International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(Research Article)

Check for updates

The possibility of applying the bioremediation process on soil contaminated with petroleum hydrocarbons

Minela Ćejvan [1] and Zlata Ibrišimović-Subašić [2,*]

[1] *Faculty of Health Studies, University of Bihać, Bihać, Bosnia and Herzegovina.*

[2] *Faculty of Technology, University of Tuzla, Tuzla, Bosnia and Herzegovina.*

International Journal of Science and Research Archive, 2025, 14(02), 170-176

Publication history: Received on 27 December 2024; revised on 01 February 2025; accepted on 04 February 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.2.0374

Abstract

Soil pollution from petroleum hydrocarbons has recently become a growing global problem. The introduction of pollutants into the soil has serious consequences in different areas of human activities. Many global oil companies have recognized this problem and have started to rehabilitate certain locations. In order to achieve the most effective remediation, various methods of remediation were considered. Bioremediation method was recognized as the main method in the remediation of soil contaminated with petroleum hydrocarbons. Bioremediation is a cost-effective technology that biologically transforms pollutants into non-toxic compounds or completely degrades them. Bioremediation is based on enzymatic reactions of microorganisms that use carbon compounds as energy for growth and development, and the final products of these reactions are water and carbon dioxide. Purification does not require the introduction of new chemicals into the environment, and no new emissions are generated. Treated soil is no longer considered waste and can be reused for landscaping purposes or as building material. All this includes bioremediation in green technology and it corresponds to the concept of sustainable development. The aim of this paper is to present the process of bioremediation and the possibilities of its application on soil contaminated with petroleum hydrocarbons.

Keywords: Contaminated soil; Oil; Bioremediation; Green technology; Environment protection

1 Introduction

Economic development has led to burdening the environment as a whole, led to damage to the soil, which represents an important environmental component and today attracts increasing attention. Soil contaminated with petroleum hydrocarbons, after benzene, represents the most common risk to human health and the environment [1]. The main causes of pollution are wastewater from settlements and industries washing away agricultural land and roads, semi-legal waste dumps, and pollution caused by various accidents. In this way, water and soil become areas where heavy metals, toxic organic compounds, nitrates, phosphates, and other hazardous substances, including petroleum hydrocarbons, are deposited. The rapid development of technology and the growth of the population. Significant investments in renewable sources are foreseen, and it is believed that by 2040, as much as 60% of the new energy demand will be met from these sources. However, oil will still be one of the dominant energy sources with a consumption of 103 mb/d [2]. Used oil, together with other harmful substances, remains in the soil and sediments for a long time after application and directly enters the food chain [3]. Also, the penetration of pollutants into the soil has serious consequences for various areas of human needs and activities, such as the use of soil in agriculture, drinking water supply, urban and rural planning, and management of natural resources. There is a need for legal protection of soil, as has already been done for water, air, and waste management [4].

Problem of soil pollution by petroleum hydrocarbons has been recognized as one of the most significant and finding solutions for soil restoration is of increasing importance in environmental protection [5]. With the development of

* Corresponding author: Zlata Ibrišimović-Subašić

waste management activities and the implementation of remediation procedures by the world's leading oil companies, the rehabilitation procedures of certain locations have begun. In order to remediate long-standing pollution and reduce the negative impact of newly created pollution, numerous remediation procedures are carried out [6].

2 Description of Bioremediation process

Physical, chemical and thermal techniques are often used to clean up oil-contaminated sites [7], [8]. These procedures proved to be expensive and very often insufficiently effective [9], [10]. This has led to a need for cheaper and more environmentally friendly methods, and today over 25% of all remediation procedures involve biological methods [6]. Oil and petrochemical products represent the most common xenobiotics, often very toxic, which can (which is often the case) pollute soil and water during storage and processing, transportation and loading, transfer by pipelines, tankers, wagons, tanks, tankers and airplanes. Despite all the precautions taken, it is almost inevitable that soil and water pollution will occasionally occur. For the purpose of effective remediation of spilled oil, it is very important to have a good knowledge of the way oil and dissolved components spread, evaporation, biodegradation, dilution, behavior of oil in heterogeneous soil, effects of oil in soil and groundwater [4]. Bioremediation is a process by which xenobiotics (chemical substances foreign to the environment) that have entered the environment are converted into less toxic forms or mineralized to inorganic forms (carbon dioxide and water) by the action of microorganisms. This process is based on the ability of microorganisms to use various chemical compounds as sources of energy [4]. Bioremediation technologies have been developed since 1940s and gained popularity after the famous Exxon Valdez oil spill in 1989, which spilled 11 million gallons of oil into the sea off the coast of Alaska [11]. These methods are economically more profitable because there is no need to build special plants, nor to introduce new chemicals into the environment.

Table 1 Example of common toxic compounds and microorganisms that metabolize them [4]

Chemicals	**Microorganisms**
Phenolic compounds	*Rhizoctonia praticola*
P-cresol	*Trametes versicolor*
Aromatic hydrocarbons	*Pseudomonas aeruginosa*
A mixture of polycyclic aromatic hydrocarbons	*Mycobacterium* sp.
Fluoranthene (FA)	*Cunninghamella elegans*
Aromatics • 3-chlorobenzoate • phenanthrene • ethylbenzene • biphenyls • naphthalene • pentafluorophenol	 - *Pseudomonas* sp. - *Pseudomonas* sp. - *Pseudomonas putida* - *Beijerinckia* sp. - *Oscillatoria* sp. - *Arthobacter* ATCC33790 *Pseudomonas*
Toluene, benzene	mixed metallogenic culture
Carbamate	*Arthobacter* sp.
Parathion	*Pseudomonas stutzeri*
Dichloro-diphenyl-trichloroethane (DDT)	*Phanerochaete chrysosporium*
Polycyclic aromatic hydrocarbons (pahs)	*Aspergillus ochraceus* *Cunninghamella elegans* *Trametes versicolor* *Phanerochaete chrysosporium* *Saccharomyces cerevisiae*
chlorinated alkenes	*Xanthobacter* sp.

Petroleum is a complex mixture of more than 1,000 different compounds primarily made of hydrocarbons [7], [12]. The energy stored in the chemical bonds of hydrocarbons is released by combustion (fossil fuels) or by chemical reactions of living organisms (enzyme reactions). The enzymes required for these reactions are found in bacteria and enable them to release the energy stored in these bonds and use it for growth and maintenance of life needs and processes. Over millions of years, bacteria have developed many different enzymatic reactions suitable for the degradation of a wide range of hydrocarbons. It is known that lighter hydrocarbons with a simpler structure and lower molecular weight are more susceptible to biodegradation, and therefore can be transformed by a larger number of different bacteria. Heavier and more complex hydrocarbon fractions pose a greater challenge for microorganisms. The enzymatic reactions required for the breakdown of these compounds have been developed only by certain bacterial strains. Therefore, the successful bioremediation of contaminated soil to target concentrations and the final degradation of petroleum hydrocarbons to water and carbon dioxide requires the synergistic action of a community of different bacterial strains. Microorganisms, using hydrocarbon compounds as a substrate for growth and development, transform hazardous compounds into less hazardous products [6]. Various types of microorganisms are known that are able to metabolize many organic compounds synthesized by man (Table 1).

3 Soil composition

Soil is a natural formation, created by complex and mostly long-lasting processes of climate, vegetation and macro and microorganisms on the parent rock, i.e. the substrate [13]. The non-living or abiotic part of the soil consists of three components: liquid, solid and gaseous. Between the solid particles there are cracks of different shapes and sizes, together forming a pore space. The total proportion of the soil covered by the pores varies greatly and depends on the type of soil (sand, clay or peat soil). Bioremediation is a complex process whose effectiveness depends on a number of factors - the existence of the necessary microorganisms, the type, availability and concentration of pollution, environmental conditions, and the characteristics of the polluted soil [6].

The first step in the analysis of the pollution site is to check whether it is suitable for the bioremediation process. This includes soil analysis, chemical and microbiological, to determine the degree of contamination, soil type, nutrient levels, and the presence of microorganisms capable of breaking down toxic compounds. If there are few autochthonous microorganisms that can degrade the pollutant or none at all, it may be necessary to inoculate (inoculate) the pollution site with microorganisms that degrade the chemical substance in question [4].

3.1 Characteristics of contaminated soil

The structure and texture of the soil affects the permeability and moisture content of the soil. Soils with a higher proportion of smaller particles (dust, clay) are characterized by lower permeability, which makes it difficult to transport moisture, nutrients and oxygen. In order to increase the flow of necessary substances, agents such as straw and sawdust can be added to the soil during bioremediation. The rate and efficiency of biodegradation is also affected by the proportion of clay and organic matter in the soil, which bind pollution very well, making it less accessible to bacteria [6].

3.2 Characteristics of polluting parameters and required microorganisms

All pollutants are not subject to biodegradation. However, most petroleum hydrocarbons are biodegradable to a greater or lesser extent [6]. Depending on the number of atoms and the structure of the molecule, the degradation of hydrocarbons takes place at different rates. It is generally accepted that biodegradation decreases along a series of n-alkanes, branched alkanes and alkenes, while the least biodegradable are polycyclic aromatic hydrocarbons [12]. In addition to the type of pollution, degradation also depends on the type and number of microorganisms. The soil found in nature contains numerous different microorganisms, of which a relatively small number have the ability to biodegrade petroleum hydrocarbons [6]. In order for bioremediation to be successful, it is necessary to increase the number of microorganisms by controlling and adjusting environmental conditions (biostimulation) and/or by inoculating new microorganisms (bioaugmentation).

3.3 Bioavailability of polluting parameters

The characteristics of the soil (low permeability, high proportion of clay and organic matter) and poor solubility of hydrocarbons sometimes make pollution inaccessible to bacteria. Hydrocarbons in the soil bind to soil particles, and the use of surfactants, substances that reduce the surface tension of water and increase the solubility of substances, support the desorption of pollution and increase their availability to bacteria [12]. Bioavailability can therefore be increased by adding commercial surfactants to polluted soil, but also by using bacterial strains that, in addition to the ability to degrade hydrocarbons, also create surfactants as a product [6].

3.4 Environmental conditions

For the efficiency of bioremediation, in addition to the above, monitoring and stimulation of the conditions in which bioremediation takes place is very important. Although some microorganisms survive in extreme conditions, the growth and development of most bacterial strains requires environmental conditions to be within precisely defined limits (Table 2). Insufficient humidity limits bacterial growth, and if the moisture content decreases below 10%, the bacteria die. Excessive humidity reduces soil aeration [14].

Table 2 Environmental conditions required for biodegradation of petroleum hydrocarbons [14]

Parameters	Minimum conditions for biodegradation	Ideal conditions for biodegradation
Moisture content [%]	25 – 28	30 – 90
pH	5,5 – 8,8	6,5 – 8,0
Oxygen content [%]	Aerobic conditions	10 – 40
Nutrients	N and P	C:N:P=100:10:1
Temperature [°C]	15 – 45	20 – 30

Biodegradation of petroleum hydrocarbons takes place most rapidly under aerobic conditions. Oxygen is one of the key elements for decomposition, so it should be continuously replenished. In soil with a higher proportion of pollution, the oxygen concentration will decrease much faster, which can ultimately lead to anaerobic conditions. A neutral pH is optimal for most bacteria. In addition to carbon and energy, bacteria also need nutrients - nitrogen and phosphorus - for cell growth. By adding these substances, the natural proportions of these elements in the biomass are established in the polluted soil, which leads to increased growth of microorganisms [12].

Although strains have been isolated today that can biodegrade pollution at temperature extremes, for most strains the optimal conditions are between 15-45 °C [14].

3.5 Nutrients

A pollutant is a source of carbon for microorganisms, so contaminated soil is generally poor in nitrogen and phosphorus. A lack of nutrients; C, N, P, K, S, and trace elements, can cause poor degradation of a chemical compound. It is common to add nutrients to the soil to establish a mass ratio of carbon: nitrogen: phosphorus (C:N :P) of about 120:10:1, which is approximately the ratio of these elements in biomass. The addition of nutrients is referred to as biostimulation, and mineral fertilizers or fertilizers of organic origin (manure, activated sludge) can be used [12].

3.6 Aerobic-anaerobic conditions

Most pollutants are degradable under aerobic conditions, but there may be a lack of a suitable electron acceptor, if not oxygen itself. There are some chlorinated organic compounds that are more susceptible to biodegradation under anaerobic conditions [4].

3.7 Soil water content

Water is necessary for the growth of microorganisms, so biodegradation cannot occur below a certain moisture content in the soil, and anaerobic conditions prevail in soil saturated with water [4]. The optimal soil moisture for the bioremediation process is 12-30%, or 40-80% of the saturation capacity [12].

3.8 Soil texture and structure

The proportions of sand, clay and silt, as well as the arrangement and organization of soil particles directly affect the passage of oxygen and carbon dioxide to the site of microbial activity [4]. Soil texture affects permeability, moisture content, and overall soil density. Fine-grained soils are less permeable than soils with coarse particles. Soils with low permeability are usually compacted and hinder the distribution and transport of moisture, nutrients and air. Agents such as straw or sawdust can be added to this soil during bioremediation to achieve the desired texture. The rate and degree of degradation is influenced by the type of contaminated soil, the content of clay and organic substances, as well as the proportion of individual sand fractions. The soil in which there is sand and gravel in the predominant amount has good drainage capacity, i.e. holds water for a very short time and it is permeable to air [12].

3.9 pH

The reactions of different types of soil can be very diverse, but most of them are somewhat acidic. Most bacteria have a limited tolerance to acidic conditions. Fungi are more resistant in this sense, and therefore the pH value of the soil often determines which type of microorganisms can participate in biodegradation [4]. The pH of the soil determines the type of microorganisms that are available for biodegradation. Most bacteria are suitable for a neutral pH, and fungi for a slightly acidic environment. Usually, the optimal pH for bioremediation is in the range of 6 to 8. If the soil is more acidic, lime is added, and if it is alkaline, the pH is adjusted by adding ammonium sulfate [12].

3.10 Temperature

Biodegradation occurs over a wide range of soil temperatures. In most soils, conditions favorable for mesophilic microorganisms prevail during at least part of the year. Some studies have shown that hydrocarbon-degrading microorganisms isolated from oil-contaminated soils in Alaska could grow at a temperature of 5°C. Mesophilic microorganisms are the most common in all soil types, while biodegradation occurs at values above 20°C (e.g. hydrocarbons). There is relatively little data on thermophilic decomposers in the soil. Some thermophilic bacteria were isolated by growing on n-tetradecane as a substrate [4].

3.11 Toxic compounds

Other toxic compounds found on the site, which are not subject to biodegradation, can prevent the action of organisms that break down the pollutant. A pollutant must be soluble to be available to microflora, but if it is combined with insoluble substances, biodegradation is difficult. If all the above factors are optimized, we can expect that the pollutant will be removed from the environment. However, even highly polluted soil still allows the growth of a large number of different microorganisms, some of which will limit the growth of decomposers by consuming nutrients. The balance of microflora on the polluted soil will change in favor of pollutant decomposers, who will thus be numerically superior during decomposition. The disappearance of pollutants, which is a growth factor for certain types of microorganisms, will change the composition of the microbial community, so their number will decrease as the content of pollutants decreases [4].

4 An example of a successfully performed bioremediation procedure

Bioremediation of soil contaminated with petroleum hydrocarbons at the Petrija gas station location, Croatia. At the location of the Petrija gas station in Moslavačka Gračenica, the ex situ, "landfarming" method of bioremediation is applied, and the combined procedure of biostimulation and bioaugmentation is used. Landfarming method (bed method) is an ex situ method characterized by the formation of beds with contaminated soil, with the aim of easier control and adaptation to bioremediation conditions. After excavation and transport, the contaminated soil is unloaded onto the prepared surface, which must be waterproof and covered. After that, "beds" of soil up to 0.8 m high are formed, and the authorized laboratory takes soil samples to determine zero (initial) pollution. To carry out the bioremediation procedure, two commercial preparations are used at the site. The first preparation consists of a mixed culture of natural microorganisms and enzymes (without GMOs), as well as nutrients necessary for the growth and development of bacteria. Another preparation is a biosurfactant that increases the bioavailability of bacterial contamination. Based on the initial concentration of contamination and the manufacturer's recipe, these two preparations are mixed with water, and after activation, they are applied to the contaminated soil. In order to distribute the prepared solution as evenly as possible, the contaminated soil is moistened and mixed with construction machines. In order to ensure the most ideal conditions for bioremediation (Table 2), loosening the soil to bring in oxygen and wetting the soil should be repeated every 7 days.

After 30 days, the soil analysis for mineral oils (TPH) is repeated to determine the degree of degradation. Also, after 30 days, a new application of commercial preparations has been made with the aim of faster and more efficient bioremediation. The entire procedure is repeated until target values are reached where the soil is no longer categorized as waste. After the bioremediation process is completed, and laboratory analysis by an authorized laboratory determines that hydrocarbon concentrations have decreased to the required values, the soil can be reused in the environment [6].

5 Results and discussion

During the reconstruction of the Petrinja gas station, 1284.8 tons of soil contaminated with petroleum hydrocarbons were excavated. After accepting and distributing the soil to the intended areas under the canopy, a laboratory analysis was made which determined the following initial concentrations of pollutants - mineral oils (TPH) 1694.6 mg/kg dry matter, BTEX 0.698 mg/kg dry matter, PAHs 0.0655 mg/kg body weight.

Table 3 Concentration of polluting parameters during the process [6].

Parameters	Initial pollution	After 30 days	After 60 days	After 90 days	After 120 days
pH	7.4	8.1	7.1	7.2	7.4
Total Petroleum Hydrocarbons - TPH - [mg/kg dry matter]	1.694.6	977.4	496.0	172.4	75.5
Benzene, toluene, ethylbenzene, xylene - BTEX - [mg/kg dry matter]	0.698	< 0.1	< 0.1	< 0.1	< 0.1
Polycyclic aromatic hydrocarbons - PAH - [mg/kg dry matter]	0.0655	< 0.001	< 0.001	< 0.001	< 0.001

In accordance with the concentrations, commercial preparations were applied, and wetting and loosening (aeration) of the soil was carried out on a weekly basis. Every 30 days, the progress of bioremediation was monitored (table 4), and the application of microorganisms and surfactant was repeated. After one month, a reduction of BTEX and PAHs below the detection limit was recorded, while the concentration of mineral oils decreased by about 40%. After 60 days of bioremediation, the soil was purified by about 70%, and after 90 days by about 90%. Target concentrations were reached after 120 days when 75.5 mg/kg dry matter was measured. Mineral oils and the bioremediation was stopped. The soil purified in this way was not disposed of in a landfill. In this way, successful results were achieved in the bioremediation of contaminated soil.

6 Conclusion

Application of bioremediation processes to petroleum hydrocarbons represents the area in which this technology is most fully developed. There are numerous locations where this technology has been successfully applied, so bioremediation is commercially available and the technology is marked by great progress.

Compliance with ethical standards

Acknowledgments

We would like to thank the researcher Barbara Bertović for making available the results of professional work on the positive application of bioremediation oil-contaminated areas using the example of a petrol pump shown in the paper "Bioremediation of soil contaminated with petroleum hydrocarbons" and INA d.d. on recognizing the importance of preserving and protecting the environment.

Disclosure of conflict of interest

M.Ć. and Z.I.S. declares no conflicts of interest.

Statement of informed consent

Both participants included in the study.

References

[1] R. G. Zytner, T. R. Brook, M. Leunissen and W. H. Stiver, Bioremediation of diesel fuel contaminated soil, Guelph, Canada: University of Guelph, 2001.

[2] I. E. Agency, "World Energy Outlook," Paris, 2016.

[3] M. Đokić, N. Bilandžić and F. Briški, "Processes for removing pesticides from the environment," Hvar Veterinary Institute and Faculty of Chemical Engineering and Technology Zagreb, vol. 61, no. (7-8), pp. 341-348, 2012.

[4] V. Bobić, "Soil contamination by petroleum hydrocarbons - bioremediation: possibilities, effectiveness, experiences," professional work, ISSN 0350-350X, GOMABN, vol. 44, pp. 3-34, 2012.

[5] E. Salehian, A. Khodadadi and B. Hosseini, "Remediation of diesel contaminated soils using surfactants : column study," American Journal of Environmental Science, vol. 8, no. (4), pp. 352-359, 2012.

[6] B. Bertović, "Bioremediation of soil contaminated with petroleum hydrocarbons," ISSN, 0350-350, GOMABN, pp. 295-305, 2016.

[7] N. C. f. S. Q. M. a. K. Transfer, "Oil in the Soil," Paris, 2016.

[8] M.W. Lim, E.V. Lau and P.E. Poh, Marine pollution Bulletin, vol. 14, no. 45, p. 109, 2016.

[9] S. Lundstedt, P. Haglund and L. Oberg, Environmental Toxicology and Chemistry, pp. 1413-1420, 2003.

[10] A. S. Roy, R. Baruah, M. Borah, A. K. Singh, H. D. Boruah, N. Saikia, M. Deka and N. Dutta, International Biodeterioration and Biodegradation, pp. 79-89, 2014.

[11] T. Branilić and M. Slišković, The Greatest Tanker Accidents, ISSN 0469-6255, Faculty of Maritime Affairs, Split, 2006.

[12] V. P. Beškoski, "Bioremediation of Oil and Petroleum Derivatives Contaminated Soil: Microorganisms, Pathways, Destruction, Technologies," Chemical Industry, pp. 275-289, 2012.

[13] T. Sofilić, Pollution and Soil Protection, Sisak: University of Zagreb, Faculty of Metallurgy, 2014.

[14] M. Vidali, "Bioremediation. An overview," Journal of Pure and Applied Chemistry, vol. 73, no. 1, pp. 1163-1172, 2001.