Innovations in Agricultural & Biological Engineering

# Bioremediation and Phytoremediation Technologies in Sustainable Soil Management

Volume 1 Fundamental Aspects and Contaminated Sites



Author Copy

Editors Junaid Ahmad Malik | Megh R. Goyal Khursheed Ahmad Wani

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### BIOREMEDIATION AND PHYTOREMEDIATION TECHNOLOGIES IN SUSTAINABLE SOIL MANAGEMENT

### Volume 1

Fundamental Aspects and Contaminated Sites

### Bioremediation and Phytoremediation Technologies in Sustainable Soil Management, 4 volume set:

ISBN: 978-1-77463-720-3 (hbk) ISBN: 978-1-77491-100-6 (pbk) ISBN: 978-1-00328-133-7 (ebk)

Volume 1: Fundamental Aspects and Contaminated Sites

ISBN: 978-1-77463-718-0 (hbk) ISBN: 978-1-77463-958-0 (pbk) ISBN: 978-1-00328-065-1 (ebk)

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Volume 4: Degradation of Pesticides and Polychlorinated Biphenyls

ISBN: 978-1-77491-038-2 (hbk) ISBN: 978-1-77491-039-9 (pbk) ISBN: 978-1-00328-120-7 (ebk)

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Volume 1

Fundamental Aspects and Contaminated Sites

Edited by Junaid Ahmad Malik, PhD Megh R. Goyal, PhD, PE Khursheed Ahmad Wani, PhD

#### First edition published 2023

Apple Academic Press Inc. 1265 Goldenrod Circle, NE, Palm Bay, FL 32905 USA 4164 Lakeshore Road, Burlington, ON, L7L 1A4 Canada

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#### CRC Press

6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742 USA 2 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN UK

Apple Academic Press exclusively co-publishes with CRC Press, an imprint of Taylor & Francis Group, LLC

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#### Library and Archives Canada Cataloguing in Publication

Title: Bioremediation and phytoremediation technologies in sustainable soil management / edited by Junaid Ahmad Malik, PhD, Megh R. Goyal, PhD, PE, Khursheed Ahmad Wani, PhD.

Names: Malik, Junaid Ahmad, 1987- editor. | Goyal, Megh R., editor. | Wani, Khursheed Ahmad, editor.

Series: Innovations in agricultural and biological engineering.

Description: First edition. | Series statement: Innovations in agricultural and biological engineering | Includes bibliographical references and indexes. | Content: Volume 1: Fundamental Aspects and Contaminated Sites.

Identifiers: Canadiana (print) 20220155194 | Canadiana (ebook) 20220155224 | ISBN 9781774637180 (v. 1; hardcover) | ISBN 9781774639580 (v. 1; softcover) | ISBN 9781003280651 (v. 1; ebook)

Subjects: LCSH: Soil remediation. | LCSH: Phytoremediation. | LCSH: Bioremediation. Classification: LCC TD878.48 .B56 2022 | DDC 628.5/5—dc23

Library of Congress Cataloging-in-Publication Data

CIP data on file with US Library of Congress

Non Commercial Use

bk)

ISBN: 978-1-77463-718-0 (hbk) ISBN: 978-1-77463-958-0 (pbk) ISBN: 978-1-00328-065-1 (ebk)

### CONTENTS

S					
S	Contributors				
(1)	Abb	vreviationsxxiii			
	For	ewordxxvii			
	Preface				
	PAI	RT I: FUNDAMENTAL ASPECTS 1			
$\mathbf{O}$	1.	Phytoremediation and Phytotechnologies: An Overview			
		Irfan-Ur-Rauf Tak and Sofi Mohammad Zuber			
	2.	Phytoremediation and Environmental Factors			
Ð		Muhammad Zubair Akram, Samreen Nazeer, Anila Sadia, and Muhammad Abdullah Saleem			
Ō	PAI	RT II: TECHNOLOGICAL ASSESSMENT			
ũ	3.	<b>Role of Phytoremediation to CleanUp Heavy Metal Polluted Soils 73</b> Joan Nyika			
AO	4.	Phytoremediation Technique for PAH-Polluted Soils: Applications, Challenges, and Opportunities			
()	5.	Assessment and Overview of Petroleum Hydrocarbon Bioremediation			
	_	Naga Raju Maddela, M. Subhosh Chandra, M. Srinivasulu, and Pallaval V. Bramhachari			
0	PAI	RT III: CONTAMINATED SITES AND XENOBIOTICS			
	6.	Phytoremediation of Soils Contaminated by Heavy Metals (Mercury, Cadmium, and Lead)147			
		Mohd. Yousuf Rather, Ashaq Ahmad Dar, and Mohammed Latif Khan			
	7.	Microbial and Phytoremediation-Based Removal of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil Environments 177			
		Podduturi Sindhura, Podduturi Vanamala, Tanaji Vasavilatha,			

Ithor

and Mir Zahoor Gul

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		M. Srinivasulu, M. Subhosh Chandra, and Naga Raju Maddela	
$\mathbf{\Omega}$	9.	Bioremediation of Petroleum Contaminated Soils	231
in		Vikrant B. Berde, Pallaval V. Bramhachari, and Chanda Parulekar Berde	
	10.	Phytoremediation of Biomedical Waste Contaminated Sites	251
g		Pimpi Sahu, Kumar Kritartha Kaushik, Caiyun Zheng, Qingyan Zeng, Zhuangzhuang Zhao, Kai Dong, and Tingli Lu	
	11.	Bioremediation of Waste Dumping Sites	271
		Trinath Biswal	
<u>S</u>	Inde	ex	305
Ο			
σ			
σ			
0			
K			
Φ			

Appl

xviii

8.

**Microbial Bioremediation and Amelioration of** 

# BIOREMEDIATION OF PETROLEUM CONTAMINATED SOILS

VIKRANT B. BERDE, PALLAVAL V. BRAMHACHARI, and CHANDA PARULEKAR BERDE

#### ABSTRACT

Petroleum is an important non-renewable energy source used for various purposes like transportation and in industries. Sometimes accidently or due to human interference, it can contaminate the soil. Cleaning of these polluted soils with mechanical or physicochemical treatments could be very expensive or difficult task. Bioremediation of these petroleum affected soils is an eco-friendly, economic, and efficient option. This chapter focuses on the use of bioremediation in treatment of petroleum-contaminated soils.

#### 9.1 INTRODUCTION

Contamination of environment with petroleum hydrocarbons (PHs) occurs on account of accidental spillage, human activities, during refining and processing, transportation, leakage from pipelines and storage tankers and oil-well waxing and overhauling of refineries [20, 115, 120]. Soil is thus affected by petroleum components like hydrocarbons.

Petroleum contaminated soil affects the environment and is hazardous to human health. Treatment of the polluted soil can be done by physical, chemical, and biological methods. The microbiological method is being preferred, because it is efficient, economical, eco-friendly, and adaptable substitute to physicochemical procedures [42]. The indigenous flora of the soil being exposed to the hydrocarbons become adapted to the pollutants. These microorganisms synthesize enzymes for the degradation of the hydrocarbons, for

utilizing these compounds for their carbon and energy requirements. Such organisms are useful in the removal of contaminants [59, 62, 84]. Improvements in the bacterial remediation technology have provided solutions for the removal of petroleum pollution from the environments [31, 32].

Bioremediation using bacteria cultures with hydrocarbon-degrading ability is a widely accepted technology. Many bacterial species have been characterized, studied, and applied in bioremediation of soil contaminated with hydrocarbons. However, numerous complications reducing the rate of biodeterioration have been encountered while practically applying the methods.

The present chapter is an overview of bioremediation techniques using various microorganisms in the treatment of petroleum contaminated soils. It describes major environmental issues created due to the petroleum industry and human use of petroleum in day-to-day life. It also highlights the role played by the microorganisms in the rejuvenation of the contaminated sites with suitable examples.

# 9.2 HAZARDOUS EFFECTS OF PETROLEUM POLLUTION ON ENVIRONMENT

harmful effects of petroleum pollution on the environment are well known [20, 92]. It affects not only humans but also animals as well as the microorganisms [30, 63, 71, 81] disturbing the ecosystems. Petroleum contaminated soil is hazardous to human health [35]. The contaminants from the soil leach out and enter the water bodies, and may even contaminate the water table, thus increasing the risk further. Petroleum contaminated soil has lower crop productivity due to the pollution [114].

Research studies have confirmed that there is a reduction in species richness and phylogenetic diversity of the affected areas following exposure to PHs due to the inhibitory effect of hydrocarbons and their metabolic intermediates, on the microflora [18, 19, 47, 56, 104]. The microorganisms that can tolerate and degrade the hydrocarbons survive in the conditions and are the dominant community in the polluted environments.

#### 9.3 **BIOREMEDIATION**

Petroleum hydrocarbon pollutants can be removed by physical and chemical methods, which however are either expensive or not effective. Microbial

bioremediation is the most promising solution to the total removal of petroleum pollution from any environment [1, 20, 46, 107]. Petroleum degrading microorganisms have enzymes for the breakdown of the complex petroleum components. PHs consist of four classes: the saturates, the aromatics, the asphaltenes and the resins [23]. Thus, hydrocarbon degradation is influenced by the nature of pollutant and its concentration. The more complex the nature of the contaminants, the more difficult and time-consuming is the bioremediation process [65], even though it is less costly as compared to other methods of remediation.

Bioremediation methodologies are largely classified as *in situ* (done at the site) or *ex-situ* (done at somewhere else) [3]. Organic pollutants are degraded to end-products carbon dioxide ( $CO_2$ ) and water at the site of pollution. This method is called *in situ*, and it is less costly and eco-friendly, as compared to the *ex-situ* bioremediation technique that requires to excavate the contaminated samples and get them to the treatment site, which increases the cost.

Bioremediation process can be carried out using stimulating the growth of indigenous hydrocarbon-degrading bacteria or by adding grown cultures, i.e., by biostimulation or bioaugmentation. Thus, the degraders having the metabolic abilities to utilize the hydrocarbons survive, while the remaining microflora that is sensitive is inhibited. However, there is a need to pay more attention to the research that will add up efficient oil degraders and also standardization of methods for efficient and quick clean-up strategies.

# 9.4 ENVIRONMENTAL COMPONENTS REQUIRED FOR BIOREMEDIATION

#### 9.4.1 ABIOTIC COMPONENTS

Bioremediation involves various mechanisms of degradation, eradication, immobilization, or detoxification of chemicals and hazardous materials that can pollute the environment. Bioremediation technology makes use of microbial cultures to get rid of toxic contaminants to harmless products (mainly CO<sub>2</sub>, water, and other inorganic compounds) [73]. It is thus the application of microorganisms that degrade and transform the pollutants including hydrocarbons, oil, heavy metal (HM), pesticides, dyes, etc.

The rate of biodegradation depends on biotic and abiotic factors, such as pollutant concentration, nature of the pollutant, environmental conditions, physicochemical parameters, etc. [33]. Biodegradation reactions are highly

influenced by temperature, nutrients, electron acceptors, and the pollutants, which play a key role in hydrocarbon bioremediation [108]. Hence, the results obtained in the laboratory and under field conditions will vary [45]. The bioremediation process also requires the availability of the substrate that the bacteria can degrade [90]. The rate at which the degradation of the hydrocarbons occur follows the order as linear [8, 58]:

alkanes > branched alkanes > low-molecular-weight alkyl aromatics > monoaromatics > cyclic alkanes > polyaromatics (such as asphaltenes)

The polycyclic aromatic compounds are degraded incompletely [6]. They are broken down into intermediate compounds; and for the complete degradation, more than one microorganism is involved. Other factors, such as soil type, presence of other contaminants, type, and amount of organic matter present, also play an important role in determining the bioremediation rate [12, 89].

Physical factors, such as, temperature affect the bioremediation process as the bacterial strains involved are temperature sensitive and also affect the hydrocarbons [2, 60, 119]. It was observed that PHs were degraded efficiently under laboratory conditions, whereas the same effects were not observed *in situ*. This was attributed to the on-site temperature variations, which could not be controlled [82, 83]. At low temperatures, petroleum products persist for a longer period. Low temperatures reduce the solubility, increase the viscosity of hydrocarbons, and delay the onset of degradation, and vice versa for elevated temperature. The increased degradation rate of hydrocarbons ensues at 30–40°C [4, 7, 11, 24, 38, 70]. However, at higher temperatures, again degradation rate is lowered as the elevated temperature will inhibit microbial growth [99].

Bacterial growth in the soil depends on nutrient availability, which includes nitrogen, phosphorus, carbon, hydrogen, etc. [21, 39]. Hydrocarbons are utilized by bacterial strains as carbon and energy sources. However, if other nutrients are deficient, the growth of the degraders is hampered, and consequently the degradation process is also affected. The addition of fertilizers is used as a stimulatory method in the bioremediation process [44, 69, 83, 84]. The use of hydrocarbon-degrading bacteria having the ability to fix nitrogen replaces the need for fertilizer supplementation [100].

The onset of hydrocarbon degradation is by incorporation of oxygen in the aromatic ring with the help of oxygenases. A step-by-step degradation process follows, whereby the enzymes act on the compound aerobically.

Thus, the presence of oxygen is the key requirement for the onset and rapid degradation of hydrocarbons [39]. In a study carried out under controlled aeration, 75% degradation was observed [41]. Aerated degradation processes however increase the cost associated with the process. Alternatively, methods to increase the soil permeability (such as the addition of a bulking agent) may be used. Another option is degradation under anaerobic conditions, which is less expensive as compared to the aerobic bioremediation method [15, 125].

# 9.4.2 BIOLOGICAL COMPONENTS: MICROORGANISMS AND THEIR ROLE IN BIOREMEDIATION

Bioremediation of petroleum hydrocarbon pollutants is an ecofriendly, less costly, and effective biological treatment. The microorganisms found in mesophilic conditions cannot function properly in harsh conditions and show reduced metabolism and hence can lower degradation abilities. Removal of pollutants from environments under extreme conditions is dependent on the microorganisms present in that niche, i.e., the extremophiles belonging to the archaea group, and they can grow in conditions unfavorable to eubacteria. Therefore, for the bioremediation of contaminated soils (such as estuarine mudflats, etc.), the focus is on members of archaea. *In situ* bioremediation can make use of a single or combination of microorganisms (i.e., consortia) [29]. This ensures complete breakdown of the pollutant.

9.4.2.1 BACTERIA

The primary degraders of petroleum during oil spills are bacteria [14, 78]. These bacterial cultures can utilize hydrocarbons as the carbon source [121], for metabolism and growth. There is an increase in their numbers as the breakdown occurs. Once the hydrocarbons are removed, the microbial count reduces with a simultaneous reduction in the physiological stress on the environment [43, 53, 124].

Several factors decide the fate of the contaminant in the environment and the degrader type and abundance will also depend on the type of petroleum product, degradation pathways/enzymes present in the organisms, physico-chemical parameters, etc. [40, 110].

The oil-degrading bacteria found in the ocean waters and sediments are subject to temperature and salinity variations. The oil-degrading organisms isolated are mostly from the domain bacteria [17] and few groups of

Archaea belonging to Marine Group II Archaea (namely: Euryarchaeota and Thaumarchaeota) [79]. Unculturable archaeal members were identified in marine sediments belonging to strains of Euryarchaeota [28, 48, 51].

These findings suggest that unculturable Archaea includes the haloarchaea and Methanogens, which are involved in the removal of oil contamination in most of the marine environments [126]. There are reports of an increase in the haloarchaeal species such as Haloferax, on the addition of oil droplets and nitrate [16, 116, 123].

According to Tremblay et al. [103], 79 genera having the hydrocarbon utilizing potential, have been studied, which includes [49, 62, 68, 87, 109, 111, 119]:

Achromobacter;
Acinetobacter;
Alkanindiges;
Alteromonas;
Arthrobacter;
Burkholderia;
Dietzia;
Enterobacter;
Kocuria;
Marinobacter;
Mycobacterium;
Pandoraea;
Pseudomonas;
Rhodococcus;
Staphylococcus;
Streptobacillus;
Streptobacillus;
Streptococcus.

Some investigators have reported the efficient bioremediation by consortia of cultures, such as OHCB (obligate hydrocarbon clastic bacteria). The bacterial members included in the consortia were: Alkanindiges sp., Alcanivorax, Marinobacter; Thallassolituus, Cycloclasticus, Oliveria, 140, 1211, The Alcanivorax, Marinobacter, Thallassolituus, Cycloclasticus, Oleispira [40, 121]. These reports also stated that almost negligible number of bacterial species were present during pre-exposure and their abundance was increased post-exposure in the presence of contaminants. A particular strain cannot use all the petroleum products or cannot breakdown the contaminants completely.

Wang et al. [118] studied the bacterial strain *Dietzia* sp. DQ12-45-1b that consumes n-alkanes (C6–C40) and related compounds utilizing them as the sole sources of carbon. Similarly, *Acinetobacter* sp. could metabolize n-alkanes having chain length C10–C40 [102]; while Ma et al. [61] reported *Achromobacter xylosoxidans* DN002 that could utilize a number of monoand polyaromatic hydrocarbons efficiently. Some more bacterial genera, namely, *Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholderia,* and *Mycobacterium, Sphingomonas*, have also been reported with petroleum bioremediation activities [11, 26, 37].

Varjani et al. [113] worked with a consortium of halotolerant bacteria, named HUBC, which included *Ochrobactrum* sp., *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa*, having very good degradation rates and similar work was reported by Tao et al. [98]; and Wang et al. [115]. These reports demonstrated that in the consortium, the bacterial strains had crude oil bioremediating potential in marine ecosystems. Szulc et al. [97] reported a hydrocarbon-degrading efficiency of 89% by consortia of bacterial cultures that included *Aeromonas hydrophila*, *Alcaligenes xylosoxidans*, *Gordonia* sp., *Pseudomonas putida*, *Pseudomonas fluorescens*, *Rhodococcus equi*, *S. maltophilia*, and *Xanthomonas* sp.

Therefore, bioremediation of petroleum waste can be improved by the application of consortia of bacteria having the ability to degrade these compounds and as a result, can degrade the pollutants completely.

#### 9.4.2.2 FUNGI

Fungal cultures with petroleum bioremediation abilities are said to be better bioremediation agents as compared to bacteria [10, 11, 37, 101]. The fungal hyphae provide an advantage of penetration into the soil contaminated with hydrocarbons, thus reaching the deeper layers of soil. Fungal cultures can adapt to different variations in the environments and have mechanisms to cope with the hydrocarbon contaminants in the environment and bring about its degradation.

Fungal cultures (such as *Amorphoteca*, *Graphium*, *Neosartorya*, *Talaro-myces*, *Candida*, *Yarrowia*, *and Pichia* sp.) were found to have hydrocarbondegrading ability [19]. In another report, terrestrial fungi *Aspergillus*, *Cephalosporium*, and *Penicillium* were isolated and found to utilize crude oil efficiently [93].

Some yeast species for their petroleum degradation ability are: *Geotrichum* sp., *Candida lipolytica*, *Trichosporon mucoides* and *Rhodotorula mucilaginosa* that were isolated from contaminated waters [13]. Barnes et

al. [10] has described studies on 10 fungal isolates that could degrade the crude oil. They were isolated from sediments from mangroves, the Arabian Sea and from tarballs. The fungal isolates were identified as Aspergillus, Fusarium Penicillium, and Acremonium species using its rDNA (recombinant deoxyribonucleic acid) sequencing.

Ezekoye et al. [36] have described the studies on petroleum hydrocarbon bioremediation using the fungal cultures, such as Aspergillus fumigates, Aspergillus terrens, Aspergillus niger, Aspergillus nidulans, Aspergillus versicolor, Acremonium sp., Bipolaris sp., Candida tropicalis, Candida globrata, Cladosporium sp., Cladophialophora carrionii, Geotrichum candidum, Gliocladium sp., Phoma sp., Paecilomyces variotii, Scopulariopsis brevicaulis, Sepedonium sp., Trichophyton tonsurans, Trichophyton *terrestre* and *Rhodotorula* sp. Additional reports describe the hydrocarbon degradation ability and their applications in bioremediation [22, 66, 75, 86, 122]. These isolates, along with bacterial cultures with good bioremediation efficiency can be utilized together for the clean-up of oil spills and other environmental contaminants, such as tarballs. Oil-Zapper is one such application used in the environmental cleanup (TERI, India).

Therefore, there is a need to conduct in depth study of the bioremediation process of both bacteria and fungi, the enzymes involved, efficiencies of different consortia, etc., to optimize the *in-situ* bioremediation of petroleum pollutants.

#### 9.4.2.3 GENETICALLY MODIFIED ORGANISMS

As compared to natural isolates, the genetically engineered microorganisms (GEMs) have a higher capacity of environment cleanup. There are several reports on the biodegradation of pollutants by GEM according to Das and Chandran [25]. The GEMs include mostly strains of Pseudomonas species [34, 72, 95].

The GEMs have applications in monitoring the bioremediation process, monitoring the response of stress on the strains, toxicity analysis, etc. [25]. For process monitoring in PCB contaminated soils, A. eutrophus H850Lr is used [105]. Other strains used in process monitoring are: P. putida TVA8 and P. fluorescens HK44 for TCE, BTEX, and naphthalene, anthracene degradation, respectively [5, 88].

B. cepacia BRI6001L was used for the strain monitoring for 2,4-D degradation [64]. There is a report on the use of GEM strain P. fluorescens 10586s/pUCD607 to study response to stress during BTEX degradation

[94]. During the degradation of 2,4-dinitrophenol hydroquinone, Kelly et al. [52] has reported the application of the *Pseudomonas* strain Shk1 for the toxicity assessment studies. Layton et al. [57] described the application of *A. eutrophus* 2050 for endpoint analysis during the degradation of non-polar narcotics.

The only constraints involved are the environmental concerns and regulatory obligations. Before the application of the GEMs, a thorough study is needed on: the ecological consequences of the release of these strains, the biochemical pathways and enzymes involved, and the possibilities of strain reversion, etc.

# 9.5 PROS AND CONS OF BIOREMEDIATION FOR PETROLEUM CONTAMINATED SOILS

Most importantly, bioremediation is known for being safe to the environment and less costly [9, 74, 77]. It may be carried out in two ways: bioaugmentation (seeding, inoculation) and biostimulation [106], wherein either there is the addition of petroleum degraders to the site or the growth of indigenous flora is encouraged by supplementing with nutrients (mostly fertilizers) [21, 91, 112].

Degradation by indigenous microflora increases the microbial count during degradation. The pollutant is utilized by the microbes for their growth. Once the compound is degraded, their number start declining.  $CO_2$  and water are the non-toxic end-products of the degradative process. Thus, the contaminants are removed completely, and not simply transferred to different environmental sites [91]. Bioremediation of pollutants by microorganisms is an eco-friendly natural way of treating the contaminants, and it is sustainable as the microbial cultures involved in the process remain in the site and can act on waste if added to the site again [27]. The sites can be used continuously. Hence the methods are non-intrusive, and there is ease of implementation [55].

The bioremediation process can be carried out *in situ*, and hence the efforts of carrying the contaminated soil can be avoided, and no transport charges are involved [67]. Physicochemical methods of bioremediation of petroleum contaminated soils are available [54, 117], but they are costly. These methods also lead to the generation of other waste and hence are not eco-friendly [85]. Successful examples are the disposal of contaminants by incineration, which leads to the production of toxic gases. More complications go with the combustion of petroleum contaminants.

Although it is known that biodegradation also leads to  $CO_2$  formation, yet the quantity produced is negligible. Some workers have reported the ill-effects of landfilling with such contaminants [50, 80]. Over time, the contaminants leach out causing further harm to the water table [76, 96].

The bioremediation process has its drawbacks also. Firstly, it can be carried out for those compounds that can be broken down by microorganisms. Some compounds may not be completely degraded and the intermediates thus formed may be more toxic than the compound itself. These are called recalcitrant compounds. Sometimes 2 or more compounds may be involved in the degradation of a particular contaminant. Through *in-situ*, the situation is very different from laboratory conditions. The concentration of the contaminants is not known, the physicochemical parameters cannot be controlled, other biotic factors are involved, the contaminated site may have more than one compounds present, and also the state in which they are present, i.e., solids, liquids, and gases. Thus, the degree of success cannot be judged.

Application method and environmental conditions also matter such as in summer the temperature will be high while in monsoon, there are chances of wash off. The biggest drawback is the time factor, as these methods are very time-consuming. Being natural and dependent on the microbial activity, the degradation processes take long. The cultures to be used in the bioremediation process need to be cultured on a large scale, and hence there is a requirement to follow all set SOPs (standard operating procedure) for culture growth and application methods, along with the requirement of skilled labor [55].

# 9.6 ECOLOGICAL AND ECONOMIC IMPORTANCE OF BIOREMEDIATION

Using the endogenous microflora of the contaminated site by the biostimulation method of bioremediation, it does not affect the biotic components of the environment. After removal of the contaminant, the number of microflora start reducing and survive on the nutrients available. This is thus a natural process with no formation of harmful components or no side effects. As it is carried out on-site, the cost and dangers of transportation can be avoided. A lot of expenses are reduced due to the almost nil energy requirements, minimal equipment or instrumentation involved, and also low maintenance. Hence, bioremediation is economically feasible. Bioremediation has a higher acceptance from regulatory authorities as well as the public.

#### 9.7 COMMERCIAL APPLICATIONS OF BIOREMEDIATION

Bioremediation can be used in several sites, where there is spillage of petroleum or its related products. It can be used in petroleum stations, where the spillage problem is well known. Another application is at the industrial sites and the sites used for landfills. Petroleum and petroleum-related compounds enter the environments through these portals. The products may leach out from the landfills and contaminate the water bodies. Thus, the bioremediation of these sites is necessary. Oils spills occurring at the sea, ports, oil rigs can affect marine life.

The removal of the spills requires the use of bioremediation. The successful implementation of bioremediation for the revival of hydrocarbon contaminated environments is the need of the day. Further, bioremediation technology should be well established with intense research and field trials with effective petroleum degrading microorganisms, including the much efficient genetically engineered organisms.

#### 9.8 SUMMARY

The overuse of petroleum products leads to contamination of soils. Soil organisms play a crucial role in the hydrocarbon degradation in contaminated environments. The present chapter is based on the hazardous effects of petroleum contaminated soils and the remedial measures taken to treat these soils with microorganisms.

#### **KEYWORDS**

- bioremediation
- genetically engineered microorganisms
- microorganisms
- obligate hydrocarbon clastic bacteria
- petroleum
- pollution
- toxicity

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