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Hydrogen peroxide as a biodegradation stimulator in remediation processes of soils heavily contaminated with petrochemicals

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The soil contaminated with petroleum products must be excluded from the crops and treated to reclamation processes. Natural processes of decomposition of hydrocarbon compounds go very slow, so it is necessary to use bioaugumentation or stimulation in order to accelerate the return of the soil to high culture. In this study the effect of hydrogen peroxide on the process of cleaning soil strongly contaminated with pertochemicals was investigated. For this purpose, a pot experiment lasting 60 days was carried. The dynamics of changes in the population of filamentous fungi, yeasts and bacteria were examined and also content of aliphatic hydrocarbons (n-alkanes), monoaromatic and polycyclic aromatic hydrocarbons (PAHs). Experimental use of hydrogen peroxide in the process of biodegradation of petroleum compounds assisted in the analyzed soil led to an increase of the number of gram-positive bacteria during the test. Stimulation of oil products biodegradation by hydrogen peroxide also increased by 35% decomposition efficiency of aliphatic hydrocarbons (C8-C40) and about 50% PAH's in comparison to control samples without hydrogen peroxide. There was no influence of hydrogen peroxide on the content of monoaromatic hydrocarbons (BTEX) with respect to controls, although in the end of experiment, the total concentration decreased by about 50% compared to the initial content.

Keywords: soil, remediation, petrochemicals, hydrogen peroxide.

INTRODUCTION

Crude oil products (petrochemicals) may transform in the environment as a result of physico-chemical as well as biological processes. Attenuation (natural processes to decrease concentrations of contaminants in soil) consists in degradation of petrochemicals by autochthonic microorganisms which use them as a main source of carbon[1, 2, 3, 4]. The process may take even several hundred years[5]. The final attenuation products are typically carbon dioxide, inorganic substances and biomass.

Biodegradation of soil-contaminating petrochemicals depends on site specific conditions such as physical and chemical properties of soil, concentration and structure of the petrochemical contamination, concentration of biogenic compounds, temperature, oxygen content, humidity, soil reaction and activity of the microbial consortia[6, 7].

Soil bioremediation processes may be enhanced by the use of such techniques as bioaugmentation and/or biostimulation[5, 8] with oxygen or/and correcting soil reaction and enrichment with biogenic compounds facilitating the development of autochthonic microbial communities.

The rate of the biodegradation process is to a large extent determined by oxygen delivery to the soil. It can be applied either directly to subsurface (oxygen injection) or in a form of chemical compounds such as ozone or hydrogen peroxide. Due to its properties and decomposing to yield only oxygen and water, hydrogen peroxide is considered environmentally friendly powerful oxidizer, often applied in chemical clean-up processes of soils with pH 3–5 (Fenton reaction)[9, 10, 11, 12]. Reactive forms of oxygen radicals may also occur at soil pH above 5[13, 14].

The aim of this study was to determine the rate of the microbial changes and the biodegradation course in a highly petrochemical contaminated soil treated with hydrogen peroxide.

EXPERIMENTAL

Material and research methods

Pot experiment was designed and conducted. Clay soil originating from a petrochemical contaminated site was used for the test. The soil was in a form of fatty black mass of a specific odor, with a high petrochemical content of ca. 17 g kg^{-1} d.m. and pH 8.

Each pot was filled with 1 kg of contaminated soil. Next hydrogen peroxide (H_2O_2) was added to the soil in the amount of 1mg O_2 g^{-1} d.m (S+O). Soil with no hydrogen peroxide application was used as control (C). Humidity was maintained at the level of 60%, the mass of the pots was controlled using scales. The pot tests were set up in three replications. Treated soil was incubated in open pots at room temperature of 22°C (±2°C) over a period of 60 days.

To determine the rate of the chemical and microbiological changes, a 10g soil sample was collected for analysis from each pot: before the experiment, after 0.25 day (6 hours), after 30 days and after 60 days.

Microbiological methods

Qualitative and quantitative determination of the microbial populations was carried out using standardized Koch's cultivation method: before the experiment, after 6 hours and after 30 and 60 days of the experiment duration in media typical for specific microorganisms:

– fungi in medium Czapek with glucose[15]
– yeast in medium YPG with chloramphenicol (0.1 g dm^{-3})[16, 17]
– bacteria in Nutrient LAB – Agar™ (BIOCORP)
– actinomycetes in Starch Casein Nitrate Agar (Difco)
– microorganisms capable to grow in medium containing hexadecane as the only source of carbon[18].

Bacterial and yeast counts were made after 5 days of experiment set up and 10 days for other investigated microbial populations. The counts of the microorganisms were expressed in cfu g^{-1} d.m.[2, 19].

Different bacterial colonies identified in microscope observations were divided into morphological groups using Gram's staining method. Filamentous fungi were determined by their morphological characteristics using selected taxonomic monographs.

Chemical analyses

– Reaction of the KCl solution (pH_{KCl}) was determined by potentiometric method using ELPO N-512 pH – meter.

– Dry mass was determined by weight at room temperature.

Samples for chromatographic determinations were weighed and dried at room temperature over anhydrous sodium sulfate[20].

Investigated compounds were extracted in an automatic fexIKA® extractor (IKA –Werke. Dichloromethane (POCH GC grade) was used for monoaromatic hydrocarbons (BTEX) extraction[21]. Polycyclic aromatic hydrocarbons (PAHs) were extracted using dichloromethane and hexane in proportion 1:9 (v/v)[22] while for alkanes – n-hexane (POCH GC grade) was applied. Samples were condensed and purified with activated aluminum oxide before extraction[23].

Qualitative and quantitative analysis of the produced extracts was carried out by gas chromatography using SHIMADZU GC 17A gas chromatograph coupled to MS-QP5000 mass detector.

Conditions for conducting the chromatographic analysis:

– BTEX – VF5 – ms capillary column 30 m x 0.25 mm x 0.25 μm; carrier gas (He) flow rate – 1cm^3 per minute, injection port operating temperature – 250°C, detector temperature – 280° C; temperature program 30-5 /5/ 170–6; MS detector voltage from 1.2 to 1.4 kV

– PAH- VF5 – ms capillary column 30 m x 0.25 mm x 0.25 μm; carrier gas (He) flow rate –1cm^3 per minute, injection port operating temperature – 300°C, detector temperature – 310°C; temperature program 80-8 /10/ 270-12/300-12; MS detector voltage from 1.2 to 1.4 kV

– n-aliphatic hydrocarbons – VF1 – ms capillary column 30 m x 0.53 mm x 1.50 μm; carrier gas (He) flow rate – 3 cm^3 per minute, injection port operating temperature – 300°C, detector temperature – 325°C; temperature program 100-3/12/320–12; MS detector voltage from 1.2 to 1.4 kV

The amount of petrochemical substances in soil extracts was determined by integrating cumulative peaks covering the boiling temperature range of 126–522°C. Aliphatic hydrocarbons of chain length of C8 (octane) to C40 (tetracontane) were determined. From the monoaromatic hydrocarbons group, the following were determined: benzene (B), toluene (T), m+p-xylene (m+p-x), o-xylene (o-x), styrene (Sty) and izopropylobenzene (Izo-pb). In the polycyclic aromatic hydrocarbons group (PAH) the following 16 compunds were deteremined which are recommended for monitoring by the US. EPA: naphtalene (Nap), acenaphtylene (Ayl), acenaphten (Aen), fluorene (Flu), fenantrene (Fen), anthracene (Ant), fluorantene (Fla), pyrene (Pur), benzo[a]anthracene (BaA), chryzene (Chr), benzo[b,k]fluorantene (BbF, BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-c,d] pyrene (IcP), benzo[g,h,i]perylene (BgP).

RESULTS AND DISCUSSION

Hydrogen peroxide (H_2O_2) applied to soil contaminated with petrochemicals (compared to control C) stimulated the growth of prokaryotic microorganisms while slightly inhibited the development of eukaryotic ones which may be attributed to the antagonistic impact. At the same time the number of microorganisms capable of petrochemical compounds degradation in a selective medium with hexadecane as the only source of carbon stayed at constant level (Fig. 1). During the experiment, bacteria dominated (95%) in the investigated microflora, with the share of grampositive bacteria in the total population on the level of ca. 60% (Fig. 2).

Microbiological changes in soil with hydrogen peroxide application were accompanied by changes of soil pH and chemical composition of the analyzed samples. Compared to control (pH 7,9), the reaction of the H_2O_2 treated soil has distinctly decreased reaching 6,4 after 60 days of experiment duration (Fig. 3). This indicated production of substances of acid character.

An increase of the effectiveness of petrochemicals removal was observed after hydrogen peroxide application to soil. (Fig. 4). At the incubation phase of the experiment i.e. after 0.25 day, the biodegradation rate was 19% compared to the initial content, whereas after 60 days it reached 58.7%. Simultaneously however, spontaneous transformations of petrochemicals in control samples were observed during incubation due to autochthonic microorganisms activity. Taking account of this fact, the biodegradation rate was by 21.7% higher compared to control.

Chemical analysis of petrochemical compounds showed the presence of n-aliphatic, monoaromatic (BTEX) as well as polycyclic aromatic hydrocarbons (PAHs). In the conditions of the experiment, PAHs turned to be the most susceptible to biodegradation and BTEX the least. (Fig. 5).

The effectiveness of the biodegradation process of n-aliphatic hydrocarbons (C8-C40) was by 35% higher than in control samples (no stimulator applied) with the highest intensity of the process observed between day 30 and 60 of the experiment. A decrease in the content of these hydrocarbons has been observed already after 6 hours since the experiment start up, it especially referred to long-chain hydrocarbons (C17-C40), except for C14 and C16 (Table. 1).

Application of hydrogen peroxide had no influence on the content of monoaromatic hydrocarbons (BTEX) in the contaminated soil. Although upon the completion of the experiment the total concentration of BTEX decreased by ca 50% compared to the initial values, however after 60 days no differences were observed between the control and the samples with hydrogen peroxide addition which proved no effect of the stimulator on the metabolism of these compounds. The content of basic benzene compounds changed over time however an unambiguous determination of the course of these changes was not possible. A distinct increase of benzene

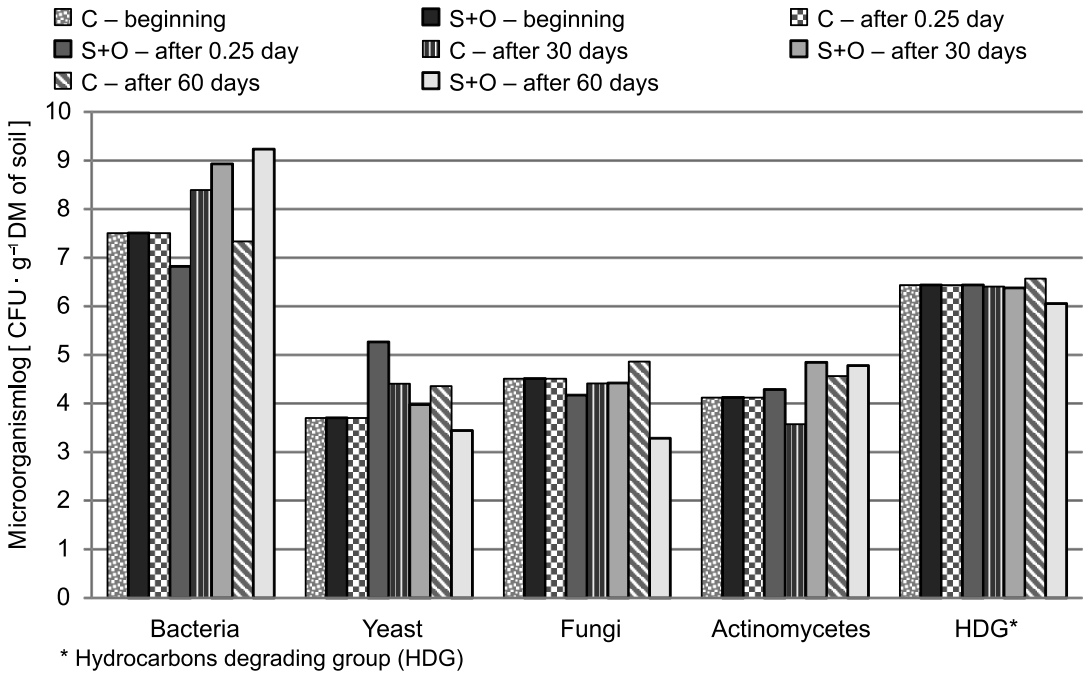

Figure 1. Dynamics of changes of autochthonous microflora number [log cfu · g^{-1} d.m.]. After 0.25 day and 30, 60 days of experiment: bacteria, yeast, fungi, actinomycetes, and microorganisms using hexadecane as a source of carbon. (C – control; S+O – soil with hydrogen peroxide)

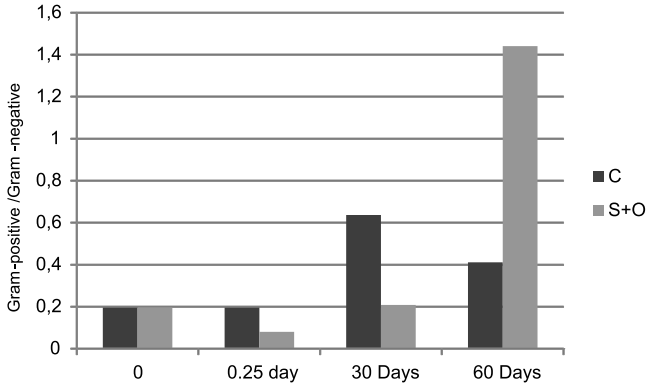

Figure 2. The share of grampositive (G+) and gramnegative (G–) bacteria in total population [%]

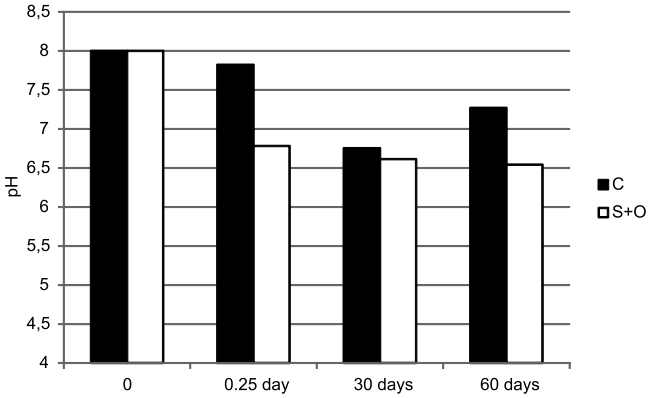

Figure 3. Changes of pH of soil samples polluted with petrochemicals after use of hydrogen peroxide. (C – control; S+O – soil with H_2O_2)

content was observed until day 30 of the experiment followed by a 10-fold decrease (Table. 1).

Compared to the initial concentration, the total PAHs content in all investigated samples with H_2O_2 addition decreased significantly. The reduction rate was 15% after

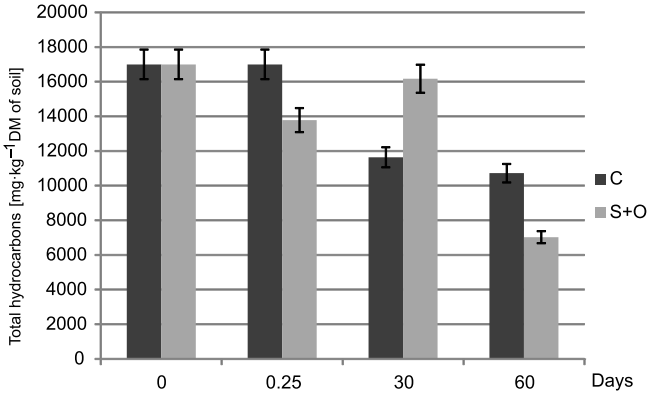

Figure 4. The total content of petrochemicals [mg kg^{-1} d.m.] in contaminated soil after biodegradation process. (C – control; S+O – soil with H_2O_2)

0.25 day and up to 85% after 60 days. The effectiveness of biodegradation stimulation was by 50% higher compared to control, although the contents of individual PAHs in H_2O_2 stimulated samples changed differently (Table 1.)

Biodegradation of petrochemicals in soil may undergo spontaneously, however to intensify this process different stimulation techniques are applied, especially with the use of environmentally friendly activators such as hydrogen peroxide. In order to use the stimulation techniques in a semi-full or full sale, it is necessary to conduct laboratory tests in order to determine the impact of a given stimulator on the number and activity of the autochthonic microbial populations and thus on the contamination reduction rate.

The experiment showed that the number of prokaryotic microorganisms initially decreased and then increased as a result of hydrogen peroxide stimulation. This effect has been also confirmed by the studies carried by Jung[15, 16]. He suggests that small changes in the bacteria number directly after stimulator application result from the use of reactive oxygen forms by hydrocarbons to

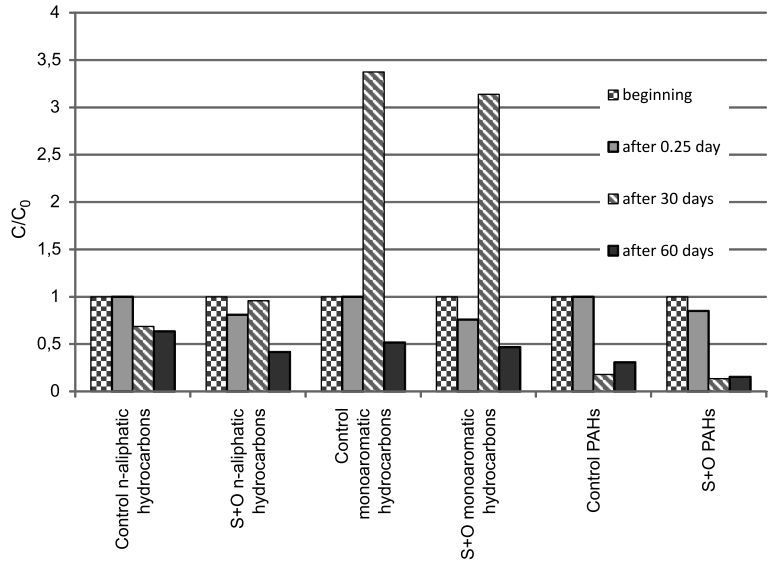

Figure 5. . Relative changes of hydrocarbons content [%] in comparison to control after 0.25 day, 30 days and 60 days of biodegradation. (C – control; S+O – soil with H_2O_2)

Table 1. Hydrocarbon content in soil during experiment [mg · kg^{-1} d.m.]

Time [days]	0		0.25 d		30 d		60 d	
Hydrocarbons	Control	Stimulation	Control	Stimulation	Control	Stimulation	Control	Stimulation
n-Aliphatic								
C8	58	58	58	32	29	64	16	29
C9	23	23	23	16	12	12	3	2
C10	57	57	57	32	32	48	4	5
C11	157	157	157	130	87	155	6	15
C12	384	384	384	329	205	459	73	71
C13	404	404	404	363	207	393	102	82
C14	584	584	584	480	299	712	73	191
C15	1021	1021	1021	821	513	994	467	302
C16	1096	1097	1097	919	556	1066	302	434
C17	1696	1696	1696	1312	856	1532	1162	700
C18	1593	1593	1593	1272	813	1442	1157	677
C19	1124	1124	1124	985	564	1104	862	589
C20	899	899	899	932	481	1008	765	417
C21	599	599	599	621	321	673	510	278
C22	566	566	566	467	932	559	387	284
C24	1126	1126	1126	625	1040	1057	545	361
C26	1574	1574	1574	1258	1129	1407	1085	692
C28	1371	1371	1371	1020	882	1139	905	556
C30	972	972	972	787	898	797	1041	531
C32	871	871	871	746	913	911	700	521
C40	603	603	603	451	789	576	486	253
Monoaromatic								
Benzene	0.6	0.6	0.57	2.4	37	35	4	4
Toluene	1.6	1.6	1.56	0.3	0.3	0.1	0.9	0.1
Ethylbenzene	0.3	0.3	0.34	0.7	0.6	0.1	0.1	0.1
m+p-Xylene	1.2	1.2	1.18	0.9	0.3	0.3	0.2	0.2
o-Xylene	1.6	1.6	1.58	1.2	0.3	0.1	0.2	0.1
Styrene	1.4	1.4	1.37	0.6	0.2	0.3	0.1	0.2
Isopropylbenzene	5.0	5.0	4.97	2.7	0.4	0.6	0.5	0.6
PAHs								
Nap	24	24	24	24	4	5	4	3
Ayl	14	14	14	13	2	3	3	1
Aen	43	43	43	45	5	7	12	3
Flu	5	5	5	6	1	1	1	1
Phe	10	10	10	12	1	2	3	2
Ant	14	14	14	14	2	2	5	3
Fla	24	24	24	13	1	1	5	4
Pur	1	1	1	1	0.5	0.1	0.6	0.2
BaA	2	2	2	11	5	3	17	0.8
Chr	12	12	12	8	1	0.7	0.5	1.7
BbF	16	16	16	7	2	0.3	6	0.1
BkF	6	6	6	12	2	1.1	1	5
BaP	5	5	5	4	2	0.5	0.5	6
IcP	28	28	28	3	4	0.8	1	0.5
DhA	1	1	1	2	4	0.7	2	0.5
BgP	2	2	2	2	0.5	0.6	0.4	0.3

degrade bindings of low bioavailability[18, 24]. An indicator expressed by the proportion of grampositive bacteria to gramnegative may reflect the degree of soil contamination with hydrocarbons. According to Margesin et al.[25] an excessive number of gramnegative bacteria in relation to grampositive ($k<1$) is typical for heavily contaminated soil samples. In this context it can be stated, that hydrogen peroxide enhanced biodegradation contributes to contaminants removal from soil.

During the experiment, a decrease of soil reaction was observed which may result from the presence of both intermediate and final products of hydrocarbons biodegradation of acid character. This phenomenon has been also confirmed by studies carried out by other authors[8, 9].

Application of biostimulation with hydrogen peroxide resulted in reduction of petrochemicals content in the investigated soil samples. This may be attributed to an increase of bioavailability of hydrocarbon bindings due to oxidation and in consequence increased the activity of the studied microorganisms[4, 7, 16, 26, 27, 28, 29].

An observed temporary increase of benzo(a)pyrene may be problematic as this compound is considered a cancerogenic factor[30, 31]. A rise of the benzo(a)pyrene content may be caused by its desorption from soil pores[32] or cumulating by *Bacillus megaterium* strains[26, 33]. Elevated contents of some polycyclic compounds may also result from co-metabolism of other compounds[34, 35]. Similar results of PAH's decomposition in 40-days experiment were obtained in incubation temperature 30°C[36].

CONCLUSIONS

Application of hydrogen peroxide in biodegradation processes of petrochemical compounds in soil led to an increase in the number of grampositive bacteria which indicated an effectively progressing degradation of contaminants in soil.

Stimulation of the biodegradation process with hydrogen peroxide increased the effectiveness of n-aliphatic hydrocarbons (C8-C40) degradation by 35% and PAHs by 50% compared to control.

Application of hydrogen peroxide to stimulate the process showed no relevant impact on the change of monoaromtic hydrocarbons (BTEX) content compared to control, though a total reduction of their mass obserrved during the experiment was 50%.

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