and products derived therefrom which triggered an increase in the environmental hazards. Mining, processing, industrial accidents, transport and storage of crude oil and its derivatives are the main causes of these products penetration into the environment. During migration of the hydrocarbon pollutants in the ground-water environment they undergo almost immediately variety of processes such as evaporation, oxidation, dissolution in ground waters and surface waters, emulsification, dispersion, adsorption, biodegradation, etc. The contamination of waters and grounds with the petroleum products (PP) including hydrocarbons of different physical, chemical and biological properties, results in serious changes in the environment. They adversely affect crop production and pose a potential risk for human and animal health [1], [2]. Aromatic hydrocarbons including benzene, toluene, xylene defined as BTEX and polycyclic aromatic hydrocarbons (PAHs) compose the biggest threat. Many of these show toxic, mutagenic and carcinogenic effects [1], [3-5]. BTEX hydrocarbons are particularly dangerous in respect of their toxicity and carcinogenic effects. Relatively high water solubility in comparison with other fuel components results in their mobility in the ground-water environment which contributes to the water pollution. Reduction of hydrocarbons concentration in the contaminated ground-water environment is the main goal of various treatment techniques involving physical, chemical, biological or mixed processes. Any case of contamination requires an individual approach depending on the site, pollution source, contamination type as well as the contamination extension [6]. Contamination recognition accuracy determines the remediation method to be applied to neutralize the pollutants. In the 90s of the 20th century new remediation methods were developed based on involvement of micro-organisms for the pollutants degradation. These technologies consist in ability of micro-organisms to degrade hydrocarbons and focus on enhancement of self-cleaning natural processes, occurring in the ground-water environment. Depending on the pollution level and environmental conditions, such bioremediation processes can be carried out by in situ or ex situ methods. The first one involves liquidation of the pollutants directly in the site where they occur, while the latter involves extraction of the contaminated ground or water from the polluted site and then application of an appropriate remediation process in specially developed treatment bench [6]. Generally, biodegradation methods are considered attractive, safe, environmentally friendly and cost-effective [6-9]. Most of the bioremediation methods consist in intensification of the natural processes using micro-organisms specialized in the utilization of hydrocarbons as a source of carbon and energy [6], [10], [11]. Efficient pollutants biodegradation proceeds thanks to variety of enzymes catalyzing metabolic

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conversion of hydrocarbons into simple compounds or other harmless products (biotransformation) [6], [10]. A major role in the petroleum hydrocarbons biodegradation play oxygenases [5], [12].

In the literature and practice there are distinguished three basic types of bioremediation processes:

natural bioremediation - it proceeds spontaneously as a result of autochthonous micro-organisms activity present in the environment.

biostimulation - the most commonly used method, based on the growth stimulation and metabolic activity of micro-organisms naturally occurring in the environment (autochthonous microflora).

bioaugmentation - introduction of appropriate species of micro-organisms to the polluted environment specially adapted to pollutants degradation, including genetically modified organisms (GMOs) [12-17].

Among the basic parameters determining promotion of the biodegradation process and its successful results, hydrocarbon's bioavailability can be mentioned. The hydrocarbons compose a source of carbon utilized by the microorganisms. Other important factors are enzymatic activity, type of the contaminant and physical-chemical parameters (temperature, humidity, pH, redox potential) [18]. The main factors limiting the bioavailability of petroleum products are their water solubility and a tendency for persistent binding with the ground, chiefly with organic matter included in the ground [9], [10], [18], [19].

Apart from the bioavailability the following conditions affect the microorganisms growth and the biodegradation process efficiency: accessibility of nutrients other than carbon that is N, P, S, Ca, Mg, K, oxidation level, hydrocarbons' type and concentration, their toxicity in relation to the microflora and presence of other toxic compounds inhibiting the process, temperature, humidity and pH.

The active microorganisms utilized in the bioremediation process mostly include the following strains: Acinetobacter, Actinobacter, Alcaligenes, Alcanivorax, Anthrobacter, Arthrobacter, Bacillus, Berjerinckia, Candida,Corynebacterium, Enterobacter, Flavobacterium, Methylosinus, Mycobacterium, Micrococcus, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctonia, Rhodococcus, Serratia, Sphingomonas, Trametes, Xanthobacter [6], [10], [20-23].

Hydrocarbon degrading microorganisms produce biosurfactants (surface-active natural agents) that improve the bioavailability [18-20], '[23, 24, 25, 26]. Thus, the natural biosurfactants enhance the hydrocarbons' degradation making the pollutants more available for the microorganism cells.

The hydrocarbons' biodegradation proceeds according to a particular scheme [27], [28] resulting from complexity of the substrates mixture, bacterial selectivity as well as preferences to specific functional groups in the chemical compounds. Recognition of microbiological decomposition paths makes it possible to select and implement an appropriate remediation method [10], [29]. Biodegradation process is affected by the chemical compounds structure and their concentration [30]. Biodegradability of the oil hydrocarbons can be ranked in the following order (arranged in the order from the most readily degradable compounds to those hard to degrade): linear alkanes, C10-C25, gaseous hydrocarbons C2-C4, alkanes C5-C9, branched alkanes up to C12, alkenes C2-C11, branched alkenes up to C12, alkenes C2-C11, branched alkanes, cycloalkanes and mono- and polyaromatic hydrocarbons.

It was proved that the hydrocarbons degradation proceeds most intensively when of one strain, microorganism culture blends are used [25], [28]. The best results are obtained in case when intentionally developed biopreparations for such purposes are involved to the ground inoculation [10], [14], [31]. It is quite understandable that pathogens have to be eliminated from the microorganisms consortia used for inoculation, as they could develop adverse effects in humans [16].

The basic drawback of the biodegradation techniques is the fact that not all the contaminants are biodegradable. Moreover, it is not sure that the biodegradation products are less harmful than the original contaminants and the process efficiency is hard to evaluate because it strongly depends on environment conditions, especially on the temperature [31].

II. MATERIALS AND METHODS

A. The site and analyses

The research was carried out on the area of former railroad transshipment station Warszawa Gdanska which was used in the past for reloading and storage of different kind of chemicals, petroleum products and organic solvents, among others. Location of the object is shown in fig. 1.

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Fig. 1. Location of the remediation piles

The ground-water environment at the site had the following features:
The main occurring contaminants were aliphatic and aromatic hydrocarbons, mainly toluene, xylene, and ethylbenzene in smaller quantities,
The content of the above mentioned contaminants varied between a few to 3000 mg/kg of soil dry mass. Mineral oils were present in small quantities,
Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and chloroorganic compounds were also found in the soil in lower concentrations,
Ground waters in the vicinity of the contaminated zones indicated presence of toluene. Its concentration was about 100 mg/ dm3, contaminated ground was found in the first 50-60 cm layer of sand, in argillaceous sands, and even in clay ground, depending on the location. On average, the particle size distribution was 35.5% sand (> 0.063 mm), 35.1% coarse silt (21–62 μm), 23% fine and medium silt (2–20 μm) and 6.4% clay (< 2 μm),
The contaminated ground included pieces of concrete lumps which was separated at the beginning of the remediation process, properties of the contaminated ground varied significantly depending on its location and depth.
The work involved physical-chemical examinations consisting in the following measurements and analyses:
Soil pHH2O (soil/water ratio a 1:5)
Humidity (gravimetric method),
Particle size distribution (sieve and sedimentation analyses)
Nitrogen (NH4 form - Kjeldahl method, NO3 - with salicylic acid)
Phosphorus (spectrophotometric method using ammonium molybdate).
Hydrocarbons determination - volatile aromatic hydrocarbons (BTEX) were determined using head-space gas chromatographic method: Perkin Elmer TurboMatrixTM HS40 automated headspace sampler and gas chromatograph Perkin Elmer Autosystem XL configured with flame ionization detector (FID).
Microbiological analyses - total number of bacteria was counted according to the surface spread method, dehydrogenase activity by TTC method (used for the determination of soil dehydrogenase activity with (+G) and without glucose (-G) as a substrate).

B. Remediation Method Applied In The Work

For remediation of the contaminated area the ex-situ method was developed and applied. The ex situ method can be realized in different systems and variants such as bioslurry system, landfarming, composting, biopile system etc. From among the mentioned variants the biopile system was chosen for the remediation. The ground excavated from the contaminated site was classified and selected in terms of the hydrocarbon contamination level based on analyses of the ground samples. Three autonomous remediation sets were formed, each consisting of 2 piles, one equipment container and 2 bioreactors. The solid surface foundations of the piles were formed directly upon the ground. They composed several layers, where the lowest one consisted of gravel with a one-sided inclination of about 15%, taking up the area of a rectangle of about 100 m x 6 m. The foundations were covered with 2mm PEHD foil. Directly upon it a layer of bark and aerial conduits of 100mm diameter were placed. The conduits were covered with a textile net with 5 mm loop wholes as well as the next layer of bark along the entire pile length. The piles height was 120 cm. The ground

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was mixed with biogenic compounds and simultaneously mechanically aerated using a rototiller. The nutrients added composed granulated NPK fertilizer (8% N (5.5% NH3-N, 2.5% NO3-N), 12% P2O5, 23% K2O) from Kemira company, Finland. Nutrients were added manually on the top of the piles before the mixing. In total about 6 thousand m3 of soil of different contamination level was used to build the 6 piles. Once the ground works were completed, the remediation plant was fitted with moisturizing system, static and dynamic aeration system, water circulation system, stripping tower. Additionally three double reactor sets, that is six bioreactors of 1000 liters each, fitted with aeration systems and temperature control were installed next to the three service containers. Each container and each double reactor set was defined for two piles. A diagram showing one of the three remediation sets of piles, containers and bioreactors is presented in fig. 2. Fig 3 shows a photograph of the remediation site with the piles, service containers and bioreactors.

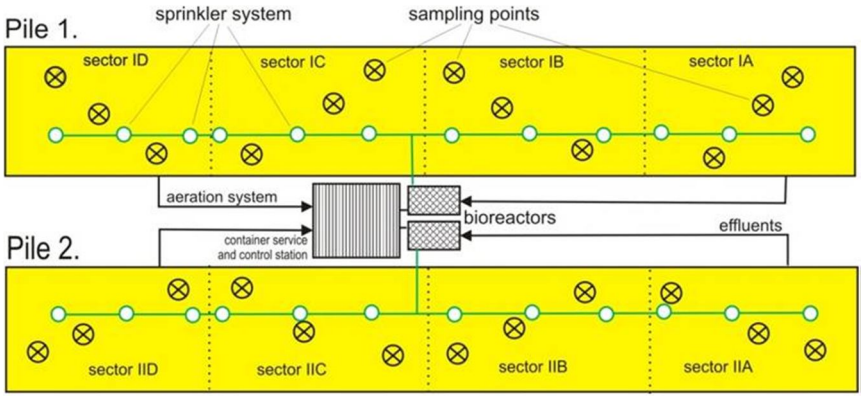


Fig. 2. Layout of the remediation piles with bioreactors, service container, sprinkling units and sampling points



Fig. 3. View of the remediation facilities with the piles, service containers and bioreactors

C. Development of Microbial Consortium

Microorganisms composing the biopreparation used for the ground remediation come from the contaminated area. They were isolated at the microbiological laboratory from the ground samples collected during project initial stage. For the bacteria isolation a mineral base containing BTEX was used. BTEX were the only source of carbon and energy for the microorganisms. To further studies the most active microorganisms demonstrating liabilities towards hydrocarbons degradation were selected by consecutive passages of isolated bacteria cultures. From the overall number of 11 strains isolated in the first stage of the work, only two strains remained after the selection process, involving the repeated passage. Standard diagnostic tests BioMérieux JD32GN, API 50 CHB were used to identify the mentioned above. The conducted tests indicated that isolated strains were not pathogenic and make up a natural soil microflora. The efficiency of hydrocarbons' decomposition by the isolated consortium of bacteria was tested. Once satisfactory results were obtained, a biopreparation intended for inoculation was prepared. The isolated strains were then frozen in the temperature of - 80oC.

Due to sanitary and environmental regulations, the microorganisms contained in the biopreparation, including *Pseudomonas putida I* and *Pseudomonas putida II*, were subjected to obligatory procedures in order to obtain required attestation. The attestation was

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obtained from the National Institute of Hygiene, Warsaw, Poland. The inoculants were multiplied in the bioreactors of 1000 liter volume and then fed into the ground during the late logarithmic bacteria growth. For the pH stabilization at 6.8 to 7.2 level, buffering agents were applied. As bacteria culture media the following chemical agents were used: Na2HPO4, KH2PO4, (NH4)2SO4, Mg(NO3) and a fertilizer product intended for special cultures delivered by Kemira company. Its composition was the following: N total – 8.0%; N NO3 – 2.5%; N NH4 – 5.5%; P2O5 – 12%; K2O – 23%; MgO – 2.5%; S – 11.0%; B – 0.1%; Cu – 0.2%; Fe – 0.1%; Mn – 0.7%; Se – 0.0006%; Ca – 1.0%.

III.REALIZATION OF THE RESEARCH

Asthe aim of the work was to study effectiveness of a native bacterial preparation isolated from the polluted ground the preparation was first tested in laboratory conditions and afterwards implemented in real polluted environment.

Scope of the work covered:

Isolation of the microorganisms capable of degrading hydrocarbons in the ground,

Determination of the taxonometric groups of the isolated strains,

Development of the technological procedures of the ground remediation,

Development and production of the bacterial inoculants,

Development of analytical and monitoring procedures,

Construction of the biopiles and its provision with technological facilities,

Application of nutrients and inoculants,

Biodegradation process monitoring and control.

The remediation process was carried out simultaneously in 6 piles. Its goal was to develop optimal remediation parameters and purify the contaminated grounds using biostimulation and bioaugmentation with involvement of bacteria isolated from the contaminated ground. The bacteria were adapted for degradation of the specific contaminants found in the environment. Assumption of the research was implementation of appropriate treatment allowing restoration of biological balance and inhibition of environment pollution and contaminants migration.

In case of the biopile method applied in the research the crucial factor was activity of the microflora used. The biological activity of microorganisms is influenced by the environmental conditions which can be controlled by enrichment of the ground with biogenic substances, by aeration as well as by inoculation with high productive microorganisms in relation to petroleum products degradability. The microorganisms were adapted and modified by the genetic engineering methods. The method and the system were patented by the authors and applied on civilian and military areas [32-36]. The bacteria preparations were isolated from the contaminated ground in each case separately, then adapted and multiplied in the field taking into account specific conditions of the environment considered. The assumptions made during elaborating the biological method were as follows:

Biological degradation of pollutants under the oxygen conditions

Implementation of the autochthonous microflora

Ex situ remediation method

Closed water circulation system.

The bioremediation process was carried out in four stages.

I stage – construction of the infrastructure composed of the heap aeration and watering systems, construction of the water circulation system consisting of draining ditches, wells, stripping column and bioreactor, assessment of the physical-chemical and microbiological properties of the ground.

II Stage – Selection of bioactive microorganism strains in relation to the pollutants in the ground. The taxonomy and pathogenic as well as relatively pathogenic properties of the strains were examined

III stage – Real bioremediation process carried out in heaps. The polluted ground was displaced in the heap equipped with aeration and drainage system. During the process the heap was sprayed with water and aerated to maintain optimal living conditions for bacteria.

Growth of micro flora was stimulated by

Inoculation of autochthonous bacteria reproduced in a bioreactor

Intensive aeration of the windrow using system of drainage and vertical air lances

Supplying the ground with biogenic substances

Maintaining proper humidity of the ground.

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IV stage - Displacing the ground from the heap to the original site after completing the bioremediation process.

The remediation process required an individual approach and development of tailored technology taking into account the local hydrological, geological, microbiological and physical-chemical conditions of the area. In the course of the processes basic physical-chemical and biological parameters of the cleaned environment were monitored that were essential to control the process and to achieve the desired purity standards.

IV. RESULTS

Due to climatic and technical factors, bioremediation process was subdivided into two seasonal periods:

I period – preparation works and preliminary bioremediation,

II period – actual bioremediation process.

The first period was started in late summer just after the piles were formed. The second period was most intensive starting in May and continued in June. It was the actual remediation stimulated by the introduction of previously prepared inoculant. The process was strongly influenced by the ambient air temperature which was the only parameter that could not be controlled. The hydrocarbons concentration decline and daily ambient air temperature changes are shown in fig. 4. Leachates drained from the pile contaminated with organic compounds were collected in a well and then sent to a reservoir. Next they were fed to a stripping column filled with ceramic rings where the hydrocarbons were separated from water. The fumes were adsorbed in a charcoal filter, whereas the purified water was sprinkled over the pile. The diagram of the process is presented in fig. 5.

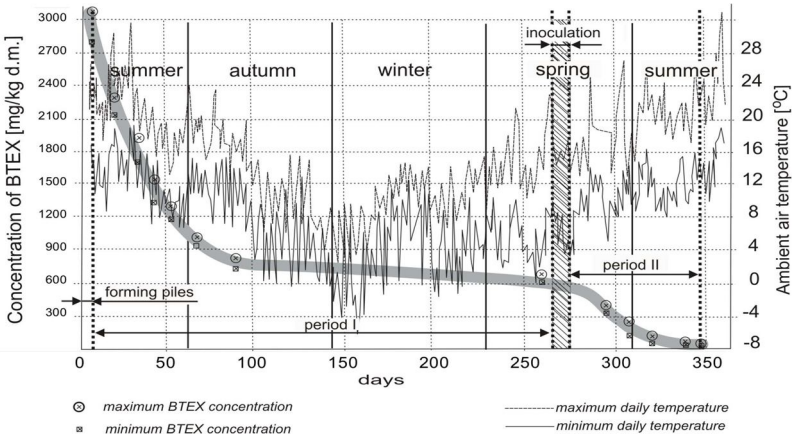


Fig. 4. Approximate hydrocarbons concentration (gray line) in the course of the bioremediation process and ambient air temperature changes

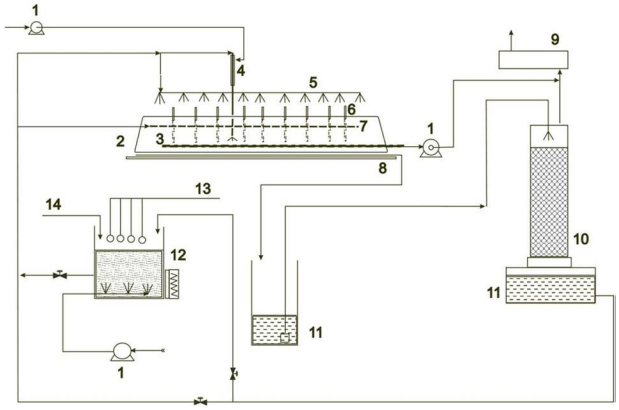


Fig. 5. Diagram of the system applied to the polluted ground bioremediation. 1 - air blast appliance, 2 - pile, 3 - drain-pipe, 4 - pressure injection, 5 - sprinkling, 6 – lance for preparations application, 7 - soil under drainage, 8 - geomebrane with water draining off, 9 - charcoal filter, 10 - stripping tower, 11 - well, 12 - bioreactor with heating system, 13 - pH-, temperature-, N- and P- control system, 14 - inoculum

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I period

The initial content of hydrocarbons in soil ranged between 2800-3050 mg/kg of dry matter. Relatively low number of bacteria and their low degradation activity was also observed. Results of microbiological analyses for the pile 1 (end of I period) are presented in tab. 1.

TABLE 1

Characteristics of the Polluted Ground Prior to The Remediation Process (a Sample Averaged from 12 Sampling Points Collected on the 6-th Process Day)

Pile	BTEX mg/kg	Dehydrogenase activity mgTF/g/24h(-G)	Dehydrogenase activity mgTF/g/24h(+G)	Number of microorganisms in soil CFU/g * 105	pH	Moisture by mass %	NNO3- mg N/g	NNH4+ mg N/g	P mg P/g
I	3050	1 .16	54 .80	101 .5	6 .2	9 .91	0 .8	18 .0	0 .9
II	3034	1 .23	48 .00	89 .8	6 .8	10 .03	0 .7	3 .2	traces
III	2884	2 .37	37 .85	78 .2	6 .0	13 .08	1 .1	21 .3	traces
IV	2972	2 .30	36 .45	137 .2	5 .8	12.34	07	15 .9	0 .4
V	2800	2 .46	25 .55	69 .2	7 .3	12 .78	0 .8	17 .4	traces
VI	2920	1 .10	49 .45	92 .0	7 .6	10 .89	0 .9	5 .9	1 .3

The process of biological purification in this stage involved only the soil's microflora, which development was stimulated by the introduced biogenic substances. During the summer months two samples were collected weekly from each sector of the soil piles. The ground left upon the piles was not additionally aerated, thus the biological decomposition of organic compounds, especially in the lower layers, proceeded under conditions with limited oxygen access. Therefore, anaerobic organisms were anticipated to take part in this process. In tab. 2 results analyses of contaminants found in the piles 1-6 after completing the first bioremediation period are shown. Period I was finalized in spring in the month of April (277-th process day).

TABLE 2

Microbiological and Chemical Analyses of the Polluted Ground after Completing the I-st Bioremediation Period (the Samples Were Averaged from 12 Sampling Points, 277-th Process Day)

Pile	BTEX mg/kg	Dehydrogenase activity mgTF/g/24h(-G)	Dehydrogenase activity mgTF/g/24h(+G)	Number of microorganisms in soil CFU/g * 105	pH	Moisture by mass %	NNO3- mg N/g	NNH4+ mg N/g	P mg P/g
I	691	2.56	64.66	101.5	7.2	9.35	4.9	15.0	traces
II	678	3.23	54.2	89.8	7.4	10.21	6.0	12.6	traces
III	664	2.77	39.95	78.2	7.5	10.80	4.7	14.5	0.001
IV	654	2.19	46.24	137.2	7.3	11.22	5.5	13.9	traces
V	621	2.84	45.76	69.2	7.5	12.67	3.1	10.9	traces
VI	628	3.12	47.86	92.0	7.7	10.11	3.8	12.1	traces

II period

During the second period the biodegradation of organic contaminants was aided by its inoculation with the biopreparation, maintenance of desired humidity and pH of the ground as well as introduction of vital biogenic substances (N, P). The process in this period was carried out from May to July (90 days) at temperature ranking from 15oC to 32 oC. Microbiological analyses showed that after inoculation of the ground with bacteria capable of decomposing aromatic hydrocarbons an intensive bacteria multiplication occurred when compared to the results from the previous year, at least by a row (tables 3 and 4). The above conclusion relates to all piles.

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TABLE 3

Microbiological and Chemical Analyses of the Polluted Ground in the Course of the II-nd Period, after Inoculation (a Sample Averaged from 12 Sampling Points – 291-th Day)

Pile	BTEX mg/kg	Dehydrogenase activity mgTF/g/24h(-G)	Dehydrogenase activity mgTF/g/24h(+G)	Number of microorganisms in soil CFU/g * 105	pH	Moisture by mass %	NNO3- mg N/g	NNH4+ mg N/g	P mg P/g
I	410	18.80	66.86	1560	7.0.	19.55	45.8	96.3	23.7
II	392	15.45	75.92	1230	7.1	25.87	44.0	93.0	23.3
III	350	23.82	53.21	3456	7.3	24.86	44.6	91.9	22.2
IV	366	21.00	34.76	2345	7.3	22.32	43.3	98.7	23.4
V	373	16.78	55.74	1245	7.2	24.21	44.6	97.9	22.8
VI	389	22.87	45.89	3045	7.4	23.25	41.5	88.8	21.8

TABLE 4

Microbiological and Chemical Analyses of the Polluted Ground in the Course of the II-nd Period (a Sample Averaged from 12 Sampling Points – 305-th Day)

Pile	BTEX mg/kg	Dehydrogenase activity mgTF/g/24h(-G)	Dehydrogenase activity mgTF/g/24h(+G)	Number of microorganisms in soil CFU/g * 105	pH	Moisture by mass %	NNO3- mg N/g	NNH4+ mg N/g	P mg P/g
I	210	42.0	73.00	2203	7.5	22.16	35.2	43.4	18.2
II	197	40.23	57.89	1535	7.7	24.34	34.3	44.5	17.9
III	176	112.37	27.65	5415	7.4	23.73	33.5	40.9	17.6
IV	172	84.80	37.85	4010	7.7	24.94	33.4	41.6	18.3
V	165	64.66	85.45	2860	7.3	23.43	35.0	45.9	18.9
VI	190	81.10	89.42	4430	7.6	24.12	31.7	38.9	16.9

In the month of May (319-th process day) bacteria count in particular piles varied from 1290·105 to 7410·105 CFU/g d.m. An increase in enzymatic activity of the ground was also observed. This fact testifies to the acceleration of biodegradation processes. Measurement of dehydrogenase ground activity made without additional substrate varied between 12.34 and 136.10 µg TF/g dry matter of soil (-G). Intensive processes of hydrocarbons decomposition promoted by the consortium of microorganisms were observed in the ground. Their metabolism was targeted mainly at the utilization of hydrocarbons as a substrate. The occurring microbiological processes resulted in a significant drop in petroleum products concentration. Chromatographic analyses conducted in June indicated almost entire elimination of the products from the grounds. The progress of hydrocarbons elimination is presented in tab. 2-5 and fig. 4.

Microbiological analyses conducted in June, that is at the end of the bioremediation process, indicated that lack of food substrate caused a drop in the count and metabolic activity of the microorganisms settled in the ground. The count of bacteria varied between 138.0to 807.5·105CFU/g d.m. Almost an entire stoppage of ground dehydrogenase activity measured without glucose was noted. Only when additional sources of carbon in the form of glucose were introduced an increase in enzymatic activity of ground was observed (+G). Value of this activity varied for particular tries between 0.0 to 71.30 µg TF/g d.m. of soil. It means that the microorganisms present in ground were most likely in the resting spore form or an anabiosic state and could activate itself after introduction of a food substrate. It can also be assumed that the makeup of the soil's microflora has changed after the biodegradation

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process. A drop in the count of previously dominating species of high degradation activity occurred. Their place was taken by microflora characteristic for pure grounds.

In the final stage of the process, series of control tests were conducted. During the II period weekly or two weekly soil samples were taken from each of the piles. Twelve subsamples were taken from different depths throughout the pile and mixed together from a single composite sample for each pile.

Based on the results of the tests, it may be assumed that the conducted in the first half of the year bioremediation work led to a drop in the concentration of BTEX contaminants down to the levels characteristic for cleaned grounds. The final results of the analyses are presented in tab. 5.

TABLE 5

Microbiological and Chemical Analyses of the Polluted Ground after the II-nd Bioremediation Period (a Sample Averaged from 12 Sampling Points – 347-th Process Day)

Pile	BTEX mg/kg	Dehydrogenase activity mgTF/g/24h(-G)	Dehydrogenase activity mgTF/g/24h(+G)	Number of microorganisms in soil CFU/g * 105	pH	Moisture by mass %	NNO_3^- mg N/g	NNH_4^+ mg N/g	P mg P/g
I	<0.005	0.48	69.76	317.7	8.0	29.05	10.2	21.4	0.05
II	0.080	0.00	38.80	146.0	7.9	20.53	8.7	22.7	traces
III	2.011	0.00	35.35	138.0	8.0	25.60	8.9	18.9	0.02
IV	3.022	0.00	46.74	445.5	7.8	27.28	7.0	19.6	0.06
V	1.868	0.00	68.32	807.5	7.7	24.88	9.3	25.7	0.04
VI	7.124	0.00	71.30	790.0	8.1	26.62	5.9	14.7	traces

V. CONCLUSIONS

It was proved that the developed biological method was very efficient, especially in eliminating hydrocarbon contaminants from the ground. A significant role in the hydrocarbons elimination from the soil was played by the natural biodegradation. During autumn and winter seasons there was still some microbial activity observed, contributing to the hydrocarbons concentration decrease in the ground.

The contaminated ground was composed mainly of a sandy fraction and small amount of organic matter, which facilitated the hydrocarbons desorption from the inter-grain spaces, particularly in case of the lighter organic compounds. This contributed substantially to speeding up the process. The multiple bioaugmentation within the second bioremediation period resulted in a rapid elimination of the hydrocarbons from the ground. In the averaged ground samples the BTEX concentration after the remediation did not exceed 0.005mg/kg d.m. It was observed that the speed of the degradation in the piles depended on the hydrocarbon structure and increased in the following order: xylenes > benzene > toluene > ethylbenzene. Originality of the implemented ex situ procedure consists in a novel method of the microorganisms cultivation with the use of the pile effluents. The effluents composed a basis in the field bioreactors, used for the cultivation of the selected bacteria resistant to metabolites originated during the organic pollutants degradation.

VI. ACKNOWLEDGEMENT

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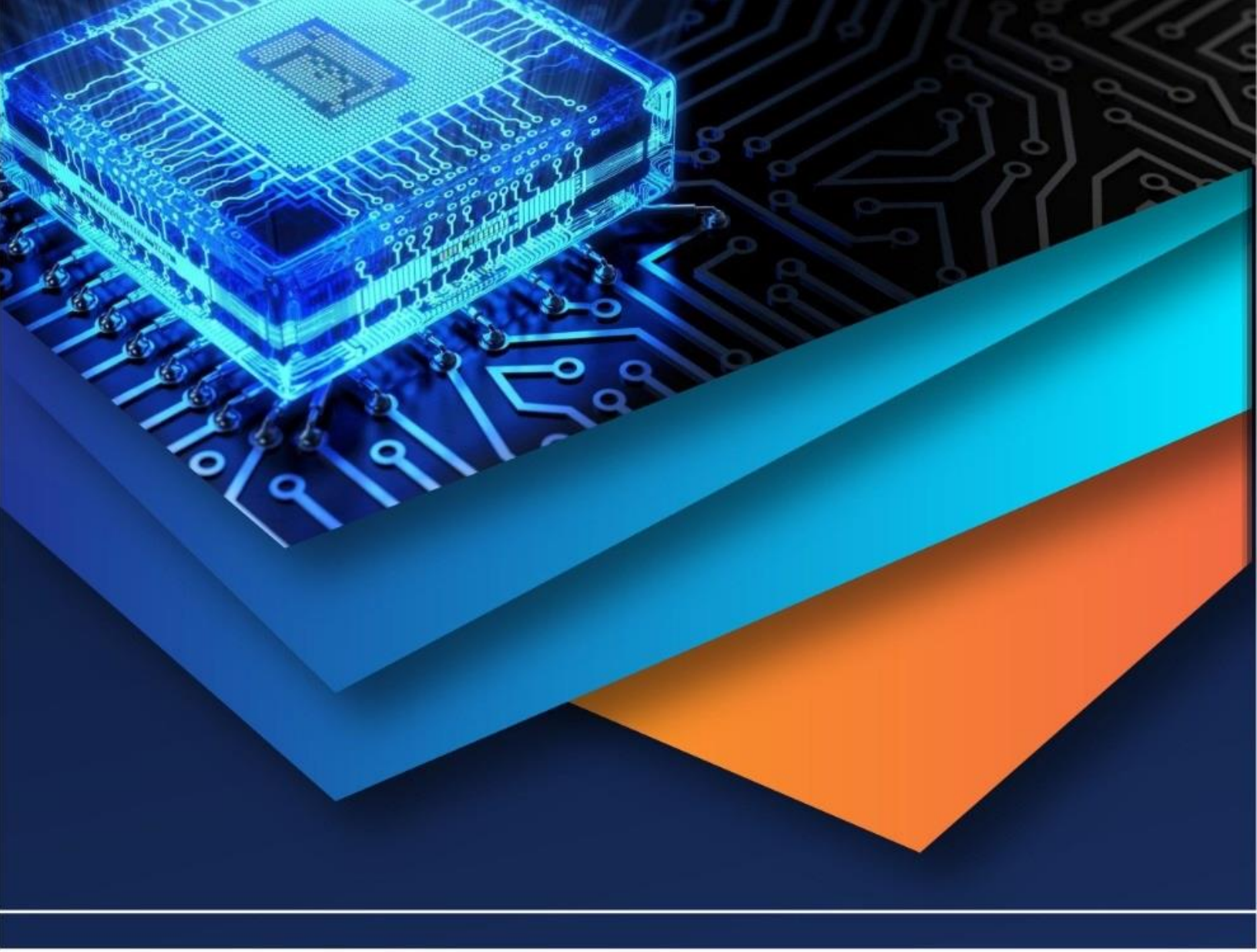
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