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

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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 (e.g. pump and treat), bioremediation is an economically cost-effective technology. The aim of this remediation approach is to biologically, most often by the microbiological activity, transform contaminants into non-toxic compounds. More precisely, it is an active remediation process that involves biostimulation (an increase of aquifer oxygenation, the 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 during a twelve months period, was highly efficient in reducing concentrations of total petroleum hydrocarbon (TPH) to acceptable levels. In the piezometer P-5 concentration of TPH has been reduced for 98.55 %, in the piezometer P-6 for 98.30 % and in the piezometer P-7 concentration of TPH has been reduced for 98.09 %. These results provide 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.

INTRODUCTION

Due to the extensive human use of petroleum hydrocarbons, this group of compounds is among the most common groundwater contaminants.1 At the same time, application of pump and treat systems for remediation of hydrocarbon-contaminated sites has proven to be less practical and reliable than first envisioned.2 Because of that, the focus of the remediation approach for groundwater
contaminated by petroleum hydrocarbons has been altered to bioremediation. Bioremediation is an efficient and cost-effective technology which biologically transforms organic pollutants into less toxic compounds, or completely degrades them to carbon dioxide and water. Microorganisms are the main biocatalysts in bioremediation, and they transform contaminants through reactions that are part of their metabolic processes. The bioremediation treatment can be conducted at the contaminated site (in situ), or outside of the contaminated site (ex situ). Groundwater in situ bioremediation has a lower cost compared to traditional site remediation approaches (e.g. pump and treat), due to the fact that it eliminates liability costs of hazardous waste transportation and storage. Currently, bioremediation accounts for approximately 25% of all remediation treatments. On the other hand, the use of this remediation technology in Serbia is in early-development stage. This paper aims to provide insight into the mechanisms and bioremediation requirements, as well as 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 the evolution, microorganisms have developed different mechanisms for degradation of petroleum hydrocarbons. If the oxygen is electron acceptor during hydrocarbon degradation, the mechanism is called aerobic. Otherwise, it is called anaerobic mechanism, and other electrons acceptors are involved (e.g. nitrate, sulfate or iron). The primary reaction in aerobic biodegradation of petroleum hydrocarbons always requires the action of oxygenase and the presence of free oxygen. Monooxygenases catalyzes the incorporation of one atom of oxygen into aliphatic hydrocarbon molecules. The resulting alcohols are enzymatically transformed into aldehydes or carboxylic acids. Carboxylic acids are further metabolized in the β-oxidation process. Generally, n-alkanes are the most biodegradable of all petroleum components. 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. New formed cis-cis diol, catechol, is further transformed to a carboxylic acid and Acetyl-CoA (Fig. 2). 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.

In the absence of oxygen, various mechanisms such as the addition of fumarate, carboxylation, hydroxylation and methylation can initially activate hydrocarbons. 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.).\(^\text{13}\)

![Equation 1](image1.png)

**Fig. 1.** Microbial degradation of \(n\)-alkane in aerobic conditions.\(^\text{8}\)

In the case of \(n\)-alkanes, the addition of fumarates usually occurs at a sub-terminal C2 position which gives (1-methylalkyl) succinate (Fig. 3A, 3B).\(^\text{13,15}\) Alkylsuccinates are then transformed by decarboxylation giving branched fatty acids that can enter into \(\beta\)-oxidation.\(^\text{15}\) 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).\(^\text{14}\)
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, (C) pathway for the biodegradation of benzene via three different mechanisms: (a) methylation; (b) hydroxylation; (c) carboxylation.

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.

**Bioremediation requirements**

Enhanced *in situ* bioremediation of groundwater contaminated by petroleum hydrocarbons is an active remediation procedure that implies biostimulation and/or bioaugmentation. Biostimulation is a more frequent approach, which involves the addition of oxygen and nutrients (nitrogen and phosphorus) in order to stimulate the growth and activity of microorganisms. On the other hand, bioaugmentation implies the addition of concentrated and specialized population of microorganisms (single strain or mixed culture-consortium). However, bioremediation is not universally applicable and it requires an understanding of site-specific limiting factors.
The presence of microorganisms with the metabolic capacity to synthesize enzymes for the contaminant degradation is a major bioremediation requirement. Thus, the indigenous bacteria at contaminated sites have a key role in the successful application of bioremediation. 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. Because of that, the aerobic biodegradation is the most efficient mechanism for degradation of petroleum hydrocarbons. The favorable environmental conditions for microbiological activity involve a sufficient amount of moisture, acceptable acidity, optimal temperature and availability of nutrients. The optimal pH values for the activity of microorganisms are neutral to base. Temperature is among the most important environmental factors since it affects the growth and development of the microbiological population. According to Venosa and Zhu, 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 indicate that the C: N: P = 30: 5: 1 ratio in mass is favorable for the growth of the microbiological population.

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

The aim of our present study was *in situ* groundwater bioremediation in the Sava river aquifer, in the vicinity of the thermo-energetic plant in Belgrade, Serbia. At the beginning of year 2015, during the regular quarterly analysis of the groundwater quality at the location of thermo-energetic plant in Belgrade, an increased concentration of mineral oils and a strong odor of oil pollutants were discovered. Due to suspected environmental incident, a large survey of the quality of the groundwaters at this location was conducted. The research included a system of 10 piezometers for groundwater sampling. Surface water samples from the Sava River between the river bank and the dock were analyzed as well. The results confirmed presence of a diesel and the heavy fuel oil in the investigated groundwaters. 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.\textsuperscript{20,21} Our present paper presents the results of the enhanced *in situ* groundwater bioremediation at this locality.

**GEOLOGICAL SETTINGS**

The thermo-energetic plant Belgrade is located in New Belgrade, on the left side of the Sava River, approximately 1 km from its confluence with the Danube (Fig. 4).

![Fig. 4. The geographical position of the thermo-energetic plant in Belgrade and spatial distribution of 10 piezometers, including Radial Collector well RC-26. From piezometers P-5, P-6 and P-7 groundwater samples were analyzed.](image)

In this area, Upper Miocene Pannonian sediments are found in the depth range between 27 and 65 m. The Pannonian sediments make the basis for the Quaternary alluvial deposits which are comprised from two geological units: a) Pleistocene fluvial sediments and b) Holocene alluvial deposits (Fig. 5).\textsuperscript{22}

![Fig. 5. A simplified geological cross-section along the investigated area.](image)

Holocene anthropogenic or technogenic sediments are present over a large surface area on the left bank of the Sava River, in New Belgrade. These modern anthropogenic deposits consist from excavated sands, construction waste and other materials. Excavated, backfilled and filled sands are the most widespread in this area, reaching a thickness of 5 m.\textsuperscript{22}
This part of New Belgrade is well known for numerous fresh water wells. Two Radial Collector (RC) wells of the Belgrade water supply system (RC-26 and RC-27) are in the vicinity of the thermo-energetic plant. On Fig. 4. is shown the position of RC-26 while RC-27 is not presented because it is about 350 m downstream away from the investigated site.

EXPERIMENTAL

The enhanced in situ groundwater bioremediation was applied at the site of thermo-energetic plant in Belgrade. This remediation approach combined both field and laboratory research activities. The field research activities included the detailed characterization of the investigated location (exploration drilling, hydrogeological mapping and water table measurements) and 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, the 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, kept and transported at 4 °C, and analyzed within 24 h. From these samples, the zymogenous consortium was cultured according to the procedure which was described in details in our previous paper.\textsuperscript{23} 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 microorganisms were optimized relative to the conditions of the location from which they were isolated. Multiplied microbial populations was then used to inoculate (approx 1 vol. %) a bioreactor originally designed by our team, in field conditions (total volume 1000 dm\(^3\); with a working volume of 800 dm\(^3\)). The biostimulation solution for optimum C/organic:N/total:P/total ratio, pH, and concentration of biodegradable surfactant consisted from: 12 g / dm\(^3\) meat peptone (Torlak, Belgrade, Serbia); 0.2 g / dm\(^3\) \((\text{NH}_4\text{)}_2\text{HPO}_4\); 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 antifoam.

Total petroleum hydrocarbons (TPH) from the groundwater samples were determined according to the ISO standard: 9377-2: 2000.\textsuperscript{24} Besides TPH determination, some physicochemical and chemical parameters were determined in order to characterize samples of groundwater in these study. Conductivity and Total dissolved solids (TDS) was measured by conductometer model 44600 Conductivity/TDS Meter, manufactured by “HACH company”.\textsuperscript{25} Dissolved oxygen was measured directly in by digital oximeter (type Oxi 330i) manufactured by “WTW” (Weilheim, Germany) with membrane electrode.\textsuperscript{26} A digital mV/pH Hanna instruments voltmeter was used for all pH measurements.\textsuperscript{27} Temperature was measured by using digital thermometer “Elite”, manufactured by “HANNA Instruments” (Padova, Italia).\textsuperscript{28} Concentrations of nitrogen and phosphorus was determined by standards: SRPS EN 12260:08\textsuperscript{29} and SRPS EN ISO 6878:08.\textsuperscript{30}
RESULTS AND DISCUSSION

The results for physicochemical and chemical parameters of groundwater samples are shown in TABLE I. The obtained results do not indicate any significant deviations. The analysed samples are pH neutral while the electrolyte content is optimal. After bioremediation treatment the increase in conductivity is result of dissolution of minerals after biodegradation of the contaminants. Therefore, the higher conductivities after bioremediation treatment can be associated with microbial activity stimulated by the presence of petroleum hydrocarbons. The same is with dissolved oxygen. Before bioremediation treatment results show that groundwater samples are poorly aerated, but after bioremediation treatment concentrations of dissolved oxygen are higher as a consequence of microbial activity. Concentration of nitrogen and phosphorus also indicate good microbial activity in bioremediation treatment.

TABLE I. Physicochemical and chemical quality parameters of groundwater samples.

<table>
<thead>
<tr>
<th>Groundwater samples</th>
<th>TDS / mg dm$^{-3}$</th>
<th>Dissolved oxygen / mg dm$^{-3}$</th>
<th>pH / $^\circ$C</th>
<th>CN / mg dm$^{-3}$</th>
<th>CP / mg dm$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before bioremediation treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-5</td>
<td>400</td>
<td>198</td>
<td>1.8</td>
<td>7.2</td>
<td>14.6</td>
</tr>
<tr>
<td>P-6</td>
<td>760</td>
<td>375</td>
<td>1.8</td>
<td>7.5</td>
<td>15.6</td>
</tr>
<tr>
<td>P-7</td>
<td>443</td>
<td>222</td>
<td>1.6</td>
<td>7.4</td>
<td>15.5</td>
</tr>
<tr>
<td>After bioremediation treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-5</td>
<td>461</td>
<td>230</td>
<td>2.2</td>
<td>7.5</td>
<td>14.5</td>
</tr>
<tr>
<td>P-6</td>
<td>852</td>
<td>426</td>
<td>2.0</td>
<td>7.5</td>
<td>13.9</td>
</tr>
<tr>
<td>P-7</td>
<td>558</td>
<td>280</td>
<td>3.5</td>
<td>7.4</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Preliminary analyses of the groundwater from this location were conducted using the system of 10 piezometers. The aim was to investigate whether the groundwater at this location was significantly contaminated with total petroleum hydrocarbons (TPH). According to the National legislation, the threshold value of TPH = 0.6 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 in the vicinity of the thermo-energetic plant in Belgrade showed that the concentrations of TPH in groundwater were in the range from 0.21 to 1.76 mg / dm$^3$. It should also be emphasized that total amount of contaminants in the study area was estimated at 8097 kg or about 8 t of petroleum products. Based on these results it was concluded that the groundwaters in six piezometers investigated were significantly polluted with petroleum hydrocarbons. Considering all these results it was concluded that the 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.
Results of the hydrogeological investigation of the research area revealed that the Quaternary deposits in the investigated area were lithological dominantly represented by sands and gravels. 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 aquifer’s upper impermeable boundary 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 $5.4 \times 10^{-2}$ cm/s.

Permeability of the aquifer formation is considered one of the most important characteristics of the subsurface environment for a successful groundwater bioremediation. The aquifer must have a 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. It is generally accepted that the minimum average hydraulic conductivity for the aquifer is $10^{-4}$ cm/s.

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 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 technologies that could be applied suggested an in situ enhanced bioremediation of the groundwaters as the best available technology for remediation of groundwater at the investigated locality.

The applied approach for enhanced bioremediation of the groundwaters in the vicinity of 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 to stimulate the naturally occurring microbial populations. In our 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, while physical oxygenation was achieved with ejector aeration systems. The nutrients were added as a biostimulation solution with predefined and strictly controlled pH and $C_{\text{organic}} : N_{\text{total}} : P_{\text{total}}$ ratio in mass. Finally, an organic biodegradable surfactant was added to reduce surface tension and stabilize emulsions, and in that way to increase bioavailability of contaminants to microorganisms.
Bioaugmentation consists of the addition of specific microorganisms to the contaminated soil or groundwater in order to supplement a native microbial community with the aim to increase biological activity in biodegradation of pollutants. In this groundwater bioremediation treatment, bioaugmentation was achieved by injection of the 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. Because of that, they were designated as extraction wells (used for extraction of the contaminated groundwater), and injection wells (used for injection of biostimulation solution, bioaugmentation solution, oxygen donors, and water after treatment in filtration/adsorption/bioreactor columns). The control wells were used for monitoring of the TPH levels in the groundwater and progress of the bioremediation. Additionally, the control wells were used as auxiliary control wells, first and foremost 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 that 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, 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, 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 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 in laboratory conditions, multiplied in a bioreactor in the 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 the appropriate submersible pumps. The average flow rate was 0.5 dm$^3$/s 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 shown in Table II.

<table>
<thead>
<tr>
<th>Groundwater samples</th>
<th>TPH concentration, mg / dm$^3$</th>
<th>Reduction of TPH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-5</td>
<td>Initial 1.39, Terminal 0.02</td>
<td>98.55</td>
</tr>
<tr>
<td>P-6</td>
<td>Initial 1.76, Terminal 0.03</td>
<td>98.30</td>
</tr>
<tr>
<td>P-7</td>
<td>Initial 1.57, Terminal 0.03</td>
<td>98.09</td>
</tr>
</tbody>
</table>

As can be seen in Table I, in the piezometer P-5, from the initial 1.39 mg / dm$^3$, concentration of TPH 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 at the end of bioremediation treatment it was 0.03 mg / dm$^3$. Finally, in the piezometer P-7 the initial concentration of TPH was 1.57 mg / dm$^3$, and at the end of bioremediation treatment it was 0.03 mg / dm$^3$. In percentage, the reduction of TPH in all three piezometers is close to 100 %.

Gas chromatograms for TPH in groundwater samples from piezometers P-5, P-6 and P-7 are shown in Fig. 6. As can be seen, 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. 6, P-5a, P-6a and P-7a).

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

CONCLUSION

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 reliable and cost-effective technology. This remediation approach eliminates liability costs of hazardous waste transportation and storage since it aims to transform contaminants into non-toxic compounds by the microbiological activity.
Fig. 6. Chromatograms before (P-5a, P-6a and P7a) and after (P-5b, P-6b and P7b) bioremediation treatment.
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 \textit{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 on the potential of this remediation approach for successful application in field conditions.

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BIOREMEDIATION OF GROUNDWATER CONTAMINATED WITH PETROLEUM HYDROCARBONS

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