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Bioremediation of groundwater contaminated by petroleum hydrocarbons

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Abstract: *Groundwater is an very important natural resource. Clean groundwater is essential for the preservation of the entire ecosystem.*

Bioremediation is a process which is based on the natural capacity of microorganisms to remove or transform hazardous substances from the environment into less toxic or harmless products.

This paper presents the bioremediation of groundwater contaminated by petroleum hydrocarbons. The contaminated area was located at the thermal energy facility, near the city of Belgrade, on the terrace sediments of the Sava River. In the process, which lasted for 18 months, groundwater was treated by in situ bioremediation. Bioremediation treatment was conducted by a combination of biostimulation and bioaugmentation. Biostimulation was conducted by injection of nutrients. Bioaugmentation was achieved by injection of a zymogenous consortium of microorganisms, previously isolated from the contaminated groundwater, which were capable of using the contaminating substances as nutrients. The treatment was carried out in a closed bipolar system,with groundwater recirculation, by combination of extraction and injection wells.

During the bioremediation, the content of petroleum hydrocarbon in groundwater decreased by 93% of the initial level. This indicates that the process of bioremediation was performed successfully.

Keywords: *bioremediation, groundwater, petroleum hydrocarbons*

1. Introduction

Groundwater represents about 98% of the Earth's available fresh water [Alvarez and Illman, 2000; Marić et al. 2015]. However, groundwater contaminated by petroleum hydrocarbons has become a one of the major problem worldwide, and is a consequence of industrial growth. During the exploitation, processing, accidental spills, transport, distribution, storage and use of crude oil and its products, these may be released into the hydrosphere in an uncontrolled manner. Due to the extent of impact and the adverse effect of oil derivatives on both the inanimate and animate environment, the development and using of sustainable technologies for the removal of petroleum substances and their derivatives from aquatic environments has therefore become extremely important today [Bandura et al. 2017].

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Bioremediation is a method of reducing petroleum pollution from environment which has been widely used in the last years. Bioremediation is a process which is based on the natural capacity of microorganisms to decompose toxic waste from the environment into harmless products. The use of microorganisms as biological agents in bioremediation is continually increasing because of their biodiversity and their great catabolic capacity. Bioremediation process enables the optimization of biodegradation of pollutants, which is achieved by aeration, biostimulation (adding nutritional substances) and bioaugmentation (adding microorganisms). Bioaugmentation implies adding microorganisms which are previously isolated form contamination sites and mulitiplied in bioreactors and returned to the site of contamination. The use of microbial populations characteristic for specific area prevents introduction of external pathogenes during the remediation process, which decreases the risk of disturbing the environmental equilibrium of the remediation site and the surrounding area [Avdalović et al. 2016; Beškoski et al. 2011; Gojgić-Cvijović et al. 2012]. The above-mentioned technology is successfully applied for treatment of water ecosystems contaminated with petroleum hydrocarbons. In additional, bioremediation procedures are economically advantageous, are categorized as "environmentally friendly" technologies, because do not form waste [Avdalović et al. 2016].

This paper presents the bioremediation of groundwater contaminated by petroleum hydrocarbons, in the process which lasted for 18 months.

2. Materials and methods

2.1. Preparation of the consortium of microorganisms for biodegradation process

A consortium of indigenous microorganisms was obtained from the groundwater contaminated by petroleum hydrocarbons in 200 ml volumes of mineral medium (10 vol. %; Loser et al., 1998), in diesel fuel (2 g dm^{-3}) was used as the only energy and carbon source, and held in Erlenmeyer flasks (1 dm^3). Suspensions of the microbial consortium were then used to inoculate four Erlenmeyer flasks (5 dm^3), each containing 2000 cm^3 of mineral medium containing 23 g of nutrient broth (Torlak, Belgrade, Serbia); 100 cm^3 of soil extract; and 20 g of diesel fuel. Commercial non-toxic and biodegradable surfactants, BioSolve CLEAR (Westford, MA, USA), were used to solubilize diesel fuel. The original solution of BioSolve CLEAR was used at a concentration of 1 ml dm^{-3}. The growth conditions were as follows: temperature, 28 °C; 120 r min^{-1} of a rotatory shaker; pH 7.0 (adjusted with 1 M HCl or NaOH); duration of growth, 96 h. The microorganisms from all four flasks were then used to inoculate a bioreactor (total volume 1000 dm^3) with a working volume of 800 dm^3, producing the microbial consortium. The medium used was as follows: 12 g dm^{-3} meat peptone (Torlak, Belgrade, Serbia); 0.2 g dm^{-3} $(NH4)_2HPO_4$; 50 g dm^{-3} of autoclave-sterilized soil extract; BioSolve CLEAR original solution (1 ml dm^{-3}); and 10 g dm^{-3} of diesel fuel. The growth conditions were as follows: non-sterile, 25 °C, aeration and agitation 0.70 volume of air/volume of medium min^{-1}, pH 7.0 (adjusted with 10 M HCl or NaOH), duration 72 h and sunflower oil (1 ml dm^{-3}) as the antifoaming agent [Marić et al. 2015; Avdalović et al. 2016].

Reinoculation with the prepared microbial consortium was performed at 30-day intervals.

2.2. Number of microorganisms

The number of microorganisms in the groundwater was determined by plating appropriate serial dilutions on agar plates incubated at 28°C. The media used were nutrient agar (15 g/L peptone, 3 g/L meat extract, 5 g/L NaCl, 0.3 g/L K_2HPO_4, 18 g/L agar; Torlak, Serbia) for total chemoorganoheterotrophs (TC) and mineral base medium (1 g/L NH_4NO_3, 0.25 g/L K_2HPO_4, 50mL soil extract, 16 g/L agar) containing 2 g/L standard D2 diesel fuel for hydrocarbon degraders (HD) [Marić et al. 2015; Avdalović et al. 2016].

2.3. The bioremediation treatment

The contaminated area was located at the thermal energy facility, near the city of Belgrade, on the terrace sediments of the Sava River. In the process, which lasted for 18 months, groundwater and sediment in contact with groundwater were treated by *in situ* bioremediation.This remediation treatment was performed by a combination of biostimulation and bioaugmentation within the closed bipolar system (one extraction and two injection wells), with adsorption in the external unit. Adsorption/filtration column, used in external unit, filled with natural inorganic hydrophobic adsorbents. During water filtration, a biofilm of zymogenous HD microorganisms was formed on the material of the adsorption column.

The cycle of bioremediation consisted of three phases:

- Injection of nutrients and chemical oxidant—biostimulation
- Injection of the zymogenous consortium of microorganisms—bio-augmentation
- Establishment of recirculation in a closed bipolar system [Marić et al. 2015; Beškoski et al. 2017].

2.4. Chemical analyses

Total petroleum hydrocarbons (TPH) from sediments and groundwater were extracted as per methods ISO 16703 (2004) and ISO 9377-2 (2000) and determined by gas chromatography The content of total petroleum hydrocarbon (TPH) in the composite samples of sediment was extracted as per method ISO 16703 (ISO 16703, 2004) and determined by gas chromatography. The gas chromatographic analyses were conducted on an Agilent 7890A gas chromatograph with a flame ionization detector (FID), equipped with a 30 m × 0.32 mm i.d. × 0.25 μm film chromatographic column HP-5. The chromatographic conditions were as follows: injection at 60 C oven temperature (injector temperature 250 °C, detector temperature 300 °C), 1 min hold, then programmed at 4 °C min^{-1} to 300 °C. The carrier gas was hydrogen at a velocity of 30 cm s^{-1}. The software used for data processing was ChemStation, Agilent Technologies [Avdalović et al. 2016].

3. Results and discussion

Stimulated in situ bioremediation was started with addition of nutrients from the reservoir through the injection well into the aquifer. Together with nutrients, in order to stimulate chemical oxidation and increase oxygenation of the aquifer, H_2O_2 was added. Finally, zymogenous HD microbial consortia was added via the same injection well. The zymogenous consortium of hydrocarbon-degrading microorganisms was prepared as previously described, and was also added to the groundwater so that it initially contained $7x10^9$ CFU dm^{-3} hydrogen-degrading microorganisms. Reinoculation with the prepared microbial consortium was performed at 30-day intervals. 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. During water filtration, a biofilm of zymogenous HD microorganisms was formed on the material of the adsorption column [Marić et al. 2015; Beškoski et al. 2017].

The gas chromatogram of TPH extracted from groundwater and sediment are shown in Figures 1-4.

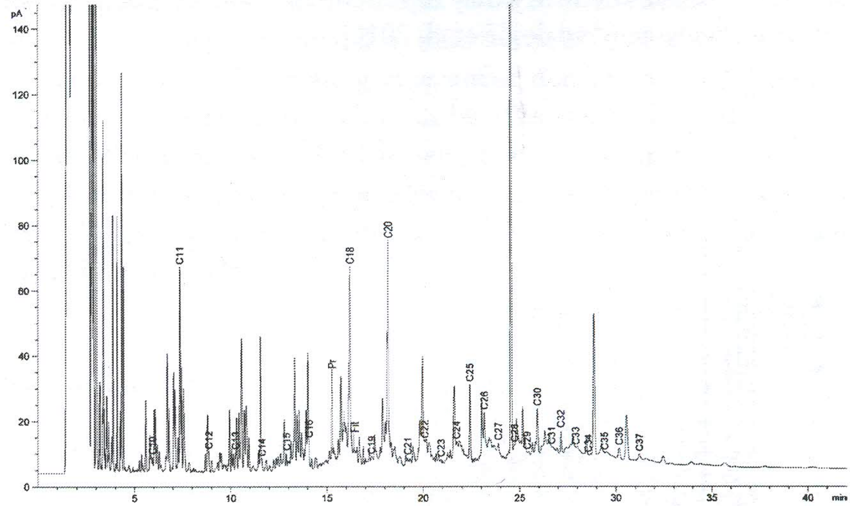

Figure 1. Gas chromatogram of TPH extracted from groundwater at the beginning of *in situ* bioremediation

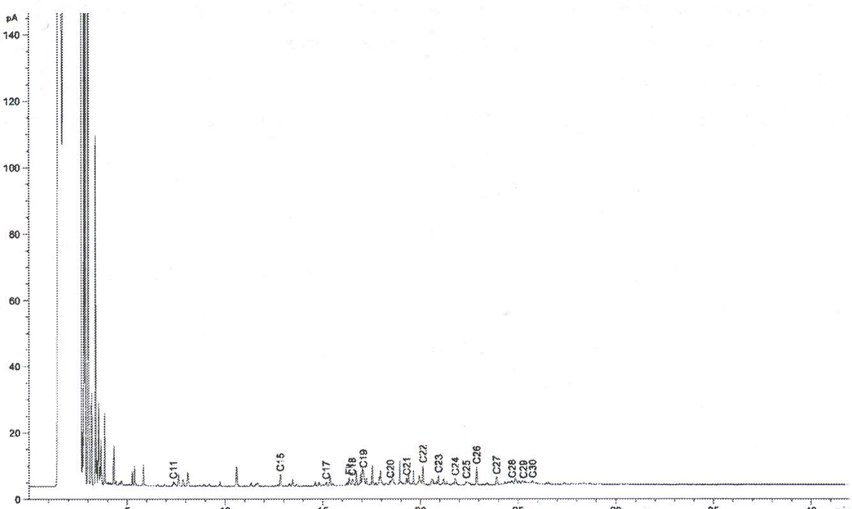

Figure 2. Gas chromatogram of TPH extracted from groundwater at the end of *in situ* bioremediation

During the bioremediation, the content of petroleum hydrocarbon in groundwater decreased by 93% of the initial level.

Numbers located next to "C", which mark the signals, indicate the number of C-atoms of *n*-paraffin. Signals marked with *Pr* and *Fit* come from isoprenoid hydrocarbons pristane (C19) and phytane (C20), which are biomarkers of petroleum and its derivates, all except benzene, and in environmental and forensic chemistry they represent an evidence that pollutants are petroleum-originated [Avdalović et al. 2016].

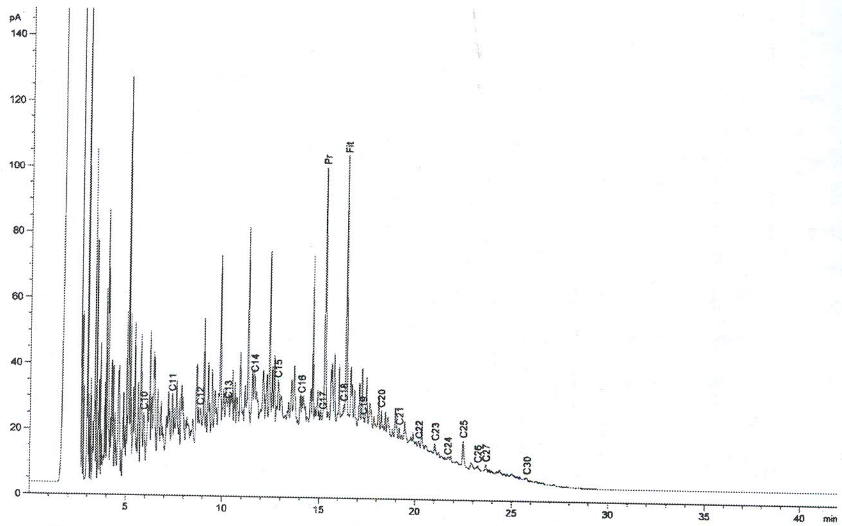

Figure 3. Gas chromatogram of TPH extracted from sediment at the beginning of *in situ* bioremediation

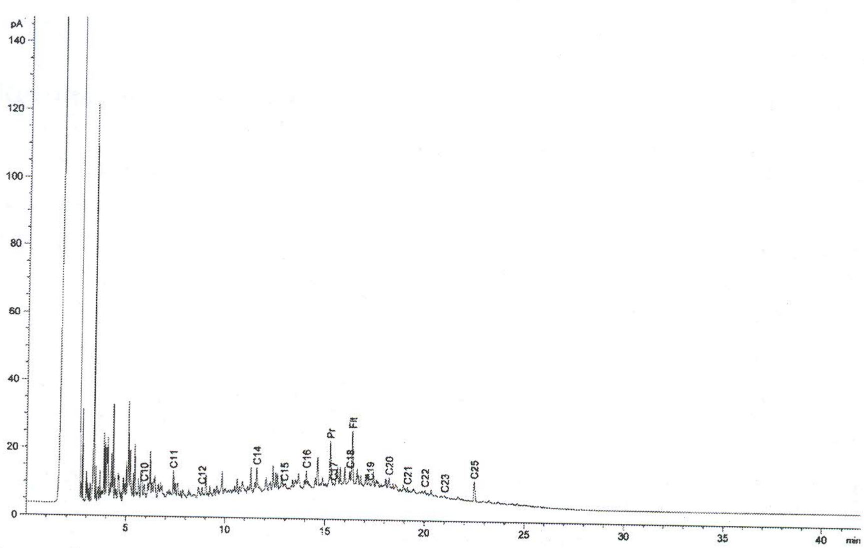

Figure 4. Gas chromatogram of TPH extracted from sediment at the end of *in situ* bioremediation

During the bioremediation, the content of petroleum hydrocarbon in the sediment decreased by 86% of the initial level.

The described groundwater treatment is more cost-effective in relation to other remediation techniques such as "pump-and-treat". Also, there is no waste production that needs to be further disposed. Additionally, the procedure described may also treat surfaces that are inaccessible to other techniques. Contaminating substances that are adsorbed or trapped in the pores of sediment particles can be effectively removed, as shown in this paper. The final level of total petroleum hydrocarbons achieved after bioremediation treatment of groundwater was lower than the level required by Serbian regulation and indicates that the process of bioremediation was performed successfully.

4. Conclusion

This study describes in situ bioremediation of groundwater and sediment contaminated by petroleum hydrocarbons. The efficiency of bioremediation was evaluated on the basis of changes in the content of TPH. During bioremediation, the TPH content in groundwater was reduced dramatically (93%). In the same tame, the TPH content in the sediment decreased by 86% of the initial level. These results provide evidence of the high efficiency of the applied method for removing petroleum pollutants from groundwater and sediment in contact with groundwater by an in situ bioremediation treatment in a closed bipolar system.

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