Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *85* (8) 1067–1081 (2020)
JSCS–5360
UDC 628.112+549.88:504.4.054:
542.92.000.57(497.11Belgrade)
Original scientific paper

Bioremediation of groundwater contaminated with petroleum hydrocarbons applied at a site in Belgrade (Serbia)

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(Received 23 October, revised 30 December 2019, accepted 3 January 2020)

Abstract: Due to their extensive use, petroleum hydrocarbons are among the most common groundwater contaminants. Compared to the traditional methods of physical pumping of contamination from the aquifer and subsequent treatment (*i.e.*, pump and treat), bioremediation is an economically cost-effective technology. The aim of this remediation approach is to transform biologically contaminants, most often by microbiological activity, into non-toxic compounds. More precisely, it is an active remediation process that involves biostimulation (increase of aquifer oxygenation, addition of nutrients) and/or bioaugmentation (injection of a concentrated and specialized population of microorganisms). Using both biostimulation and bioaugmentation, enhanced *in situ* groundwater bioremediation was applied at a hydrocarbon-contaminated site in Belgrade. The bioremediation treatment, applied over twelve months, was highly efficient in reducing the concentrations of total petroleum hydrocarbon (TPH) to acceptable levels. The concentration of TPH in the piezometer P-5 was reduced by 98.55 %, in the piezometer P-6 by 98.30 % and in the piezometer P-7 by 98.09 %. These results provided strong evidence on the potential of this remediation approach to overcome site-limiting factors and enhance microbiological activity in order to reduce groundwater contamination.

Keywords: enhanced *in situ* bioremediation; hydrocarbon-contaminated groundwater; biodegradation.

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https://doi.org/10.2298/JSC191023003B

INTRODUCTION

Due to the extensive human use of petroleum hydrocarbons, this group of compounds is among the most common groundwater contaminants.[1] Simultaneously, application of pump and treat systems for the remediation of hydrocarbon-contaminated sites has proven less practical and reliable than first envisioned.[2] As a result, focus on the remediation approach for groundwater contaminated by petroleum hydrocarbons has been changed to bioremediation. Bioremediation is an efficient and cost-effective technology in which organic pollutants are biologically transformed into less toxic compounds, or completely degraded to carbon dioxide and water.[3] Microorganisms are the main biocatalysts in bioremediation, and they transform contaminants through reactions that are part of their metabolic processes.[4] The bioremediation treatment can be conducted at the contaminated site (*in situ*), or outside of the contaminated site (*ex situ*).[5] Groundwater *in situ* bioremediation is cheaper compared to traditional site remediation approaches (*e.g.*, pump and treat), because it eliminates liability costs for transportation and storage of hazardous waste. Currently, bioremediation accounts for approximately 25 % of all remediation treatments worldwide.[4,6] On the other hand, the use of this remediation technology in Serbia is in the early-development stage. This paper aims to provide insight into the mechanisms and bioremediation requirements, as well as the results of the application of enhanced *in situ* groundwater bioremediation on the industrial level at a hydrocarbon-contaminated site in Belgrade (Serbia).

Bioremediation mechanisms and requirements

Mechanisms of bioremediation. During evolution, microorganisms have developed different mechanisms for the degradation of petroleum hydrocarbons. If oxygen is an electron acceptor during hydrocarbon degradation, the mechanism is called aerobic. Otherwise, it is called anaerobic, and other electrons acceptors are involved (*e.g.*, nitrate, sulfate or iron).[7] The primary reaction in aerobic biodegradation of petroleum hydrocarbons always requires the action of oxygenase and the presence of free oxygen.[8] Monooxygenases catalyzes the incorporation of one atom of oxygen into aliphatic hydrocarbon molecules.[4] The resulting alcohols are enzymatically transformed into aldehydes or carboxylic acids. Carboxylic acids are further metabolized in the β-oxidation process.[4,8] Generally, *n*-alkanes are the most biodegradable of all petroleum components.[8,9] The pathways of the *n*-alkane aerobic biodegradation are shown in Fig. 1. In most aromatic hydrocarbons, the diol group is formed in the first stage of degradation, when dioxygenases incorporate two atoms of oxygen into the aromatic hydrocarbon molecules.[4] The newly formed *cis–cis* diol, catechol[10,11] is further transformed to a carboxylic acid and Acetyl-CoA (Fig. 2).[10] For aromatic hydrocarbons, the efficiency of biodegradation depends on the number of rings present in the molecule. More aromatic groups result in higher resistance to degradation and *vice versa*.[11]

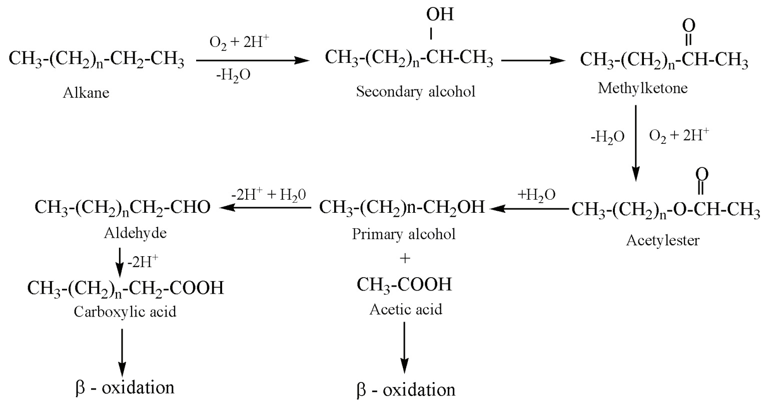

Fig. 1. Microbial degradation of *n*-alkane under aerobic conditions.[8]

In the absence of oxygen, various mechanisms, such as the addition of fumarate, carboxylation, hydroxylation and methylation, can initially activate hydrocarbons.[13,14] The addition of fumarate is the most common mechanism used by different anaerobic microorganisms to activate alkanes (linear and cyclic) or alkyl-branched aromatic compounds (alkylbenzenes, methylnaphthalenes, *etc.*).[13]

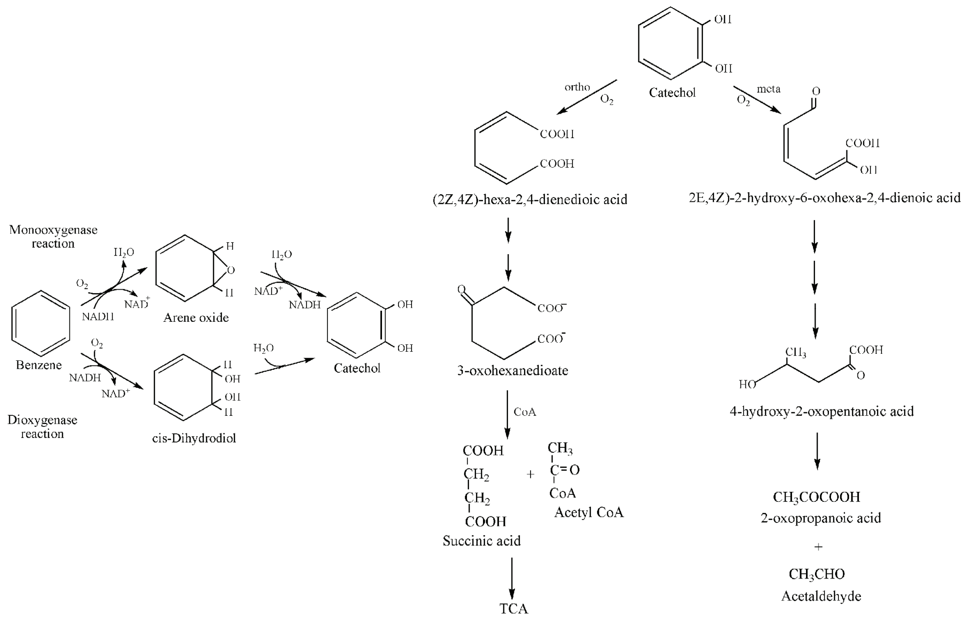

Fig. 2. Microbial degradation of benzene under aerobic conditions.[10,12]

In the case of *n*-alkanes, the addition of fumarates usually occurs at a subterminal C2 position which gives (1-methylalkyl) succinate (Fig. 3A and B).[13,15] Alkylsuccinates are then transformed by decarboxylation giving branched fatty acids that can enter into *β*-oxidation.[15] In anaerobic catabolism, the central intermediate in the degradation of aromatic compounds is benzoyl-CoA, which is then completely decomposed to carbon dioxide (Fig. 3C).[14]

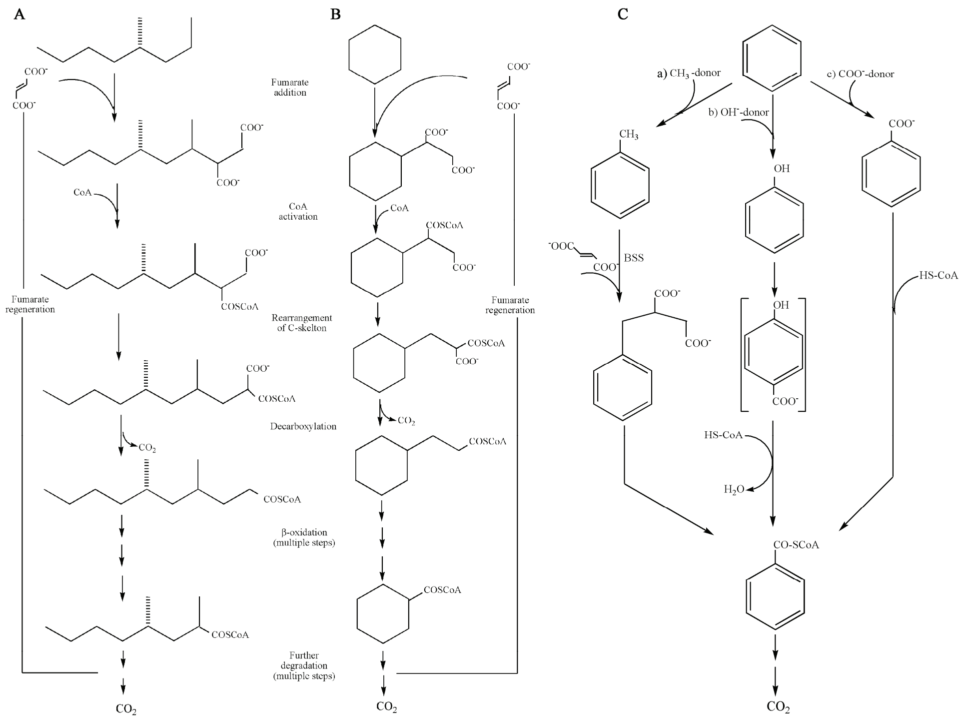

Fig. 3. Anaerobic biodegradation of alkanes *via* fumarate addition: A) pathway for the biodegradation of *n*-alkanes, B) pathway for the biodegradation of cyclic alkanes [15] and C) pathway for the biodegradation of benzene *via* three different mechanisms: a) methylation; b) hydroxylation; c) carboxylation.[14]

However, it should be emphasized that organic compounds will be degraded to a measurable extent only if the organism has enzymes that catalyze its conversion to a product that can be incorporated into an existing metabolic pathway.[8]

Bioremediation requirements. Enhanced *in situ* bioremediation of groundwater contaminated by petroleum hydrocarbons is an active remediation procedure that implies biostimulation and/or bioaugmentation.[6] Biostimulation is the more frequent approach, which involves the addition of oxygen and nutrients (nitrogen and phosphorus) in order to stimulate the growth and activity of microorganisms. Whereas, bioaugmentation implies the addition of a concentrated and specialized population of microorganisms (single strain or mixed culture-consortium).[3] However, bioremediation is not universally applicable and it requires an understanding of site-specific limiting factors.[6]

The presence of microorganisms with the metabolic capacity to synthesize enzymes for the degradation of contaminant is a major bioremediation requirement.[16] Thus, the indigenous bacteria at contaminated sites play a key role in the successful application of bioremediation.[17] Microorganisms obtain energy through oxidation–reduction reactions, where a contaminant is being used as an energy source (*i.e.*, electron donor), while inorganic components are electron acceptors. Compared to the other electron acceptors, the reduction of dissolved oxygen yields the highest amount of energy. Due to this, aerobic biodegradation is the most efficient mechanism for the degradation of petroleum hydrocarbons.[6,17] The favorable environmental conditions for microbiological activity involve a sufficient amount of moisture, acceptable acidity, optimal temperature and availability of nutrients.[3,6] The optimal pH values for the activity of microorganisms are neutral to base.[3] Temperature is among the most important environmental factors since it affects the growth and development of the microbiological population. According to Venosa and Zhu,[9] the highest degradation rates in freshwater are achieved at temperatures from 20 to 30 °C. The most common elements used by microorganisms are carbon, phosphorus, nitrogen and trace metals. Paul and Clark[18] indicated that a C:N:P mass ratio of 30:5:1 is favorable for the growth of a microbiological population.

In addition to the above-mentioned requirements, the effectiveness of groundwater bioremediation systems will depend on the hydraulic conductivity of the aquifer. The hydraulic conductivity is an important parameter that defines the ability of the aquifer to distribute nutrients and electron acceptors.[6] For a successful application of a bioremediation system, hydraulic conductivity values should be higher than 10^{-4} cm s^{-1}.[19] Overall, the application of bioremediation under field conditions is a complex task, since it requires the understanding and improvement of site-specific limiting conditions.

The aim of the present study was *in-situ* groundwater bioremediation in the Sava River aquifer near the thermo-energetic plant in Belgrade, Serbia.

At the beginning of 2015, during a regular quarterly analysis of the groundwater quality at the location of the thermo-energetic plant in Belgrade, an increased concentration of mineral oils and a strong odor of oil pollutants were discovered. Due to a suspected environmental incident, a large survey of the quality of the groundwaters at this location was conducted.[20] The research included a system of 10 piezometers for groundwater sampling. Surface water samples from the Sava River between the riverbank and the dock were analyzed as well. The results confirmed the presence of diesel and heavy fuel oil in the investigated groundwaters.[21] The volumetric analysis indicated that the total predicted volume of contaminated water was approximately 105 m^3. Considering the fact that the concentration of petroleum hydrocarbons in some of the investigated piezometers was higher than the remediation value for these contaminants (according to national legislation), *in situ* bioremediation of the groundwater at this locality was recommended as the most appropriate remediation procedure.[20,21] The present paper presents the results of enhanced *in situ* groundwater bioremediation at this locality.

Details related to geological settings are given as Supplementary material to this paper.

EXPERIMENTAL

Enhanced *in-situ* groundwater bioremediation was applied at the site of the thermo-energetic plant in Belgrade. This remediation approach combined both field and laboratory research activities. The field research activities included a detailed characterization of the investigated location (exploration drilling, hydrogeological mapping and water table measurements) and the installation of the specific infrastructure needed for the enhanced *in-situ* groundwater bioremediation. For this purpose, 15 bioremediation wells (10.10 cm diameter PVC pipes, fully screened across the saturated zone), 9 control wells (having the same construction and the same depth as the bioremediation wells), and filtration/adsorption columns were installed at the investigated location.

During the installation of the necessary infrastructure, from the zone of the groundwater table, groundwater and sediment samples were collected for isolation of the active consortium of zymogenous microorganisms to be used in the *in situ* bioremediation. The soil and groundwater samples were transferred into glass jars, and kept and transported at 4 °C, and analyzed within 24 h. From these samples, the zymogenous consortium was cultured according to the procedure described in details in a previous paper.[22] The obtained suspensions of the cultured microbial consortium were inoculated in Erlenmeyer flasks (5 dm^3), each containing 2000 cm^3 of the medium composed from: 23 g of nutrient broth (Torlak, Belgrade, Serbia); 100 cm^3 of groundwater extract; and 20 g of mazut (added as an additional carbon source but also as a model compound, added to stimulate the flourishing of the most active hydrocarbon degrading species from the zymogenous consortium). The growth conditions for the microorganisms were optimized relative to the conditions of the location from which they were isolated. Multiplied microbial populations were then used to inoculate (approx. 1 vol. %) a self designed bioreactor, under field conditions (total volume 1000 dm^3; with a working volume of 800 dm^3). The biostimulation solution for the optimum $C_{organic}$:N_{total}:P_{total} ratio, pH and concentration of biodegradable surfactant consisted of 12 g dm^{-3} meat peptone (Torlak, Belgrade, Serbia), 0.2 g dm^{-3} $(NH_4)_2HPO_4$, 50 cm^3 dm^{-3} soil extract, BioSolve Clear original solution (1 cm^3 dm^{-3}) and 10 g dm^{-3} of mazut. The growth conditions were: non sterile, at 25 °C; aeration and agitation: 0.70 volume of air/volume of medium with a minimum of 1 dm^3; pH 7.0 (adjusted with 10 M HCl or NaOH); duration 48 h; and sunflower oil (1 cm^3 dm^{-3}) as an antifoam agent.

The total petroleum hydrocarbons (TPH) from the groundwater samples were determined according to the ISO standard: 9377-2: 2000.[23] Besides TPH determination, some physicochemical and chemical parameters were determined in order to characterize the samples of groundwater in these studies. Conductivity and total dissolved solids (TDS) was measured by conductometer model 44600 Conductivity/TDS meter, manufactured by the HACH company.[24] Dissolved oxygen was measured directly by a digital oximeter (type Oxi 330i) manufactured by "WTW" (Weilhem, Germany) with a membrane electrode.[25] A digital mV/pH Hanna instruments voltmeter was used for all pH measurements.[26] The temperature was measured using a digital thermometer "Elite", manufactured by "Hanna Instruments" (Padova, Italia).[27] The concentrations of nitrogen and phosphorus were determined according to the standards: SRPS EN 12260:08[28] and SRPS EN ISO 6878:08.[29]

RESULTS AND DISCUSSION

The results for physicochemical and chemical parameters of the groundwater samples are given in Table I. The obtained results do not indicate any significant deviations. The analyzed samples were pH neutral while the electrolyte content was optimal.[20] After bioremediation treatment, the increase in conductivity is the result of dissolution of minerals after biodegradation of the contaminants. Therefore, the higher conductivities after bioremediation treatment could be associated with microbial activity stimulated by the presence of petroleum hydrocarbons.[30] The same was the case with the dissolved oxygen. Before bioremediation treatment, the results showed that groundwater samples were poorly aerated, but after bioremediation treatment, the concentrations of dissolved oxygen were higher because of microbial activity. The concentrations of nitrogen and phosphorus also indicated good microbial activity in the bioremediation treatment.

TABLE I. Physicochemical and chemical quality parameters of groundwater samples

Groundwater sample	σ µS cm^{-1}	c_{TDS} mg dm^{-3}	$c_{dissolved\ oxygen}$ mg dm^{-3}	pH	T °C	c_N mg dm^{-3}	c_P mg dm^{-3}
Before bioremediation treatment							
P-5	400	198	1.8	7.2	14.6	0.13	0.01
P-6	760	375	1.8	7.5	15.6	0.24	0.02
P-7	443	222	1.6	7.4	15.5	0.20	0.02
After bioremediation treatment							
P-5	461	230	2.2	7.5	14.5	0.12	0.01
P-6	852	426	2.0	7.5	13.9	0.18	0.01
P-7	558	280	3.5	7.4	15.4	0.15	0.01

Preliminary analyses of the groundwater from this location were conducted using a system of 10 piezometers.[21] The aim was to investigate whether the groundwater at this location was significantly contaminated with total petroleum hydrocarbons (*TPH*). According to the national legislation,[31] the threshold value of TPH = 0.60 mg dm^{-3} indicates a significant pollution of the groundwater with petroleum pollutants and indicates that remediation of the investigated location is needed. The results of the preliminary analyses of the groundwater samples near the thermo-energetic plant in Belgrade showed that the concentrations of *TPH* in the groundwater were in the range from 0.21 to 1.76 mg dm^{-3}. It should also be emphasized that the total amount of contaminants in the study area was estimated at 8097 kg or about 8 t of petroleum products.[20] Based on these results, it was concluded that the groundwaters in six investigated piezometers were significantly polluted with petroleum hydrocarbons. Considering all these results, it was concluded that remediation of the groundwater at this location was necessary. Microbiological analyses of these groundwater samples demonstrated that the bioremediation potential, expressed as percentage of hydrocarbon degraders relative to the total number of microorganisms, was approximately 5 % or higher, which indicated an acceptable condition for microbiological remediation.

The results of a hydrogeological investigation of the research area revealed that the Quaternary deposits in the investigated area were lithologically dominantly represented by sands and gravels.[20] Within these sediments, a confined aquifer with intergranular porosity was formed. The Quaternary clays form both, the upper and the lower impermeable boundary of the aquifer. The thickness of the upper impermeable boundary of the aquifer within the study area ranges from 0.5 to 10 m. The clay interbeds within sandy–gravelly deposits is in the range from several centimeters to several meters. The sandy–gravelly deposits have significant porosity. Their hydraulic conductivity values range from 10^{-1} to 5.4×10^{-2} cm s^{-1}.[32]

Permeability of the aquifer formation is considered one of the most important characteristics of the subsurface environment for a successful groundwater bioremediation.[33] The aquifer must have sufficient permeability to allow adequate transfer of the nutrients and/or microorganisms through the formation. In permeable aquifers, such as sandy and gravelly, bioremediation is usually effective.[34] It is generally accepted that the minimum average hydraulic conductivity for an aquifer is 10^{-4} cm s^{-1}.[19]

According to the results of the hydraulic conductivity measurement at the location of the thermo-energetic plant in Belgrade, it was concluded that the investigated aquifer fulfilled the hydraulic requirements for a successful groundwater bioremediation.

Considering all the characteristics of the investigated location, such as, a large number of facilities and their technical and technological characteristics, quantities of material contaminated with petroleum hydrocarbons, security hazards, and proximity of the two radial collector wells of the Belgrade water supply system, a comparative analysis of the technologies that could be applied suggested an *in-situ* enhanced bioremediation of the groundwaters as the best available technology for remediation of the groundwater at the investigated locality.[20]

The applied approach for enhanced bioremediation of the groundwaters near the thermo-energetic plant in Belgrade used biostimulation, bioaugmentation and treatment of the contaminated groundwater within a closed system.

The term "biostimulation" refers to the addition of electron donors, electron acceptors, and/or nutrients with the aim of stimulating the naturally occurring microbial populations.[35] In the present research, the biostimulation was performed through the oxygen enhancement (with application of chemical and physical oxygenation), and with the addition of nutrients. For chemical oxygenation, hydrogen peroxide was chosen due to its high oxygen-releasing potential,[36] while physical oxygenation was achieved with ejector aeration systems. The nutrients were added as a biostimulation solution with predefined and strictly controlled pH and $C_{organic}$:N_{total}:P_{total} mass ratios in. Finally, an organic biodegradable surfactant was added to reduce surface tension and stabilize emulsions, and in that way to increase bioavailability of contaminants to the microorganisms.[36]

Bioaugmentation consists of the addition of specific microorganisms to the contaminated soil or groundwater in order to supplement the native microbial community with the aim of increasing the biological activity in the biodegradation of pollutants.[37] In this groundwater bioremediation treatment, bioaugmentation was achieved by the injection of a pre-grown active consortium of zymogenic microorganisms isolated from the same location.

The groundwater treatment was conducted using the engineered constructed network containing 15 bioremediation wells, 9 control wells, and filtration/adsorption/bioreactor columns. All bioremediation wells were the same in construction. However, not all of them were used for the same operations during the enhanced bioremediation of the groundwaters. As a result, they were designated extraction wells (used for the extraction of the contaminated groundwater), and injection wells (used for injection of the biostimulation solution, bioaugmentation solution, oxygen donors, and water after treatment in filtration/adsorption/bioreactor columns). The control wells were used for monitoring the *TPH* levels in the groundwater and progress of the bioremediation. Additionally, the control wells were used as auxiliary control wells, first for chemical aeration but also for corrections of groundwater flows. The filtration/adsorption/bioreactor columns were filled with natural inorganic hydrophobic adsorbents. The purpose of this material was twofold: 1) to filter and adsorb oil pollutants from the extracted groundwater and 2) to provide a large specific surface area, and in this way to intensify biodegradation/mineralization of oil pollutants. In these columns, the concentration of TPH was monitored daily and due to the intense microbial activity within the columns, the TPH concentrations were drastically decreased over bioremediation treatment. The filtration/adsorption/bioreactor columns were also equipped with an appropriate ejector aeration system. In these columns, the concentration of TPH was monitored daily and due to the intense microbial activity within the columns, the TPH concentrations were drastically decreased over bioremediation treatment.

The construction of the bioremediation network was organized into several bioremediation units. Each bioremediation unit consisted of one extraction and two injection wells, with a filtration/adsorption column between them. The nutrients were added from the reservoir through the injection well into the aquifer. In order to increase the oxygen level in the aquifer and stimulate aerobic biodegradation, hydrogen peroxide (as an oxygen donor) was added in the same way. Bioaugmentation was achieved using laboratory-grown consortia of zymogenous hydrocarbon-degrading microorganisms, previously isolated from contaminated groundwater and sediment from the same location. These microorganisms were grown under laboratory conditions, multiplied in a bioreactor under field conditions, and finally added into the aquifer through the injection well. Recirculation was achieved by extraction of contaminated groundwater using the extraction well followed by filtration through the filtration/adsorption column filled with natural inorganic hydrophobic adsorbents, and finally injection to the subsurface through the injection well. The process was managed and controlled with appropriate submersible pumps. The average flow rate was 0.5 $dm^3 s^{-1}$ per injection well.

For monitoring of the *TPH* levels in the groundwater and progress of the bioremediation, three wells with the highest *TPH* levels measured during the preliminary investigation were chosen. The TPH concentrations before and after bioremediation treatment in different groundwater samples are given in Table II.

TABLE II. Concentration of TPH before and after bioremediation treatment in the groundwater samples

Groundwater sample	TPH concentration, mg dm^{-3}		Reduction of TPH, %
	Initial	Terminal	
P-5	1.39	0.02	98.55
P-6	1.76	0.03	98.30
P-7	1.57	0.03	98.09

As can be seen in Table II, in the piezometer P-5, from the initial 1.39 mg dm^{-3}, the concentration of TPH had decreased to 0.02 mg dm^{-3} at the end of the treatment. In the piezometer P-6, the initial concentration of TPH was 1.76 mg dm^{-3}, while it was 0.03 mg dm^{-3} at the end of bioremediation treatment. Finally, in the piezometer P-7, the initial concentration of TPH was 1.57 mg dm^{-3}, and it was 0.03 mg dm^{-3} at the end of bioremediation treatment. In percentage, the reduction of TPH in all three piezometers was close to 100 %.

Gas chromatograms for TPH in groundwater samples from piezometers P-5, P-6 and P-7 are shown in Fig. 4. As can be seen, the peaks had negligible intensity at the end of the bioremediation treatment (Fig. 6, P-5b, P-6b and P-7b), compared to the beginning (Fig. 4, P-5a, P-6a and P-7a).

It could be concluded that the applied bioremediation treatment was very successful. After twelve months of enhanced bioremediation of the groundwaters, the *TPH* levels were reduced and lowered to well below the threshold level regulated by National legislation. It should be emphasized that this reduction of contamination was achieved under field conditions.

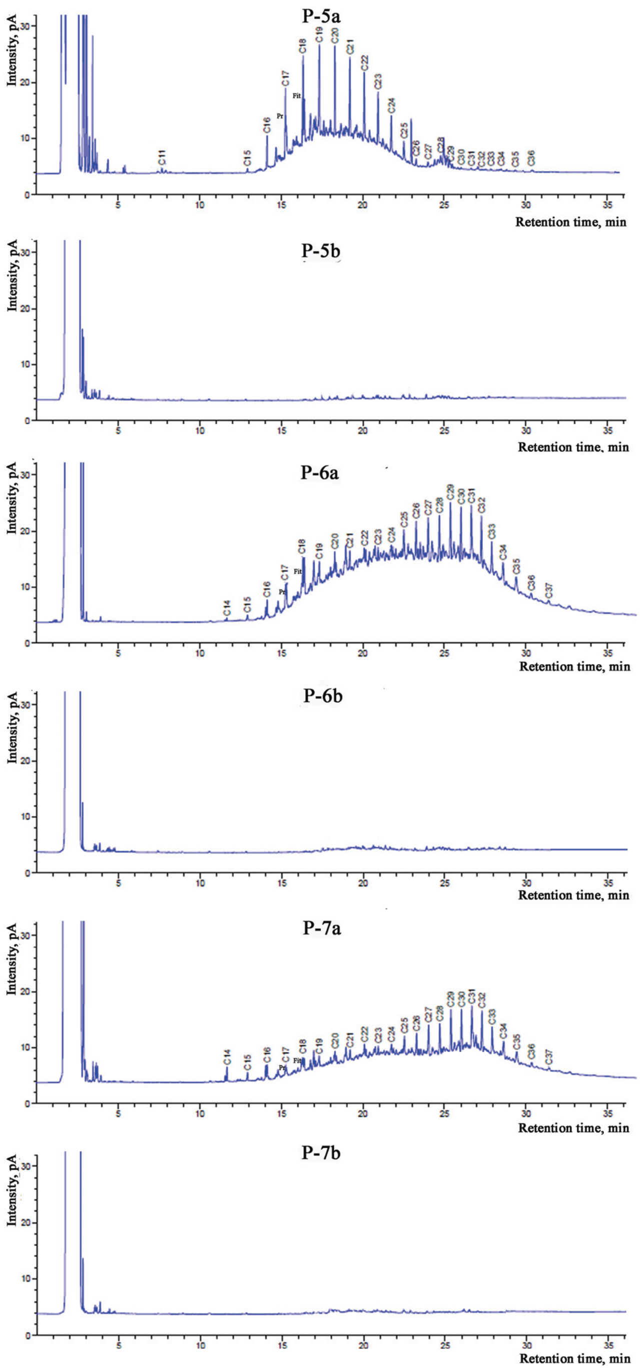

Fig. 6. Chromatograms before (P-5a, P-6a and P7a) and after (P-5b, P-6b and P7b) bioremediation treatment.

CONCLUSIONS

Due to their widespread use, petroleum hydrocarbons are among the most common groundwater contaminants. Compared to the traditional remediation methods for groundwater contaminated by petroleum hydrocarbons (*e.g.*, pump and treat), bioremediation is both a reliable and cost-effective technology. This remediation approach eliminates liability costs of hazardous waste transportation and storage since it aims at transforming contaminants into non-toxic compounds by microbiological activity.

On the other hand, its application requires a site-specific approach, in order to satisfy physiological and nutritional requirements for the activity of the indigenous bacteria in contaminated sites. The enhanced *in-situ* bioremediation approach presented in this paper included both biostimulation (chemical oxidation, the addition of nutrients) and bioaugmentation (addition of laboratory-grown zymogenous hydrocarbon-degrading microbial consortia previously isolated from contaminated groundwater/sediment) in order to overcome site-limiting factors for the microbiological activity. The reliability of this remediation approach at the industrial level has been proven at the hydrocarbon-contaminated sites in Belgrade. The applied remediation treatment was highly efficient in reducing *TPH* to acceptable levels. These results provide strong evidence for the potential of this remediation approach for successful application under field conditions.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

Acknowledgment. This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under Grant No. III 43004.

И З В О Д

БИОРЕМЕДИЈАЦИЈА ПОДЗЕМНИХ ВОДА ЗАГАЂЕНИХ НАФТНИМ УГЉОВОДОНИЦИМА ПРИМЕЊЕНА У БЛИЗИНИ ТЕРМО-ЕНЕРГЕТСКОГ ПОСТРОЈЕЊА У БЕОГРАДУ

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Услед широке употребе, нафтни угљоводоници спадају међу најчешће загађујуће супстанце у подземним водама. У поређењу са конвенционалним методама физичког црпења загађења из издани ("пумпај и третирај"), биоремедијација је економски исплатива технологија. Циљ овог ремедијационог поступка је да се биолошки, најчешће микробиолошком активношћу, трансформишу загађујуће супстанце до нетоксичних једињења. Прецизније, ово је активан ремедијациони процес који укључује биостимулацију (повећање оксичности средине, додавање нутријената) и/или биоаугментацију (додавање концентроване и специјализоване популације микроорганизама). Користећи биостимулацију и биоаугментацију, стимулисана *ин ситу* биоремедијација подземних вода примењена је на локацији загађеној угљоводоницима у Београду. Биоремедијациони третман, примењиван током периода од дванаест месеци, био је веома ефикасан у смањењу концентрација укупних нафтних угљоводоника до прихватљивих вредности. У пиезометру P-5 концентрација TPH смањена је за 98,55 %, у пиезометру P-6 за 98,30 %, а у пиезометру P-7 концентрација TPH смањена је за 98,09 %. Добијени резултати потврђују потенцијал примењеног ремедијационог поступка у превазилажењу ограничавајућих услова средине и стимулисању микробиолошке активности у циљу смањења загађења подземних вода.

(Примљено 23. октобра, ревидирано 30. децембра 2019, прихваћено 3. јануара 2020)

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