Innovations in Agricultural
& Biological Engineering

Bioremediation and Phytoremediation Technologies in Sustainable Soil Management

Volume 2

Microbial Approaches and Recent Trends

Editors
Junaid Ahmad Malik | Megh R. Goyal

CRC Press
Taylor & Francis Group
APPLE ACADEMIC PRESS

BIOREMEDIATION AND PHYTOREMEDIATION TECHNOLOGIES IN SUSTAINABLE SOIL MANAGEMENT

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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)

Volume 2: Microbial Approaches and Recent Trends
ISBN: 978-1-77463-719-7 (hbk)
ISBN: 978-1-77463-892-7 (pbk)
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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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AAP APPLE ACADEMIC PRESS

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

CRC Press
6000 Broken Sound Parkway NW,
Suite 300, Boca Raton, FL 33487-2742 USA

4 Park Square, Milton Park,
Abingdon, Oxon, OX14 4RN UK

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 2: Microbial Approaches and Recent Trends.
Identifiers: Canadiana (print) 20220155194 | Canadiana (ebook) 20220155224 | ISBN 9781774637197 (v. 2 ; hardcover) | ISBN 9781774638927 (v. 2 ; softcover) | ISBN 9781003280682 (v. 2 ; 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

ISBN: 978-1-77463-719-7 (hbk)
ISBN: 978-1-77463-892-7 (pbk)
ISBN: 978-1-00328-068-2 (ebk)

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CHAPTER 2

ROLE OF ARCHAEA IN THE REMEDIATION OF CONTAMINATED SOILS

CHANDA PARULEKAR BERDE and VIKRANT B. BERDE

ABSTRACT

Bioremediation has emerged as an effective and alternative method of treating contaminated soils, and it mainly depends on microorganisms. Besides bacteria, archaea can also be used for the remediation purpose to remove hydrocarbons, because archaea can work in very harsh conditions, such as halophilic and acidophilic environments. The industries, which release their effluents with heavy metals (HMs) in soil, rivers, or ocean, can use Archaea for pre-treatment of waste effluents. In the present chapter, the use of various Archaeal domain organisms in remediation of contaminated natural resources has been discussed.

2.1 INTRODUCTION

Industrial pollution and its impact on soil, water, and sediments are becoming a main threat to the environment and health of living organisms. Biostimulation (involves nutrient addition or adjusting the conditions) and bioaugmentation (involves the adding of microbes) both can help in complete removal of contaminants. Bacterial domain plays a crucial role in bioremediation, and it has some limitations in extreme thermophilic and halophilic environments.

Many of *Archae*bacteria are inhabitable to environments, where most other organisms cannot survive. If such extreme environments get contaminated, it is a difficult task to remove pollutants from these environments. The chemical effluents or wastewaters from many industries have high salt contents, high temperatures, extremely alkaline or acidic waters, or even heavy metals (HMs) released from the production process. Under such conditions, extremophilic archaebacteria possess the ability to function in the elimination of pollutants.

Bioremediation involves mechanisms of degradation, eradication, immobilization, or detoxification of chemicals and hazardous materials that pollute the environment. Bioremediation technology involves the use of microorganisms to breakdown toxic compounds to harmless ones, such as CO_2, H_2O, and other inorganic complexes [86]. It is thus the application of microorganisms to degrade and transform the pollutants including hydrocarbons, oil, HMs, pesticides, dyes, etc. The rate of biodegradation depends on biotic and abiotic factors, such as pollutant concentration, nature of the pollutant, environmental conditions (EC), the physicochemical parameters, etc. [33]. The microorganisms that harbor enzymes required for degradation of the pollutants can utilize or degrade these compounds.

Bioremediation of organic pollutants is a very effective biological method that is less costly and does not harm the environment. The microorganisms found in normal EC cannot function efficiently under adverse environments. Their metabolic and degradation abilities are not optimal in extreme habitats. Whereas, the extremophilic microorganisms are adapted to survive and metabolize hydrocarbons under stressed conditions.

Such microorganisms are called extremophiles belonging to the archaea group, and they can grow in conditions unfavorable to eubacteria. Therefore, for the bioremediation and removal of contaminated soils, the focus is on an archaebacterial domain.

This chapter provides a holistic overall view of the role of Archaea in bioremediation. It explicitly covers archaeal distribution, bioremediation mechanisms, and applications in bioremediation and the future of research to reinforce our understanding of the applications of Archaea in the field.

2.2 BACKGROUND INFORMATION ON ARCHAE DOMAIN

Extremities of temperature, pressure, salinity, dryness, radiation, pH, or concentrations of HMs make the survival of organisms difficult. Microorganisms that are adapted and can tolerate the extreme conditions can survive in these ecosystems. These microorganisms are extremophiles classed under Archaebacteria. Depending on the extreme conditions, the archaea may be termed as: halophiles (salt), barophiles (pressure), thermophiles/

psychrophiles (temperature), acidophiles/alkalophiles (pH), etc. Extremophiles harbor unique cell walls and extremozymes that help them in the survival process [6, 15]. Thus, being acquainted with the harsh conditions, the extremophiles with pollutant degradation abilities can be utilized for bioremediation of contaminated soils in such conditions. These archaea have great capacity as new platforms, which can exploit the extreme environments and thrive.

Hypersaline environments constitute high salt concentrations, which create unfavorable stressful conditions for the survival and existence of the majority of the living organisms [52]. Natural hypersaline environments comprise of salty lakes, pans, marshes, flats, and effluents of production units of oil and gas. They are contaminated with crude oil and effluents of various industries; and are highly saline [65]. The biological communities that exist in such environments are the ones that can tolerate high salt concentrations. These organisms are called halophiles, and the presence of the high salts is a must for their survival [81], while the halotolerant strains can survive in the absence of high salt stress [69].

Members of Archaea are found to dwell in these extreme environments, and these are grouped as haloarchaea that are clustered in one class (Halobacteria in the phylum Euryarchaeota), requiring high salt concentrations of 1.8–5.0 M of NaCl [5, 30]. Along with high salinity, some hypersaline environments (such as salterns also experience high temperature). The haloarchaea found in such niches are thus called “thermophilic halophiles.”

The thermophilic microorganisms grow optimally at 40°C or above; while the extreme thermophiles and hyperthermophiles grow best at >70°C and >80°C, respectively. Thermophiles belong to eubacteria and archaea [102].

2.3 ROLE OF ARCHAE IN BIOREMEDIATION

Coastal marine sediments are subjected to anthropogenic activities leading to the accumulation of contaminants in these environments. Hydrocarbon degrading archaea are ideal strains for bioremediation of habitats that are contaminated with oils and hydrocarbons. The conditions in these environments are hypersaline, extremely high temperature, or strictly anaerobic. There are very few studies on the application of bacteria from archaeal populations in pollution management.

2.3.1 REMEDIATION OF HYPERSALINE ENVIRONMENTS

Hypersaline environments have saturating salt concentrations of about 350 gL^{-1} as compared to seawater with 35 gL^{-1} [63]. These environments support the organisms that can survive the high salinity. These are the halophilic and halotolerant microorganisms belonging to haloarchaea [82]. Oil pollution in hypersaline environments thus is dependent on the archaeal components. Numerous reports have indicated the members of class Gamma-proteobacteria [12, 37, 40, 63, 73, 76, 99, 111] with degradative activities under saline stress. However, studies also show the negative influence of increase in salinity of the habitat to affect the breakdown of hydrocarbons by the archaea, thus affecting the bioremediation process [1, 28, 96].

The exact reason for the decreased rate of degradation cannot be pinpointed yet. Unavailability of hydrocarbons in higher salt concentrations due to the salting-out effect may result in reduced biodegradation under hypersaline environments [68, 72, 105]. The hydrocarbon metabolizing ability of halophiles is seen in isolates from hydrocarbon-contaminated and uncontaminated environments (Tables 2.1 and 2.2).

The Archaebacterial strains not exposed to oils or aromatic compounds (being isolated from uncontaminated environments) show good degradation properties. The presence of growth supplements along with the oil or aromatic compound was found to have a beneficial effect on the degradation by haloarchael communities [2, 19]. Surfactants (such as Tween 20, Tween 80) play a significant role in the degradation of oils; and are used in remediation of oil spills and hydraulic fracturing mixtures [77, 95].

Large diversity of haloarchael strains in hypersaline environments through requiring high temperature as well as requirements of growth supplements have tremendous metabolic degradation capabilities for oil, crude oil, hydrocarbons [3, 4, 10, 30]. Due to high aromatic degradation rates of these communities, they are excellent and promising candidates for bioremediation of hydrocarbons in hypersaline environments.

TABLE 2.1 Oil Degradation by Archaea Isolated from Contaminated Environments

Bacteria	Carbon Source	Salinity	References
Dietzia maris	Hydrocarbons, paraffin	58 to 175 $g.L^{-1}$ NaCl	[5]
Fusarium lateritium Drechslera sp.	Crude oil	10% NaCl	[78]

TABLE 2.1 *(Continued)*

Bacteria	Carbon Source	Salinity	References
Gammaproteobacteria	Monoaromatic	140 to 230 g.L^{-1}	[76]
	–	290 g.L^{-1}	[99]
Haloarchaea	Mixture of polyaromatic hydrocarbons	200 g.L^{-1}	[10]
Haloarcula Haloferax	Heptadecane	225 g.L^{-1}	[104]
Haloarcula vallismortis	Hydrocarbons	310 g.L^{-1} (31%)	[104]
Haloferax *Halobacterium Halococcus*	Aliphatic and aromatic hydrocarbons	58 to 175 g.L^{-1} NaCl	[5]
Halomonas strain	Crude oil, diesel, lubricant oil, or hexadecane	Saline water	[73]
Halophilic bacteria	Phenanthrene	5% NaCl	[107]
Marinobacter hydrocarbon oclasticus	Intermediate chain length aliphatics	35 g.L^{-1} NaCl	[40]
Planococcus	BTEX	0.5% to 25% NaCl	[66]

TABLE 2.2 Aromatic Compound Degraders from Uncontaminated Environments

Bacteria	Carbon Source	Source of Isolation	References
33 strains of *Halobacteriaceae*	Crude oil, tween 80	Hypersaline lakes in Turkey	[83]
Haloarcula st. D1	4-Hydroxybenzoic acid	Industrial wastewater	[36]
Haloarcula st. MSNC 2, *Haloferax* st. MSNC 2, *Haloferax* st. MSNC 14, *Haloferax* st. MSNC 16	Heptadecane	Salt crystallization pond in Camargue, France	[104]
Haloarcula vallismortis	Aliphatic and aromatic hydrocarbons	Saltmarsh in France	[104]
Halobacteriaceae L1	Benzoic acid	Dead sea	[20]
Halobacterium	Alkanes	Hypersaline wastewater in Russia	[61]

TABLE 2.2 *(Continued)*

Bacteria	Carbon Source	Source of Isolation	References
Haloferax st. MSNC 14,	n-Heptadecane, pristane, and phenanthrene	Salt crystallization pond in Camargue, France	[31]
Haloferax mediterranei st. M-11	Hydrocarbons	Kalamkass oil field [Mangyshlak, Kazakhstan]	[115]
Haloferax sp., *Halobacterium piscisalsi*, *Halobacterium salinarum*, *Halorubrum ezzemoulense*, *Halorubrum* sp.	Naphthalene, phenanthrene, and pyrene	Çamaltı Saltern, Turkey	[35]
Haloferax st. HA-1, *Haloferax* st. HA-2, *Halobacterium* st. HA-3, *and Halococcus* st. HA-4	Alkane and aromatic degradation abilities	Coast of Arabian Gulf	[05]
Haloferax strains	Polycyclic aromatic hydrocarbons and crude oil	Salt marshes, salterns, salt flats, crystallizer ponds and the dead sea	[30]
Haloferax volcanii strain D1227	Monoaromatic carboxylic acids, 3-phenylpropionate	Saline oil-brine from Michigan	[39]
Halopenitus	4-Hydroxybenzoate	Great Salt Lake [Utah, USA]	[21]
Haloterrigena mahii sp. H13	1,2-Dichloroethane, naphthalene/anthracene, γ-hexachlorocyclohexane, 1-/2-methylnapthalene, and benzoate	Saltern pond in San Diego, CA, USA	[29, 30]
Natrialba sp. st. C21	Phenol, pyrene, and naphthalene,	Contaminated saline water in Ain Salah, Algeria	[56]

2.3.2 ROLE OF ARCHAEA UNDER THERMOPHILIC CONDITIONS

The majority of oil degraders being mesophilic are unable to efficiently bring about oil degradation at elevated temperatures (i.e., under thermophilic conditions). Therefore, thermophilic archaea are of abundant significance in the bioremediation of oil contamination in these regions. Some workers have reported the application of thermophilic microorganisms in the bioremediation process.

The research study by Jiang et al. [50] on bacterial isolates from soil contaminated with oil at Daqing Oilfield, China showed the ability of the bacterial isolates to degrade crude oil at 52 to 80°C. Audrius et al. [7] studied thermophilic bacteria isolated from an oilfield in Lithuania, having high temperature. These isolates could degrade naphthalene, anthracene, benzene, phenol, benzene-1,3-diol, protocatechuic acid at high temperatures. Thermophilic strains *Sulfolobus solfataricus* P2 and *Sulfolobus solfataricus* P1 degrading phenol were studied for the degradative pathways involved in the degradation at 80°C and highly acidic pH [16–18].

2.4 ROLE OF ARCHAEA IN OIL DEGRADATION: OCEANIC WATERS AND MARINE SEDIMENTS

The oil-degrading bacteria found in the oceanic waters and sediments are subjected to temperature and salinity variations. Mostly the oil-degrading organisms isolated are from the domain bacteria [14]. Few groups of Archaea were found including Marine Group II Archaea, Euryarchaeota, and Thaumarchaeota [90].

Archaeal members were indicated in marine sediments near Rio de Janeiro (Brazil) when heptadecane, naphthalene, or crude oil was added. The archaea detected belonged to uncultivable strains of Euryarchaeota [51]. Similar findings were also reported from oil-contaminated mangrove sediments [27, 49]. These findings suggest that uncultivable Archaea are involved in the removal of oil contamination in most of the marine environments, and this includes the haloarchaea and methanogens also [114]. These studies indicated that the increase in methanogens of the groups Methanosarcinales and Methanomicrobiales occurred on the addition of stimulants (such as methanol, acetate, etc.) [113]. There are similar reports of an increase in the haloarchaeal strains such as *Haloferax*, on addition of oil droplets and nitrate [13, 108, 109, 112]. The role of the haloarchaea in oil degradation in sediments has been documented from these results, which is important because the sediments being more contaminated as compared to the ocean waters.

2.5 ROLE OF ARCHAEA IN HEAVY METAL (HM) TREATMENT

HM contamination even at low concentration as well as depending on its oxidation/reduction state poses a serious problem to the environment, due to its toxicity to biotic factors in the environment [94]. Hence, it is crucial to remove the metal contamination. Several physical methods (such as ion exchange, precipitation, filtration, electrochemical treatment, or reverse osmosis) are being employed for the purpose [110]. However, being costly, there is a need for cost-effective and safe methods. Bioremediation using metal tolerant haloarchaea is an alternative.

Ranawat and Rawat [89] have reviewed the metal-tolerant thermophiles in bioremediation. Kashefi et al. [54] have described the role of *P. islandicum* in the Fe[III] oxide reduction and conversion U[VI] to magnetite and uraninite. This conversion takes place in hyperthermophilic habitats along with the reduction of several other metals, such as Tc[VII], Cr[VI], Co[III], Mn[IV], and Au[III] [53, 55]. The Kashefi and his colleagues also reported a reduction of Gold and Fe[III] by archaea *Pyrococcus furiosus* [55]. *Methanobacterium bryantii* was reported to exhibit copper chelating properties [57].

Some thermophilic archaea could cause oxidation of Arsenite [AsIII] to arsenate [AsV], which is a less toxic form. Some of the strains reported belong to Crenarchaeota and Euryarchaeota [48], *Sulfolobus acidocaldarius* st. BC [98], *Aeropyrum pernix* st. K1, *Pyrobaculum calidifontis* strain JCM 11548, and *Sulfolobus tokodaii* strain 7 [43, 64], species belonging to genus *Halorubrum* [47, 79]. Mercuric mercury [HgII] is highly toxic that is reduced to zero-valent mercury [Hg0] (which is volatile) by Crenarchaeota and Euryarchaeota [11], *Sulfolobus solfataricus* [97], *Halococcus*, *Halobacterium*, *Haloferax* [2].

There are several reports of archaeal strains involved in the reduction of U[VI] to U[IV], which resulted in precipitation of Uranium [75]. Some of the examples are hyperthermophile *Pyrobaculum* sp. [54], *Sulfolobus acidocaldarius* [91]. Haloarchaea has been reported to bioconvert cadmium. Examples of these haloarchaea are *Halobacterium noricense* [100] and *Haloferax* st. BBK2 [23].

2.6 ROLE OF ARCHAEA IN SOIL AND DRAINAGE AFFECTED BY THE MINING INDUSTRY

The mines are responsible for the release of large amounts of metals in the environment. These sites are habitat for the metal tolerant microbial communities that react with the metals leading to the generation of acid mine drainage. It is seen that microorganisms involved in mining also have utility in bioremediation of metal-contaminated sites, due to their higher tolerance levels to the metals. The bioremediation process involves the conversion of toxic metals into insoluble non-toxic or less toxic metal precipitates, resulting in the deposition of minerals of the corresponding metal ores. This is indeed an indication of presence of microbial activity.

Studies carried out at mining sites have shown the presence of microbial communities that are involved in the transformation of metals in these environments. Baker and Banfield [8] described thermoplasmatales that were found associated with environments, such as the iron mines. Ferroplasma, Thermoplasma. The five groups A-plasma, B-plasma, C-plasma, D-plasma, and E-plasma were found to colonize these sites. Archael communities were also reported by Druschel et al. [32] from Iron Mountain in California, an iron mining site. Archaea belonging to Thermoplasmatales was found to constitute the microbial community.

The presence of Thermoplasmatales was also reported by Golyshina et al. [41] from bioleaching pilot plants as well as shallow submarine and continental solfataras. Studies on acid mine drainage and the incidence of archaea have also been reported by other workers [103, 106]. Isolation of Archaeal iron reducers from very diverse environments has been reported. One such site is the marine geothermal regions. Both culturable and non-culturable archaebacterial have been encountered, such as, strains belonging to Crenarchaeota and Euryarchaeota [54] and *Aciduliprofundum boonei* strain T469 [93].

2.7 ROLE OF ARCHAEA IN THE TREATMENT OF EFFLUENTS FROM INDUSTRIAL PLANTS

There are numerous reports on the use of extremely thermophilic archaeal in industrial effluent treatment. Thermophilic enzymes find application in the removal of various contaminants from the industrial effluents before their discharge in the water bodies or wastelands. These wastewaters are characterized by high temperatures and high salinities. Removal of phosphate and arsenate, nitrogen, organophosphorus compounds by thermophilic enzymes have been described by several investigators [24, 44, 92].

2.8 ADVANTAGES AND DISADVANTAGES OF ARCHAE IN REMEDIATION

There are several disadvantages to the application of archaea for bioremediation purposes. Firstly, for using thermophilic archaeal strains, the temperature at the site should be optimal for growth. At this temperature, the hydrocarbons are volatile and oxygen availability is less.

Secondly, there are still lacunae in the information about anaerobic degradation by archaeal strains. In the hypersaline habitats, anaerobic conditions exist. Hence, contamination of these sites with hydrocarbons or oils will require the knowledge of tolerant organisms. According to Ramos et al. [88], having halo tolerance or temperature tolerance may still restrict the bioremediation of contaminants by this microflora. Extremophilic microbes can be employed in the cleanup of metal and radionuclide contaminants in hypersaline ecosystems. The metal and radionuclide recycling and recovery ability of archaea can be exploited for remediation purposes [71].

The use of archaea for bioremediation of hypersaline environments is cost-effective as compared to the other treatment methods of oil-contaminated sites [74]. It is a completely eco-friendly and sustainable method that makes use of natural inhabitants of the hypersaline ecosystem. Either indigenous or organisms that can survive in the harsh conditions are used for the purpose [26]. Another advantage of the use of archaea for bioremediation is that it is non-intrusive and has implementation ease [62].

2.9 COMMERCIAL APPLICATIONS

Halophilic archaea find applications in industrial processes, hypersaline wastewater treatment, evolutionary studies, fossil studies, possible involvement in petroleum product synthesis, etc. [63]. Microbial bioremediation technology has been successfully used for the elimination of environmental pollutants converting toxic to non-toxic compounds at low cost [86]. According to Holden et al. [45], the natural attenuation (NA) process was utilized for bioremediation of 25% of petroleum-contaminated land.

Bioremediation of hypersaline environments makes it mandatory to use halophilic or halotolerant microorganisms [85]. Application of the non-halophiles in bioremediation of contaminated hypersaline regions would require dilution of salt-laden soil and water, which increases the cost of the process.

Xenobiotic bioremediation is the most important application of halophilic archaea. Oil and gas recovery processes result in the production of saline wastewater as effluents [80]. Treatment of these wastewaters biologically requires the agents to be tolerant to salinity as well as the aromatic components in the waste. Hence, haloarchaea find application in bioremediation of contaminated saline soils, toxic effluents, and hydrocarbon-polluted environments [29]. *Hfx. mediterranei* is able to grow using oil as a carbon and energy source, while *Natrialba* sp. strain C21 can utilize hydrocarbons (such as phenol, naphthalene, and pyrene) as the sole source of carbon [56]. Similarly, several halophiles isolated from saline habitats with oil and hydrocarbon-degrading properties are enlisted in Tables 2.1 and 2.2. These haloarchaeal members are apt microbial agents for application in the biodegradation of hydrocarbon-polluted saline environments.

2.10 FUTURE PERSPECTIVES FOR USE OF ARCHAEA IN BIOREMEDIATION

The presence of archaebacteria is widespread in environments, where the conditions are unfavorable (such as hypersaline regions, polar regions, hydrothermal vents, etc.), for the growth of eubacteria [34, 59]. They are involved in the transformation processes in these habitats that influence the biogeochemical cycles, including degradation of organic compounds, and metal transformations, etc.

Most of the in-depth studies of these processes involving bacteria are numerous, while there are lacunae in the case of haloarchaea and archaea in general. There is a need to apply gene analysis and expression methods to understand the metabolism and utilization of transformation processes.

Studies on the unculturable archaea will help in generating the complete picture of the entire archaeal communities in the extreme habitats. The information will be applicable in the understanding of the enzymatic pathways involved in biodegradation and biotransformation of contaminants. Some workers have reported microflora with hydrocarbon-degrading abilities using culture-independent molecular techniques [22, 42, 46, 70]. These studies show that aerobic and anaerobic organisms together bring about the hydrocarbon biodegradation in the extreme habitats [58].

Several investigators have researched on the utilization of various aromatic contaminants including oils. These research reports indicate abundance of hydrocarbon-degrading archaea in these habitats focusing on their degradation potential [38, 42]. However, most of the research is laboratory-based. It is high time to have field-based (*in-situ*) research. It is thus a challenge to carry out *in situ* bioremediation of oil and petroleum-contaminated waste containing aromatics, aliphatics, and HMs [84].

There are few reports on the *in-situ* bioremediation of contaminated sites using the indigenous microorganisms, describing the difficulties encountered [67, 101]. Bioremediation carried out with biostimulation results in the growth of the required microflora and successful removal of contaminants has been reported [9, 24, 101, 114]. Thus, the indigenous microbial flora with bioremediation properties needs to be characterized to develop the bioremediation technology.

In most of the studies, there are reports on the less abundance of detection of Archaea. Since the methods used for non-culturable and culturable archaea are PCR (polymerase chain reaction) based and the primers used are universal primers, which are more apt for eubacteria. There is a very high possibility of missing archaeal strains. Hence the amplification post PCR should be done more cautiously not to miss the clades of Archaea [87]. Thus, more research to monitor and to study the degradation process and detect the application of haloarchaea in *in-situ* degradation is required. This will increase the understanding of biodegradation processes of archaeal strains in the contaminated hypersaline environments.

2.11 SUMMARY

Archaea are extremophiles that can live in environments that do not allow other organisms to survive. These are uninhabitable extreme environments with high salt concentrations, high and low temperatures, highly acidic and alkaline pH, etc. The remediation of these environments requires microorganisms that can survive the hostile conditions. Furthermore, effluents of many industries have high salinity, temperature, metal content; and the pH is either too acidic or too alkaline. Thus, when the effluents enter water bodies or are discharged on land, the environment gets polluted. It is the extremophilic Archaea that can survive the conditions as well as have the potential to remove the contaminants.

KEYWORDS

- **archaea**
- **bioremediation**
- **contamination**
- **extremophiles**
- ***Methanobacterium bryantii***
- **microorganism**

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