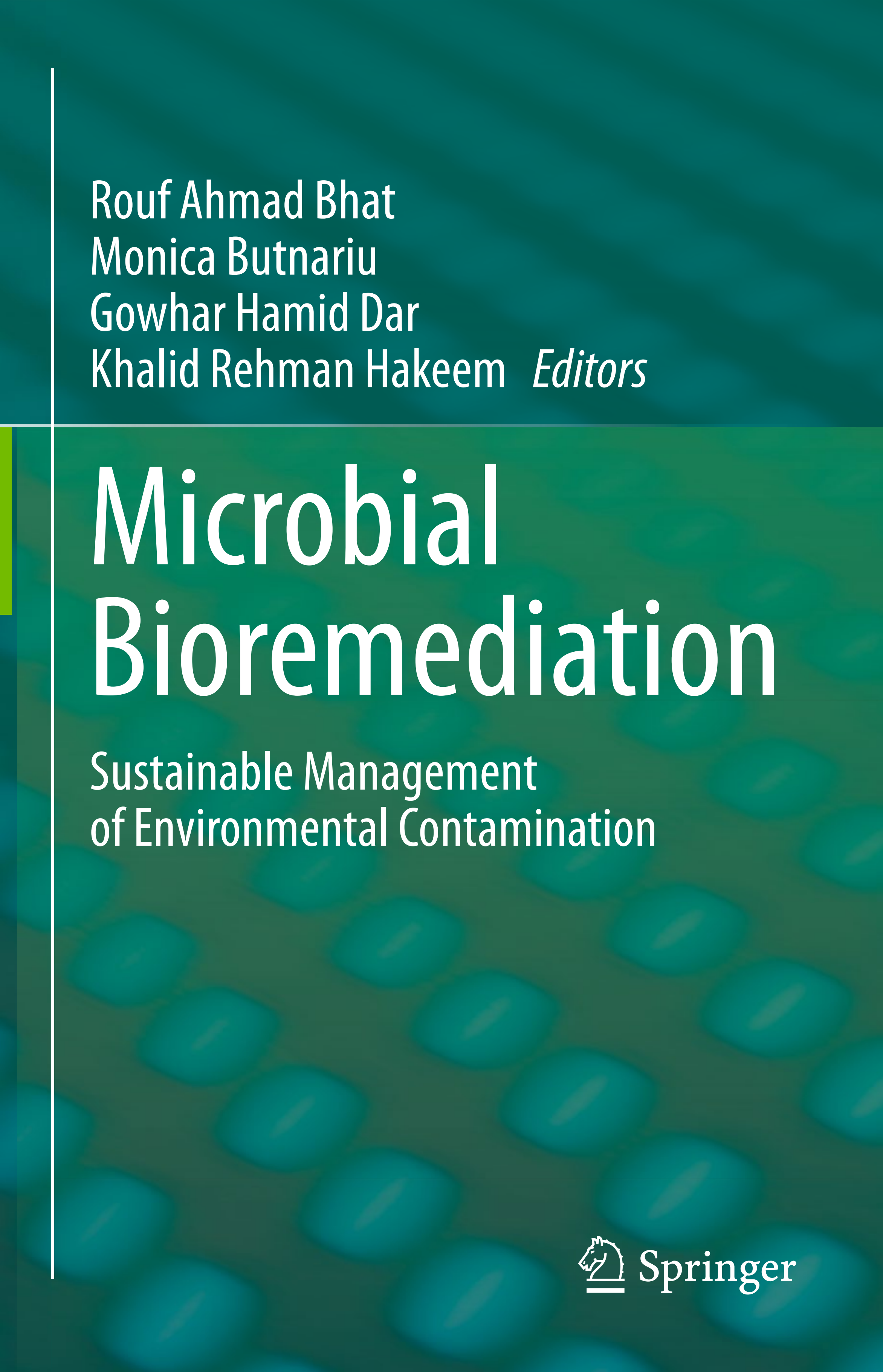

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Microbial Bioremediation

Sustainable Management of Environmental Contamination

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ISBN 978-3-031-18016-3 ISBN 978-3-031-18017-0 (eBook)
https://doi.org/10.1007/978-3-031-18017-0

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Foreword

Bioremediation processes have numerous advantages from economic, environmental, and practical aspects when applied to remediate polluted and degraded environs. They offer environment-friendly approaches which can be implemented effectively for the removal of contamination in the environment. Adsorption and biodegradation of organic contaminants and the immobilization, mobilization, and/or transformation of metals and metalloids are the main remediation processes mediated by the action of several microorganisms. This has been protracted scientifically. The present book has designated a good quantity of space to the aspects of microbial-mediated bioremediation processes beneficial to the environment and abilities of microorganisms to convert noxious compounds into utilizable intermediates and value-added products.

The book at the same time is evaluating and documenting the bio-techniques and technologies that are and can be used to address the problems of pollution in a sustainable manner. These technologies are not only eco-friendly but also cost effective. The latest research pertaining to biotechnology and bioremediation has been presented in a lucid manner.

A total of 16 chapters have been included, and the first chapter addresses the microbial mechanisms of degrading toxic metal compounds and their utilization in bioremediation processes. Chapter “Aerobic Processes: Best Approach for the Treatment of Wheat Starch Effluents” highlights the necessity of aerobic processes having potential to reduce the quantity of wastes and their ability to recover the useful substances from starch effluents, and their reuse for other purposes.

Although the harmful effects of numerous pesticides are well known, even then, they are still in use in many regions of the world. In the light of these activities, chapter “Banned Pesticides with High Persistence: The Impact of Their Use in Agriculture and Their Removal by Microbial Biodegradation” highlights the toxicity impacts of banned pesticides on animals and humans together with the ability of microorganisms to remove these compounds from the environment.

Bioremediation is the most effective technique to overcome the contamination of petroleum and other petroleum products; therefore, among other physical and chemical methods, this topic has been expounded in chapter "Bioremediations for Oil Spills by Utilizing Microbes".

The application of GEMs in bioremediation has been covered in depth, along with the creation of recombinant strains with desirable characteristics via "route design" and "enzyme specificity" alterations. All the findings related to this theme have been discussed at length in the chapter "Genetically Engineered Microorganisms for Bioremediation Processes". Chapter "Microbial Modifications and Biochemical Pathway: Mechanism for Ecosystem Decontamination" focuses on types of genetically engineered microorganisms having high potential for the degradation of toxic pollutants and the future perspectives of GMMs as an alternative to enhance the process of efficacy for its successful application at a wider scale. In chapter "Innovative Biofilms Mediated as Empiricist of Bioremediation for Sustainable Development", "bacterial biofilms" as a promising technique for decontamination of environmental pollutants has been discussed in full detail. Detailed information pertaining to the importance of microorganisms in pollution remediation, their advantages over other processes, and the influence of such microscopic organisms on quality environs have been evaluated in chapter "Major Groups of Microorganisms Employed in Bioremediation". Microbial exploration and its metabolic capacity for detoxification and restoration of natural ecosystems have been discussed in chapter "Microbial Exploration and Their Metabolic Capacity for Detoxification and Restoration of Natural Ecosystems". Multidisciplinary research groups are and have been dedicating their efforts to develop and propose innovative and eco-friendly solutions to re-establish quality environment as well as avoid further damage to the environment. Among these, the use of microbes as tools to efficiently remediate polluted areas has been presented in chapter "Microbes in Restoration of Polluted Ecosystems". In chapter "Microbial Biotechnology: Energy Generation Approach from the Environmental Waste", the topic of bioprospecting for microorganisms adapted to landfill conditions for later use in anaerobic digesters has been discussed at length together with the treating of the degradable fractions of municipal solid wastes, worth exploring for future studies. Chapter "Microbial Degradation of Industrial Pollutants From Different Environments" focuses on the degradation pathway of microbes in wastewater treatment, petroleum hydrocarbons, DDT degradation, and pesticide residues which are used in cosmetics, pharmaceuticals, and day-to-day household products. An emphasis on the importance of various microbial enzymes in pollution decontamination has been presented in chapter "Microbial Enzymes and Their Importance in the Environmental Decontamination". Plant-associated bacteria and other micro-organisms are believed to be the important natural resources which can serve as an ultimate alternative to face the global demand for food. In this context, chapter "Structural and Functional Dynamics of Bacterial World for Sustainability" has reviewed and highlighted the significance, composition, and structural and functional dynamics of plant-associated bacteria which profoundly benefit from plant growth and development in particular as well as ecosystems in general. In relation to the development and growth of plants, microorganisms are essential for soil processes such as nutrient recycling, functional stability, and soil ecosystem sustainability. In this regard, chapter

"Plant-Associated Bacteria in Ecosystems Functioning and Sustainability" discusses the recent advances in the plant-bacterial interactions, as well as enlightening the considerable understanding on the new ways to employ beneficial microbes in ecosystem functioning, restoration, and environmental sustainability. Chapter "The Science of Microbial Enzymes as Detoxification Tool for Inorganic and Organic Pollutants" is the last chapter, and it focuses on the most widely used bio-enzymes (dehydrogenases, cytochrome P450s, proteases, lipases, and dehalogenases) for the degradation of environmental toxicants, that is, aromatic hydrocarbons, dyes, detergents, and agrochemical compounds.

I feel that this book will be a prized resource for researchers at the graduate level vis-a-vis scientists working on bioremediation and related sciences. The authors and editors have worked tremendously to build a noteworthy support to the area of bioremediation. I congratulate them and would like to wish all the best for their future endeavors.

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Preface

The book entitled *Microbial Bioremediation: Sustainable Management of Environmental Contamination* presents a set of microorganism-prompted technologies for environmental hygiene. These technologies are based on the use of metabolic capabilities of microorganisms to remove contaminants from soils and waters. The concept of the book starts from the idea that through microbial remediation, a wide variety of polluting and toxic organic compounds can be bio-transformed until they become harmless. Microbial bioremediation can even degrade some organic compounds to their simplest forms, such as CH_4 and CO_2. The technologies discussed under this theme can extract or immobilize environmental toxic substances and will be no longer available in toxic forms to living organisms. Furthermore, reducing the bioavailability of a toxic substance has been reconnoitred well, although it does not involve removing the substance from the environment. In the practice of "microbial bioremediation," several concepts are used to reflect the bioremediation process, depending on the type of action of microorganisms involved in the remediation process and bio-restoration of disturbed ecosystems.

We are enormously thankful to all the authors who have contributed to this book, and to the Springer team for their substantial teamwork and for printing this volume.

Srinagar, Jammu and Kashmir, India — Rouf Ahmad Bhat
Timisoara, Romania — Monica Butnariu
Srinagar, Jammu and Kashmir, India — Gowhar Hamid Dar
Jeddah, Saudi Arabia — Khalid Rehman Hakeem

About the Book

Microbial communities can play a pivotal role in the conversion of agro-industrial wastes to useful products. Bioremediation and biodegradation by microbes in environmental monitoring offer environmental-friendly approaches that can be implemented effectively for the removal of contamination in the environment. These biological processes have numerous advantages from economic, environmental, and practical aspects when applied to remediate polluted and degraded environs. Microbial-mediated bioremediation process can benefit the environment by applying the abilities of microorganisms that enable the utilization of noxious compounds and transforming them into utilizable intermediates and value-added products. Recent advancements in this field (biochemical engineering, OMICS and genetic engineering tools, and synthetic biology) paved the way for achieving these desired criteria for bioremediation of environments.

Microbial Bioremediation: Sustainable Management of Environmental Contamination summarizes the role of microbial consortium in environmental monitoring and presents some facets regarding their use in ecological engineering, ecological modeling, and ecological management, which allow obtaining consistent results in environmental protection and management. This book provides important information on microbial remediation and highlights the benefits of using combinations of microorganisms selected for their synergistic capabilities to improve water, soil, and air quality. The book significantly highlights the latest research in this field and offers fascinating information on the attitude of microorganisms to improve the environment quality and therefore opening new dimensions of their use.

Key Features

- Provides information on the utility of microorganisms for environmental monitoring and bioremediation of polluted environs

- Details the indicator microbial strains, route of pollution, and development of tailor-made microbe-metabolites that can effectively be applied for the management of fragile environments
- Focusses in detail on new technologies applied to bioremediate polluted environs, specially discussing the role of biological treatment, bioremediation, rehabilitation, or ecological reconstruction

Contents

About the Editors

Rouf Ahmad Bhat, PhD, has pursued his doctorate at Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir (Division of Environmental Sciences) and presently working in the Department of School Education, Government of Jammu and Kashmir. Dr. Bhat has been teaching graduate and postgraduate students of environmental sciences for the past 3 years. He is an author of more than 50 research articles (h-index 21; i-index 32; total citation 1400) and 40 book chapters, and has published more than 30 books with international publishers (*Springer, Elsevier, CRC Press Taylor and Francis, Apple Academic Press, John Wiley, IGI Global*). He has his specialization in limnology, toxicology, phytochemistry, and phytoremediation. Dr. Bhat has presented and participated in numerous state, national, and international conferences, seminars, workshops, and symposium. Besides, he has worked as an associate environmental expert in World Bank-funded Flood Recovery Project and also the environmental support staff in the Asian Development Bank (ADB)–funded development projects. He has received many awards, appreciations, and recognition for his services to the science of water testing, air, and noise analysis. He has served as an editorial board member and a reviewer of reputed international journals. Dr. Bhat is still writing and experimenting with diverse capacities of plants for use in aquatic pollution remediation.

Monica Butnariu, PhD, heads the Romania Biotechnology and Environmental Sciences Group-Council for Nutritional and Environmental Medicine; is the national correspondent of the International Association for Plant Biotechnology; has 79 articles published in the Web of Science Core Collection-Clarivate Analytics that have been cited over 1730 times, with H-index = 32 (WoS); and has been serving as an editorial board member of reputed journals and is also involved in peer-review activity. Prof. Butnariu has written several books and book chapters in different areas of chemistry. Her research interests focus on agricultural chemistry (biotechnology, environmental protection); applied chemistry (instrumental analysis, biocides, biochemistry, agrofood waste); material chemistry (biomaterials); nutritional biochemistry; pharmacology and toxicology; natural products (different aspects); medical biochemistry; and chemical ecology.

Gowhar Hamid Dar, PhD, is currently working as Assistant Professor of Environmental Science, Sri Pratap College, Cluster University Srinagar, Department of Higher Education (J&K). He has a PhD in environmental science with specialization in environmental microbiology (fish microbiology, fish pathology, industrial microbiology, taxonomy, and limnology). He has been teaching postgraduate and graduate students for the past many years in the Postgraduate Department of Environmental Science, Sri Pratap College, Cluster University Srinagar. He has more than 70 research articles (h-index 13; i-index 15; total citation >500) in international and national journals of repute and a number of books with international publishers (Springer, Elsevier, CRC Press Taylor and Francis, Apple Academic Press, John Wiley, and IGI Global) to his credit. Moreover, he is supervising a number of students for the completion of degrees. He has been working on the isolation, identification, and characterization of microbes, their pathogenic behavior, and impact of pollution on development of diseases in fish fauna for the last several years. He has received many awards and appreciations for his services towards science and development. Besides, he also acts as a member of various research and academic committees. Further, Dr. Dar is principal investigator and co-principal investigator for different R&D projects sanctioned by the Govt. of India and Govt. of Jammu and Kashmir.

Khalid Rehman Hakeem, PhD, FRSB, is presently working as a professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completed his PhD (botany; specialization in plant ecophysiology and molecular biology) from Jamia Hamdard, New Delhi, India, in 2011, he worked as an assistant professor at the University of Kashmir, Srinagar, for a short period. Later, he joined Universiti Putra Malaysia, Selangor, Malaysia, and worked there as a postdoctoral fellow in 2012 and fellow researcher (associate professor) from 2013 to 2016, respectively. He joined King Abdulaziz University in August 2016 and was promoted to professorship recently.

Dr. Hakeem has more than 12 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant-microbe-soil interactions, as well as in environmental studies. He is the recipient of several fellowships at both national and international levels. He has recently been elected as the Fellow of the Royal Society of Biology, London. Prof. Hakeem has served as the visiting scientist at Fatih Universiti, Istanbul, Turkey, as well as at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organizations.

So far, Dr. Hakeem has authored and edited more than 60 books with international publishers, including Springer Nature, Academic Press (Elsevier), and CRC Press. He has also to his credit 121 research publications in peer-reviewed international journals and 65 book chapters in edited volumes with international publishers.

At present, Dr. Hakeem is serving as editorial board member and reviewer of several high-impact international scientific journals from Elsevier, Springer Nature, Taylor and Francis, Cambridge University Press, and John Wiley Publishers.

A Diverse Array of Microbial Taxa Affianced in Bioremediation to Counteract Environmental Pollution

Vandana Singh and Tahseena Naaz

1 Introduction

Microbes are found in almost every place or environment on the earth because of their metabolic ability, which allows them to grow in various environmental conditions. Bioremediation employs microbes because of their nutritional versatility, and they have the ability to alter toxic pollutants for the production of biomass and energy gain (Abatenh et al., 2017). Humans have been using the resources of the earth for about more than two million years, which has led to the release of pollutants and wastes into the environment, and hence, the pileup of these pollutants is a serious threat to the ecosystem. These organic pollutants may also harm animals, plants, the health of humans, and aquatic ecosystems. The majority of these recalcitrant pollutants are carcinogenic and can accumulate in biological tissues by entering the food chain (Baghour, 2017). Some of these pollutants can even cause alteration in hormone homeostasis by blocking, interfering, or mimicking the hormone's function (Wielsøe et al., 2017). Similarly, pollutants like organochlorine pesticides (OCPs), polybromodiphenyl ethers (PBDEs), and polychlorobiphenyls (PCBs) are potential endocrine disruptors in wild animals (Vanden Berghe et al., 2013). There are multiple ways through which these pollutants are released into the environment such as oil spills during transportation, industrial and household effluent removal in the water bodies, pesticides used in agriculture, and so on.

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_1

Oil spilling adversely affects the life of aquatic animals as when marine animals ingest it, oils travel to their liver and PAHs get activated by the enzymes, which makes the oil more reactive and toxic (Saadoun, 2015). Similarly, pesticides and heavy metals are contaminating both underground and surface water by runoff (Chekroun & Baghour, 2013). Hence, instead of collecting the effluents and pollutants, microbial bioremediation will be a well-organized procedure to convert the toxic elements into nontoxic ones. The biological agents used for the removal of pollutants are called bio-remediators.

The nutritional versatility of microbes can be used to aid in the biodegradation of contaminants. Bioremediation is the process by which some microbes convert, alter, and consume hazardous contaminants in order to produce energy and biomass production. The archaea, bacteria, fungi, and algae were found to be the prime bioremediators (Strong & Burgess, 2008).

Microbes require a variety of nutrients, including carbon, nitrogen, and phosphorus, to survive and continue their activity. Bio-optimization of the bacterial C:N:P ratio can be enhanced by providing an additional source of nutrients such as N and P. The addition of nutrients adjusts the critical nutritional balance for microbial growth and reproduction (Abatenh et al., 2017). This, in turn, impacts the rate and efficacy of biodegradation. Microorganisms combat pollutants when they have access to a variety of materials and substances that assist them in generating energy and nutrition to build additional cells (Nancharaiah et al., 2015). The efficiency of bioremediation is determined by a number of factors, including the chemical nature and concentration of contaminants, the physicochemical environmental attributes, and their availability to microorganisms (Abatenh et al., 2017).

The control mechanism and optimization of microbial remediation is one of the complicated systems that may require to own several dynamics, such as the presence of a microbiome capable of reducing or degrading the contaminants, availability of noxious waste to the microbial inhabitants, and the most important environmental factors such as nature of the soil, pH, nutrition, temperature, and the presence of gases like oxygen or other electron acceptors.

- Biological factors: Innumerable factors are involved in the degradation process of organic compounds, such as antagonism between microbial flora due to constrained carbon sources, antagonistic concords between microbes by bacteriophages and protozoa, pollutant concentration, and the amount of catalyst (number of microorganisms efficient in the breakdown process of the pollutants along with the quantity of enzymes synthesized by each microbial cell) (Kumar et al., 2011). The expression of specific enzymes by the organisms determines the degree of contaminant degradation. The key biological factors that affect the contamination are mutation, enzymatic activities, upright gene transfer, all type of interactions (succession, predation, and competition), size of population, and composition(Abatenh et al., 2017).
- Environmental factor: The metabolic features of the microbes and the physicochemical properties of the targeted pollutants determine the complex association that may occur during the bioremediation process. The environmental factors

such as pH, moisture, temperature, nature of the soil, nutrients, solubility in water, redox potential, and oxygen level mainly affect the growth and activity of bioremediants (microorganisms). This also takes into account the contaminant's physicochemical bioavailability, concentration, solubility, type, structure, and level of toxicity. These are the key factors that influence the kinetics of degradation studies (Sharma, 2020). In most aquatic and terrestrial environments, the optimal pH for biodegradation ranges from 6.5 to 8.5. Along with pH, moisture also plays an important role in the degradation of contaminants, as it influences the metabolic rate of contaminants and helps in their availability to microorganisms (Tahri et al., 2013).

- Availability of nutrients: Although microorganisms can be found in contaminated soil, they are unlikely to be in sufficient numbers to allow for the bioremediation process. Nutritional elements are one of the critical elements of microbes for their growth and reproduction, which, in turn, impacts the rate and efficacy of biodegradation.
- Temperature: Temperature is one of the most critical physical elements that impact microbial viability and hydrocarbon composition. An increase and decrease in temperature may affect the biochemical reaction rates between microbes and pollutants. The temperature at which biological enzymes participate in the degradation pathway has an optimum value. They don't have the same metabolic turnover at all temperatures since each compound's degradation mechanism requires a different temperature. Temperature impacts the physiological features of the organism; hence, it can either speed up or slow down the bioremediation process (Kim et al., 2014).
- Concentration of oxygen: Varying microorganisms have different oxygen requirements. The amount of oxygen accessible in the system determines whether it is aerobic or anaerobic. Some species require oxygen, while others do not, and this requirement varies by organisms. The biodegradation rate depends on the oxygen requirement of organisms, because oxygen is a gaseous necessity for most living organisms. Biological degradation occurs in both aerobic and anaerobic conditions and by both anaerobic and aerobic organisms. The presence of oxygen can improve hydrocarbon metabolism in the majority of situations. Under aerobic conditions, hydrocarbons are easily degraded, whereas chlorate compounds can only be decomposed in anaerobic conditions (Kim et al., 2014).
- Metal ions: Metals are required by microorganisms in very small amounts, and their presence in larger amounts may hinder cell metabolism. To overcome the higher amount of metals, microbes either adopt or devolve metabolism. The presence of metal compounds also has an effect on the rate of degradation, either directly or indirectly. Metals and their toxic compounds can slow down the process of degradation (Kanamarlapudi et al., 2018).

This chapter will explain the role of different microbes employed in the bioremediation of these pollutants and their working mechanism.

2 Principle of Bioremediation

The inclusion of certain substances to boost autochthonous microbial assemblages (biostimulation) and/or the addition of specific microbial populations with effective biodegradation/detoxification ability (bioaugmentation) are two common microbial-based bioremediation techniques. Microbial species that may be useful for bioremediation of contaminated sediments can be isolated from the same location. Autochthonous microorganisms are expected to be more effective and environmentally friendly than the one which may require manipulation of the natural environment to improve their performance (e.g., adjusting oxygen and/or nutrient concentration, pH) (Sharma, 2020). Microbes (fungi, bacteria, and algae) in bioremediation converts the hazardous pollutants into microbial biomass, metabolites, CO_2, and H_2O. These microbes can be indigenous or can be added from outside to the contaminated sites for bioremediations. The microbes use these pollutants for their growth by degrading and transforming them using their metabolic reactions. Large number of microorganisms are required for the complete degradation of these pollutants. Hence, various potential microorganisms are brought from outside to the contaminated site for the proper degradation process, and this process is known as bioaugmentation (Tyagi & Kumar, 2021).

Bioremediation depends on favorable factors like pollutants' concentration and chemical nature, suitable environmental conditions, microbes' availability, electron acceptor and energy source, pH and moisture, and nutrients. Therefore, an appropriate environment is provided for the proper growth of microbes and the effective degradation of pollutants (I. Sharma, 2020).

2.1 Types of Bioremediations

Bioremediation is classified into ex situ remediation and in situ bioremediation and based on transportation, origin, and pollutant removal from the contaminated sites (Azubuike et al., 2016).

2.1.1 In Situ Bioremediation

This process involves the pollutant treatment without disturbance or excavation in the actual contaminated area and is further divided into intrinsic in situ bioremediation and enhanced in situ bioremediation (Kumar et al., 2018; Tyagi & Kumar, 2021). Intrinsic bioremediation involves passive and unassisted remediation of contaminated sites without any human involvement. This technique contains both anaerobic and aerobic microbial actions for the treatment of recalcitrant and biodegradable pollutants (Azubuike et al., 2016). The enhanced in situ bioremediation involves polluted site enhancement by adding microbes, nutrients, and air to supplement microbial growth for the process of bioremediation. Some examples of enhanced in situ bioremediation are bio-stimulation, bio-sparging, bio-slurping, bioaugmentation, and bioventing (G. Singh, 2014). In situ bioremediation is mainly used for the treatment of areas contaminated with heavy metals, chlorinated solvents, hydrocarbons, and dyes (S. Kim et al., 2014).

2.1.2 Ex Situ Bioremediation

This method requires transportation and excavation of pollutants from the original site to another site for its treatment utilizing multiple techniques of bioremediations. This technique is then again divided into different categories like bioreactors, landfarming, biofilters, bio-piling, and composting that is based on treatment cost, degree and depth of pollutants, types of pollutants, and the geological and geographical features of polluted sites (Tyagi & Kumar, 2021).

3 Mechanism of Microorganism for Bioremediation

It is already stated above that the major groups of microorganisms employed in bioremediation are bacteria, algae, and fungi. There are two categories of bioremediation. The first is biosorption, and the second is bioaccumulation. Biosorption refers to the mechanism of passive adsorption that is reversible and fast (Wang et al., 2021). The metals on the cell surface are retained by physiochemical interactions like complexation, ion exchange, crystallization, adsorption, and precipitation (Khadiga et al., 2017). The biosorption of metals is affected by several factors like ionic strength, the concentration of biomass, size of particles, temperature, pH, etc. (Vilar et al., 2005). There can be both dead and living biomass for biosorption because it is cell metabolism independent. On the other hand, bioaccumulation contains both extra- and intracellular processes. Here limited role is played by the passive uptake. So, for bioaccumulation, only living biomass can occur (Chojnacka, 2010).

The cell wall of the microorganisms is comprised of multiple macromolecules like proteins and polysaccharides, along with several charged functional groups (imidazole, ester sulfate, carboxyl, sulfhydryl, phenol, hydroxyl, thioether, and amino groups) (Rosca et al., 2015; Akar & Tunali, 2006). The adsorption occurs in a solution when the positively charged metal present drifts towards the functional groups present in the solution. The cell wall composition gets influenced by the method of microbe cultivation. Therefore, this property can be used to increase the capacity of adsorption in microbes (Coelho et al., 2015) (Fig. 1).

Pollutants like heavy metals from wastewater can be removed by using bacteria through functional groups like aldehydes, ketones, and carboxyl groups existing in their cell wall (Kanamarlapudi et al., 2018). It will help in producing less sludge. Heavy metals can be removed by employing both gram-positive and gram-negative

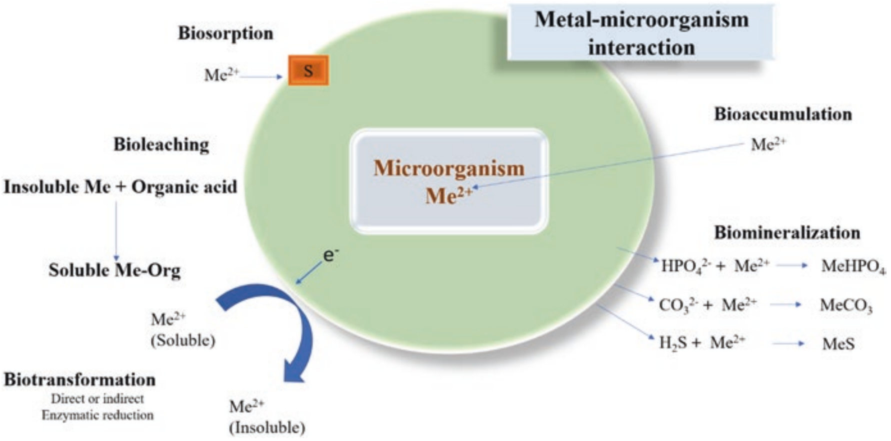

Fig. 1 Mechanism of microbes utilized in bioremediation

bacteria. Algae are also found helpful in bioremediation as brown, red, and green algae are employed as biosorbents (Bwapwa et al., 2017). Functional groups like xylans, alginic acids, uronic acid of sulfate groups, carboxyl groups, and galactans of bacteria can perform ion exchange and can produce toxic material, whereas algae usually do not produce toxic materials, so it’s an advantage of using algae as biosorbents instead of other microbes like fungi and bacteria (Coelho et al., 2015). Fungi are one of the bioremediants used in the degradation of benzo(a)pyrene. However, limited information is available about it. It was discovered that, for the degradation of HMW PAHs like BaP, fungi use enzymes that are different from bacteria. A few studies have also shown that the fungal degradation process is also affected by selective pressure. Similarly, *P. chrysosporium* was found to have the ability of BaP degradation, but its ecological niche is given as a lignin degrader (Syed & Yadav, 2012).

4 Different Types of Pollutants

As stated above, microbes can degrade various pollutants like heavy metals, hydrocarbons, etc. Hydrocarbons (HCs) contain carbon and hydrogen in their structure, and they enter the environment from many sources (Table 1). With the flourishment of petrochemical industries, there is an increase in HC contamination, which has become a major environmental issue worldwide. Hydrocarbon groups of compounds can be neurotoxic and carcinogenic to various forms of life (Kothari et al., 2014). Besides this, heavy metals that are released from pesticides and fertilizers also contaminate the environment. Biological degradation of heavy metals is not possible as they can only be transformed from one organic or oxidation state to another. It was found that microbes can protect themselves from the toxicity of

Table 1 Source of different heavy metals. (Medfu Tarekegn et al., 2020)

Anthropogenic activities	Heavy metals
Electroplating, smelting	Zinc
Leather tanning, manufacturing, chromium salts, industrial coolants, mining	Chromium
Spent catalyst	Molybdenum
Fuel combustion, zinc melting, paint sludge, e-waste, incinerations, waste batteries	Cadmium
Smelting operations, electroplating, mining	Copper
Fuel burning, thermal power plants, smelting operations, natural/geogenic processes	Arsenic
Hospital waste, fluorescent lamps, thermal power plants, Chlor-alkali plants	Mercury
Bangle industry, ceramics, smelting operations, E-waste, paints, lead-acid batteries	Lead

metals by their multiple mechanisms like methylation, adsorption, reduction and oxidation, and uptake. In the bioremediation process of heavy metals, methylation plays an important part as methylated compounds are usually volatile (Abatenh et al., 2017). There are also several other types of pollutants and some are listed below for reference.

- *Polycyclic aromatic hydrocarbons (PAHs)*: These are hydrophobic organic components mainly found in soil, air, and sediments. It is mainly released from industries. It can accumulate in marine habitats and can sorb to organically rich soils (Tahri et al., 2013).
- *Pesticides*: These are mixtures of materials that are mainly made to prevent destruction from any pest in the agriculture field. Pesticides are divided into two categories. One is those that are degraded easily and are called nonpersistent, and the other is those that resist degradation and are called persistent (Tahri et al., 2013).
- *Polychlorinated biphenyls (PCBs)*: These are synthetic organic chemical mixtures. Polychlorinated biphenyls are used in various industries due to their high boiling points, chemical stability, non-flammability, and electrical insulating properties. They are toxic compounds that can cause cancer and endocrine disruption (Seeger et al., 2010).
- *Dyes*: They are extensively used in paper, textiles, printing, rubber product, pharmaceuticals, color photography, cosmetics, and various other industries (Rafii et al., 1997). The highest class of synthetic dye is the Azo dye, which is an aromatic compound. The dyes are not easily biodegradable due to their structures. Hence, wastewater treatment containing dyes generally involves chemical or physical methods like oxidation, adsorption, flocculation, filtration, etc. (Verma & Madamwar, 2003) (Table 2).

Table 2 Microorganisms involved in the bioremediation of dyes

Compounds	Microorganisms	References
Effluents of textiles and vat dyes	*Staphylococcus aureus*, *Bacillus macerans*, *Bacillus firmus*, and *Klebsiella oxytoca*	Adebajo et al. (2016)
Effluents of azo dyes	*Acinetobacter baumanii*, *Exiguobacterium indicum*, *Bacillus cereus*, *Exiguobacterium aurantiacums*	Kumar (2016)
Oil-based based paints	*Bacillus subtilis* strains NAP1, NAP2, and NAP4	Phulpoto et al. (2016)
Industrial dyes	*Penicillium ochrochloron*	Shedbalkar and Jadhav (2011)
RNB dye, sulfonated di-azo dye, Reactive Red HE8B, textile dye (Remazol Black B)	*Bacillus pumilus* HKG212, *Pseudomonas aeruginosa*, *Bacillus* spp. ETL-2012	Das et al. (2016); Patel and Gupte (2016)
Textile azo dyes	*Nocardia atlantica*, *Listeria denitrificans*, *Micrococcus luteus*	Hassan et al. (2013)

5 Group of Microorganisms Employed in Bioremediation

Bioremediation is mainly based on the catabolic action of the microbes (Hazen, 2018). And in this process, contaminant transformation doesn't provide any advantage to the cell. Hence, this process is called nonbeneficial biotransformation (Kumar et al., 2011; Wasilkowski et al., 2012). Multiple studies have proved that several microorganisms can naturally absorb the ions of toxic heavy metals (Wasilkowski et al., 2012). Typically, eukaryotes are different from prokaryotes based on their cellular structure. The interaction between heavy metals and microorganisms partially depends on whether the organism is eukaryotic or prokaryotic. It is because prokaryotes are less sensitive to heavy metals than eukaryotes (Perpetuo et al., 2011). In eukaryotes, metals can interact via ether by intracellular chelation or by active metal extrusion, and transformation process to convert into less hazardous chemical forms. Microbes convert toxic pollutants enzymatically into harmless products to increase the efficiency of bioremediation (Sharma, 2012). It was also seen that bacteria have developed resistance to heavy metals (Table 3) and render them harmless (Mejáre & Bülow, 2001). Various microbes (anaerobes, aerobes, and fungi) are involved in this enzymatic degradation.

As multiple pollutants can be present at the site of contamination, various groups of microbes are required for efficient bioremediation. Some microbes are capable of degrading petroleum hydrocarbons and utilize them as a source of energy and carbon. As microbes can only remain alive in a limited range of chemical contaminants, the organism used in the remediation process needs to be selected carefully (Table 4). It is the potential of microbes to introduce oxygen into the hydrocarbon for the metabolic pathway of the cell that affects the degradation process. Some bacteria exhibit a chemotactic response through which they search for contaminants and move toward them.

Table 3 Microorganisms employed in bioremediation of heavy metals

Compounds	Microorganisms	References
Cd, Cr, Pb	*Rhodopseudomonas palustris*, *Aerococcus* sp.	Sinha and Biswas (2014); Sinha and Paul (2014)
Cr, Ni, Cu, U	*Aeromonas* sp., *Pseudomonas aeruginosa*	Sinha et al. (2011)
Cadmium	*Bacillus safensis* (JX126862) strains (PB-5 and RSA-4)	Priyalaxmi et al. (2014)
Cadmium	*Cladosporium* sp., *A. fumigatus*, *Microsporum* sp., *Terichoderma* sp., *Paecilomyces* sp., *Aspergillus versicolor*	Mohammadian Fazli et al. (2015)
Lead, copper, cobalt, and chromium	*Lysinibacillus sphaericus*	Peña-Montenegro et al. (2015, p. 5)
Cu^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, and Fe^{2+}	*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*	Paranthaman and Karthikeyan (2015)
Heavy metals	*Cunninghamella elegans*	Tigini et al. (2010)
Nickel, mercury, lead, and heavy metals	*Saccharomyces cerevisiae*	Chen and Wang (2007); Tálos et al. (2021)

5.1 Bioremediation by Bacteria

Several studies have been done on degradation through bacteria. It was also seen that bacteria feed on hydrocarbons (Yakimov et al., 2007). Bioremediation of hydrocarbons by bacteria can occur in anaerobic or aerobic conditions (Grishchenkov et al., 2000). The most common genera of bacteria participating in bioremediation processes include *Alcaligens*, *Acinetobacter*, *Arthrobacter*, *Achromobacter*, *Burkholderia*, *Alteromonas*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Bacillus*, etc. (Ghattas et al., 2017), and some of the examples are listed in Table 5 (Hassanshahian et al., 2012). Likewise, genera, for instance, *Alcanivorax*, *Thallassolituus*, *Oleispira*, *Cycloclasticus*, and *Marinobacter*, along with obligate hydrocarbonoclastic bacteria (OHCB), are well acknowledged for their ability to remediate or degrade hydrocarbons.

5.2 Bioremediation by Fungi

Fungi have been recognized for their potential to synthesize enzymes (e.g., catalases, laccases, peroxidases, etc.) that breakdown organic pollutants and/or immobilize inorganic contaminants. Fungi can survive in freshwater as well as in marine habitats with complex soil matrix (Deshmukh et al., 2016). Fungi can remain alive in various climatic conditions, including harsh ones, and aid in the ecosystem balance (Anastasi et al., 2013). Some studies also reported that they could survive in

Table 4 Interaction of hydrocarbons and microorganisms

Pollutants	Microorganisms	References
Anthracene, striatum Pyrene, dibenzothiophene lignin peroxidase, 9-methylanthracene	*Gleophyllum striatum*	Yadav et al. (2011)
Monocyclic hydrocarbons like xylene and benzene	*Pseudomonas putida*	Bahadure et al. (2013); Idris et al. (2015)
Toluene, benzene, phenol compounds, monocyclic aromatic hydrocarbons, xylene, and ethyl benzene	*Penicillium chrysogenum*	Abdulsalam (2012); Pereira et al. (2014)
Benzopyrene, phenanthrene	*Candida viswanathii*	Hesham et al. (2012)
Aromatic hydrocarbons	*Ralstonia* sp., *Pseudomonas* sp., *Acinetobacter* sp., and *Microbacterium* sp.	Simarro et al. (2013)
Naphthalene	Diatoms and green algae, cyanobacteria, and *Bacillus licheniformis*	Sivakumar et al. (2012)
Methylnaphthalenes, PAHs, and dibenzofurans	*Coprinellus radians*	Aranda et al. (2010)
Hydrocarbons	*F. solani*, *A. fumigatus*, *A. niger*, and *P. funiculosum*	Jawhari (2014)
Triphenylmethane and biphenyl	*Phanerochaete chrysosporium*	Wolski et al. (2013)
Toluene, diesel, and petrol polycyclic aromatic hydrocarbons	*Acinetobacter*, *Flavobacterium*, *P. putida*, *P. mendocina*, *P. alcaligenes*, *P. veronii*, and *Achromobacter*	Idris et al. (2015)
Phenol	*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Alcaligenes odorans*, *Corynebacterium propinquum*	Singh (2013)

wastewater treatment plants (Badia-Fabregat et al., 2015). They are potential candidates for bioremediation as they can survive in various habitats and secrete multiple enzymes. Fungi are capable of breaking polymeric compounds with their extracellular multienzyme complexes. With the aid of their hyphal system, they can penetrate the substrate and colonize. It can transfer and redistribute the nutrients to their mycelium (Tahri et al., 2013). In aerobic environments, fungi metabolic pathways implicated in hydrocarbon breakdown may begin with oxidation mediated by alkane-oxygenase enzymes and cytochrome P450 monooxygenases. *Aspergillus*, *Drechslera*, *Curvularia*, *Fusarium*, *Mucor*, *Lasiodiplodia*, *Rhizopus*, *Trichoderma*, and *Penicillium* are a few genera of fungi that have mostly been found to be capable of degrading aromatic hydrocarbons. A few examples of fungi able to degrade pollutants are listed in Table 6.

Table 5 Heavy metal degradation by bacteria

Microorganisms	Metals	References
Bacillus circulans MN1	Cr	Chaturvedi (2011)
Bacillus cereeus		Nayak et al. (2018)
Sporosarcina saromensis (M52)		Zhao et al. (2016)
Acinetobacter sp.		Bhattacharya et al. (2014)
Immobilized *B. subtilis* (B bead)		Benazir et al. (2010)
Pseudomonas aeruginosa (P)		
Streptomyces sp.	Pb	Kumar et al. (2011)
Staphylococcus sp.		
Bacillus firmus		Salehizadeh and Shojaosadati (2003)
Methylobacterium organophilum		Bharagava and Mishra (2018)
Cellulosimicrobium sp. (KX710177)		
Desulfovibrio desulfuricans	Cu, Ni	Kumar et al. (2011)
Bacillus firmus		Kim et al., (2015)
Flavobacterium sp.		Kumar et al. (2011)
Desulfovibrio desulfuricans (KCTC5768)		Congeevaram et al. (2007)
Vibriofluvialis	Co	Jafari et al. (2015)
Vibrio parahaemolyticus (PG02)	Hg	
Pseudomonas aeruginosa		
Klebsiellap neumoniae		
Enterobacter cloacae		Al-Garni et al. (2010)
Pseudomonas sp.	Zn	Kumaran et al. (2011)
Bacillus firmus		Salehizadeh and Shojaosadati (2003)

5.3 Bioremediation by Algae

Microalgae from the genera *Scenedesmus platydiscus*, *Chlorella vulgaris*, and *S. capricornutum* have been shown to be effective in the breakdown of polycyclic aromatic hydrocarbons like naphthalene, phenanthrene, and pyrene, along with the immobilization of metals. Algae are capable of utilizing mixed hydrocarbon substrate and crude oil. It can degrade aromatic hydrocarbons, iso-alkanes, and n-alkanes. Researchers also stated about diatoms, brown alga, red alga, green algae, and cyanobacteria that could oxidize naphthalene. A few more examples are listed in Table 7. The synthesis of exopolysaccharides, which can facilitate the absorption of contaminants on the cell surface and/or their complexation into less accessible forms, is crucial to microalgae's ability to remove hazardous chemicals, thus lowering their bioavailability and toxicity. Depending on the microalgal species, the contaminant bound to the membrane or cell wall (exopolysaccharides) can remain adherent or be absorbed and chelated by phytochelatin molecules.

Table 6 Degradation of different pollutants by fungi

Microorganisms	Compounds	References
Saccharomyces cerevisiae (Y)	Chromium	Benazir et al. (2010)
Aspergillus sp.		Congeevaram et al. (2007)
Candidapara psilosis	Mercury	
Aspergillus niger	Nickel	Taştan et al. (2010)
Aspergillus sp.		
Aspergillus versicolor		
Phoma eupyrena, *Myceliophthora thermophila*, *D. purpureofuscus*, *Doratomyces nanus*	Polychlorinated biphenyls	Mouhamadou et al. (2013)
T. viride, *A. foetidus*, *Aspergillus niger*	Decolorization of textile dyes	Jebapriya and Gnanadoss (2013)
Exophiala xenobiotica	Gasoline	Isola et al. (2013)
Rhizopus sp., *A. niger*, *Mucor* sp., *Penicillium* sp.	Crude oil	Damisa et al. (2013)
R. stolonifera and *Gongronella* sp.	Folpet and metalaxyl	Martins et al. (2013)
Aspergillus terreus	Chloropyriphos	Silambarasan and Abraham (2012, p. 1)
Pythyme, *Acrimonium*, *Curvularia*, *Aspergillus*	Heavy metals	Akhtar et al. (2013)
Pleurotus eryngii	Naphthalene	Hadibarata et al. (2013a)
Armillaria sp.	Anthracene	Hadibarata et al., (2013b, p. 022)

Table 7 Degradation of different pollutants by algae

Microorganisms	Pollutants	References
Portieria hornemannii	2,4,6-trinitrotoluene	Cruz-Uribe et al. (2007)
Acrosiphonia coalita		
Macrocystis integrifolia	Phenol	Navarro et al. (2008)
Lessonia nigrescens		
Ulva lactuca	Polybrominated diphenyl ethers	Qiu et al. (2017)
Ulva lactuca	Polycyclic aromatic hydrocarbons	NET et al. (2014)
Cladophora sp.	Malachite green	Khataee et al. (2011)
Cystophora sp., *Ulva* sp.	Dichlorodiphenyltrichloroethane	Sudharshan et al. (2013)
Ulva lactuca	Chloramphenicol	Leston et al. (2013)

6 Genetically Engineered Microorganisms (GEMS) in Bioremediation

Molecular biology is rendering tools to enhance the ability of the microorganisms employed in the remediation process. It is accelerating evolution of new activities and making new pathways by collecting the catabolic segments of various microorganisms (Ramos et al., 1994). Genetically modified microorganisms have been shown to have the ability to degrade pollutants in groundwater, soil, and activated sludge (Abatenh et al., 2017). Multiple genes subjected to the degradation of various environmental pollutants like chloro-benzene acids, toluene, toxic wastes, and other halogenated pesticides have been identified. For every single toxic compound, one separate plasmid is required. One plasmid can't degrade all groups of toxic compounds. The plasmids are divided into four groups: (a) hexane-, octane-, and decane-degrading plasmids are called OCT; (b) toluene- and xylene-degrading plasmids are called XYL; (c) camphor-decomposing plasmids are called CAM; and (d) naphthalene-degrading plasmids are called NAH (Ramos et al., 1994). One study has shown the potential to modify the gene of a bacterial strain to degrade different groups of hydrocarbons. They made a multiplasmid having pseudomonas strain in it (Markandey, 2004). It is capable of oxidizing polyaromatic, terpenic, aromatic, and aliphatic hydrocarbons. The utilization of genetically modified microbes in heavy metal removal has increased many interests. For instance, for simultaneous expression of the metallothionein and mercury transport system for the removal of Hg^{2+} from heavy metal wastewater, *Rhodopseudomonas palustris* was developed (Deng & Jia, 2011). Similarly for chromium removal, *Alcaligenes eutrophus* AE104 (pEBZ141) was developed (Srivastava et al., 2010). A few more examples of genetically modified organisms are listed in Table 8. Using genetically modified organisms is a very useful and effective approach for removal of toxins that indigenous bacteria cannot breakdown. GMOs play a crucial role in industrial-waste remediation, reducing the toxicity of some hazardous substances, and assisting in the removal of pollution caused by hydrocarbons. For the successful production of GMOs in a short amount of time, a variety of molecular methods such as horizontal DNA transfer in bacteria, molecular cloning, electroporation, protoplast transformation, homologous recombination transformation, conjugation, and

Table 8 Genetically modified organisms involved in bioremediation

Genetically engineered bacteria	Heavy metals	References
Ralstonia eutropha CH34	Cd^{2+}	Azad et al. (2014)
Achromobacter sp. AO22	Hg	
Sphingomonas desiccabilis and *Bacillus idriensis* strains	As	
Escherichia coli and *Moraxella* sp.	Cd and Hg	
P. fluorescens 4F39	Ni	
Mesorhizobium huakuii B3	Cd^{2+}	
Deinococcus radiodurans strains	Hg	
Pseudomonas fluorescens OS8	Cd, Hg, Zn, Pb	
Staphylococcus aureus RN4220 and *P. putida* 06909	Cd	
Pseudomonas K-62	Hg	
Acidithiobacillus ferrooxidans strain	Hg	
E. coli SE5000	Ni	
P. putida strain	Cr	

transformation of competent cells are available. The bioremediation methods incorporating GMOs have more benefits when it compared to the conventional means, because it can be easily applied to the contaminant place.

7 Conclusion

Microbial processes are necessary for global carbon cycle maintenance, and these processes are one of the key parts of bioremediation. It's an attractive and fruitful technique to eliminate pollutants from the environment as a continuous accumulation of these pollutants will destroy the environment and different habitats due to their toxicity. The degradation speed can be determined against the addition of biological agents like nutrients and appropriate conditions. It has been seen that this process is less effective in the natural environment, which gives minimum results. Therefore, this problem can be minimized if an appropriate atmosphere can be provided at the contaminated sites. Genetic engineering is also playing a great role in improving the ability of microbes employed in bioremediation. Microbial bioremediation has a promising future due to its eco-friendly nature and sustainability. It has more advantages than disadvantages as very less toxicity is generated in this procedure. The number of sites is increasing day by day that is utilizing this technique worldwide. Different species are still getting explored from multiple sites for the development of this process.

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Aerobic Processes: Best Approach for the Treatment of Wheat Starch Effluents

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1 Introduction

There are various types of polysaccharides present in wheat starch, which helps to produce a viscous jelly layer during the filtration of wheat starch wastes. Various tests were carried out by using different enzymes to reduce the effluent from wheat starch discharge by decreasing its viscosity by using Brew-n-Zyme Pentosanase. It becomes stable at 70 °C and does not exhibit any proteolytic activity. Wheat starch and its effluents are hydrolyzed by two models i.e., pressure-driven and mass transfer models. During hydrolysis, enzymes play essential roles in reducing the viscosity of the gel layer that helps remove the outer layer by shear stress. In fresh wheat starch discharge, the main reason for resistance to permeate flux is adsorption of big or complex molecules that occurs on the membrane surface, which further contributes to the total resistance of about 57% and 78% for wheat starch effluent and enzymatically hydrolyzed wheat starch discharge respectively. When the thickness of polysaccharides decreases, it results in the exposure of wheat starch effluent gel layer and leads to increased adsorption of macromolecules.

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_2

2 Industrial Production of Starch

Starch is produced commercially in different industries such as food industries and pharmaceutical industries that generate approximately 150 million tons of starch and may increase up to 165 million tons in 2026 (Radley, 1976). It has been reported that about 11 million tons of starch and its derivatives are produced in the EU, which requires about 25 million tons of raw materials. It has been reported that annually about nine million tons of starch and its derivatives are utilized and starch sweeteners are the main products (Onyenwoke and Simonyan, 2014). In the entire world, corn, wheat, cassava, and potato are the leading crops broadly used for the extraction of starches as per the availability and economics in a given region (Desta & Tigabu, 2015). It has been reported that in the world paper starch consumption comprises about 67% corn, 15% potato, 8% tapioca, and 3% waxy maize. There is no general toxicity during the process of starch formation; purification of soluble starch having some amount of protein shows zero effect or toxicity. Validation of this with real processing of starch enterprises of sewage treatment and wastewater analysis produced from starch processing is combined. The starch factory is a big enterprise of "starch corn." Glucose crystallization and large-scale production of its byproducts are the main processes from which huge waste is generated, which cannot be managed properly due to the lack of sewage treatment stations.

3 Treatment Processes

"Transformation is the basic principle for the treatment of wastewater to decrease the treatment and operational cost, encourages to use the resources of wastewater as required." It also helps to increase economic benefits. However, proper use of technology is required in the in situ transformation process. The new anaerobic system has a 3000 m^3/d ability to gradually process the starch effluents (Radley, 1976). Potato starch is mostly used in food, chemicals, pharma, and other industries. In the food industry, potato starch is used as a "thickener for sauces and stews, a binding agent in cake mixes, dough, biscuits and ice-cream." More than 40% of the potato starch generated in the European Union is used for nonfood purposes such as "paper and bio-plastics manufacture, pharmaceuticals and cosmetics, textile and adhesive manufacturing." It is broadly used in "pharmaceutical, textile, wood and paper industries as an adhesive, binder, texter agent and filler, and by the oil drilling firms to wash boreholes." It is known due to its neutral taste, good clarity, and high binding strength. Preferably potato starch is used in the food industry, due to its paste form having good clarity "(little quantity of lipids and protein) and neutral flavor." In the paper industry, potato starch is mostly used due to its high molecular weight amylose and its higher solubility. About 15% of the total world paper starch (five million tons in 3 years, 2005 estimate) comes from potato. "Potato starch is used to improve the quality of fabrics and textiles by providing abrasion-resistance and smoothness."

Moreover, potato starch is also used in pharmaceuticals in the preparation of cosmetics creams, pastes (stick, glue, or adhesives), and powders. Use of potato starch minimizes the use of chemical additives in beauty and pharmaceutical products. Potato starch dextrins are having benefits over other starches as an adhesive, having good remoistenability and a desirable rheology resulting in a perfect direct tack. Textiles are manufactured better with potato starch because of its film producing properties, paste penetration depth and adhesive power. A key reason for considering the supply of potato starch is its purity and of better quality than cereal starch, due to the presence of low fat and protein content and devoid of color, taste, or smell. Nonetheless, potato starch is used as a precursor material for the production of biodegradable sugar detergents. Starch acts as a better absorbent by absorbing the stains from fabrics in comparison to other cleaning substances. There are various steps on the basis of feedstock and the different technologies such as feedstock washing, steeping, refinement, scarification, etc. (Belfort, 1984). Water pollution occurs in large quantities due to industrial processes, and in this, both starch and gluten processes from wheat flour have a significant role (Belfort, 1984). The mixture of proteins, pentosans, hexosans, suspended starch particles, and monosaccharides has 0.35, 0.35, 0.35, 0.13, and 1.0 wt.% in wheat starch effluent (WSE), respectively (Matthiasson, 1983). Ultrafiltration of waste is one of the best methods to recover the protein-rich fraction in the market. Its economic cost–benefit depends upon wheat starch effluent (WSE) and high permeate flux rates having more than 16 wt.% solids (Harris, 1985).

3.1 Martin Process

In 1835, a process, namely Martin or dough ball, was proposed in Paris (Beretta et al., 2019). This process is very popular to date (Mittleider, 1978), and the raw material used was wheat flour, rather than wheat grain. This process comprises five steps:

1. Mixing of flour and preparing a dough
2. Washing out the starch
3. Drying the gluten
4. Starch refining
5. Drying the starch

The ratio of flour and water is 2:1, blended to form a uniform, lumps-free, and smooth dough. Its ratio depends upon the type of flour used; e.g., if hard wheat flour is used, then the dough will be strong and elastic that requires more water than soft wheat flour. The water that is used in it should have mineral salts because the water having fewer mineral salts than gluten becomes slimy (Knight, 1965). Before the washing step, the dough could be fully hydrated and then washed out to separate starch from gluten without gluten breaking into small pieces. Various instruments are used to operate this process such as ribbon blenders, twin-screw troughs, rotating drums, and agitated vessels (Knight, 1965). The ribbon blender is boat-shaped, deep and narrow, having twin open paddle rotors, and in rotor beds groove is present that controls the paddle action having different speeds and rotating in opposite directions. Paddles are covered with the dough by adding the dough in continuous manners. Both the fresh and treated water put into the vessel, water becomes suspended and starch gets overflow from it. Meanwhile, about 70% of water and 70–80% of proteins get discharged through the pipe with some amount of starch. About 8% of moisture can be dried with spray, flash, or drum dryers, and among these mainly flash dryers are utilized to dry it. There is a need for impulsive or controlled temperature to dry the gluten because it loses its vitality due to continuous exposure to extreme temperatures. The wet gluten is mixed with dried material following its exposure to a hot air stream. About 10% of solids are found in the starch slurry on the sieve when washed in a dough washer. After separating it, all the macro-particles (like bran) are removed through equipment like Dorr-Oliver D.S.M. screen. A wedge wire media is used, having 100-μm openings of vibratory sieves or centrifugal conical filters. The starch slurry refinement occurs through washing it in nozzle discharge centrifuges, and then the batch is dewatered in centrifuges having a basket-like shape and having about 40% moisture in them. Some pieces of equipment such as solid bowl, scroll-discharge, and decanter centrifuges are utilized for continuous dewatering of it. The supply of flesh dryer with starch is mixed with the other dry products to maintain 36% moisture. Starch is dried continuously in hot air to reduce its ability to gelatinize. The minimum moisture content, i.e., 31%, is required for wheat starch gelatinization, and at last it contains about 10–12% moisture and 0.3% protein content. Moreover, starch is produced when micro-granules of starch are dried by prime starch refining stage to separate other particulates. Nozzle-type centrifuge plays an important role to minimize the insoluble material in the wastewater stream due to the huge requirement of water to wash the starch (15 parts by wt. of water/part of flour). The stream has about 10–13% and 0.85–1.2% dry flour substance and solids, respectively. The solid effluents are either discarded through waste or are reutilized.

3.2 Biodegradation of Starch Stillage

There are various methods that are capable of using the completely starch stillage volume. Mostly the stillage effluent contains organic matter content. It cannot be discharged into the environment, i.e., water bodies and soil. Nagano et al. (1992) reported that in sweet potato stillage load is about COD 12.1 g O_2/l in which the feedstocks contain about 80% and 20% starch and potato starch, respectively (Cibis, 2004). It completely shows different chemical properties. A feedstock is not only the reason which affects the COD content, it is also affected by the technology of spirit production, its methods, and storage of stillage. Sour fermentation was shown by the distiller's stillage because of the production of organic acid (lactic acid) (Wilkie et al., 2000). All these processes concluded that stillage is a biodegradable material (Fargey & Smith, 1965; Smith & Fargey, 1965). Various researchers have reported starch stillage gets decomposed by anaerobic processes (Weiland & Thomsen, 1990; Nagano et al., 1992; Goodwin & Stuart, 1994; Laubscher et al., 2001; Gao et al., 2007; Tang et al., 2007). Hutnan et al. (2003) reported that the initial COD range in the anaerobic treatment of wheat stillage was 91–107 g O_2/l. On the other hand, the COD of malt whisky distillery wastewater was in the range of 30.5 and 47.9 g O_2/l, which helps reduce about 90% of pollution (Goodwin & Stuart, 1994). The total COD in the grain distillation ranges from 20 to 30 g O_2/l with UASB (up-flow anaerobic sludge bed) system having the efficiency in removing about 80% of COD reported by various researchers (Laubscher et al., 2001). Shin et al. (1992) reported that when both barley and sweet potato stillage of primary organic matter of about 29.5 g O_2/l are processed continuously, then about 80% of COD gets reduced. The different studies reported that starting pollution load is about 40 g O_2/l of wheat and sweet potato stillage, which help attain about a 98% reduction of COD (Nagano et al., 1992). Weiland and Thomsen (1990) reported that the organic content of potato stillage was between 20 and 55 g O_2/l, and it shows about an 80–95% reduction of COD.

Biological techniques have been used for the treatment of wastewater reclamation for over a century. Out of the many different processes employed, the activated sludge system has proven to be the most popular. The implementation of membranes within the treatment sequence of a water pollution control facility was initially limited to tertiary treatment and polishing. Ultra-filtration, micro-filtration, or reverse osmosis units were utilized in areas where discharge requirement was very stringent or direct reuse of the effluent was desired. High capital and operational costs as well as inadequate knowledge to use membrane for the treatment of waste water. However, with the emergence of less expensive and more effective membrane modules and the implementation of the ever-tightening water discharge standard, membrane systems regained interest.

In distillery processes, stillage is a major byproduct; the volume of distillery wastewater is more than that of ethanol produced and it has also become a serious problem throughout the world. Different methods were used to solve this problem. About 90% of ethanol is produced mostly from rye, triticale, wheat, mainly potatoes in root crops, and different agricultural feedstock (Dzwonkowski et al., 2010) and grains; potatoes and starch are also utilized for the production of spirit in the entire world, particularly in European countries. Moreover, both the starch stillage utilization mode and comparison methods (aerobic as well as anaerobic) help in the biodegradation of stillage. About 5% of ethanol is produced through a synthetic approach, and about 95% or more is produced from feedstock produced in agricultural areas in which about 42% sugar and 58% non-sugar-based feedstock, respectively, produce ethanol. From 2000 to 2007, production of potato-based feedstock dropped from 8% to 3.1% (Dzwonkowski et al., 2010). Generally, the most dominant feedstock is starch-based, in which rye acts as raw material for the production of ethanol (90%). Starch-containing wastes such as frozen potatoes, peelings, potato slops, waste flour, etc. are used in the production of spirit, i.e., up to 50–60% (Dzwonkowski et al., 2010).

3.3 Methods of Stillage Utilization

Distillery stillage produced from the fermentation of starch-based feedstock by using the yeast contains feedstock components along with degradation of yeast cells (Sweeten et al., 1981–1982; Davis et al., 2005; Sanchez et al., 1985). Various substances contain higher quantities of nutrients, i.e., having higher nutritional values, including vitamins (with huge amounts of those classified as group B), proteins rich in exogenous amino acids (Mustafa et al., 1999), and mineral components. When we compare barley and wheat stillage, it can be observed that barley contains more calcium, iron and sodium than wheat-based stillage (Mustafa et al., 1999). Considering the chemical composition of the dry matter, total protein content of potato stillage has been given more importance as a valuable fodder (despite high water content, 90–95.3%) (Maiorella et al., 1983; Larson et al., 1993; Ham et al., 1994; Fisher et al., 1999; Mustafa et al., 2000). Nonetheless, it should be considered that feeding value of the potato stillage (on the basis of the estimation of protein and vitamin content) is less than that of the grain stillage (Maiorella et al., 1983). The stillage obtained from wheat, rye, triticale, and barley contains a lower nutritive value; however, barley has the lowest nutritive value among all the three in both liquid and solid fractions (Mustafa et al., 2000).

Raw warm stillage has higher feeding value but also has serious disadvantages; for example, it cannot be stored for a prolonged period due to the presence of proneness to souring and occurrence of mold growth. This indicates that the animals might be fed within the short time when stillage has been produced and makes this method of stillage really difficult. Feeding farm animals with raw stillage is cost-effective only if the users live in close proximity to the distillery. Due to the higher water quantity, the transport of the stillage for longer distances is beneficial (The Mother Earth News, 1980; Ganesh & Mowat, 1985; Aines et al., 1986). The best resolution to this issue is to integrate a small rural distillery with a large animal farm that should be capable of consuming the whole quantity of the manufactured stillage (Carioca et al., 1981; Ganesh & Mowat, 1985). However, in Poland an opposite pattern has been detected. These rural distilleries have become self-dependent economic units interested in their own progression. on the other hand, higher animal farms are deficient. Under standard conditions the issues of using the stillage volume produced completely as animal fodder remains unsolved. Meanwhile, raw stillage cannot be stored for a prolonged time; it must be processed as otherwise its nutritive value is lost. One of the approaches to extending the storage life of starch-based stillage is souring by the addition of corn or hay. The fodder derived from this method can be stored for numerous months; however, its nutritive value declines with time (McCullough et al., 1963; Hunt et al., 1983; Muntifering et al., 1983). One more disadvantage inherent in stillage is the presence of a higher quantity of crude fibers, which restricts its utility as fodder, particularly for nonruminant farm animals (Kienholz et al., 1979a, b). Current research reports suggested that lactic bacterial inoculants increase the preservation of feed (Garcia & Kalscheur, 2004). Starch stillage is fermented using yeast and this composite biomass is used as fodder (Murray & Marchant, 1986; Jamuna & Ramakrishna, 1989). The examination reported in literature involved mainly vinasse (Tauk, 1982; Malnou et al., 1987; Moriya et al., 1990; Cibis et al., 1992), although yeast was cultivated at the industrial level. Inappropriately, the COD level in the effluent from cultivation was so high that the decrease in this pollutant approached 70% at the most.

4 Biodegradation of Starch Stillage

The methods regarded in the previous section make them unable to utilize the entire starch stillage quantity generated. This results in distilleries facing a difficult issue. The stillage is a high-power effluent which, due to the significant organic matter quantity, can neither be sent to the sewer system nor be cleared into a watercourse or soil, at least some part of the COD load must be eliminated at the foundation of origin. The COD load of the liquid phase differs between 12.1 and 122.33 g O_2/l for the sweet potato stillage and stillage from a waste feedstock containing wheat starch (80%) and potato starch (20%) (Nagano et al., 1992; Cibis, 2004). The chemical composition of stillage obtained from feedstock differs considerably in chemical properties.

Distiller's stillage displays a proneness to sour fermentation. This might be because of the production (within a comparatively short period) of organic acids, particularly lactic acid, which frequently controls in that type of stillage. Considering the other sources of carbon, reducing substances and glycerol are present in higher quantities. The occurrence of total and phosphate phosphorus and total nitrogen is present in large quantities, which might be discussed as being related with the greater quantity of proteins in the feedstock from which stillage is obtained (Wilkie et al., 2000). This kind of chemical composition indicates that starch stillage is biodegradable up to some extent. Aerobic and anaerobic fermentation of thin stillage was primarily reported four decades before (Fargey & Smith, 1965; Smith & Fargey, 1965).

This problem has not got too much attention until recent past. However, during the last 15 years, various research reports have been published reporting that starch stillage is biodegradable in nature by using the anaerobic processes (Weiland & Thomsen, 1990; Nagano et al., 1992; Goodwin & Stuart, 1994; Laubscher et al., 2001; Gao et al., 2007; Tang et al., 2007). Laboratory examination during the treatment of stillage under anaerobic treatment, in which initial COD level ranges between 91 and 107 g O_2/l (Hutnan et al., 2003), and of malt whisky distillery wastewater with an initial COD between 30.5 and 47.9 g O_2/l (Goodwin & Stuart, 1994) has revealed an about 90% decrease of this pollutant. The processing of grain distillation wastewater in which initial COD level varies from 20 to 30 g O_2/l with the aid of an up-flow anaerobic sludge bed (UASB) system has produced about 80% of COD elimination potency (Laubscher et al., 2001).

During the continuous processing of barley stillage and sweet potato stillage containing initial organic matter content of 29.5 g O_2/l, a decrease in COD was found to be about 80% (Shin et al., 1992). It has been observed that about 98% decrease in COD was obtained with wheat stillage and sweet potato stillage having initial pollution load of about 40 g O_2/l (Nagano et al., 1992). When potato stillage was treated having an initial quantity of organics ranges from 20 to 55 g O_2/l, the content of COD decrease ranges from 80% to 95% (Weiland & Thomsen, 1990). With the treatment of potato and sugar beet stillage having an initial organic pollution load of about 40 g O_2/l, the decrease in COD was about 90% (Wilkie et al., 2000).

In comparison to anaerobic processes, aerobic biodegradation of starch stillage with thermo- and mesophilic bacteria has been carried out at the industrial level. The findings derived at the laboratory-scale aerobic thermophilic and mesophilic biodegradation of distillery wastewater revealed that the impact of this process was the same as that for other microorganisms. In contrast to anaerobic methods, aerobic biodegradation of starch stillage with thermo- and mesophilic bacteria has not yet been conducted on an industrial scale. The results obtained reveal that laboratory-scale aerobic thermophilic degradation (Anastassiadis & Rehm, 2006; Battestin & Macedo, 2007; Choorit & Wisarnwan, 2007) is affected by the aerobic conditions, pH, and temperature (Cibis et al., 2002; Krzywonos et al., 2002, 2008).

5 Conclusion

Wheat starch effluents contain higher concentrations of toxic chemicals. There are various methods, such as biochemical, flocculation, and sedimentation methods, available for the treatment of wheat starch effluents. However, aerobic processes are required to minimize the content in the wastes to recover the useful substances from starch effluents and reuse them for other purposes. The available and commonly used methods are not fully efficient in removing the toxic substances and retaining the useable ones; therefore, aerobic processes are very essential to retain all the useful substances as sources for future utilization.

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Banned Pesticides with High Persistence: The Impact of Their Use in Agriculture and Their Removal by Microbial Biodegradation

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1 Introduction

The world population growth leads to an increase in demand for food, and by default, it will require greater inputs: land, water, and/or energy (Mohammad Fakhrul Islam & Karim, 2020). The United Nations Food and Agricultural Organization (FAO) specifies that in 2050 the world's food needs will be about 70% higher compared to 2009 (Tilman et al., 2011; FAO, 2021a). In order to make agriculture more productive and profitable in accordance with the costs and standards for human health and the environment, the appropriate combination of available technologies is mandatory (Popp et al., 2013). Pesticides used in agriculture help the farmers increase the crop productivity, usually by 20–50%. In addition, due to the use of pesticides, farmers obtain high-quality seeds, reduce fertilizer consumption, and conserve water resources. Therefore, pesticides have become indispensable for the sustainable production of food and fiber of high quality (Popp et al., 2013; Stoleru et al., 2015).

The Environmental Protection Agency of the United States (EPA) defined a pesticide as "*any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest*." Depending on the target pest species that cause harm during the production and storage of crops, pesticides are divided into

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_3

21 groups: acaricide, algicide, attractant, bactericide, desiccant, elicitor, fungicide, herbicide, insecticide, molluscicide, nematicide, plant activator, plant growth regulator, pruning, repellant, rodenticide, safener, soil treatment, synergist, virus inoculation, and other treatment (European Commission, 2021). Also, the pesticides could be classified according to the sources of origin (e.g., chemical pesticides or biopesticides) (Kaur et al., 2019; Hassaan & El Nemr, 2020) or by hazard (Ia, – extremely hazardous; Ib, highly hazardous; II, moderately hazardous; III, slightly hazardous; or U, unlikely to present acute hazards), as proposed by the World Health Organization (WHO and FAO, 2021). Based on chemical structures, the pesticides are grouped into ionic (inorganic) and non-ionic (organic) pesticides (Kaur et al., 2019). A detailed classification of pesticides according to their origin as well as their chemical structure, with examples for each class/subclass, is presented in Fig. 1.

The database managed by the European Commission considers 1462 active substances safe and synergists that can be used as pesticides. Of the total pesticides included in the database, 454 pesticides are approved for use, 927 are not approved, 64 are under evaluation, and 17 pesticides have other statuses. More than half of not-approved substances belong to herbicide (209 active substances), insecticide (194 active substances), and fungicide (164 active substances) classes (European Commission, 2021). According to the World Health Organization, Rotterdam Convention, Stockholm Convention on Persistent Organic Pollutants, and other organizations, the most harmful pesticides for both human health and the environment belong to the inorganic class and organochlorine, organophosphorus, carbamate, organotin, and thiazine chemical subclasses. The World Health Organization and Food and Agriculture Organization of the United Nations classify 13 organophosphorus, 1 organochlorine, 3 coumarin, and 2 carbamate pesticides as extremely hazardous (Class Ia). In class Ib (highly hazardous) are included 24 organophosphorus, 1 organochlorine, 9 coumarin, 9 carbamates, 4 pyrethroid, and 27 organophosphorus; 10 organochlorines, 14 carbamates, 4 organotin, 3 triazine, and 2 pyrazole pesticides are part of class II (moderately hazardous). Many of these pesticides have been banned for use either globally or regionally and even locally.

According to the Stockholm Convention on Persistent Organic Pollutants, the parties and signatories of this convention must take measures to eliminate the production and use of 16 pesticides (aldrin, endrin, hexachlorobenzene (HCB), hexabromobiphenyl, beta hexachlorocyclohexane, pentachlorobenzene, chlordane, dicofol, heptachlor, lindane, chlordecone, dieldrin, alpha hexachlorocyclohexane, mirex, pentachlorophenol and its salts and esters, technical endosulfan and its related isomers, toxaphene) and 10 industrial chemicals, and for DDT and perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride used as pesticides must be taken measures to restrict their production and use (http://chm.pops.int/). By Rotterdam Convention, other 25 pesticides have been banned or severely restricted due to the negative effects on human health or on the environment (e.g., alachlor, carbofuran, parathion, tributyltin compounds, methyl-parathion, methamidophos, monocrotophos, etc.).

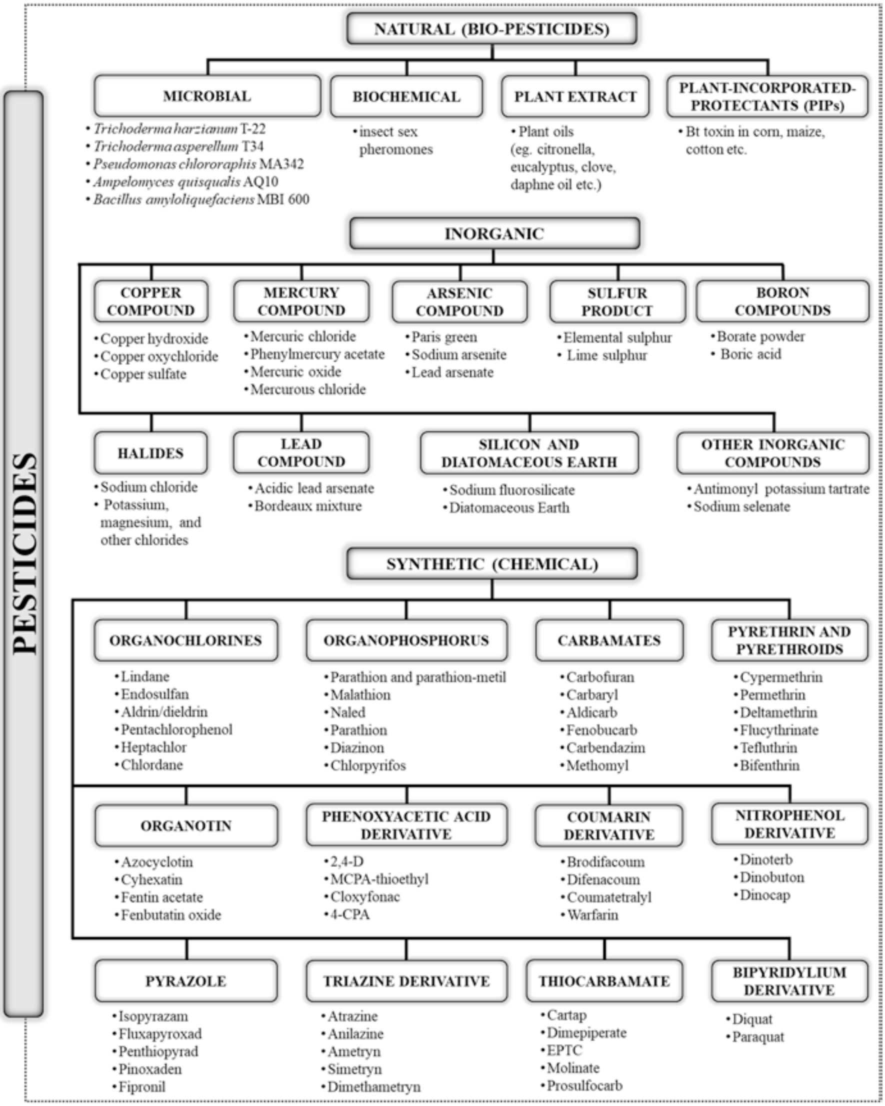


Fig. 1 Pesticide classification based on origin

At the European level, in addition to pesticides banned by the Stockholm Convention and Rotterdam Convention, the active substances such as fipronil, paraquat, atrazine, carbendazim, 1,3-dichloropropene, cyanamide, ethoxysulfuron, triasulfuron, oxadiargyl, acetochlor, propargite, and others were also banned due to their toxicity on human health and the environment (PAN Germany, 2011). For example, the fipronil used as an insecticide is suspected to be a carcinogen agent and is highly toxic for bees, paraquat is very toxic for humans if inhaled, and atrazine interferes with reproduction and development and may cause cancer (Bethsass & Colangelo, 2006). Carbofuran insecticide is highly toxic for bees, highly hazardous, very toxic for humans if inhaled, and a potential endocrine disruptor (PAN Germany, 2011). Also, these four pesticides have been shown to have high persistence in the environment. For example, carbofuran has a half-life of 450–1200 days in water (Hanson et al., 2020), antrazine 168 days in water and 60–75 days in soil (FAO, 1997), and paraquat 1000 days in soil (Vogue et al., 1994) and between 2 and 820 years in water (Thi Hue et al., 2018).

Although many pesticides are harmful to the environment and their production and use in agriculture have been banned in many parts of the world, they are still produced and used either for other purposes or even in agriculture by the third-world countries. According to the FAOSTAT database (2021), lindane, dieldrin, aldrin, endosulfan, DDT (dichloro-diphenyl-trichloroethane), pentachlorophenol, chlordane, and heptachlor whose production and use are no longer allowed but are still on the market. In 2019, 16.331 tons of lindane were exported and 74.14 tons of lindane were imported by FAO Members. During 2017–2019, the FAO members imported 47,005 tons of DDT and exported 18,782 tons. Currently, DDT is still used in India and other tropical countries to control malaria and leishmaniasis diseases (van den Berg et al., 2017).

In this framework, this chapter is a literature review that highlights (i) the role in agriculture and the actually annual production of 15 banned pesticides with high persistence (lindane, dieldrin, aldrin, endosulfan, parathion and parathion-metil, tributyltin oxide, DDT, pentachlorophenol, chlordane, heptachlor, paraquat, carbofuran, fenbutatin oxide, fipronil, and atrazine), (ii) its spreading in soil, water, air, and products, (iii) its toxicity on animals and humans and its fate in the environment compartments, and (iv) the ability of microorganisms to remove these compounds from the environment. Through the information provided in fulfilling the proposed objectives, decision-making will be facilitated in order to reduce the health and environmental risks induced by these pesticides.

2 Pesticide Production and Use in Agriculture

Annually, worldwide pests destroy up to 40% of global crops and are recorded around 220 billion of dollars of losses. Without the use of pesticides, the losses would be higher, maybe even double (FAO, 2021b). To combat these losses have been used in agriculture a wide variety of pesticides, including the banned pesticides with high persistence (e.g., lindane, dieldrin, aldrin, endosulfan, parathion and parathion-metil, tributyltin oxide, DDT (dichloro-diphenyl-trichloroethane), pentachlorophenol, chlordane, heptachlor, paraquat, carbofuran, fenbutatin oxide, fipronil, and atrazine). The main benefits of pesticides used in agriculture and other fields of activity consist in the following (Aktar et al., 2009):

- Improving land productivity
- Preventing crop losses/yield reduction

- Vector disease control including those not related to agricultural crops
- Improving the quality of food
- Other benefits

According to the FAOSTAT database (2021), in 2019 the FAO members applied in agriculture 5,964,661 tons of pesticides, of which 999,641 tons were insecticides, 3,159,511 tons were herbicides, 1,379,713 tons were fungicides and bactericides, 2129 tons were fungicides for seed treatment, and the rest are other categories of pesticides. Of the total insecticides used in agriculture in 2019, 0.014% were from chlorinated hydrocarbons class, 1.17% were organophosphates substances, 0.145% carbamates, 0.801% pyrethroids, and 97.87% other types. Also, in 2019, in agriculture were used 15,731 tons of triazines herbicides, 2851 tons of carbamates herbicides, and 33,606 tons of dithiocarbamates fungicide and bactericide. As can be seen in Table 1, in 2019, at the European level, insecticides based on chlorinated hydrocarbons, organophosphorus and carbamate insecticides, triazine herbicides, and organotin and pyrazole (phenyl-) insecticides were still used for the protection of crops against pests (EUROSTAT, 2021). These pesticides have been applied mainly for the protection of common winter wheat and spelt, grain maize and corn-cob-mix, sunflower seeds, rape and turnip rape seeds, potato, grapes for wine, orange, apple, tomato, and other crops in Hungary, Poland, Greece, Spain, Czech Republic, Croatia, Cyprus, and Malta (EUROSTAT, 2021).

Organochlorine pesticides are chlorinated compounds widely used in various fields of activity for pest control. Organochlorine pesticides have high persistence in the environment, most of them being included by Stockholm Convention in the persistent organic pollutant (POP) class (Parada et al., 2016). In agriculture were used to fight against insects, such as worms, beetles, termites, Colorado beetles, whiteflies, aphids, etc. (http://chm.pops.int/). Organochlorine insecticides such as DDT have been used successfully for the control of malaria, typhus, and other diseases after the World War II (Parada et al., 2016). Their persistence in the environment components differs from compound to compound, some of them having a very high persistence. For example, the half-lives of lindane, dieldrin, and DDT in soil are over 1000 days (Vogue et al., 1994; Stoleru et al., 2016).

Organophosphates are the compounds resulted from the esterification process between phosphoric acid and alcohol. In agriculture, these chemicals are mainly used as herbicides and insecticides. The most commonly used organophosphate pesticides are chlorpyrifos, malathion, parathion and methyl parathion, azamethiphos, and azinphos-methyl (Adeyinka et al., 2021). Many organophosphorus pesticides have been banned for use in agriculture in many parts of the world (e.g., chlorpyrifos, chlorpyrifos-methyl, parathion and parathion-metil, and others). For example, parathion and parathion-metil were banned in 2003 in EU (Kalipci et al., 2010) and 2012 in the USA (Pope, 2014). These two pesticides are highly toxic to birds, honey bees, and aquatic invertebrates. According to Neslen (2018), as a result of exposure to organophosphates, the memory of children is affected, attention deficit occurs, and the risk of reduced IQs increases.

Table 1 Pesticide use in agriculture in 2019 at the European level on crops (EUROSTAT, 2021)

Category of pesticides	The quantities of pesticides used for crop protection
Insecticides based on chlorinated hydrocarbons	Common winter wheat and spelt – 617 kg by Hungary Grain maize and corn-cob-mix – 328 kg by Hungary Rape and turnip rape seeds – 13 kg by Hungary Sunflower seeds – 1854 kg by Hungary Cabbages – 40 kg by Poland
Organophosphorus insecticides	Common wheat and spelt – 3437 kg by Greece and 19354.5 kg by Spain Common winter wheat and spelt – 23,522 kg by Czech Republic, 8636 by Hungary, and 333 kg by Estonia Grain maize and corn-cob-mix – 697 kg by Czech Republic, 8311 by Greece, 5397 kg by Croatia, and 28,940 kg by Hungary Rape and turnip rape seeds – 84,490 kg by Hungary Winter rape and turnip rape seeds – 94,095 kg by Czech Republic Potatoes – 555 kg by Czech Republic, 43,429 kg by Greece, 1572 kg by Spain, 10,914 kg by Italy, and 390 kg by Cyprus Sunflower seeds – 21252.8 kg by Spain, 1349 kg by Croatia, and 16,931 kg by Hungary Cabbages – 450 kg by Croatia and 220 kg by Poland Tomatoes – 336.8 kg by Spain, 1474 kg by Italy, 14 kg by Cyprus, and 15 kg by Malta Carrots – 31 kg by Cyprus Olive – 88,724 kg by Greece and 7454 kg by Croatia Grapes for table use – 681.2 kg by Spain Grapes for wine – 44184.9 kg by Spain Orange – 19,172 kg by Greece and 18756.9 by Spain Apples – 706 kg by Czech Republic, 10,640 kg by Greece, 5740.1 kg by Spain, and 1750 kg by Hungary onions Onions – 3005 kg by Spain and 67 kg by Cyprus Sugar beet – 4991 kg by Czech Republic and 1349 by Croatia Spring barley – 1599 kg by Czech Republic Winter barley – 3592 kg by Czech Republic Barley – 6132.6 kg by Spain Durum wheat – 10,984 kg by Greece and 2964 kg by Spain
Carbamate insecticides	Common winter wheat and spelt – 66 kg by Czech Republic and 422 kg by Hungary Common spring wheat and spelt – 21 kg by Poland Spring barley – 10 kg by Czech Republic Grain maize and corn-cob-mix – 395 kg by Hungary Potatoes – 18 kg by Italy Sugar beet – 34 kg by Czech Republic Rape and turnip rape seeds – 72 kg by Hungary Sunflower seeds – 2 kg by Croatia and 1356 kg by Hungary Cabbages – 46 kg by Poland Tomatoes – 89.1 kg by Spain, 264 kg by Italy, and 27 kg by Cyprus Onions – 76.1 kg by Spain Apples – 510 kg by Czech Republic, 524 kg by Greece, 275.8 kg by Spain, and 701 kg by Hungary Olive – 37 kg by Greece

(continued)

Table 1 (continued)

Category of pesticides	The quantities of pesticides used for crop protection
Triazine herbicides	Common winter wheat and spelt – 250 kg by Hungary Barley – 408.8 kg by Spain Grain maize and corn-cob-mix – 14,851 kg by Czech Republic, 14,987 kg by Greece, 33,618 kg by Croatia, and 184,032 kg by Malta potato Potato – 3 kg by Italy rape Rape and turnip rape seeds – 951 kg by Hungary Sunflower seeds – 6974.7 kg by Spain and 49,755 kg by Hungary Green maize – 56,099 kg by Czech Republic Tomato – 1037.9 kg by Spain Apple – 58 kg by Hungary
Organotin insecticides	Tomato – 1 kg by Italy Grapes for wine – 244.3 kg by Spain
Pyrazole(phenyl-) insecticides	Common wheat and spelt – 448.7 kg by Spain Common winter wheat and spelt – 3 kg by Hungary Grain maize and corn-cob-mix – 37 kg by Czech Republic and 1423 kg by Hungary Potato – 27 kg by Czech Republic, 219 kg by Greece, 14.2 kg by Spain, 8 kg by Croatia, 248 kg by Italy, and 273 kg by Cyprus Sugar beet – 5 kg by Croatia Sunflower seeds – 102 kg by Spain Green maize – 192 kg by Czech Republic Cabbage – 1 kg by Croatia, 6 kg by Cyprus, and 74 kg by Poland Tomato – 2070.3 kg by Spain, 2527 kg by Italy, and 44 kg by Cyprus Apple – 91 kg by Czech Republic, 357 kg by Greece, and 403 kg by Hungary Grapes for table use – 100.2 kg

Carbamate pesticides are derived from carbamic acid and can be grouped into herbicides, insecticides, fungicides, nematicides, and inhibitors. In carbamate insecticides, the functional group is carbamate ester, which acts as a reversible inhibitor of the enzyme acetylcholinesterase (AChE) of the nervous system. Among the most used carbamate pesticides are fenobucarb, carbofuran, carbendazim, aldicarb, oxamyl, propoxur, and carbaryl. Due to the potentially dangerous effects on both wildlife and human health, the use of some carbamates in agriculture was banned, one of these being carbofuran (Silberman & Taylor, 2021; Malhotra et al., 2021). Carbofuran has a broad spectrum of activity being banned in the world due to numerous cases of bird poisoning (Sim et al., 2019).

Organotin pesticides are organometallic compounds with tin in their structure. This class of pesticides includes biocides, fungicides, miticides, molluscicides, nematicides, and ovicides. Even in low levels, the organotin compounds are toxic for marine invertebrates but also for mammals and humans, being immunotoxic, teratogenic, neurotoxic, and carcinogenic agents in mammals (Okoro et al., 2014). Among the most toxic pesticides in this class are tributyltin oxide and fenbutatin oxide, which have been banned from being used in the EU since 2002 and 2014, respectively (EC, 2021).

A central pyrazole ring characterizes the chemical structures of *phenylpyrazole insecticides*, the phenyl group being attached to one of the nitrogen atoms of the pyrazole. Fipronil belongs to the phenylpyrazole family and acts on insects by blocking GABA receptors at the entrance channel of the chlorine neurons. The mammals are less susceptible to its effects due to the lack of this type of chloride channel (Vidau et al., 2009).

Triazines are aromatic compounds with three carbons and three nitrogens in the heterocycles. Triazine herbicides act against weeds and grasses by binding to the D-1 protein in photosynthetic electron transport in the chloroplast and thus destroying the photosynthetic tissues (Battaglino et al., 2021). According to Klementova and Keltnerova (2015), the herbicides belonging to this class are classified as persistent organic compounds since they have high resistance to biological and chemical degradation. Among the most used active substances in agriculture are found atrazine, cyanazine, propazine, and simazine.

Bipyridyl or bipyridinium pesticides are quaternary ammonium compounds commonly used in agriculture as contact herbicides and desiccants. The bipyridyl herbicides control the grasses and broad-leafed weeds by interfering with the electron transfer system and inhibiting the reduction of nicotinamide adenine dinucleotide phosphate to reduced nicotinamide adenine dinucleotide phosphate (NADPH) during photosynthesis. Examples of some bipyridyl herbicides commonly used include paraquat and diquat, but also cyperquat, diethamquat, difenzoquat, and morfamquat, which are no longer marketed or whose uses have been considerably reduced (Roede & Miller, 2014; Eddleston, 2016).

The lindane, dieldrin, aldrin, endosulfan, DDT, pentachlorophenol, chlordane, and heptachlor belong to the organochlorine class, parathion and methyl parathion are organophosphate insecticides, tributyltin oxide and fenbutatin oxide are organotin compounds, paraquat is a bipyridyl herbicide, carbofuran is a carbamate insecticide, fipronil is a phenylpyrazole insecticide, and atrazine belongs to the triazine class. For these active substances, in Table 2 are included their half-life in environmental components, the main uses in agriculture, and when their uses were banned in the same parts of the world.

Although the Stockholm Convention on Persistent Organic Pollutants requires actions to eliminate or restrict the production and use of lindane, dieldrin, aldrin, endosulfan, DDT, pentachlorophenol, chlordane, and heptachlor, according to FAOSTAT database these pesticides are still on the market. Following the analysis of the FAOSTAT database, it was identified that the member countries started again to import or export DDT and endosulfan in 2017. Between 2017 and 2019 was imported 47,005 tons and exported 18,782 tons of DDT. In 2019, the leading import countries of DDT were Thailand (3680 tons), the Philippines (1576 tons), the United Kingdom of Great Britain and Northern Ireland (990 tons), and Indonesia (581 tons) and the leading export countries were South Africa (1157 tons), the Netherlands (819 tons), the Philippines (704 tons), Indonesia (471 tons), and Spain (335 tons). Almost all FAO members have imported DDT in 2019, with the exception of

Table 2 Banned pesticides with high persistence

Name of pesticide	Type of pesticide	Half-life	Uses	Ban of use in agriculture
Lindane	Organochlorine insecticide	88–1146 days in soil WHO (2004a)	As a seed treatment for barley, corn, oats, rye, sorghum, and wheat (http://chm.pops.int/)	Since 2000 in the EU EC (2021) and 2002 in the USA ATSDR (2021)
Dieldrin	Organochlorine insecticide	1000 days in soil Vogue et al. (1994)	As a pesticide for corn, cotton, and citrus crops (http://chm.pops.int/)	Since 1991 in the EU EC (2021) and 1987 in the USA for all uses ATSDR (2021)
Aldrin	Organochlorine insecticide	365 days in soil Vogue et al. (1994)	To control the worms, beetles, and termites in soils and to protect the corn and potato crops against pests Berntssen et al. (2012)	Since 1991 in the EU EC (2021) and 1987 in the USA for all uses ATSDR (2021)
Endosulfan	Organochlorine insecticide	50 days in soil Vogue et al. (1994); 6 years in groundwater and sediments Rosales Landeros et al. (2019)	To control a wide variety of insects such as Colorado potato beetles, whiteflies, aphids, leafhoppers, and cabbage worms Berntssen et al. (2017). Usually used to protect the coffee, cotton, rice, sorghum, and soy crops (http://chm.pops.int/)	Since 2005 in the EU EC (2021) and 2010 in the USA ATSDR (2021)
Parathion and parathion-metil	Organophosphate insecticide	14 days in soil Vogue et al. (1994); 95–237 days in water at 6 °C and 18–46 days at 22 °C WHO (2004b)	To control the insect pests in cotton, pome and stone fruit, vegetable crops, tomatoes, beans, potatoes, tobacco, and clover seed crops. The target insects are light brown apple moth, codling moth, long-tailed mealybug, and oriental fruit moth NRA (1999)	Since 2003 in the EU Kalipci et al. (2010) and 2012 in the USA Pope (2014)

(continued)

Table 2 (continued)

Name of pesticide	Type of pesticide	Half-life	Uses	Ban of use in agriculture
Tributyltin oxide	Organotin biocide	15–20 weeks in soil; 6 days and 35 weeks in water and water-sediment mixtures PubChem (2021)	As fungicides for the protection of potato, celery, sugar beet, coffee, and rice crops Fent (1998)	Since 2002 in the EU EC (2021)
DDT (dichloro-diphenyl-trichloroethane)	Organochlorine insecticide	2000 days in soil Vogue et al. (1994) >20 years in soil Stoleru et al. (2016)	As insect pests control in agricultural crops, gardens, orchards, and forests and bloodsucking insects and disease vectors Li et al. (2006)	Since 1972 in the USA ATSDR (2021) and 1986 in the EU EC (2021)
Pentachlorophenol	Organochlorine fungicide and bactericide	<4 weeks in water; <20 weeks in sediments and <10 weeks in soil UNEP (2013)	As a pre-harvest defoliant in cotton and as a general pre-emergence, non-selective contact herbicide IARC (1991)	Since 2002 in the EU EC (2021) and since 1984, the purchase and use have been restricted and now it is no longer available to the general public in the USA ATSDR (2021)
Chlordane	Organochlorine insecticide	4 years in soil WHO (2004c)	As a fumigating agent and for controlling a wide variety of insects in agricultural crops, lawns, and gardens Bidleman et al. (1998)	Since 1991 in the EU EC (2021) and 1988 in the USA for all uses ATSDR (2021)
Heptachlor	Organochlorine insecticide	6–9 months in soil; 6.3 hours in air; 1–3.5 days in water Reed and Koshlukova (2014)	For termites, ants, and soil insects control in seed grains and various crops WHO (2006)	Since 1984 in the EU EC (2021) and 1988 in the USA ATSDR (2021)

(continued)

Table 2 (continued)

Name of pesticide	Type of pesticide	Half-life	Uses	Ban of use in agriculture
Paraquat	Bipyridyl herbicide	1000 days in soil Vogue et al. (1994); 2–820 years in water Thi Hue et al. (2018)	As a defoliant and desiccant on cotton, tomatoes, beans, soybeans, potatoes, sunflowers, and sugarcane to aid in harvesting and in controlling broad-leaved weeds and grasses in crop and fruit yields in plantations and orchards Tsai (2013)	Since 2003 in the EU EC (2021)
Carbofuran	Carbamate insecticide	50 days in soil Vogue et al. (1994); 450–1200 days in water Hanson et al. (2020)	To control insects on a wide variety of field crops, including potatoes, corn, and soybeans De Gerónimo et al. (2014)	Since 2007 in the EU for all uses EC (2021) and since 2009 in the USA on all crops grown for human consumption
Fenbutatin oxide	Organotin acaricide	90 days in soil Vogue et al. (1994)	For fight against mites, whiteflies, aphids, thrips, and mealybugs, and scale on citrus, apples, stone fruits, nut trees, several other food crops, and ornamentals USEPA (1994)	Since 2014 in the EU EC (2021)
Fipronil	Phenylpyrazole insecticide	4–12 hours in water; 122–128 days in aerobic soils Vogue et al. (1994)	As seed treatments of canola, rice, sorghum, sunflower, and cotton to control mites, worms, and thrips. As foliar sprays to control insect pests in bananas, sugarcane, brassicas, cotton, potatoes, grapes, pasture, sorghum, and mushrooms and to control locusts in pasture and sorghum. To control the pests in soil APMVA (2012)	Since 2017 in the EU EC (2021)

(continued)

Table 2 (continued)

Name of pesticide	Type of pesticide	Half-life	Uses	Ban of use in agriculture
Atrazine	Triazine herbicide	168 days in water; 14 hours in air; 60–75 days in soil FAO (1997)	To control the grass and broadleaved weeds in various crops (e.g., sugarcane, sorghum, maize, lupins, canola, and also in eucalypt and in pine plantations) APVMA (2010)	Since 2004 in the EU EC (2021)

Armenia, Belarus, Benin, Bermuda, Bosnia and Herzegovina, Chile, China, Taiwan Province, Croatia, Estonia, Greece, Guyana, India, Kazakhstan, Latvia, Madagascar, Switzerland, Tunisia, and Zimbabwe. Romania during 2017–2019 imported a quantity of 79 tons of DDT. According to the information provided by Kimutai (2017), nowadays, DDT is used indoors for mosquito control in order to reduce the risk of malaria by countries such as Ethiopia, South Africa, Uganda, and Swaziland. Regarding endosulfan, the countries that imported this compound during 2017–2019 were Costa Rica (7 tons), France (11 tons), Germany (288 tons), Guatemala (40 tons), Hungary (62 tons), Italy (1 tons), Malaysia (4 tons), Netherlands (4 tons), Portugal (2 tons), Slovakia (2 tons), Spain (28 tons), the United Kingdom of Great Britain and Northern Ireland (21 tons), and Cambodia (2 tons). The exporting countries for endosulfan are Austria, the Republic of Korea, Spain, Turkey, the USA, China, Czech Republic, Netherlands, and South Africa (FAOSTAT, 2021).

As can be seen from Fig. 2, pentachlorophenol production was quite high in 2019, the imports and exports by the FAO member being 160,406.49 tons and 80,505.08 tons, respectively. The highest sold amount of pentachlorophenol reported on the market was in 2011 (285,491.32 tons). Also, in Fig. 2 are shown the trends in export and import of tributylin compounds between the years 2012 and 2019 and of aldrin, chlordane, heptachlor, lindane, dieldrin, and parathion and parathion-methyl starting with 2007 and until 2019. From Fig. 2, it can be easily seen that aldrin, chlordane, heptachlor, and parathion and parathion-methyl exports and imports have been considerably reduced over the years (over 90% in 2019 compared to 2007). Lindane exports and imports were also reduced after its introduction in the Stockholm Convention on Persistent Organic Pollutants list, but based on the FAO database, it was found that lindane imports by FAO members in 2015 were higher by more than 88.1% compared to 2008 and over 98.5% compared to 2019. The Stockholm Convention banned the production and the agricultural use, but not its pharmaceutical use as a second-line treatment for scabies and lice. The imports and exports of these pollutants also differ from country to country; therefore, in Fig. 3 are presented the leading export countries of lindane, dieldrin, endosulfan, parathion and parathion-metil, and tributylin compounds in 2019. For lindane in 2019, Kenya is the first largest exporter with 44.06 tons followed by Belgium with 26.67

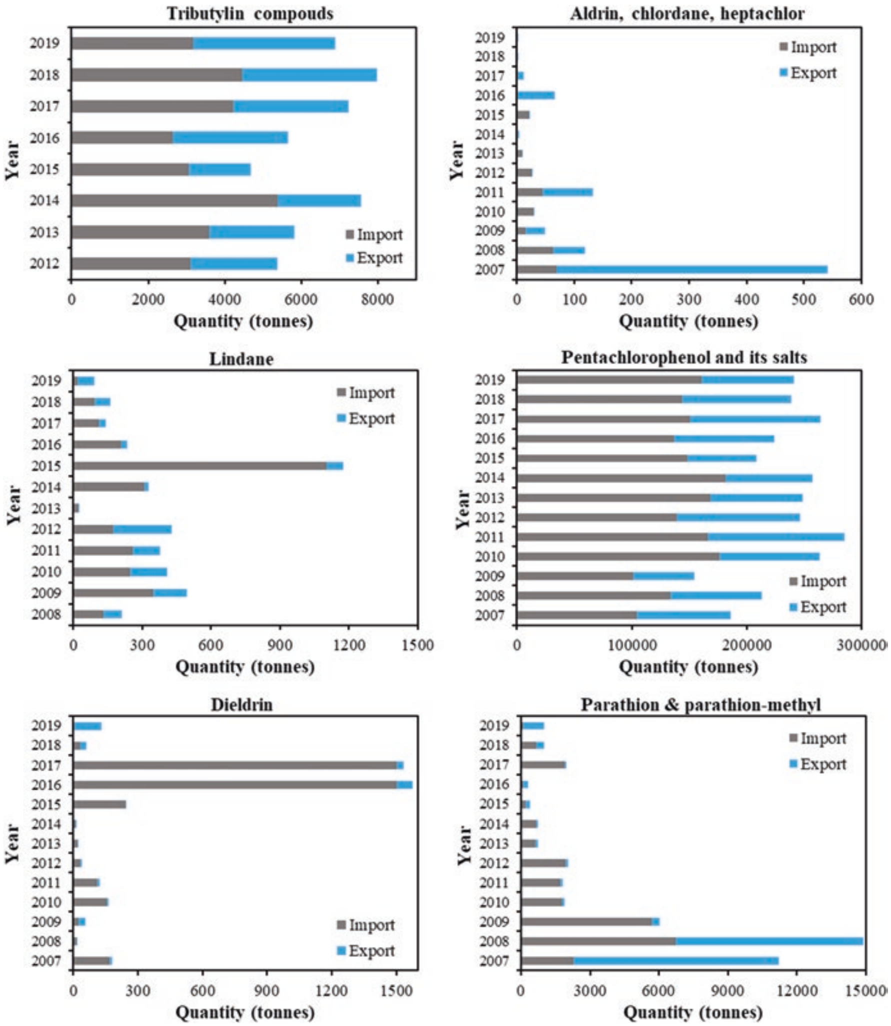


Fig. 2 Lindane, dieldrin, endosulfan, parathion and parathion-metil, and tributylin compounds import and export trade according to the FAOSTAT database

tons. The first largest exporters for dieldrin endosulfan, parathion and parathion-metyl, and tributing compounds are South Africa, Austria, the Netherlands, and Germany, respectively (FAOSTAT, 2021).

Although paraquat, atrazine, carbofuran, fenbutadin oxide, and fipronil pesticides have high persistence in the environmental compartments and have been banned for use in agriculture in many parts of the world, they are still produced and traded. Paradoxically, these pesticides are produced and marketed even by member states of the European Union, where their use has been banned. In Fig. 4, the destinations of these pesticides depending on the exporters are shown. The paraquat

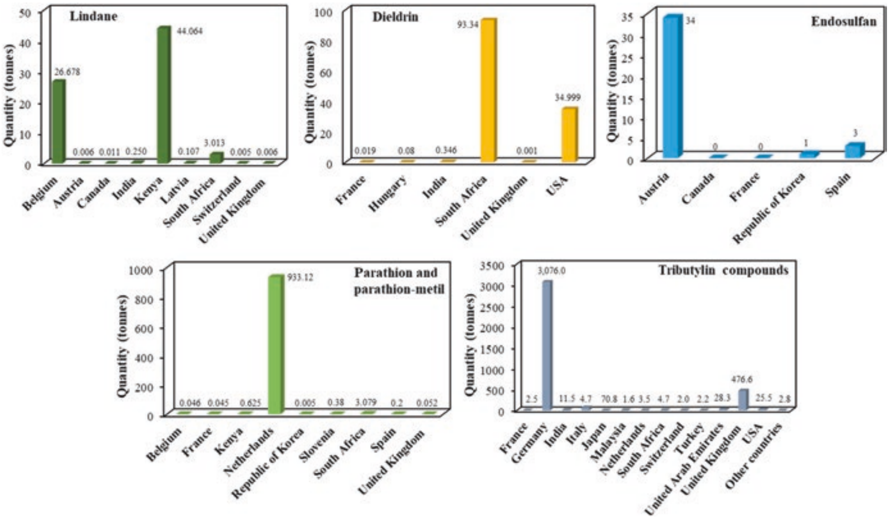
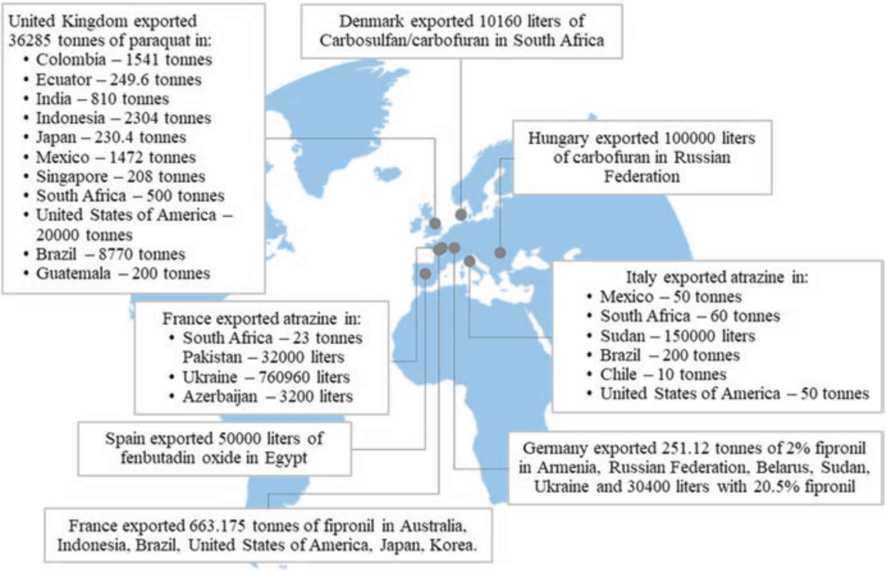


Fig. 3 Leading export countries of lindane, dieldrin, endosulfan, parathion and parathion-metil, and tributylin compounds in 2019 (FAOSTAT, 2021)



United Kingdom exported 36285 tonnes of paraquat in:
- Colombia – 1541 tonnes
- Ecuator – 249.6 tonnes
- India – 810 tonnes
- Indonesia – 2304 tonnes
- Japan – 230.4 tonnes
- Mexico – 1472 tonnes
- Singapore – 208 tonnes
- South Africa – 500 tonnes
- United States of America – 20000 tonnes
- Brazil – 8770 tonnes
- Guatemala – 200 tonnes

Denmark exported 10160 liters of Carbosulfan/carbofuran in South Africa

Hungary exported 100000 liters of carbofuran in Russian Federation

Italy exported atrazine in:
- Mexico – 50 tonnes
- South Africa – 60 tonnes
- Sudan – 150000 liters
- Brazil – 200 tonnes
- Chile – 10 tonnes
- United States of America – 50 tonnes

France exported atrazine in:
- South Africa – 23 tonnes
  Pakistan – 32000 liters
- Ukraine – 760960 liters
- Azerbaijan – 3200 liters

Spain exported 50000 liters of fenbutadin oxide in Egypt

Germany exported 251.12 tonnes of 2% fipronil in Armenia, Russian Federation, Belarus, Sudan, Ukraine and 30400 liters with 20.5% fipronil

France exported 663.175 tonnes of fipronil in Australia, Indonesia, Brazil, United States of America, Japan, Korea.

Fig. 4 Paraquat, carbofuran, atrazine, fenbutadin oxide, and fipronil export by the Member States of the European Union in the world in 2019 (Dowler, 2020)

herbicide was exported in 2019 by the United Kingdom in countries such as Colombia, Ecuador, India, Indonesia, Japan, Mexico, Singapore, South Africa, the USA, Brazil, and Guatemala. On the market, it is sold under different brand names (more than 75 names), the most common being Gramoxone. In the USA, starting in 2016, the use of paraquat was restricted, its application being carried out under the strict supervision of an authorized applicator. This measure was taken in order to minimize accidental paraquat ingestions and to reduce the exposure of workers to this pesticide (Dowler, 2020).

According to Fig. 4, atrazine is still used in countries such as Mexico, South Africa, Sudan, Brazil, Chile, and the USA, which in 2019 imported together from Italy a quantity of 370 tons. From France, South Africa, Pakistan, Ukraine, and Azerbaijan imported 23 tons and 796,160 liters of atrazine. Although atrazine is widely detected in the US surface waters bodies and drinking-water sources, it is the second-most used herbicide in the USA and its use was reapproved by US Environmental Protection Agency in 2020 for the next 15 years (USEPA, 2020). European producers of carbofuran in 2019 were Denmark and Hungary, which exported 10,160 liters to South Africa and 100,000 liters to Russian Federation. Fipronil in 2019 was exported by France and Germany under liquid solution, whose concentration in the product varied between 2% and 20.5% (Dowler, 2020). Even though the mentioned pesticides have toxic effects on the environment but also on living organisms, including humans, they are still used in agriculture. The fact that they are still produced and used only increases the risk of contamination of environmental components (water, air, soil) and the manifestation of toxic effects caused by it.

3 Spreading of Banned Pesticides with High Persistence in Soil, Water, Air, and Food

High persistent pesticides like low persistent pesticides are known for their ability to enter the environment once they are applied in agriculture for plant protection. Their transfer takes place through processes such as absorption by crops, leaching in groundwater, volatilization in air, spray drift, and runoff to surface water (lakes and rivers). As a result, human health can be severely affected by the consumption of foods originating from contaminated soil or water or by inhaling toxic air (Fig. 5) (Yadav et al., 2015; Ashraf, 2015; Tudi et al., 2021). Many diseases like cancer, reproductive, neurological, respiratory, or gastrointestinal disorders have been linked to exposure to pesticides (Rought et al., 2000; Zou & Matsumura, 2003; Keswani et al., 2021; Nicolopoulou-Stamati et al., 2016).

The main disadvantage of highly persistent pesticides is their inability to break down due to their stable nature. For this reason, their half-lives can reach decades (e.g., DDT degradation can range from 4 to 30 years), leading to their accumulation in the environment (soil, water, air), causing long-term damage (Filote et al., 2021; Stoleru et al., 2016; Yadav et al., 2015).

In soils, highly persistent pesticides due to their hydrophobic, bioaccumulative, and persistent properties create very strong bonds with the soil particles, threatening

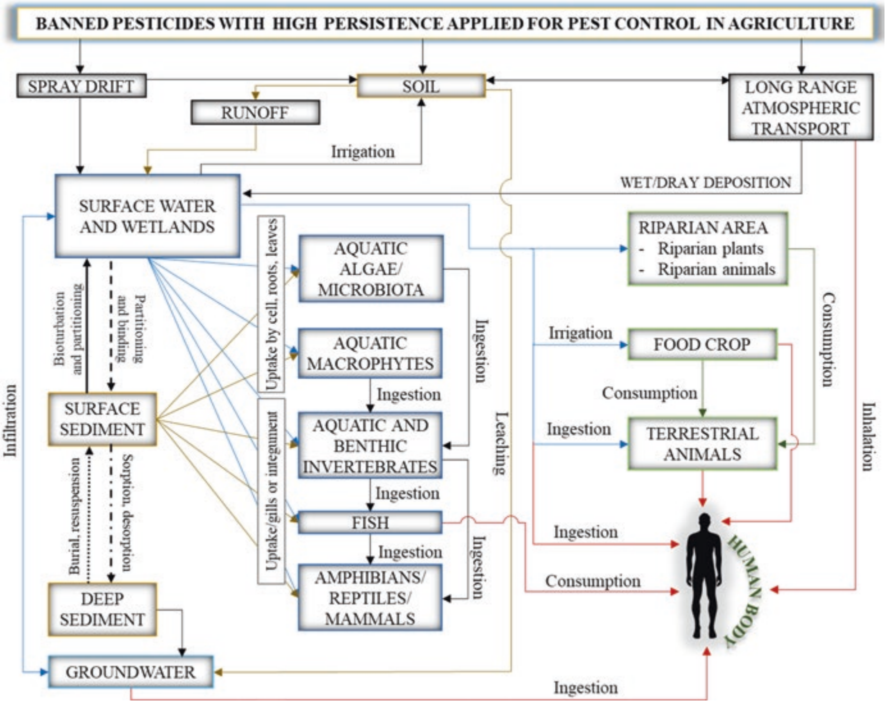


Fig. 5 Movement of banned pesticide with high persistence in the environment compartments

the food chain (Yadav et al., 2015). Residues of banned pesticides with high persistence have been reported worldwide, despite the soil type. For instance, Yang et al. (2012) in a study carried out in Zhangzhou City, Fujian Province, China, regarding the residues of organochlorine pesticides in various agricultural soils (paddy fields, vegetable lands, orchards, and tea plantations) showed high concentrations of DDTs in the surface soil of the whole studied region, with values ranging between 0.64 and 78.07 ng g^{-1} dry weight. In different regions of India (Korba, Assam, Tripura, Manipur, Keoladeo National Park, and NCR Delhi), research studies demonstrated the presence of banned pesticides with high persistence such as endosulfan and DDT, in several types of soil like forest soil, National park sediment, residential soil, or surface soil. The concentrations of endosulfan varied from 0.009 to 0.47 ng g^{-1} in forest soils, 4569 ng g^{-1} in National Park sediment and 0.95 ng·g^{-1} in surface soil. Regarding the DDT, the concentrations ranged from 2.1 to 315 ng g^{-1} in residential soil, 0.017–2.68 ng g^{-1} in forest soils, 1321.8 ng g^{-1} in National park sediment, and 1.61 ng g^{-1} in surface soil (Devi et al., 2011; Kumar et al., 2011, 2014; Bhadouria et al., 2012). Residues of banned pesticides with high persistence were also found in Australia, where residues of atrazine were detected in high concentrations in the field soils (Chowdhury et al., 2021). In Europe, various studies reported important concentrations of banned pesticides with high persistence in agricultural soils of Almeria, Spain (DDT, dieldrin, endosulfan), Nothern France (aldrin, dieldrin, DDT, endosulfan, lindane, heptachlor, atrazine), Italy (aldrin, dieldrin, endosulfan, heptachlor, chlordane, DDT), Poland (DDT), Germany (lindane, DDT), or Romania (lindane, DDT or chlordane) (Manz et al., 2001; Villanneau et al., 2011; Plaza-Bolaños et al., 2012; Tarcau et al., 2013; Thiombane et al., 2018; Malusá et al., 2020). In Romania, under vegetable soils, OCP residues such as heptachlor epoxide, alpha chlordane, endosulfan I, 4,4-'DDT, endrin, endrin aldehyde, and ketone were detected although no longer used for over 20 years, and although they are in small amounts in fruits, this means that they are not decomposed and cannot be neglected (Stoleru et al., 2016).

Taking into account the wide spread of banned persistent pesticides in soil, regardless of the continent, soil is considered the main reservoir of pesticides (Tudi et al., 2021). From soil, the pesticides are spreading into other environments having negative impacts on different ecosystems and also on human health through food.

In water systems (groundwater, rivers, lakes, estuaries, etc.), the presence of pesticides with high persistence is mostly determined by industrial and agricultural practices through the processes of leaching, runoff, and drift (Fig. 5). Leaching takes place by the transfer of pesticides from the upper level of the soil into the depths, in groundwater, and from there to other water bodies. One of the main factors contributing to the leaching is temperature, which influences the composition of the soil, enhancing this process. Runoff and drift occur as a result of a combination of factors such as climatic conditions (precipitation, temperature) and the characteristics of the soil (type, texture, slope, structure) (Yadav et al., 2015; Tudi et al., 2021). It is estimated that, on average, 46% of the pesticides used in agriculture enter the groundwater, while 76% go to the water surface (Vasseghian et al., 2021). Research done between 1970 and 2020 in different parts of the world (Asia, Africa, Europe, America, and Oceania) reported the presence of banned pesticides with high persistence in water resources such as groundwater, surface water, seawater, or drinking water. The most studied banned persistent pesticides were lindane, aldrin, DDT, and heptachlor. According to these studies, the variation over time (2000–2020) of the above-mentioned pesticides showed a decrease in seawater and drinking water with no incidence between 2015 and 2020, while in surface water the concentration of DDT and aldrin increased during 2015–2020 (89.21 ng L^{-1} – DDT, 73.58 ng L^{-1} – aldrin) as compared to the interval 2010–2015 (6.4 ng L^{-1} – DDT, 59.39 ng L^{-1} – aldrin), 2005–2010 (15.35 ng L^{-1} – DDT, 4.43 ng L^{-1} – aldrin), 2000–2005 (23.2 ng L^{-1} – DDT, 10.62 ng L^{-1} – aldrin), or before 2000 (35.72 ng L^{-1} – DDT, 46.6 ng L^{-1} – aldrin). For lindane, the concentration reported in the surface water for 2015–2020 (5.76 ng L^{-1}) was lower than for 2010–2015 (9.72 ng L^{-1}), 2000–2005 (20.18 ng L^{-1}), and before 2000 (11.91 ng L^{-1}), but higher than in the years' interval 2005–2010 (1.93 ng L^{-1}). Approximately, the same trend was observed for heptachlor (8.55 ng L^{-1} (2005–2010) or 10.56 ng L^{-1} (before 2000) > 23.78 ng L^{-1} (2015–2020) < 64.65 ng L^{-1} (2010–2015) or 33.49 ng L^{-1} (2000–2005)) (Vasseghian et al., 2021). Other banned persistent pesticides detected in water bodies were endosulfan, dieldrin, chlordane, atrazine, or carbofuran. These pesticides were reported mainly in surface water (rivers, lakes, sea, or other reservoirs of water), but also in groundwater and sediments (Leong et al., 2007; Singh et al., 2007; Kucuksezgin & Tolga Gonul, 2012; Herrero-Hernández et al., 2013; Unyimadu et al., 2018; Miglioranza et al., 2021; Oginawati et al., 2021; Sarker et al., 2021; Zhang et al., 2021). In general, a higher concentration of pesticides is found in the surface water as compared to the groundwater because of the fast translocation through runoffs (Syafrudin et al., 2021). Research has demonstrated that most of the banned persistent pesticides were reported in developing countries, where despite the fact that most of them are forbidden to be used, they are applied in high amounts in order to provide enough food (Sarker et al., 2021). Also, in most of these countries, the water from the rivers is used for consumption. So the risk of contamination in humans is very high. In order to reduce the risk, the World Health Organization (WHO) and some national governments (USA, Australia, European Union, New Zealand, Canada, and Japan) imposed some regulatory limits for pesticides in drinking water, which can differ depending on several parameters such as geographical, economical, industrial, or social conditions. For instance, the limits imposed by WHO for atrazine, lindane, and DDT is 2 μg L^{-1}, for chlordane is 0.2 μg L^{-1}, for aldrin and dieldrin is 0.03 μg L^{-1}, or for carbofuran is 7 μg L^{-1} (Hamilton et al., 2003; Syafrudin et al., 2021).

Persistent prohibited pesticides reach the air by volatilization from the soil and crops where they are applied or from deposits that are not stored according to an appropriate and safe procedure or by spray drift from equipment used in agriculture (Pribylova et al., 2012; Yadav et al., 2015). It is estimated that approximately 90% of the pesticides are volatilized in just a few days after application on crops or soils, while the pesticide losses resulting from the spray drift account for 2–25% during agricultural practices (Yadav et al., 2015; Tudi et al., 2021). The main consequence of these processes is air pollution, which takes place not only on the site of application, but over long distances, having a negative impact on the flora, fauna, and human health (Tudi et al., 2021). Despite the fact that some pesticides have been banned for use because of their toxicity for over 20 years, analyses done on air samples have demonstrated their presence. For instance, DDT, chlordane, endosulfan, lindane, or heptachlor were found in the air samples from different regions of India. The concentrations registered varied depending on the analyzed area and season. For instance, the concentration of endosulfan and chlordane in a rural region during the summer season was higher (260 pg m^{-3}) than in the rainy seasons (44 pg m^{-3}) (Devi et al., 2011). In seven metropolitan Indian cities, the lindane concentration was the highest reported across the globe between the years 2006–2007, on average 5400 pg m^{-3}. High concentrations were also registered for DDT, chlordane, and endosulfan (on average 1470, 1530, and 1040 pg m^{-3}, respectively) (Chakraborty et al., 2010). Banned persistent pesticides were also found in the air samples taken from some agricultural areas of India in concentrations classified as high: 292 pg m^{-3} for lindane, 2770 pg m^{-3} for endosulfan, and 247 pg m^{-3} for DDT. Lindane, chlordane, heptachlor, aldrin, dieldrin, DDT, and endosulfan were also registered in air samples collected from countries in West Africa (Isogai et al., 2018). Apart from India and Africa, worrying levels of banned persistent pesticides were found in Central and Eastern Europe. For instance, the highest concentrations of DDT in these regions were found in the emission sites of Romania, Kyrgyzstan, Ukraine, Serbia, and Czech Republic, with values ranging from 0.1 to 173 ng sample^{-1} (Pribylova et al., 2012). Banned persistent pesticides were also detected at high altitudes (2650, 2663, and 3106 m a.s.l.) by stations situated in Alps. Pesticides like DDT, lindane, chlordane, heptachlor, dieldrin, aldrin, and endosulfan were detected in higher concentrations in the air masses coming from northeast and south (Bavaria, Eastern Central Europe, and Mediterranean countries) than from the Atlantic Ocean (Kirchner et al., 2016).

Studies done on the occurrence of highly persistent pesticides in foods showed that fruits, vegetables, cereals, dairy products, meat products, chicken eggs, and aquatic organisms can be contaminated with these substances. The main source of pesticides with high persistence in food is agriculture and its related practices. Over time, the application of important amounts of pesticides in order to increase food production and to decrease the risk of vector outspread has led to their accumulation in soil. Processes like volatilization, spray drift, leaching, runoff, or absorption have contributed to their spread in water, air, and also in foods of different origins. Among the banned pesticides found in food samples were DDT, aldrin, dieldrin, heptachlor, chlordane, lindane, endosulfan, pentachlorophenol, or carbofuran. These were registered in all the food categories mentioned above, in different concentrations and regions. For instance, in a study done in Lagos, Nigeria, the concentration of aldrin found in fruits and meat products was 0.12 ng g^{-1}, in aquatic food and dairy products 0.16 ng g^{-1}, in eggs 0.02 ng g^{-1}, in vegetables 0.15 ng g^{-1}, and in cereals 0.14 ng g^{-1}. In the same study, the concentration of DDT, regardless of the type of food, was by far the highest among all tested pesticides (Adeyi et al., 2021). In another study done in China, DDT and lindane were detected in all the tested food categories (eggs, fish and shrimps, meats, dairy products, grains, fruits, vegetables, and oils). Also, in China, fipronil was found in fruits and vegetables such as litchi, banana, grape, apple, dragon fruit, plum, leaf lettuce, cabbage, common bean, eggplant, and many others in concentrations ranging from 0.0014 to 1.8644 mg kg^{-1} (Singh et al., 2021). Pentachlorophenol was recorded in grains, vegetables, eggs or fish, and shrimps, in concentrations ranging from 0.08 μg kg^{-1} for grains to 77 μg kg^{-1} for fish and shrimps. The highest concentration registered among all the tested pesticides was for heptachlor, in grains, at 111 μg kg^{-1} (Fan et al., 2021). In Europe, according to European Food Safety Authority (2020), carbofuran was detected in plants like eggplants or sweet peppers in concentrations that exceeds the legal limits. The same situation was found for dieldrin in melons. However, of all the pesticides analyzed, DDT and lindane, because of their high persistence in the environment, were detected in most of the food samples considered (EFSA et al., 2020). The presence of banned persistent pesticides in food has a direct negative impact on human health through their consumption. These compounds due to their lipophilic character have the tendency to bioaccumulate in adipose tissues, breast milk, and blood, having a long-term effect on health. Studies conducted in Asian, European, African, and South and North American countries have demonstrated the presence of persistent pesticides in breast milk samples. The most frequent pesticides recorded regardless of the country were DDT, lindane, and endosulfan. Other banned pesticides with high persistence detected in breast milk samples were aldrin, heptachlor, and dieldrin (Keswani et al., 2021). These pesticides are toxic to the human body, having negative effects on the central nervous system, endocrine system, or immune system by blocking the function of different enzymes, receptors, or the transport of ions. Also, carcinogenic effects were associated with some pesticides like DDT, lindane, heptachlor, or endosulfan. For instance, it was observed that in the presence of DDT and lindane, the proliferation of MCF-7 breast cancer cells was increased. Heptachlor and endosulfan experiments have suggested that both can induce apoptosis in lymphocytes (Rought et al., 2000; Zou & Matsumura, 2003; Keswani et al., 2021).

4 Banned Pesticides with High Persistence Impacts on the Environment and Living Organisms

After the Second World War, more and more persistent organic substances were synthesized and used as pesticides in agriculture. The intense use of these chemicals has generated unwanted byproducts and negative effects on both ecosystems and human health. Due to the lipophilic characteristics, the POPs tend to remain in fat-rich tissues of organisms, being biomagnified along the food chain and thus becoming highly toxic to humans. Unwanted side effects of using pesticides in agriculture usually come from a lack of understanding of the impact of the substance used on the environment, worsened by excessive and inappropriate use of the product. Side effects of pesticide use do not always occur immediately after they have been applied (Aktar et al., 2009; Özkara et al., 2016). Some of these effects may be

- Reduction of beneficial species
- Drift of sprays and vapor during application
- Residues in animal and human food
- Groundwater contamination by leached chemicals
- Development of the resistance to the pesticide by target pests due to overuse and incorrect use of the chemical
- Poisoning hazards and other health effects
- Other possible health effects

Due to the chemical properties of pesticides as well as environmental factors, the pesticides exert their toxic effects not only on the area of application but also on distant aquatic environments or other lands, grazing areas, human settlements, and undeveloped areas mainly by runoff and pesticide spray drift. The environment contamination with banned pesticides with high persistence also results from the production, transport, storage, and disposal (Kent, 1992).

Repeated application of pesticides has also led to an increase in pests' resistance, while its effects on other species may facilitate the pests' resurgence. The applied pesticides act not only against the target species but also affect the non-target species, such as plants, animals, and humans (Kent, 1992). According to Amaeze et al.

(2020), more than 98% of sprayed insecticides and 95% of herbicides end up in a destination other than the target species because they are sprayed or spread over the whole surface of agricultural fields.

In aquatic ecosystems, the banned pesticides with high persistence affect not only the aquatic plants (aquatic vascular plants, seaweeds, algae, and other sources of aquatic plants) but also the aquatic invertebrate and vertebrate (e.g., fish, crabs, mussels, fish communities, etc.). The adipose tissue of aquatic bodies is more sensitive to POPs, and as a result, they are bioaccumulated in aquatic organisms' tissues and propagated along the food chain. The POPs, including the banned pesticides with high persistence, can have a wide range of effects on wildlife, such as decreased brain size and neurotoxicity, causing behavioral changes, hormonal disorders, carcinogenesis, cellular and tissue damage, and reproductive problems (Porta et al., 2010).

Human exposure to these substances can be classified as acute or chronic, depending on the dose, duration, and types of chemicals and resulting in a wide variety of negative effects on humans (Gavrilescu, 2005). The adverse health effects, including various diseases and even death, are caused by occupational or accidental exposure or as a result of inhalation, ingestion, and dermal contact with these pollutants (Gavrilescu, 2005; Roșca et al., 2017). According to Aktar et al. (2009), production workers, formulators, sprayers, mixers, loaders, and agricultural farm workers are the groups with the highest risk of exposure to pesticides. Due to the negative effects on human health, the International Agency for Research on Cancer (IARC) based on clinical trials included lindane and pentachlorophenol in group 1 (carcinogenic to humans) and aldrin, dieldrin, and DDT are classified as "*probably carcinogenic to humans*" (group 2A). The pesticides such as parathion, chlordane, and heptachlor are considered possibly carcinogenic agents to humans (group 2B) and methyl parathion and atrazine are included in group 3 – not classifiable as to its carcinogenicity to humans (IARC, 2020).

The specific effects on human health but also on mammals, aquatic and terrestrial organisms, and plants, as well as birds of each type of banned pesticide with high persistence considered in this study, are listed in Tables 3 and 4. According to Mitra et al. (2021), birds, particularly the carnivorous species (e.g., bald eagles, hawks, and owls), are mostly affected by persistent pesticides. Nowadays, these birds are often rare, endangered, and susceptible, their reduction in the number being caused even by persistent pesticides. The number of partridges, grouse, and pheasants, which are insect-eating birds, have decreased, mainly caused being the decrease in insect population in agricultural fields through insecticide use. One of the pesticides that cause these effects is DDT. Mitra et al. (2021) in their paper also state that DDT and its DDE metabolite are linked with the decline of numbers in fish-eating birds, such as sparrow hawks, mallards, and brown pelicans. Annually, worldwide are recorded cases of human pesticide poisoning. According to the World Health Organization, about one million unintentional pesticide poisonings occur annually, leading to approximately 20,000 deaths (Boedeker et al., 2020). One of the biggest tragedies for humans related to the use of endosulfan in agriculture was in Kerala, India. In this area, the use of endosulfan began as early as 1976 and was

Table 3 The effects of banned pesticides with high persistence on aquatic and terrestrial plants and organisms and birds

Pesticide name	Aquatic and terrestrial plants and organisms and birds	References
Lindane	Vitellogenin induction in male fish affects the immune systems of fish The epithelial necrosis and rupture of the gill epithelium Biochemical changes in liver, brain tissue, and hyperglycemia of terrestrial organisms Adverse effects on the immune systems of birds Reduction in hemoglobin, increase in kidney weight, and liver toxicity in addition to histopathological changes in liver and kidney	Ortiz et al. (2003); Sandu and Virsta (2015)
Dieldrin	Hepatic lesions in benthic organisms Reduce phallus size and sex reversal in alligators and in red snapping turtles Are linked to decreases in hatching success rate in osprey birds	Honeycutt and Shirley (2014)
Aldrin		
Endosulfan	Cause lethargy, weakness, and diarrhea in Japanese quail Is a potential endocrine disrupter in birds	Mitra et al. (2021)
Parathion and parathion-metil	Toxic effect for birds, freshwater fish, hydrophilous organisms as well as sea organisms in a low rate Alter the biochemical constituents in various tissues of *H. fossilis* Cause metabolic dysfunction Decrease in carbohydrate content in all the tissues Produce alterations in brain acetylcholinesterase activity of birds Inhibit the egg production and hatchability in Japanese quail	Hertel and International Programme on Chemical Safety (1993); Kalipci et al. (2010); Rao et al. (2018)
Tributylin compounds	Causes imposes in gastropods, characterized by the development of male sex characteristics in females	Ostrakhovitch (2015)
DDT (dichloro-diphenyl-trichloroethane)	Influences the health and promotes defects in birth and in wildlife	Kabasenche and Skinner (2014)
Pentachlorophenol	Produce DNA damage, endocrine disruption, alter the activities of antioxidant enzymes, are linked with deformities, changes in serum testosterone, antiestrogenicity, damage the reproductive system, induce immunotoxicity and gene mutation in aquatic invertebrates and fish	Singh and Chaube (2019)

(continued)

Table 3 (continued)

Pesticide name	Aquatic and terrestrial plants and organisms and birds	References
Paraquat	Affects the histology of gills, liver, and kidney of fishes Cause curling of secondary lamellae, aneurysm, gill bridging, and enlargement of the cartilaginous core and tissue damages in fishes Is embryotoxic to sensitive species of birds	Badroo et al. (2020)
Carbofuran	Inhibit the cholinesterase in nerve synapses in the central nervous system Disrupt the immune cells and biochemical and enzymatic activities in aquatic organisms Damage the enzymatic activities of plants	Dobšíková (2011); Mishra et al. (2021)
Fenbutatin oxide	Is nontoxic to birds and has no effect on their reproduction. It also is practically nontoxic to mammals and honey bees	USEPA (1994)
Fipronil	Damage the nervous system, such as the optic thalamus in animals Highly toxic for bees	Wu et al. (2021)
Atrazine	Induce changes in sex ratio in fish and amphibian Produce hermaphroditism in frogs A potent endocrine disruptor in wildlife and can induce reproductive cancer in animals. Is linked with a wide range of reproductive abnormalities including hypospadias, undescended testes, developmental testicular changes, and apoptosis of cells in testis Toxic effects on hypothalamus Is a hepatotoxic agent	Solomon et al. (2008); Victor-Costa et al. (2010); Hussain et al. (2017)

aerial spraying in the cashew plantations. After over 20 years of use, the Calicut Medical College and the National Institute of Occupational Health (NIOH), through the Kerala State Department of Health and Family Welfare, identified 6728 persons with different types of diseases and 779 deaths as the cause of endosulfan poisoning (Sreekumar & Prathapan, 2021).

5 Microbial Biodegradation of Banned Pesticides with High Persistence

Pesticides with high persistence are known as being resistant to degradation due to their stable chemical structure. As a consequence, their presence in the environment can be found for long periods of time after their application. The excessive use of persistent pesticides in agriculture has led to the accumulation of these compounds in soil, water, air, or food (Filote et al., 2021; Yadav et al., 2015). Even though some

Table 4 The effects of banned pesticides with high persistence on mammals and human health

Pesticide	Hazard class by WHO	Mammals	Humans	References
Lindane	Class II	Affects the central nervous system, the behavior, motor activities, and brain chemical levels. High doses induce convulsions and seizures	The acute effects induced are headache, dizziness, seizures, and aplastic anemia, and the long exposure affects the liver, blood, gastrointestinal tract, and cardiovascular, musculoskeletal, nervous, and immune systems. It is considered a possible human carcinogen	National Research Council (1982)
Dieldrin	Class Ia	Affects the central nervous system, produces irritability, tremors, and convulsions, increases incidences of liver tumors, fetal deaths, and anomalies	The short exposure causes major motor convulsions, malaise, incoordination, loss of consciousness, headache, dizziness, and gastrointestinal disturbances	National Research Council (1982); Honeycutt and Shirley (2014)
Aldrin	Class Ib			
Endosulfan	Class II	Affects the nervous system and produces hyperactivity, tremors, decreased respiration, dyspnea, and eventually death	The long exposure induces congenital physical disorders, mental disabilities, and deaths, and the acute effects consist of headaches, dizziness, nausea, vomiting, mental confusion, convulsions, hyperactivity, seizures, coma, and respiratory depression	Amizadeh and Amizadeh (2011)

(continued)

Table 4 (continued)

Pesticide	Hazard class by WHO	Mammals	Humans	References
Parathion and parathion-metil	Class Ia	Produce body weight decrease and increase the weight of livers and cause cancer in animals. The acute effects include excessive salivation, hypermotility of gastrointestinal tract, abdominal cramping, vomiting, diarrhea, sweating, dyspnea, cyanosis, miosis, muscle fasciculations, and convulsions	The short exposure causes inhibition of cholinesterase enzyme in the brain, neurological problems, muscle weakening, loss of memory short period, anxiety or irritability, and depression. As a result of short-term exposure are fear, sleep withdrawal, excess working of secretory glands, diarrhea, nausea and vomiting, headache, dizziness, blurred vision, tightness in the chest, sweating, nausea, and vomiting	Kalipci et al. (2010); Pope (2014)
Tributylin oxide	Class II	Produces immunotoxicity, increases the incidence of tumors of the endocrine system, and is highly embryotoxic	The short exposure at high levels causes loss of memory and seizure, including death, severe dermatitis, difficulty in breathing, and flu-like symptoms	Boyer (1989); Ostrakhovitch (2015)
DDT (dichloro-diphenyl-trichloroethane)	Class II	Causes reproductive and neurological diseases and developmental abnormalities	Causes reproductive and neurological diseases, developmental abnormalities, and cancer	Kabasenche and Skinner (2014)

(continued)

Table 4 (continued)

Pesticide	Hazard class by WHO	Mammals	Humans	References
Pentachlorophenol	Class Ib	Causes increases in liver tumors (hepatocellular adenomas and carcinomas) and adrenal medulla pheochromocytomas, hemangiosarcomas, and hemangiomas and accelerates respiration, vomiting, and increased body temperature, and causes tachycardia, neuromuscular weakness, and cardiac failure	Affects the cardiovascular system, blood, liver (jaundice), and eyes (visual damage and irritation); causes lethargy, tachypnea, tachycardia, delirium, convulsions, loss of appetite, respiratory difficulties, hyperpyrexia, sweating, dyspnea, and coma	National Research Council (1982)
Chlordane	Class II	Causes liver and kidney damage, appetite loss, growth retardation, and unspecified signs of poisoning	The effects of short-term exposure are intermittent clinic convulsions, loss of coordination, and increased excitability. The chronic effects include aplastic and acute leukemia and neuroblastoma	National Research Council (1982)
Heptachlor	Class II	Causes hepatic necrosis and increases the incidence of benign hepatomas	The effects as a consequence of short exposure are headache, dizziness, incoordination, tremors, and seizures	National Research Council (1982)
Paraquat	Class II	Alter the synthesis of proteins, DNA, collagen, and pentose phosphate metabolism and causes vomiting, diarrhea, abdominal pain, ulcers, and death	Causes cellular damage in many organs, pneumonitis and lung fibrosis, renal and liver injury, irritation and corrosive effects to the mucous membranes, cornea, and skin	Gawarammana and Buckley (2011); Roede and Miller (2014); Gao et al. (2020)

(continued)

Table 4 (continued)

Pesticide	Hazard class by WHO	Mammals	Humans	References
Carbofuran	Class Ib	Affects the central nervous system by inhibiting the cholinesterase in nerve synapses, produces carcinogenic, teratogenic, and genotoxic effects, crosses the placental barrier, and affects the placenta and the fetus	The effects of short-term exposure are weakness, sweating, nausea and vomiting, abdominal pain, blurred vision, muscle twitching, and loss of coordination and may even cause shortness of breath. It is a probable human carcinogen	Dobšíková (2011); Mishra et al. (2021)
Fenbutatin oxide	Class III	Causes anorexia, gastric lesions, and abortions	Low acute toxicity but is a severe eye irritant. It caused redness of the skin and swelling	USEPA (1994)
Fipronil	Class II	Causes neurotoxicity, hepatotoxicity, reproductive toxicity, disruption of endocrine function, abnormal emotional and cognitive behaviors, irritation, lethargy, incoordination, and convulsions	The effects induced by short exposure are sweating, nausea, vomiting, headache, stomach pain, dizziness, weakness, and seizures	Wu et al. (2021)
Atrazine	Class III	Causes changes in blood hormone levels and affects the reproductive system. Also, causes liver, kidney, and heart damage	It is an endocrine disruptor, possibly carcinogenic, and can be a connection to low sperm levels in men. Affects the heart, lungs, and kidney by their congestion and produce low blood pressure, muscle spasms, weight loss, adrenal glands and cardiovascular damage, retinal degeneration, and muscle degeneration. In addition, it can cause birth defects	Pathak and Dikshit (2012)

of the pesticides with high persistence have been banned or restricted for use in agriculture (Table 1), there are countries that continue to produce and export these categories of pesticides to developing countries that still use them in agriculture

(Fig. 1). For instance, in 2019, France exported atrazine to South Africa, Pakistan, Ukraine, and Azerbaijan (Dowler, 2020). After their application in agriculture, high persistent pesticides depending on their nature and the environmental conditions can be degraded by biotic (microorganisms) and abiotic factors (chemical reactions or light) or can stay unaltered (Fig. 6) (Sarkar et al., 2020).

Biotic degradation is one important part of pesticide degradation, which implies the action of microorganisms. The degradation in which microorganisms are involved is called biodegradation. Through this process, microorganisms use pesticides as nutrients to grow and develop and at the same time degrade them into simple compounds like carbon dioxide and water. In addition to carbon dioxide and water, other secondary products can result from being further degraded by other microorganisms through metabolism or mineralization (Fig. 6) (Ye et al., 2018; Bose et al., 2021).

The main categories of microorganisms known to be capable of persistent pesticide degradation are bacteria, yeast, and fungi. Studies have demonstrated that these microorganisms can degrade persistent pesticides like aldrin, dieldrin, lindane, endosulfan, DDT, chlordane, paraquat, heptachlor, fipronil, or pentachlorophenol.

Lindane has been restricted or banned by the Stockholm Convention on Persistent Organic Pollutants since 2019 because of its harmful effects on human health and the environment, also for the potential to be transported on long distances (the Artic or Antarctica). Once introduced into the environment, lindane cannot be degraded by light because of the lack of chromophores; also, chemical degradation through hydrolysis is not important if the pH is neutral, as in the case of water. Regarding microbial degradation, it was demonstrated that bacteria, yeast, and fungi are capable of degrading lindane. Among the bacterial genus detected to degrade this pesticide was *Microbacterium*, *Pandoreae*, *Streptomyces*, *Paracoccus*, or *Sphingobium* (Okeke et al., 2002; Benimeli et al., 2008; Zheng et al., 2011; Sahoo et al., 2019;

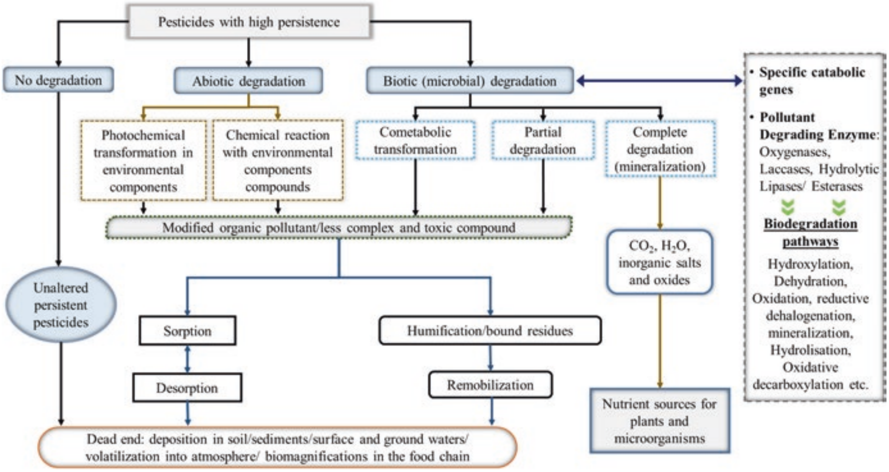


Fig. 6 Pesticide removal by biotic and abiotic factors

Singh & Singh, 2019). The highest rates of degradation were registered for *Paracoccus*, which removed lindane in a proportion of 90% in 8 days, and by *Microbacterium*, which degraded in 15 days 82.7% of the lindane found in the medium (Sahoo et al., 2019; Singh & Singh, 2019). For *Streptomyces* and *Pandoreae*, it was observed that their ability to degrade lindane increased along with the concentration of pesticide until a limit point when their ability decreased (Okeke et al., 2002; Benimeli et al., 2008). For instance, after 4 weeks of incubation, *Streptomyces* degraded 29.1% of lindane from spike soil when its concentration was 100 μg kg^{-1}, 78.0% at a concentration of 150 μg kg^{-1}, 38.8% at a concentration of 200 μg kg^{-1}, and 14.4% at a concentration of 300 μg kg^{-1} lindane (Benimeli et al., 2008). Regarding the action of *Pandoreae*, lindane was removed until its concentration in the substrate was 150 mg L^{-1}, and then starting with 200 mg L^{-1}, the concentration of degraded lindane decreased (Okeke et al., 2002). The authors of the experiments with *Strepromyces* stated that the cause of degradation decrease was not the bacterial growth inhibition, but the fact that lindane was used as a secondary source of nutrients, the soil already having organic matter available (Benimeli et al., 2008). Even if the optimum temperature for lindane degradation is 30 °C, it was demonstrated that strains of *Sphingobium* sp. had the ability to remove in 24 h, 98% of the lindane present in the medium at 4 °C, being considered a candidate for the decontamination of lindane from cold regions (Zheng et al., 2011). In addition to bacteria, yeast from the *Rhodotorula* or *Candida* genus was identified as a lindane degrader. Both the yeasts degraded 100% of the lindane at a concentration of 600 mg L^{-1}, in 10 and 6 days after incubation, respectively (Abdul Salam et al., 2013; Salam & Das, 2014). At higher concentrations of lindane, the growth of *Rhodotorula* VITJzN03 was inhibited. Regarding the ability of fungi to degrade lindane, strains of *Fusarium* were reported to remove in 10 days, 100 μg lindane mL^{-1}, or to degrade it into other metabolites (Sagar & Singh, 2011; Guillén-Jiménez et al., 2012).

Aldrin and dieldrin are two extremely persistent pesticides with a half-life of more than 1 year. Both were banned in 1987 because of the environmental and health problems that they can cause. However, residues can be found in soil, water, air, or food. Biodegradation of these pesticides was detected in bacteria and fungi. The efficiency of the degradation depends on many factors such as pH, temperature, moisture, structure, and composition of the soil, light, or the microorganism species involved. Bacteria like *Pseudomonas* sp., *Bacillus* sp., *Clostridium* sp., *Micrococcus* sp., or *Flavobacteria* sp. were reported as having the ability to degrade aldrin and dieldrin at different rates. For instance, a strain of *Pseudomonas fluorescens* degraded both pesticides in a proportion of 94.8%; three strains of *Clostridium* sp. degraded 80% of the dieldrin, through dehalogenation, in 54–80 days; *Flavobacterium* sp. and *Micrococcus* sp. had a degradation rate of 27% and 24.2%, respectively, regarding aldrin; *Bacillus polymyxa* removed from medium 48.2% of the total aldrin added, after 12 days of incubation (Purnomo, 2017; Doolotkeldieva et al., 2021). Among fungi, *Fusarium* sp., *Trichoderma* sp., *Penicillium* sp., *Mucor* sp., or *Aspergillus* sp. were reported as having the ability to degrade aldrin or dieldrin or to transform aldrin to dieldrin by epoxidation. For instance, *Fusarium* sp. converted in 6 weeks 9.2% of the total aldrin added to dieldrin, while a strain of

Penicillium miczynskii degraded in 14 days after incubation, 90% of the dieldrin from the liquid media. The same degradation rate was also recorded for a strain of *Mucor racemous* at different pH values (Kataoka et al., 2010; Birolli et al., 2015; Purnomo, 2017). Observations regarding the transformation of aldrin to dieldrin by soil microorganisms were done years ago, in 1960, when dieldrin residues were found in places where it was never used (Purnomo, 2017). Comparative studies between the capacity of bacteria or fungi to degrade aldrin and dieldrin showed that fungi are better degrader than bacteria.

Endosulfan is an insecticide that has been widely used for over 60 years for plant protection. Because of the toxicity shown on different aquatic organisms, it was banned from being used in 2011 by the Stockholm Convention (Berntssen et al., 2017). Residues can still be found in the environment, causing health issues for humans. Through degradation by oxidation or hydrolysis, endosulfan decomposes in metabolites that can be toxic like endosulfan sulfate or less toxic like endosulfan diol (Narkhede et al., 2015). Microorganisms like bacteria and fungi are capable of degrading endosulfan or its metabolites. Studies have shown that bacteria like *Pseudomonas* sp., *Klepsiella* sp., *Bacillus* sp., *Flavobacterium* sp., *Acinetobacter* sp., *Bordetella* sp., *Burkholderia* sp., *Arthrobacter* sp., *Mycobacterium* sp., *Achromobacter* sp., or *Pandoraea* sp. can remove the pesticide from the medium in rates ranging from 43% to 100% (Hussain et al., 2007; Romero-Aguilar et al., 2014; Bose et al., 2021). A complete degradation of endosulfan was reported for a strain of *Pseudomonas aeruginosa*, which removed the pesticide in 504 h or for a strain of *Pseudomonas fluorescens*, in 288 h (Jesitha et al., 2015; Narkhede et al., 2015). Regarding the fungi, *Fusarium ventricosum* was reported as having the ability to degrade in 15 days the alpha-endosulfan, in a proportion of 91.1%, or the beta-endosulfan to a rate of 89.9% (Siddique et al., 2003). *Mucor thermohyalospora* was also registered for the ability to remove the alpha- and beta-endosulfan but in a longer period of time (20 days) and a lower proportion (70% and 50%, respectively) (Shetty et al., 2000). The same compounds, in the same proportion, were degraded by *Mortierella* sp., after 28 days of incubation (Kataoka et al., 2010). Another fungus capable of endosulfan degradation was a strain of *Penicillium* sp., which in 6 days removed 94.87% of the pesticide from the medium (Romero-Aguilar et al., 2014). Bhalerao and Puranik (2007) in a study done on the ability of *Aspergillus niger* to degrade endosulfan showed that in 12 days the fungi removed all amount of pesticides from the contaminated soil.

DDT is known for its highly efficient insecticidal properties, but in the same time for the important hazard that is having on the environment and the organisms that live in it. Harmful effects have been detected on terrestrial organisms, as well as on aquatic or aerial organisms. Since 2001, the production and use of DDT has been controlled by the Stockholm Convention on Persistent Organic Pollutants, its indoor application being the only one accepted. Taking into account that DDT started to be used in 1939, when it was discovered, and its half-life can reach 10–15 years, residues are still present in soil, water, or atmosphere (Zaranyika et al., 2020).

Degradation of DDT and its metabolites have been observed in different research studies done on bacteria or fungi. Strains of Gram-positive (*Arthrobacter* sp., *Streptomyces* sp., *Bacillus* sp., *Rhodococcus* sp., *Staphylococcus* sp.) or Gram-negative bacteria (*Stenotrophomonas* sp., *Sphingobacterium* sp., *Alcaligenes* sp., *Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp.,) were reported to degrade DDT or its metabolites in various proportions. High levels of degradation were registered for strains of *Streptomyces* sp. (84.5% in 14 days), *Rhodococcus wratislaviensis* (99.7% in 70 days), *Bacillus* sp. (73% in 5 days), *Stenotrophomonas* sp. (55% in 5 days), or *Rhodococcus* sp. (50–60% in 18 days) (Pant et al., 2013; Xie et al., 2017; Egorova et al., 2017; Ito et al., 2021; Panormo, 2017). Lower rates of degradation were registered for strains of *Pseudomonas* sp., *Bacillus circulans*, *P. aeruginosa*, *Bacillus* sp., and *Flavobacterium* sp., after 10 days of inoculation (29.8%, 22.7%, 19.5%, 13.5%, and 7%, respectively) (Egorova et al., 2017). Regarding the fungi identified as a DDT degrader, strains of *Cladosporium* sp., *Trichoderma* sp., *Rhizopus* sp., *Mucor* sp., *Galactomyces* sp., or *Fusarium* sp. (Purnomo et al., 2011; Russo et al., 2019; Mitra et al., 2021) were detected. Depending on the temperature (30 or 60 °C), strains of mesophilic *Mucor* sp. degraded DDT at rates ranging from 81.9% to 95.3% and 72.9% to 88.7%, respectively. In the same conditions of temperature, a mesophilic strain of *Galactomyces* sp. removed 83.6% and 90.1%, respectively, of the added DDT (Purnomo et al., 2011).

Paraquat was first synthesized herbicide in 1882 and was prohibited in 2007 in European Union. As the rest of the persistent pesticides, paraquat has high toxicity to human health and the environment. It was estimated that the normal microbiota of soil could degrade less than 1% of the paraquat absorbed in soil particles (Huang et al., 2019). According to Alexander (1999), paraquat is completely degraded by soil microorganisms within 6 years into ammonia, carbon dioxide, and water. Bacterial strains with potential to degrade paraquat are *Pseudomonas putida* (47.3% in 3 days), *Clostridium prazmowski* (80.3% in 3 days), *Sporohalobacter orenetal* (86.2% in 3 days), *Enterobacter cloacae* (95% in 7 days), or *Micrococuss* sp. (20% in 48 h). Other bacterial strains like *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, or *Bacillyus cereus* were reported to use paraquat as the sole source of carbon and nitrogen between 1 and 5 days (Tu & Bollen, 2006). Besides bacteria, fungi like *Aspergillus tamarii* and *Cunninghamella* sp. were registered to have the ability to degrade 80% and 68%, respectively, of the paraquat from a synthetic liquid medium (Wongputtisin et al., 2021).

Fipronil, an insecticide developed in 1990, was banned in 2013 in European Union because of its toxicity (Simon-Delso et al., 2015). Since the first report regarding the ability of microorganisms to degrade fipronil, only nine bacteria species (*Paracoccus* sp., *Bacillus thuringiensis*, *Stenotrophomonas acidaminiphila*, *Acinetobacter calcoaceticus*, *Acinetobacter oleivorans*, *Burkholderia thailandensis*, *Streptomyces rochei*, *Escherichia coli*, and *Staphylococcus arlettae*) and one fungi species (*Aspergillus glaucus*) were identified as having this property. The minimum days needed by bacteria for degradation below the detectable limit were 20 days (*Paracoccus* sp.), while the maximum were 90 days (*Acinetobacter* *calcoaceticus* and *Acinetobacter oleivorans*). Compared to bacteria, the fungi *Aspergillus glaucus* took only 5 days to degrade fipronil (Gajendiran & Abraham, 2017).

Limited information about the microbial degradation of heptachlor, chlordane, or pentachlorophenol is available. Heptachlor was reported to be degraded mainly by fungi. A strain that successfully removed the heptachlor and its residues from liquid media was *Aspergillus niger*. Regarding other fungi strains from the *Trichoderma*, *Penicillium*, *Fusarium*, or *Rhizopus* genus, they degraded heptachlor to heptachlor epoxide, a compound that is more toxic than heptachlor (León-Santiesteban & Rodríguez-Vázquez, 2017). Pentachlorophenol was also described as being mostly degraded by fungi like *Byssochlamys nivea*, *Scopulariopsis brumptii*, *Trichoderma harzianum*, or *Rhizopus oryzae* (Bokade et al., 2021).

Chlordane, as in the case of the rest of the persistent pesticides described, is restricted to be used or even banned in most of the developed countries, but residues are still found in soil or other environments (Zhu et al., 2021). Studies regarding the ability of bacteria or fungi to degrade chlordane are still scarce. The existing data show that the most studied bacterial genus with good results regarding chlordane degradation is *Streptomyces*. The degradation rate registered for different strains of *Streptomyces* was high. For instance, in a study done by Fuentes et al., six different *Streptomyces* strains removed the chlordane from the medium in a proportion ranging from 91.3% to 95.5% (Fuentes et al., 2016). In another study, Cuozzo et al. (2012) demonstrated that after 24 h of incubation 6 strains of *Streptomyces* sp. removed between 97% and 99.8% of the chlordane from liquid medium and one of them 56% from the soil after 28 days. Other bacteria genera identified as capable of growing in chlordane contaminated soil, and probably having the ability to degrade it, are *Flavobacterium*, *Bacteroides*, *Clostridium*, *Bacillus*, *Sphingomonas*, or *Deinococcus* (Zhu et al., 2021). Regarding the ability of fungi, *Aspergillus niger* was able to remove in 8 days between 87.5% and 100% of the chlordane from the medium (León-Santiesteban & Rodríguez-Vázquez, 2017).

6 Conclusions

To combat the losses in agriculture have been used a wide variety of pesticides, including banned pesticides with high persistence, such as lindane, dieldrin, aldrin, endosulfan, parathion and parathion-metil, tributyltin oxide, DDT (dichloro-diphenyl-trichloroethane), pentachlorophenol, chlordane, heptachlor, paraquat, carbofuran, fenbutatin oxide, fipronil, and atrazine. Most organic banned pesticides belong to the insecticides based on chlorinated hydrocarbons, organophosphorus, carbamate, triazine, organotin, and pyrazole(phenyl-) insecticides classes. Although these pesticides are harmful to the environment and their production and use in agriculture have been banned in many parts of the world, they are still produced and used either for other purposes or even in agriculture by the third-world countries. As a consequence of their presence, they can be found for long periods of time after their application in the environment. The excessive use of persistent pesticides in agriculture has led to the accumulation of these compounds in environment compartments (air, water, soil). Furthermore, these pesticides are detected in fruits, vegetables, cereals, dairy products, meat products, chicken eggs, and aquatic organisms, thus increasing the risks to human health. Microorganisms have the great potential to degrade persistent pesticides. The main categories of microorganisms identified to biodegrade the persistent pesticides are bacteria, yeast, and fungi. Several studies have demonstrated that microorganisms belonging to *Microbacterium*, *Pandoreae*, *Streptomyces*, *Flavobacterium*, *Bacteroides*, *Clostridium*, *Bacillus*, *Sphingomonas*, *Deinococcus*, *Trichoderma*, *Penicillium*, *Fusarium*, *Rhizopus* and others can degrade aldrin, dieldrin, lindane, endosulfan, DDT, chlordane, paraquat, heptachlor, fipronil, or pentachlorophenol. Taking into account that the microbial degradation of persistent pesticides is a slow process, further studies should focus on finding a better formula to speed up the degradation, which combines the use of microorganisms and physicochemical methods. Also, information about the mechanisms involved in the degrading processes should be of interest, in order to obtain genetically engineered microorganisms with high abilities regarding the degradation of persistent pesticides.

Acknowledgments The authors contributed equally.

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Bioremediations for Oil Spills by Utilizing Microbes

Richa Verma, Vijay Kumar, Indu Bhardwaj, Ashish Jaiswal, and Sahil Kumar

1 Introduction

An oil spill is a seepage from ocean-going tankers, pipelines, or any other oil sources. It happens mostly and becomes a reason for immense ecological harm. Bioremediation for oil spills is a technique during which microorganisms are used to eliminate the contamination of hydrocarbons from soil and water and so make them sheltered for terrestrial and aquatic species. Bioremediation can be done by using bacterial species, fungal species, and plant species. Bioremediation that is made by utilizing fungal species is called mycoremediation, and the remediation that is made by the plant species is called phytoremediation. The spilled oil in the oceans destroys the earth's ecosystem and also have an extremely negative impact on the existing creature. The contaminant materials present in oil affect the entity of marine life. We know about the oil rigs and also how the oil is detracted from the sea bedding.

This extracted oil is utilized for multiple purposes, such as carriage, construction, and processes in various industries. During loading or unloading, ballasting, and tank cleaning, the oil spilled by tankers causes ocean contamination. An oil lapse is the leakage of fluid petroleum hydrocarbons into the climate by the action of humans. Contamination of marine water depends on which type of oil is suddenly dropped into the marine water. Any type of crude oil that spills into the ocean can be any type of crude oil or pure petroleum products such as oil mixed in waste, or oily refuse, gasoline, or diesel fuel. Whether light oil such as diesel oil is spilled, this oil does not remain in the atmosphere for a prolonged period of time because it evaporates readily, although it is toxic and highly flammable (Liu et al., 2010).

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_4

2 Petroleum

Petroleum is a much more complicated combination of a large variety of high- and low-molecular-weight hydrocarbons. This complicated combination of petroleum contains branched alkanes, saturated alkanes, naphthenes, alkenes, and aromatics, including aromatics containing hetero atoms like oxygen, sulfur, nitrogen, and different heavy metal complexes, large aromatic molecules like resins, asphaltenes, naphthene aromatics, and the hydrocarbon containing various functional groups like ethers and carboxylic acids. Heavy metals are present in unrefined oil, which is linked to pyrrolic structures known as porphyry (Tang et al., 2019). Petroleum is exposed to living matter in numerous ways, indirectly or directly.

There are certain products that are built up during petroleum rectification and processing that are utilized for the generation of different products that are supreme toxicants. Incessantly, these toxicant compounds are unintentionally liberated into the atmosphere and so on; hydrocarbons constitute the prime cause of atmospheric contamination. The toxicity of hydrocarbon particles and their utility to microbial metabolism depends upon their physical and chemical nature. Chemicals present in unrefined oil causes a variety of hazardous health impacts on human and animals, depending upon the degree of exposure and sensibility. The toxicant chemicals present in unrefined oil are able to harm organs of the human body such as the immune, nervous, respiratory, reproductive, circulatory, endocrine, and sensory systems and hence cause a broad spectrum of illness and deformation (Liu et al., 2010; Tang et al., 2019). The deformation due to the toxicity of unrefined oil to body systems can be instant, or this can take up numerous months. In spite of that, oil refineries produce a large amount of oily slush, which is a hydrocarbon waste. The Exxon Company and the US Environmental Protection Agency utilize microbes to clean Alaskan beaches that get contaminated by the Valdez oil spill, the process called bioremediation (Tang et al., 2019). There are physical, biological, and chemical methods. Physical modes comprise truck vacuums, skimmers, and booms. Chemical modes comprise surface collecting agents, dispersants, and surface washing agents. In the biological mode, microbial cultures, nutrient additives, and enzyme additives are used to enhance the rate of degradation of the pollutant (Tang et al., 2019; Atlas, 1981). In India, bacterial species have evolved to change oily slime and oil spills. Oil zapper or inoculant is impressive in spacious field trials as well (Singh & Singh, 2007). Bacteria and fungi chiefly biodegrade the hydrocarbons in the atmosphere. For soil fungi, the range of biodegradation is 6–82%, and for soil bacteria, it is 0.13–50% and 0.003–100% range for marine bacteria. Several scientists reported that mixed populations with enzymatic efficiency are expected to degrade complex combinations of hydrocarbons like unrefined oil in soil, marine environments, and fresh water. In petroleum degradation, bacteria are the most effective agents, and they chiefly work to degrade the spilled oil in the environment (Atlas, 1981). Bioremediation is done with the help of bacterial species, plant species, and fungal species (Wang et al., 2008; Bahadure et al., 2013).

3 Components That Influence Petroleum Hydrocarbon Degradation

There are a different number of factors that influence the biodegradation of petroleum hydrocarbons. Many of them are discussed here (Brusseau, 1998).

1. The conformation and the implicit biodegradation capability of the petroleum hydrocarbon contaminants is the first and predominant important consideration when the eligibility of a remediation approach is to be judged.
2. Among all the physical factors, temperature plays a significant role in the degradation of petroleum hydrocarbons by directly affecting the pollutants as well as by affecting the physiology and diversification of the microbial flora. At low temperatures, the viscosity of the oil is enhanced, while the volatility of low-molecular-weight hydrocarbons is reduced, so the degeneration rates decrease (Atlas, 1975). The solubility of hydrocarbons is also affected by temperature (Foght et al., 1996). Even the biodegradation of hydrocarbons can occur over a broad range of temperatures; the rate of biodegradation of petroleum commonly decreases with decreasing temperature. The temperature range at which the degradation is highest is in the range between 30 and 40 °C in the soil, in fresh water it is 20 and 30 °C, and in the marine environment it is 15 and 20 °C (Bartha & Bossert, 1984). Venosa and Zhu (2003) have stated that the vast temperature of the atmosphere impresses the characteristics of lapsed oil and also the activities of microbes (Pelletier et al., 2004; Delille et al., 2004).
3. Nutrients are also significant ingredients in the prosperous degeneration of hydrocarbon pollutants, particularly phosphorus, nitrogen, and iron (Cooney, 1984). Atlas (1985) reports that when a large oil spill occurs in freshwater and marine environments, supplies of carbon are enhanced and the availability of nitrogen and phosphorus commonly becomes the limiting factor for oil degeneration. In oceanic environments, it gets more clear due to low levels of nitrogen and phosphorous in seawater (Floodgate, 1984). Freshwater wetlands are typically nutrient deficient because of the bulky demands for nutrients by the plants. So, the summation of nutrients is necessary to increase the biodegradation rate of oil pollutants (Choi et al., 2002; Kim et al., 2005). In spite of that, intense nutrient concentrations may also prevent the degradation rate (Chaillan et al., 2006).
4. The negative effects of high NPK levels on the biodegradation of hydrocarbons have also been reported (Oudot et al., 1998; Chaineau et al., 2005), specifically on aromatics (Carmichael & Pfaender, 1997). The impact of fertilizers on unrefined oil bioremediation has also been studied (Pelletier et al., 2004).
5. Utilization of poultry compost as a biofertilizer in polluted soil was also studied (Okolo et al., 2005), and the biodegradation rate also increased in the presence of poultry manure. Photo-oxidation also enhanced the degradation rate of petroleum hydrocarbons by enhancing their bioavailability (Maki et al., 2001).

4 Mode of Action of Petroleum Hydrocarbon Degradation

Under aerobic conditions, very fast and complete degeneration of biotic pollutants occurs. Activation as well as inclusiveness of oxygen is the enzymatic reaction catalyzed by the enzymes oxygenases and peroxidases. The preparatory intracellular invasion of biotic contaminants is an oxidative process (Fig. 1).

The peripheral degradation pathways modify biotic pollutants into intermediates of the central intermediary metabolism, for instance, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, succinate, acetyl-CoA, and pyruvate. The degradation of petroleum hydrocarbons can be mediated by a specific enzyme system.

5 Bioremediation

The word bioremediation consists of two parts: bio and remediation. “Bios” means live organisms and the word “remediate” means to resolve an issue. So, the term “bioremediation” means utilizing biotic organisms to resolve an environmental issue such as contaminated soil, oil spills, or polluted groundwater. Bioremediation is the application of live microbes to degrade atmospheric contamination. Bioremediation is a technique to recapture environmental pollutants and so restore pure natural surroundings and prevent the environment from further contamination (Sasikumar & Papinazath, 2003). Bioremediation can easily be defined as a biotic process of cleaning the contaminated atmosphere. The environment can be terrestrial, aquatic, or both (Sardrood et al., 2013). In bioremediation, microbes have been applied for the treatment and alteration of waste products. Bioremediation is considered a new technology for the eco-friendly decontamination of contaminated atmospheres (King et al., 1997). Bioremediation is a naturalistic process and so it is understood by most people as an admissible waste treatment process for depraved

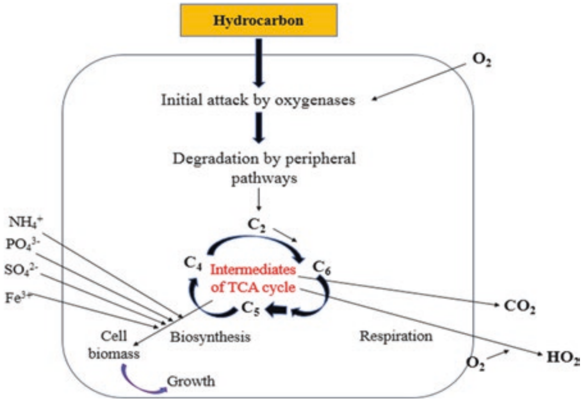

Fig. 1 Mechanism of degradation of petroleum hydrocarbon

materials such as soil and the ocean. Microbes have the potential to degrade pollutants. They increase in numbers when the contaminant is present, and when the pollution is degraded, the biodegradative microbe population falls. The remaining residues from the treatment are commonly harmless products and comprise carbon dioxide, water, and cell biomass. In nature, there are sufficient bioremediants that are used against a wide range of pollutants, and bioremediation is considered a practical technique for the overall degradation of a broad range of contaminants. Numerous compounds that are legally considered injurious and dangerous can be changed into harmless products. Bioremediation saves the bioweb and prevents the passage of hazardous and dangerous contamination from one ecosystem to another. Most of the bioremediation can be carried out onsite, often without causing a general disintegration of normal activities. This also displaces the need to transport the waste to the site and the potential risk to human health and the environment, which can rise during transportation. Bioremediation has proved less costly than other technologies that are used to clean up risky waste (Vidali, 2001). Yet, bioremediation technology bears two drawbacks. One drawback is that only certain bacteria and fungi work on a wide range of organic compounds. So there are not enough microbes to destroy chemical contamination in nature. Another drawback to bioremediation is that it takes a prolonged time to act and inflict its effect. Certain solutions are there to make us free of such a limitation. By using genetic manipulation techniques, an invaluable opportunity has been obtained to enhance new strains of bioremediation. Bioremediation works only on those compounds that are biodegradable. Biological processes are frequently highly specific. Significant factors required for well-turned bioremediation comprise the existence of metabolically able microbial populations, appropriate levels of nutrients and contaminants, and suitable environmental growth conditions. Contaminants can exist in the form of solids, liquids, and gases. Frequently, bioremediation takes longer than other treatment options, like excavation and incineration (Vidali, 2001). Although bioremediation is considered a credible technique for present environmental problems, it can also be considered problematic since additives used to encourage the activity of special microbes may disrupt the habitats of other microbes living in the same environment. Furthermore, genetically modified microorganisms that are liberated into the environment for a fixed period of time become difficult to remove. Bioremediation is very costly, and it takes several months of labor to complete the remediation of polluted environments. Nutritional imbalance can prevent biodegradation. An inadequate diet of nitrogen, phosphorus, potassium, and sulfur can limit the rate of degradation of hydrocarbons in the environment (McGill & Nyborg, 1975). There are adequate hydrocarbon-utilizing microorganisms in the soil that help in bioremediation as soon as nutrient limitation is reduced (Stone et al., 1942). Soybean lecithin, natural phospholipids, and ethyl allophanate are the best available nitrogen and phosphorus sources for the microbial bioremediants of oil contamination (Olivieri et al., 1978). No doubt, bioremediation is a necessity in the current world and can lead to the maintenance and preservation of natural resources.

6 Types of Bioremediation

Bioremediation is of two types on the basis of the place where wastes are removed, i.e., in situ bioremediation and ex situ bioremediation.

1. *In Situ Bioremediation*: In situ bioremediation is applied to eliminate the pollutants in contaminated soils and from groundwater. It is a preferable method to clean up contaminated environments since it saves transportation costs and, in it, harmless microorganisms are used to eliminate chemical contamination. These microbes have a better positive chemotactic affinity toward contaminants or pollutants. The next advantage of in situ bioremediation is the workability of isochronous treatment of soil and groundwater. Yet, in situ bioremediation also has some disadvantages, such as the fact that this method is more time-consuming than other remediation methods (Fig. 2).

   There are two types of in situ bioremediation that are distinguished based on the origin of the microorganisms applied as bioremediants.

   (1) *Intrinsic bioremediation*: It is carried out without direct microbial amendment and via intermediation in the ecological conditions of the contaminated region and the metabolic activities of naturally existing microfauna by improving nutritional and ventilation conditions.
   (2) *Engineered in situ bioremediation*: In this kind of in situ bioremediation, certain microbes are introduced to a contaminated site.

2. *Ex Situ Bioremediation*: Ex situ bioremediation is a process of bioremediation that takes place anywhere away from the contamination site, and so it needs transportation of contaminated soil or pumping of groundwater to the site of

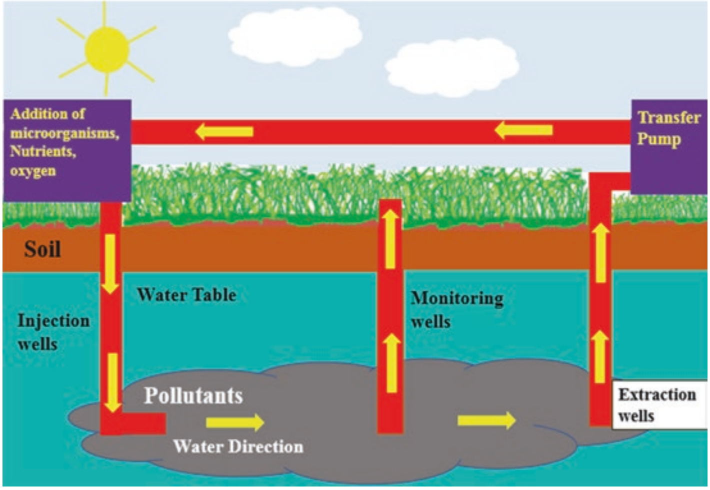

Fig. 2 In situ bioremediation to decontaminate groundwater and soil

bioremediation. This ex situ bioremediation technique has more disadvantages than advantages, as the steps of ex situ bioremediation are classified as follows:

(1) *Solid phase system*: Solid phase treatment includes treatments of land and soil. The system is used to cure domestic and industrial wastes, organic wastes, sewage sludge, and municipal solid wastes. In solid-phase soil bioremediation, these processes include land-farming and composting.
(2) *Slurry phase systems*: Solid–liquid suspensions in bioreactors are an example of slurry phase systems. Slurry phase bioremediation is a comparatively faster process than the other treatment processes. Contaminated soil is assorted with water and additives in a big tank called a bioreactor and combined to bring the indigenous microbes in nearby contact with soil contaminants. The optimum conditions in the bioreactor are adjusted so that an optimal environment for microbial bioremediation is provided. After completing the process in the bioreactor, the water is removed, and the remaining waste solids are disposed of.

7 Different Bioremediation Methods to Cure Oil Spills

Various techniques are employed either in situ or ex situ to eliminate toxic substances from the soil. The utility of techniques depends on the form and the intensification of the pollution. With the help of enzymes that are secreted by microbes, breakdown of the toxic compounds takes place. So, the water or soil becomes clear when the chemicals are taken up by the microbes (Wang et al., 2008). Bioremediation for oil spills is a technique that removes the contamination of hydrocarbons from water and soil. Oil leakage occurs mostly from ships, causing significant dangers to aquatic life. Because of the leaking of oil, including petrol, diesel, and other forms of hydrocarbons, from shipwrecks, mismanagement, and calamities, oceans have been discovered to be polluted with hazardous substances. When dirty water comes into touch with the soil, it pollutes it even more. The process of removing hazardous chemicals from the sea and soil is both difficult and costly. One of the most efficient ways of removing oil from soil and water and making them safe for aquatic and terrestrial organisms is bioremediation. Bacterial, plant, and fungal species are used in bioremediation techniques (Wang et al., 2008; Bahadure et al., 2013). There are three ways of cleaning up oil spills: physical, chemical, and biological. The biological approach, also known as bioremediation, is more beneficial than the physical and chemical methods since it saves time and money. Toxic chemicals are accumulated on the site via chemical processes, resulting in environmental contamination. Oil spills, whether they occur accidentally or on purpose, have a significant impact on environmental contamination. Oil spills from ships have long been recognized as a significant environmental risk. The spilled oil is thought to have mostly dispersed the habitat of marine animals, fish, and seabirds. The thick, sticky crude oil flows may immediately harm fish and marine species, seas, and coastal ecosystems and endanger human health over time (Tansel, 2014; Safiyanu et al., 2015). Because of its extensive usage, as well as the related disposal procedures and unintentional spills, crude oil pollution is relatively frequent.

8 Bacterial Bioremediation

Bacterial species such as *Pseudomonas* species are employed in bioremediation because they have the ability to break down hydrocarbons from gasoline and diesel, minimizing the impact of oil spills. *Pseudomonas alcaligenes* can break down polycyclic aromatic hydrocarbons, while *Pseudomonas mendocina* and *Pseudomonas putida* can eliminate toluene. The bacterium *Pseudomonas veronii* can destroy a wide range of aromatic chemical compounds. These oil-based chemicals are consumed by bacteria. They use the chemicals as substrates for metabolic processes. Oil spills may be cleaned up with these microorganisms, which are prevalent in soil and water bodies. Other bacteria that help with bioremediation include *Achromobacter*, *Flavobacterium*, and *Acinetobacter* (Atlas, 1981; Wang et al., 2008) (Fig. 3).

Toluene and other monocyclic aromatic hydrocarbons, such as benzene, toluene, and xylene, can be degraded by *Pseudomonas putida*. The production of diol and breakage of the aromatic ring, as well as the formation of diacids such as cis–cis muconic acid, occur when aromatic hydrocarbons are degraded by bacteria (Wang et al., 2008; Bahadure et al., 2013). Using bacterial species, many *Pseudomonas* species have the ability to breakdown hydrocarbons from gasoline and diesel, minimizing the impact of oil spills. *P. alcaligenes*, for example, can degrade polycyclic aromatic hydrocarbons, but *P. mendocina* and *P. putida* can degrade toluene. *P. veronii* is capable of degrading a wide range of aromatic chemical compounds. Bacteria consume these oil-based molecules and use them as substrates for metabolic processes. These bacteria may be found in large numbers in water bodies and soil, and they are capable of cleaning up oil spills. The process of bioremediation is

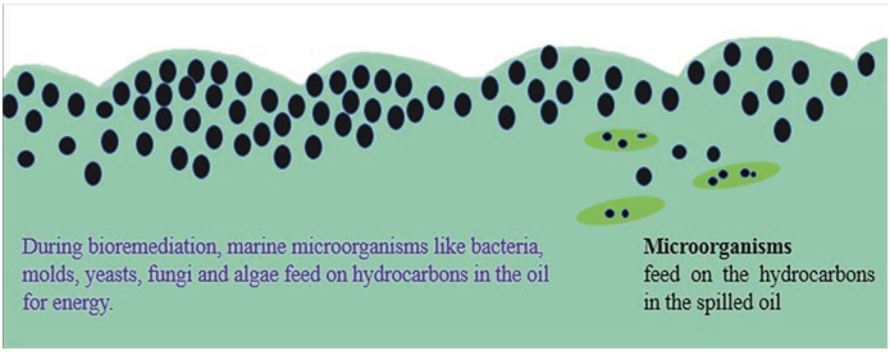

Fig. 3 Bioremediation processes by microbes

accelerated as the density of these microorganisms rises. *Acinetobacter*, *Flavobacterium*, and *Achromobacter* are some additional bacteria that aid in bioremediation. *Pseudomonas putida* is capable of degrading toluene as well as other monocyclic aromatic hydrocarbons such as benzene and xylene. The production of a diol followed by breakage of the aromatic ring and the generation of diacids such as cis–cis muconic acid is typical of bacterial degradation of aromatic hydrocarbons (Wang et al., 2008; Bahadure et al., 2013).

Algae, which are important components of the microbial community in both aquatic and terrestrial environments, have been found to participate in hydrocarbon biodegradation. Walker et al. (Walker et al., 1975) isolated *Prototheca zopfi*, an alga that can use crude oil and hydrocarbon substrates and degrades n-alkanes, isoalkanes, and aromatic hydrocarbons extensively. Cerniglia et al. (Cerniglia et al., 1980) looked at nine cyanobacteria, five green algae, two diatoms, one brown algae, and one red alga, to see if they could oxidize naphthalene.

9 Mycoremediation

It is the process of degradation of environmental toxicants with the help of fungi. In the natural world, fungi are the most significant decomposers. Fungi generate enzymes that aid in the degradation of cellulose and lignin. These two chemicals provide structure to plants and are extremely long-lasting. Fungi may also break down a few hazardous chemicals via similar processes (Wolski et al., 2012). It's used to clean up polluted soil, contaminated surface water, oil spills, industrial pollutants, and farm waste, among other things. *Lentinus edodes* (shiitake mushrooms) is an example of mycoremediation since it can breakdown pentachlorophenol (PCP), a broad-spectrum biocide that is more harmful than DDT (Fig. 4).

Pleurotus pulmonarius (Italian oyster mushroom) has capability to break down atrazine, a pesticide that contaminate groundwater in many Midwestern states. The white rot fungus, *Phanerochaete chrysosporium*, degrades chemicals like biphenyl and triphenylmethane (Wolski et al., 2012).

In the kingdom of Fungi, *Penicillium* species belong to the phylum Ascomycota. *Penicillium* species can be found in the air, on surfaces, and in food. *Penicillium chrysogenum* is generally present in salted meat, dried cereals, indoor air environments, salty soils, and marine water. *Penicillium* strains have been identified as the best hydrocarbon assimilates, with several publications demonstrating their capacity to convert xenobiotic chemicals such as phenol into less mutagenic products. Many companies that damage the environment create phenol (Atlas, 1981). Benzene, toluene, ethyl benzene, xylene, phenol compounds, and heavy metals including nickel, lead, and iron are all removed and degraded by the *Penicillium chrysogenum* strain. *Penicillium chrysogenum* and other fungi usually oxidize aromatic hydrocarbons and produce trans-diol (Pereira et al., 2014; Abdulsalam et al., 2012).

Fig. 4 Mycoremediation process by using mushroom

10 Phytoremediation

It is a new method that uses specific plant species to remediate various types of pollution in the environment, such as cleaning up groundwater and soils polluted with hydrocarbons and other harmful chemicals. Hydraulic control, phytovolatilization, rhizoremediation, and phytotransformation are all processes that may be utilized to remediate a wide range of pollutants.

For large sites with shallow residual levels of contamination by organic, nutrient, or metal pollutants, phytoremediation may be cost-effective if contamination does not pose an immediate danger and only "polishing treatment" is required, and vegetation is used as a final cap and closure of the site (Schnoor et al., 1995).

Phytoremediation has several advantages, including economic effectiveness, aesthetic benefits, and long-term application. Even then, using phytoremediation as a secondary or polishing in situ treatment stage reduces land disturbance and eliminates the transportation and liability costs of offsite treatment and disposal. Over the last 15 years, research and experimentation in phytoremediation for the treatment of petroleum hydrocarbons has yielded a lot of useful knowledge that can be used to design effective remediation systems and manage ongoing progress and innovation. Phytoremediation may be used to clean up a wide range of polluted areas. The possibility of using phytoremediation on hydrocarbon-contaminated areas was examined. The Alabama Department of Environmental Management approved a site with roughly 1500 cubic yards of soil and 70% of the baseline tests indicating total petroleum hydrocarbons above 100 ppm. Following a year of vegetative integument, over 83% of the samples had less than 10-ppm total petroleum hydrocarbon. Expulsion of total petroleum hydrocarbons from different field locations contaminated with petroleum refinery wastes, crude oil, and diesel fuel at initial TPH concentrations ranging from 1700 to 16,000 mg/kg has also been studied (Das & Chandran, 2011).

Plant growth has been discovered to be species-dependent. The presence of certain species resulted in a higher loss of total petroleum hydrocarbons than the absence of other species. Milo (*Thespesia populnea*), kou (*Cordia subcordata*), kiawe (*Prosopis pallida*), and the native shrub beach naupaka (*Scaevola serica*) have all survived field conditions and aided in the cleanup of diesel-polluted soils in the Pacific Islands (Kamath et al., 2004). When organic pollutants are present, the grass is frequently planted alongside trees as an initial remediation strategy. The presence of a large number of fine roots on the soil surface was discovered to have an impact on the binding and transformation of hydrophobic contaminants. Grasses are frequently planted between rows of trees to help stabilize the soil and guard against wind-blown dust, which can carry pollutants away. Some legumes, such as alfalfa (*Medicago sativa*), alsike clover (*Trifolium hybridum*), and peas, can help replenish nitrogen in depleted soils (*Pisum* sp.). Plants such as fescue (*Vulpia myuros*), rye (Elymus *sp*.), clover (*Trifolium* sp.), and reed canary grass (*Phalaris arundinacea*) are utilized successfully in many locations that have been contaminated with petrochemical wastes. The grass from these plants may be composted when they are harvested. It might be a very useful method for removing diesel-range organics from contaminated, vegetated soils (Miya & Firestone, 2001).

11 Use of Genetically Modified Bacteria for Bioremediation

The use of genetically modified microbes (GEMs) in bioremediation has got a lot of interest as a way to enhance the degradation of hazardous wastes in the lab. Genetic engineering technology has been used to revolutionize the bioremediation of hydrocarbon pollutants utilizing bacteria in numerous cases. The degradative effectiveness of the genetically engineered bacteria was greater. For effective in situ bioremediation utilizing genetically engineered bacteria, a combination of microbiological and ecological understanding, as well as biochemical processes, is required.

12 Conclusion

When it comes to cleaning up marine oil spills, bioremediation offers a lot of advantages over traditional physical and chemical approaches. One of the main benefits of bioremediation is that it is both cost-effective and time-saving when it comes to cleaning up a contaminated environment. When applied appropriately, the cost savings of bioremediation outweigh the disadvantages of standard cleaning methods. Bioremediation, unlike chemical techniques, does not require the use of harmful or foreign substances to clean up contaminated areas. Bioremediation does not cause the natural habitat environment to be disrupted, as physical and chemical cleanup approaches sometimes do. Bioremediation allows microorganisms to breakdown complex hydrocarbons into simple hydrocarbons with no negative environmental consequences. The world's major challenge in the subterranean environment is cleaning up petroleum hydrocarbons. By using different physical and chemical approaches, the microbial degradation process aids in the removal of spilled oil from the environment. It's possible because bacteria have enzyme systems that can breakdown and use various hydrocarbons as a carbon and energy source.

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Genetically Engineered Microorganisms for Bioremediation Processes

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1 Introduction

Although a diverse and particular microbial population can completely eradicate specific contaminants from the environment, the majority of toxins disintegrate slowly and so therefore tend to accumulate. Many of these contaminants have chemical characteristics that make it difficult for bacteria to degrade them (Dejonghe et al., 2000). Microorganisms have yet to develop relevant catabolic mechanisms to remove these chemicals due to their distinctiveness. Complex mixtures of pollutants are resistant to standard degradation mechanisms, or the communities of microbes that are responsible for this degradation are too small or inactive to properly convert these compounds (Bruins et al., 2000).

Using exogenous microorganisms to boost indigenous populations is one way to expand populations of microorganisms capable of precise pollutant breakdown. Bioaugmentation is a technique that involves introducing microorganisms that have been genetically modified or those that have been naturally endowed with the necessary genes (Gentry et al., 2004). This method can also be used to deliver plasmids containing sufficient genetic material to native microbes. New strains with beneficial bioremediation characteristics have been created as a result of recent advancements in molecular biology adapted to microorganisms. One of these is the development and control of novel pathways:

- Extending the substrate intervals of pathways without producing harmful metabolites
- Modifying the selectivity and affinity of catabolic enzymes
- Improving the genetic stability of catabolic activities (Paul et al., 2005)

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_5

The instability of the infused genetic material may restrict their application in the environment, despite the efficiency of GEMs in terms of bioremediation (Velkov, 2001). GEMs' ability to transport genetic material in a regular manner is essential to their function. Second, despite the fact that genetic material transmission is a common occurrence among indigenous species, it is regarded as a negative phenomenon. Scientists are investigating GEM durability, tenacity, and competitiveness, as well as the risks associated with their release into the environment. Table 1 shows how genetic engineering techniques can be used to improve bioremediation. In limited environments, these genetically modified bacteria have been shown to break down a variety of contaminants. However, biological and environmental issues, as well as bureaucratic constraints, make field testing GEM complicated. Before GEM can deliver a suitable clean-up solution at a cheaper cost, these challenges must be addressed.

2 Advancement and Implication of Genetically Engineered Microorganisms in Bioremediation

Pathway design and change of substrate affinity, enzyme specificity, expression and cellular location have resulted in innovative strains with important properties. It's also led to the development of new technologies for detecting GEMs and pollutants in the environment.

2.1 Gene Transfer Strategies

Xenobiotic substances can remain in the environment due to a variety of factors, and organisms are not involved in the degradation processes of such molecules. There is a lack of proper catabolic routes, inferior catabolic ability of pre-existing pathways, completely inadequate potential for substance uptake due to retention and hydrophobicity in soil due to progressing (Suidan et al., 2005). To circumvent these constraints, microorganisms can be genetically engineered, with the potential to develop strains capable of enormous in situ bioremediation (Furukawa, 2000a, 2000b).

Table 1 Genetic engineering for biodegradation of pollutants (Paul et al., 2005)

Microorganism	Modification	Contaminants
Pseudomonas sp. B13	Pathway	Mono/dichlorobenzoate
P. putida	Pathway	4-ethyl benzoate
P. putida KT2442	Pathway	Toluene
Pseudomonas sp. FR1	Pathway	Methylbenzoate
E.coli JM109	Substrate specificity	Benzene, toluene

Using traditional methods, bacterial strains with enhanced potency to bioremediate harmful chemical metals have indeed been established.

(a) *Catabolic Pathways*

Aromatic compounds are a diversified collection of pollutants in soil and water, making them a strong contender for bioremediation with modified bacteria. Reineke (1998) investigated how patchwork assembly may be employed to create chloro-aromatic breakdown-complete recombinant strains. This technique gathers a comprehensive system capable of mineralizing a given chemical by combining pathways from several bacteria into a single recombinant host. Hrywna et al. cloned and produced the ohb operon from *P. aeruginosa* and the fcb operon from *Arthrobacter globiformis* (both encode enzymes that may metabolize chloro-benzoic acids) in *Comamonas* testosterone strain VP44 (1999).

The genes that metabolize chlorinated biphenyls into ortho- and para-CBAs are identified in the host strain. Using plasmids with the ohb and fcb operons to evolve the host resulted in a mono-chlorobiphenyl mineralizing strain. A *Burkholderia* sp. strain transferred DNT genes for the 2,4-dintirotoluene breakdown pathway into *Pseudomonas fluorescens* ATCC 1740 (Monti et al., 2005). When 2,4-DNT was employed as the primary nutritional supply for the recombinant strain, it was entirely digested, and the carbon produced was co-metabolized by the cell. The recombinant strain outperformed *Burkholderia* in terms of breaking down DNT at relatively low temperatures and non-toxicity to a particular species under specific surroundings. Genes from the *Comamonas* sp. strain CNB1 have also been transcribed and generated in *E. coli* to establish a purported preferred oxidation–reduction pathway for 4-chloronitrobenzene and nitrobenzene (Wu et al., 2006).

(b) *Engineered Bacteria: Enhanced Bioremediation of Mixed Waste and Metals*

Radionuclides, heavy metals and organic compounds are a few of the pollutants found in trash heaps. Bioremediation of organics in such environments is arduous due to the radiation from these radionuclides, which is hazardous to most microorganisms. *Deinococcus radiodurans* is an excellent host for genetic engineering procedures using mixed waste because of its increased tolerance to rapid ionizing radiation exposure. The modified strain was shown to successfully oxidize 3,4-dichloro-1-butene, chlorobenzene and toluene in a highly incinerating environment (Lange et al., 1998).

Renninger et al. (2004) utilized an integral strategy for uranium bioremediation by upregulating polyphosphate kinase in modified *Pseudomonas aeruginosa*. Underneath the impact of the tac-lac promoter, endogenous genes for polyphosphate synthesis and degradation were reproduced in a plasmid with a broad host range. When compared to the control strain, the transformed strain accumulates 100 times the quantity of polyphosphate. A substantial amount of phosphorus is liberated when the polyphosphate is degraded, which couples with the uranyl group and condenses at the cell membrane. Heavy metal bioremediation genetic change has also been carried out on *E. coli*. Crameri et al. (1997) used the arsenate resistance operon from *Staphylococcus aureus* and DNA shuffling techniques to build an

Table 2 Engineered bacteria involved in remediation of heavy metals

Microorganisms	Gene	Heavy metals	Reference
HgR *E. coli*	*mer A*	Hg	Gomes et al. (2013)
Salmonella choleraesuis strain 4 A	*SmtAB*	Pb	Naik et al. (2012)
Deinococcus radiodurans strains	*mer A*	Hg	
Achromobacter sp. AO22	Hg reductase expressing mer gene	Hg	Nagata et al. (2009)
Enterobacter sp. CBSB1	*Gcsgs*	Pb, Cd	Qiu et al. (2014)

arsenate detoxification route. The genetic modified bacteria have a significant metabolizing capability and have been shown in controlled settings to assimilate a range of pollutants. The genetically modified bacteria used in bioremediation are listed in Table 2.

2.2 *Variations of Genes That Encode Biodegradative Enzymes*

Engineered strains with superior bioremediation capabilities can be created since genes can be transferred from one species to another. These genes can be managed to support degradative proteins by increasing their specialized activity, quantity and site-directed mutation as well as directed evolution (Ang et al., 2005). In vitro mutagenesis and recombinant DNA can also be applied to generate hybrid genes that code for beneficial fusion proteins, or to contribute different transcriptional promoters and translational start sites to enhance enzyme expression.

(A) *Alterations of Enzyme Affinity and Specificity*

Oxygenases are enzymes that aid in the reduction of oxygen by adding one or two oxygen atoms to the oxidized substrate. They can use this method to ionize a range of organic pollutants (Parales et al., 2002). Anaerobic breakdown pathways, such as the anaerobic conversion of TCE to vinyl chloride, can generate toxic metabolites (Ensley, 1991). Oxidase protein engineering can be used to improve the effectiveness of oxidative pollution elimination. Furukawa et al. (2004) highlighted research that demonstrated how oxygenases involved in the dissolution of a wide range of aromatic compounds might be adjusted in terms of substrate specificity relaxation and degradation rate acceleration. Exchanging genes encoding homologous components from adjacent animals, swapping parts of such genes, DNA shuffling and site-directed mutagenesis of critical amino acids were among the biochemical processes used to achieve these changes. The laccase gene (mtL) from the fungus *Myceliophthora thermophia* is expressed by *S. cerevisiae*. Laccase can assist in the biodegradation of polyaromatic hydrocarbons and the breakdown of lignin (PAHs). Through error-prone PCR and in vivo shuffling, direct evolution produced a 170-fold more active mtL encoded laccase than the wild type (Bulter et al., 2003).

Methods for Upregulating Non-modified Coding Regions

The Palk promoter from *Pseudomonas* sp. P51 was used to make *E. coli* with the chlorobenzene dioxygenase (CDO) gene. In this strain, the lac promoter regulated CDO gene transcription, resulting in three times the amount of CDO produced by an identical recombinant *E. coli* strain. Furthermore, when compared to the lac promoter, the Palk promoter has better transcriptional control. The resultant strain was capable of catalysing benzonitrile and other aromatics cis-dihydroxylation as well as contributing to biodegradation (Yildirim et al., 2005). Utilizing PCR primers arbitrarily inserted 4–17 bp upstream of the dszB start codon, Reichmuth et al. (2004) generated a list of dszB hybrids with distinct ribosome binding sites. When integrated with the rest of the dsz operon, a proportion of these alterations resulted in nine-fold greater dibenzothiophene to hydroxybiphenyl transformation than the wild-type dszB ribosome-binding site strain.

(B) *Fusion proteins having a distinct significance*

The enzyme organophosphorus hydrolase (OPH) degrades organophosphate insecticides. Using genetically modified *E.coli*, OPH fusion proteins with domains that allow them to be produced on the cell surface were developed (Wang et al., 2005). In a model bioreactor, the cloned cells digested organophosphorus insecticides well, providing them a significant advantage over intracellular expression because the cell membrane hindrance to substrate transit would not have been an issue (Mulchandani et al., 1999). In recombinant *E. coli*, a heterologous bacterial OPH gene was coupled to a signal sequence to stimulate its extrusion to the periplasm. When contrasted to a modification in cytosolic OPH expression, this resulted in a 1.8-fold rise in OPH activity (Kang et al., 2006). It means that during organophosphate breakdown, OPH's periplasmic expression defies substrate dispersion restrictions.

3 Stability and Survivability of Genetically Engineered Microorganism and Genetic Transmission to Bacteria

The propensity of a strain to reproduce and disseminate its modified genotype in naturalistic situations and the extent to which it can transmit undesired genes to native species are the key considerations when using bacterial GEMs as biosensors or bioremediators (Pieper & Reineke, 2000). As a result of these considerations, studies into the survival, tenacity and conflict of GEMs released into the wild have been conducted.

4 Survivability of Genetically Engineered Microorganisms

In order to be effective under the environment, a bacterial GEM must be able to live and reproduce in such settings. In this regard, inoculum size, growth rate and environmental circumstances such as spatial dispersion and the presence of competing microorganisms and predators are all key determinants. The spatial distribution of injected GEMs in the environment is important because it affects how they interact with the indigenous microbial population and other ecology variables (Dechesne et al., 2005). A bacterium that has been taken from its natural habitat is more likely to survive when placed back into that ecosystem than one that has spent days in a laboratory. A plasmid-bearing GEM's rate of multiplication is a crucial predictor in its survival and implantation in the milieu. Plasmid-free cells are thought to have a significant advantage over plasmid-bearing cells due to plasmids' ability to boost metabolic strain (Diaz-Ricci & Hernandez, 2000). While they compete for nutrients with natural flora, this could be a tripping topic in the field when it comes to creating substantial and lengthy GEM colonies for bioaugmentation (Top et al., 2002).

4.1 Acquisition and Structural Fragility of Recombinant DNA

Plasmid stability is strongly related to phenotypic stability in the field when a bacterium is genetically transformed using a plasmid. Medium composition, pH, oxygen availability, copy number, temperature and variation are all factors that affect the stability of plasmid vectors. Segregational plasmid instability occurs when one or both daughter cells refuse to endorse at least one plasmid during cell division. Due to alterations, deletions, and insertions to regions inside the plasmid vector, structural instability can interrupt the information on the plasmid vector without enabling the overall plasmid to be abandoned (Sharp et al., 1998).

4.2 Effect of Genetically Engineered Microorganisms on Microbiota

The impact on ecosystem structure and function while transporting synthetic bacteria into agricultural regions is a critical challenge. Although molecular methods have been applied in research work in some instances, miniature investigations provide the preponderance of known information in this sector, indicating that the latter technique has a lot of potential.

4.3 Horizontal Transfer of DNA in Bacteria

Horizontal recombinant DNA transfer is sometimes confused with horizontal gene transfer, which is a widespread occurrence. Numerous studies have demonstrated the importance of horizontal gene transfer in bacterial evolution (Dennis, 2005). The exposure of microbial communities to organic contaminants is thought to be a crucial step in the development of unique biodegradative potential. Transposable elements, conjugative plasmids and integrative and conjugative transposons all appear to aid in the transmission of genes that code for biodegradative activity. Both the plasmid-containing cells, specific growth rate and different concentrations of substrate had a massive effect on the rate coefficients, showing that the cells' energy to facilitate transference was restricted (Rittmann et al., 2006). Horizontal gene transfer of recombinant genes can be done swiftly using the same methods as before. However, any horizontal DNA transfer from GEMs is predicted to occur mostly in non-recombinant individuals with the same genes at such reduced levels that any influence from GEMs is likely to be minimal.

4.4 Effects of Horizontal Recombinant DNA Transfer as Well as Other Heterologous Species on Native Flora

Horizontal recombinant DNA transfer occurs when GEMs are placed in polluted regions to induce bioremediation, and there is fear that this could have harmful environmental repercussions. Even if the imported strain does not sustain, plasmid transfer from an acquired GEM to an indigenous microbe can occur in extreme circumstances (Peters et al., 1997). In general, the impact of GEM importation on native microbial populations appears to be inconsistent, and each case must be evaluated separately. Dejonghe et al. (2000) investigated the exchange of two recombinant plasmid vectors that incorporate the 2,4-D breakdown pathway from host *P. putida* UWC3 to bacteria isolated in a 2,4-D-contaminated sandy-loam soil microcosm. Following the conjugative transmission of these genes to a variety of native bacteria and trans-conjugant growth, different communities emerged that were more effective in removing 2,4-D from the soil. DeFlaun et al. (1987) used recombinant copies of two naturally produced plasmids that generate 2,4-D breakdown enzymes to explore hgt in soil microcosms.

5 Suicidal Genetically Engineered Microorganisms

Because environmental safety is necessary, transposition vectors without antibiotic resistance genes must be developed, as antibiotic vectors are unsuitable for this purpose. Combining lethal genetically modified microorganisms with bacterial contaminant systems is the most efficient strategy for mitigating risk coupled with transgenic microorganism ecological emission. GEMs have the power to destroy; nevertheless, fast advances in the manufacture of suicidal genetically altered bacteria will make it possible in the near future to use GEMs expressing suitable P450 for bioremediation of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) contaminated soil.

Paul and his colleagues created a genetic model to anticipate the unpredictable behaviour of genetically modified microbes. Killer genes are activated when the chemical is no longer present, killing the GEM. Killer genes on plasmids have been proven to stop horizontal gene transmission by killing the microbial recipient during the transfer. The potential issues connected with introducing genetically engineered bacteria into the ecosystem are eliminated with this strategy. This is an approach for lowering the risks of genetically modified bacteria while also avoiding uncontrolled microorganism expansion for successful bioremediation. Authorities and scientists have failed to take into account most of these pollution technologies while constructing bioremediation microorganisms, which is unfortunate. GEMs can have one of two outcomes: the organism can do the required action and then totally remove the GEM from the surroundings, which is the desired conclusion. The organism can survive and grow instead of being killed, which is a less well-known possibility. Because recombinant bacteria that survive in the environment may have negative consequences on ecosystems, the first option is recommended (Paul et al., 2005).

6 Bacterial Plasmid Addiction System

Plasmids are recognized for containing genes that code for a number of helpful properties for the host in specific situations, such as resistance to hazardous substances, chemical breakdown ability, pathogenicity and toxin production. Plasmid copies are exchanged between generations during cell division, and plasmid-free isolates are unable to survive due to plasmid-encoded processes or plasmid addiction. Koyama et al. (1975) emphasized the importance of an addiction mechanism for proper plasmid maintenance in cells.

Poison–antidote, post-segregational killing, toxin–antitoxin and plasmid–addiction system are two terms used interchangeably. The terms killing–anti-killing and planned cell death are used to describe scenarios in which the host cell is purposely destroyed such that no plasmid survives cell division. The killing–anti-killing system requires the expression of two genes: a toxin/poison gene and a antitoxin/antidote gene. The half-life of the killer toxin is lengthy, while the half-life of the anti-killing toxin is brief. Antidotes work by neutralizing or suppressing the production of the toxins they're meant to counteract. In plasmid-free cells, toxin inactivation is based on the fact that toxin and antidote degradation rates differ. Toxin–antidote combos act as plasmid addiction mechanisms by eliminating plasmid-free cells from the population of plasmid-bearing cells (Pandey et al. 2005).

7 Techniques for Tracking GEMs

It's vital to locate and measure GEMs in a variety of microbial specimens to assess the potential liability of gene segments and their potential horizontal gene transfer to other existing microbial communities. There are several options in this field, but one that is real-time, convenient, reputable and cost-effective should be considered.

7.1 PCR-Based Techniques

Counting the number of colonies that have formed on plates is a common way to identify GEMs. This method is straightforward, although it has limitations in terms of sensitivity and accuracy. These limitations can be solved with the use of molecular technology. A southern hybridization-based approach has limited sensitivity for detecting soil organism DNA, but PCR-based nucleic acid amplification of a sample measures both dead and live cells. The MPN-PCR method requires diluting soil samples in triplicate and comparing the occurrence of microbes in each concentration to a database. The viability of an organism is determined by the proficiency of target gene sequence amplification rather than the quantity of living cells. Similar to MPN-PCR, cPCR compares the value of final DNA in a sample to standard templates, providing details (Widada et al., 2002).

7.2 Fluorescent-Based DNA Hybridization Technique

To detect the presence of a specific bacterium, unique fluorescent-labelled DNA probes of a particular strain are used. Researchers employed fluorescent labelling of a precise ribosomal RNA probe to distinguish and count *P. fluorescens* cells after they were introduced into a microcosm. Because ribosomal RNA rises with cell growth rate, the metabolic condition of the cells at any given time can be easily determined. Due to the hybridization phase, this method has a disadvantage in that it takes a lengthy time to finish the procedure (Boye et al., 1995).

7.3 Bioluminescence-Mediated Technique

GEM's phenotypic features are detected through the selective abilities of recombinant organisms, such as bioluminescence or the formation of coloured compounds. The enzymes xyl E, lac A and gus A, respectively, are encoded by the genes xyl E, lac A and gus A. By encoding uroporphyrinogen III methyl transferase, a genetically modified bacterium cloned with the lux, luc and cob A genes produced a bioluminescent product (Feliciano et al., 2006).

7.4 DNA Microarray Technique

Non-recombinant cells and GEMs are identified and counted using DNA microarrays, which use both DNA and rRNA as probes. The sensitivity and specificity of this approach limit the degree of quantification (Cho & Tiedje, 2002). A new detection approach can be used to identify GEMs from indigenous people. By comparing the gene sequences of GEM and the 5S rRNA gene of *Vibrio proteolyticus*, the engineering of *E. coli* with the 5S rRNA gene of *V. proteolyticus* may be easily confirmed (Hedenstierna et al., 1993). Single cells interacting with monoclonal antibodies can identify GEMs with unique surface protein genes *phoE-caa* (Zaat et al., 1994).

8 Molecular Techniques for Generating Genetically Modified Microorganisms for Bioremediation

8.1 Molecular Cloning

Cloning is a technique for making multiple copies of a gene, investigating gene function and making multiple copies of a gene. For molecular cloning, a plasmid vector is required, as is the copying or synthesis of a DNA fragment with a defined purpose. Plasmids are small circular DNA molecules that proliferate autonomously of their host bacteria's chromosomal DNA. Plasmids that have been redesigned are inserted into the host species and permitted to multiply. The inserted DNA fragment is replicated along with the majority of the bacterial genome during cell division. The vector is made up of a lot of small DNA sequences that restriction endonucleases can digest. Endonucleases that recognize and cleave a specific region in DNA sequences to produce sticky DNA are known as restriction endonucleases. Palindrome sequences, which are four to eight nucleotide sequences, are recognized by the majority of restriction enzymes in plasmid DNA. This means that the complementary nucleotide sequences in the forward and reverse directions are nearly identical. Using the enzyme DNA ligase, digested DNA fragments with sticky ends of both foreign and host DNA are annealed together to generate double-strand DNA. As a result, plasmids with a foreign gene are referred to as recombinant DNA, and the proteins they create are referred to as recombinant proteins. Certain environmental conditions can boost or stifle protein production, giving scientists more control over how a protein is expressed.

8.2 Electroporation

Electroporation is a quick and easy way to get a foreign gene into a bacterial host. To take the DNA in, high-voltage electric pulses are employed, inducing transient penetration of the plasma membranes. In a tank containing an appropriate buffer, foreign DNA and the protoplast of the host cells are held between two electrodes. The protoplasts are positioned using a 1 MHz electric current in di-electrophoresis. The electrostatic force causes membranes to disintegrate and openings to form, allowing DNA to pass through efficiently. Dc power pulses of 1–3 kV are being used to induce fusion after the DNA has been delivered into the host organism. Electroporation can be used to electroporate foreign DNA with a molecular size of up to 240 kb. Because genome sequencing demands long DNA segments, this characteristic gives this technique an advantage.

Field strength is determined by several factors:

(i) Electric pulse voltage, resistance and capacitance
(ii) Temperature and pH
(iii) Density and protoplast size
(iv) Host cell and genetic features
(v) post-pulse therapy impact

8.3 Protoplast Transfusion

The protoplast transformation process involves PEG-induced DNA absorption in protoplasts and subsequent cell wall rebuilding. This method can change up to 80% of plasmids and is better suited and efficient for even the most esoteric plasmids. Protoplast transformation involves the following steps: hypertonic DNA therapy and PEG treatment. The following are the essential elements that govern protoplast transformation mediated by PEG:

(a) Culture conditions and cell density – late log phase cells are ideal for transformation.
(b) DNA concentrations of 0.1–1 g trigger swift transformation.
(c) Tonicity – sucrose, sorbitol, potassium and sodium chloride, lithium and ammonium chloride all induce transformation at particular doses.
(d) pH of 3.5–5 was shown to be optimum for PEG mediation protoplast transformation.
(e) The influence of temperature and reaction time – effective DNA uptake in protoplast is caused by a 10-minute reaction period and a temperature of 22 °C.

8.4 Biolistic Transformation

The gene of interest is encapsulated in amorphous tungsten or gold beads with a diameter of 0.36–6 m and transferred to recipient bacterial cells through helium gas stimulation through a halting screen in biolistic transformation. The foreign DNA is maintained inside the bacterial cells after the pellet DNA molecule passes through them. When the helium pulse sweeps the microcarrier-coated DNA in the specimen cartridge through the barrel, the target is retained, enabling it to reach the host cell properly. This method uses a simple transformation mechanism and does not require the use of a binary vector. The method's significant shortcomings include the complexity of establishing single-copy mutant events, ridiculous prices of apparatus and microcarriers, random intracellular localization and the inability to fulfil single-copy transgenic events.

9 Obstacles Associated with Use of GEMs in Bioremediation

While genetic engineering has resulted in a plethora of strains capable of dissolving ordinarily inaccessible pollutants in a Petri plate or bioreactor, in situ bioremediation techniques have seen little use of this expertise (Sayler & Ripp, 2000). A major cause of concern in this research is the rising recognition that the strains and bacterial species most typically used in traditional enrichment approaches do not conduct the majority of biodegradation in naturalistic conditions and may even be ineffective as bioremediation mediators. According to stable isotope probing (SIP) and analogous initiatives in microbial ecology, *Pseudomonas*, *Rhodococcus* and the typical aerobic prompt growers that are typically selected as hosts of biodegradation linked chimeric genes are substantially less pertinent under benchmark instances (Wackett, 2004). When fast-growing plants are used as biodegradation agents, excess biomass will inevitably accumulate. The best clean-up agent has the highest catalytic ability and the lowest cell mass on either hand. Biodegradation gene expression can be segregated from proliferation using stationary phase or restricted promoters (Matin, 1994). Moreover, substantial advances in recombinant DNA technology have paved the path for the development of suicidal genetically engineered microbes (S-GEMS) to eliminate such risks and allow for more secure and reliable removal of pollutants (Pandey et al. 2005).

It doesn't matter if the bacteria transplanted are recombinant or not in some cases because the concern is the implantation of foreign germs in a new environment. The insertion of bacterial biomass into a pre-existing niche may provide protozoa with a favourable environment, inhibiting bacterial overgrowth (Iwasaki et al., 1993). To get around this problem, creative solutions have been proposed, such as encasing the inoculum in plastic tubing or encapsulating it in a polymeric matrix. Its efficacy is determined by the presence of sufficient in-situ enzyme activity in the target area (Foster et al., 2002).

A field release of *P. fluorescens* HK44 for bioremediation application was successfully carried out on a reasonably large scale and in controlled field settings (Ripp et al., 2000). However, there will be concerns about the discharge of genetically modified bacteria into the atmosphere if they are employed to clean up pollution in the future. The risk of using other changed microbes in the foreseeable is still unknown. As a consequence, researchers will investigate the future opportunities of genetically modified bacterial strains in field conditions, which will aid in determining the hazards associated with utilizing genetically modified bacteria in ecological bioremediation. The microorganisms that are used in productive bioremediation technology are exposed to extreme field conditions, which is the technique's principal disadvantage. In order to create modified microorganisms, researchers must investigate other bacterial strains. The distinctive characteristics of open biotechnological applications have obviously prompted the creation of modified bacterial strains to solve new challenges.

The fundamental difficulty is to develop genetically engineered bacteria that can be employed for bioremediation in the field while being ecologically friendly. In the vast majority of situations, bacteria used in bioremediation techniques were created in the lab for a specific purpose, ignoring field conditions and other demanding scenarios. There is no indication, on the other hand, that using genetically engineered bacteria for bioremediation has any discernible negative influence on the natural microbial community. The overblown idea of risk assessment has sparked a lot of debate and inquiry in the field of environmental microbiology from the beginning. According to a new assessment, the survival of genetically modified microorganisms in complex environments is a major concern that must be dealt (Singh et al., 2011).

10 Advantages and Disadvantages of GEMs

The following are some of the advantages and disadvantages of using GMOs:

10.1 Advantages

- Faster crop development and production, as well as larger yields, less fertilizer, less herbicides and much more micronutrients are all partly attributable to GMO technology.
- Traditional breeding includes the transformation of multiple genes at random to the generation, whereas genetic engineering entails the mobility of a block or specialized grouping of genes at a specified period.
- Even though genetically modified organisms (GMOs) are not natural, they are not always efficient. Despite the fact that lethal mushrooms exist in nature, they can be genetically modified to become edible.

10.2 Disadvantages

- The majority of GMOs have not been properly evaluated, and a standard GMO test only takes 90 days.
- GMOs generated by transgenic modification are not considered natural, and their consequences remain unknown.
- Regardless of the fact that GMOs were aimed to minimize pesticide consumption, there is no assurance that the crop will be acceptable if these recombinant microorganisms are utilized.
- GMO testing frequently employs animal testing, which some claim is a violation of animal rights.
- GMO-based products are not adequately labelled, and it is still impossible to know whether products generated from genetically modified organisms are safe for human consumption.

11 Conclusion and Future Aspects

The eventual promise of GEMs in bioremediation may be constrained to difficulties that are simply not cost-effectively addressed by chosen field therapeutic approaches. Alternative options exist, and manipulated microbes could be used in restricted reactor technologies for bioremediation or waste treatment in the years ahead. In a wider sense, greenhouse gas reduction, carbon sequestration and waste conversion to value-added commodities are instances of these application scenarios. Pollution prevention has been proved to be more cost-effective and environmentally friendly, and demand for waste site remediation technologies is envisaged to drop significantly. Components must be reused or recycled for pollution prevention to be efficient, and this presents a new opportunity for the use of GEMs in bioremediation. Before such noticeable developments can be accomplished, however, a fundamental knowledge collection on GEM effectiveness under severe environmental conditions must be formed. Only thorough field research and a comparative life cycle analysis that considers both risk and biotechnology benefits will be able to implement this.

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Microbial Modifications and Biochemical Pathway: Mechanism for Ecosystem Decontamination

Gulzar A. Rather and Madhu Raina

1 Introduction

Microorganisms or microbes are cosmopolitan in distribution. The feature owes to their easy growth in diverse environmental conditions coupled with their quite striking metabolic capabilities. The nutritional adaptability of microbes paves a window for them to be utilized in the biodegradation of highly hazardous pollutants, a process known as bioremediation. A large amount of toxic chemicals is being discharged into the environment during different processes, which contaminate the same. This is done either purposely during pesticide application or fortuitously, for instance, during oil spills. Being one of the "Top 10 Biotechnologies to Improve Global Health" (Daar et al., 2002), bioremediation employs the capabilities of numerous microorganisms like bacteria to degrade, amend/alter, and exploit hazardous organic compounds/pollutants in the atmosphere and transform the same into less or nontoxic compounds or elemental forms. Such toxic compounds include benzene, dioxins, toluene, and nitro-aromatics. Being a microbiological and well-organized technical activity, bioremediation accounts for biomass and energy production (Tang et al., 2007). The biological agents used as significant tools for the removal of pollutants from the soil, sand, sediments, and water are termed as bio-remediators. Bacteria, fungi, and archaea act as emblematic bio-remediators (Strong & Burgess, 2008), reinstating the unique expected surroundings and averting secondary pollution (Demnerova et al., 2005). Notwithstanding the isolation and engineering of a myriad collection of bio-remediators, the unambiguous tender of these

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_6

microorganisms toward the bioremediation process has not been advanced with the same impetus as their discovery. However, as our understanding of science and technology developed more, there have been some key advances in the microbial research fields to design genetically engineered microorganisms (GEMs) for the purpose of bioremediation (van der Meer et al., 1992; Pieper & Reineke, 2000; Dua et al., 2002). Even various bioremediating microbes have also been successfully isolated.

Genetic engineering technique has emerged as a way to improve the utilization for purging of hazardous redundant wastes under controlled laboratory conditions by producing GEMs (Jain et al., 2011). These organisms are capable of carrying a gene suitable for the production of specific enzymes, which can easily degrade various chemical pollutants (Jain et al., 2010). Such organisms have publicized the latency for bioremediation applications in groundwater, soil, and triggered slush environments, demonstrating superior debasing capabilities, encircling a broad range of hazardous chemical contaminants. In recent times, myriad prospects have been brazening for convalescing the destructive performance via genetic engineering strategies. The most important are the manipulations brought in the rate-limiting phases in various metabolic pathways. Through genetic engineering, the enzyme-catalyzed reactions can be hastened so as to achieve increased degradation rates. Alternatively, an altogether new biosynthetic pathway can be integrated into microbial strains for the disintegration of formerly obstinate compounds. For this, GEMs work on different strategies: (a) modifying substrate affinity toward enzymes, (b) constructing a pathway and regulation of the same (Demnerova et al., 2005), and (c) monitoring, bioprocess development, and control (Fantroussi & Agathos, 2005), applications of bio-affinity, bioreporter sensors for chemical detection, toxicity attenuation, and finish face analysis. Genes specifying enzymes particularly important during catabolism of different remarkable substrates are carried on plasmids. Development of GEMs can thus be used sharply for bio-degradation purposes (Kulshreshtha, 2013). Several field trials for the use of such organisms have already been carried out (Sayler & Ripp, 2000).

Compared to various biotechnological breakthroughs over the past two decades, genetically modified crops, drugs, etc., have proven to be a boon owing to their successful commercialization. This is unlike that of genetically engineered microorganisms employed for bioremediation purposes (Sayler & Ripp, 2000; Watanabe, 2001). Since the United States Environmental Protection Agency (EPA) commenced the legalization of genetically engineered microorganisms nearly more than 25 years back, there has been no commercialization of any such transgenic microorganism for bioremediation to date. The possible reasons conjectured for the same have been the complexity, cost, and burdensome regulation (Watanabe, 2001). However, that explanation apparently does not present the full picture.

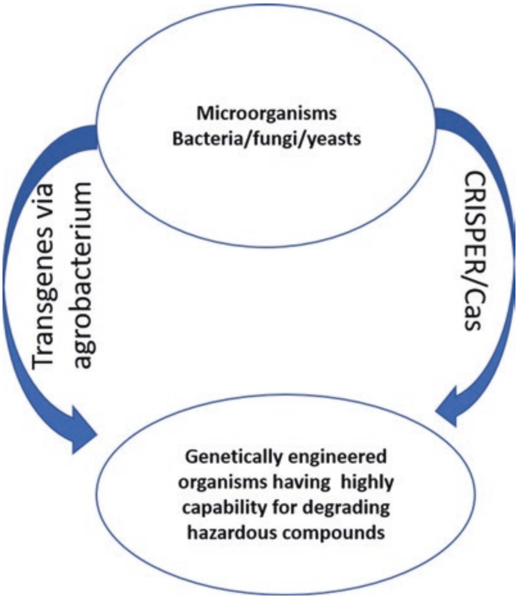

Fig. 1 In many plant species, agrobacterium-mediated insertion of transgenes specific for degradation of several compounds and taking advantage of advanced technologies like CRISPER/Cas for the genetic modification of microorganisms to make them capable of degrading hazardous organisms

2 Basic Principle of Bioremediation

As mentioned earlier, bioremediation involves the bio-degradation of hazardous waste products under controlled conditions (Fig. 1). This converts the noxious components to an inoffensive state or to low levels under concentration frontiers laid down by concerned regulatory authorities. This task of contaminant obliteration has been well suited to microbes. Reasonably, microorganisms are rich in different enzymes that allow them to exploit environmental contaminants as their food resource.

Bioremediation thus aims to persuade them to work by contributing threshold nutrients and other essential metabolites so as to degrade the substances responsible for toxifying the environment and the life therein. All metabolic processes are enzyme-catalyzed reactions, and different enzymes are destined to accomplish different but specific reactions. Accordingly, six classes of enzymes are available: oxidoreductases, hydrolases, transferases, isomerases, lyases, and ligases. Despite specific substrate affinity, many enzymes tend to be slightly nonspecific and have a surprisingly broad degradation competence. Bioremediation works on the principle that microorganisms ought to employ their enzymes to act on pollutants and convert the same to nondetrimental products. For bioremediation to be operational and quite effective, the environment must provide conditions favorable for microbial growth and activity. It has been seen that bioremediation frequently engrosses the maneuvering of environmental factors so as to tolerate microbial growth and the degradation process and to ensue at a faster pace (Kumar et al., 2011; Meenambigai et al., 2016). Bioremediation is principally based on the biodegradation of naturally occurring processes and gains confidence during the addition of fertilizers. It is the process of producing naturally occurring composites like inorganic compounds, water, and carbon dioxide; all are harmless and safe for human, animal, plant, and aquatic life (Jain & Bajpai, 2012).

3 Types of Bioremediations

Bioremediation process encompasses the use of microorganisms or plants, doable or not, natural or genetically modulated, to unload environmental contaminants like organic xenobiotics that are quite complex to degrade. The process also alleviates lumber due to lethal heavy metals by transformation and formation of harmless products (Dobson & Burgess, 2007; Li & Li, 2011). With the aim of recuperating the process of bioremediation, diverse tactics can be used, depending on the conditions prevailing in the polluted environment. Here we focus on two such strategies:

(a) *Biostimulation:* In this process, the rate of bioremediation/biodegradation can be enhanced by introducing pH alteration substances, surfactants, nutrients, and oxygen at the contaminated site. This, in turn, promotes the growth of autochthonous microorganisms, which play a pivotal role in degrading the toxic compounds to undisruptive ones.
(b) *Bioaugmentation:* Also called bio-addition, this strategy takes into account the environment with a dearth of indigenous microorganisms and facilitates indigenous use of microbial organisms, alien or GMO. Such areas typically have eco-physiological characteristics, which are compatible with the habitat conditions encouraging the endorsement of the bioremediation process (Vidali, 2001; Silva et al., 2004; Li & Li, 2011).

On the basis of the mode of application, bioremediation of toxic products is a flexible process. It can either be pragmatic in situ or ex situ. While the former becomes functional at the contaminated site, the latter inculcates the removal of harmful and contaminated ingredients to be treated elsewhere. Comparatively, in situ biodegradation technologies are more cost-effective and discharge few numbers of pollutants into the environment; nevertheless, it is expected that they entail longer management durations than the ex situ techniques (Vidali, 2001; Tabak et al., 2005).

Presently, there is an ample assortment of microbes, including bacteria, yeasts, fungi, and algae that are being premeditated for exploitation in biodegradation processes. Many of these by now have been engaged as biosorbents of heavy and hazardous metals (Machado et al., 2008; Bogacka, 2011). Nowadays, biosorption is preferred over conventional treatment methods since the former includes low cost; high efficiency; dissertation of specific metals; curtailment of chemical and biological mire; resurgence of the biosorbent; no supplementary nutrient prerequisite; and the likelihood of metal upsurge (Kratochvil & Volesky, 1998). Literature is flooded with studies carried out on bioremediation involving the removal of heavy metals. These studies typically focus on two phases of the biphasic biosorption process: (a) reversible adsorption forming a rapid initial phase and independent of metabolism or temperature and (b) intracellular accumulation forming a slower phase with metabolism dependence. This stage is intolerant to several environmental factors like metabolic inhibitors and temperature. The optimum temperature for the biosorption process works between 25 and 35 °C. When the range exceeds, the altered temperature specifically dwindles the biosorption potential since it directly hits the target sites (Malik, 2004; Tabak et al., 2005). Similarly, biodegradation for the treatment of waste waters is fittingly proficient by desorption. The process allows the recuperation of adsorbed metallic ions, in addition to the salvaging and reclaiming of biomass, for a fresh cycle of metallic recovery. Perceptibly, the technique offers a magnificent awareness in the expansion of a route that facilitates the revitalization of confiscated metallic ions, in addition to the cellular steadfastness of the biosorbents to be upholded, thus allocating for their renaissance and reclaim in succeeding cycles of sorption desorption. This grades the synchronized attainment of two prized products: the treated water and the process of cost-effective recovery of metal (Volesky, 2001; Yu & Kaewsarn, 2001).

4 The Potential of a Genetically Engineered Microbe for Bioremediation

The period between the 1980s and 1990s shined unsteadily with respect to an exponential growth in the research on genetically engineered microorganisms for bioremediation (Zwillich, 2000). This era witnessed the establishment of various bioremediation companies. Researchers in genetic engineering and microbiology across the globe augmented their passion for research in this new and emerging field (Chen et al., 1999). However, due to the authoritarian snags with the amplified technical cost required to gratify regulation, many of these companies stepped back. Consequently, trials on genetically engineered microbes got confiscated to research institutions only and the related research lingers between companies and academics.

In 1971, Ananda Chakrabarty patented the first genetically engineered microbe working as a microbiologist and genetic engineer. As per the report published, a variant of the genus *Pseudomonas* and competent enough to degrade crude oil, the patent was named “oil-eating microbe.” These microbes have the potential to degrade oil 10–100 folds faster, and it takes an edge over other nongenetically engineered independent strains. The bacteria contain different enzymes that can hydrolyze different hydrocarbons. Chakrabarty worked on four different strains of *Pseudomonas* and recognized that the genes for oil-degrading enzymes do not follow Mendelian segregation since they occupy extra-chromosomal space, i.e., the plasmids. Taking this advantage, he isolated these plasmids and introduced them into a single strain of *Pseudomonas* bacteria. In 1980, the discovery gained worldwide gratitude of the United States Supreme Court. Unfortunately, due to various bylaws and communal apprehensions about utilizing microbes for bioremediation, Chakrabarty's breakthrough oil-eating microbe still sits fallow on a sill.

Not only Chakrabarty's oil-eating microbe but also numerous genetically engineered microorganisms have been formulated but not made functional in bioremediation (Pieper & Reineke, 2000; Jiang et al., 2005; Yang et al., 2010). One such example can be *Deinococcus radiodurans*, a bacterium with the potential to resist radiation. With this feature, the bacterium was successfully engineered to degrade toluene. Yet, this has not been commercialized for bioremediation (Lange et al., 1998). Understandably, failure of advancement is expected to be related to numerous factors.

5 Factors Affecting Microbial Bioremediation

Bioremediation, an important microbial procedure to debase, eradicate, shift, halt, or de-venom assorted chemicals and many physical wastes from the environment, is accomplished through the action of bacteria, fungi, and plants. Since microbes utilize their enzymes in the process, they serve as biocatalysts and facilitate the progress of biochemical reactions that mortify the required contaminant. These microorganisms remain active while being aligned with the pollutants. This is possible merely while they have admittance to a range of compounds to facilitate them engender energy and nutrients so as to fabricate additional cells. Thus, the competence of bioremediation relies on several factors like chemical composition and concentration of pollutants, the physicochemical features of the environment like soil type, temperature, pH, availability of oxygen or other electron acceptors, and most importantly nutrients, with uninterrupted accessibility of pollutants to microorganisms. More importantly, for the bioremediation process to get realized, it is quite important that both bacteria and pollutants ought to establish contact with each other. Furthermore, microbes and pollutants do not have a uniform distribution in the environment. Thus, domineering and optimizing bioremediation is an intricate organization with the accessibility of contaminants to the microbial population and the potential of the latter to degrade the former.

6 GEM: A Tool to Sense Ion Explosive Residues

The widespread fabrication and high-speed use of explosives for both local and armed forces during the precedent century have bent miscellaneous environmental inconveniences. One such incident can be exemplified by soil and groundwater contamination with explosive residues. Such contaminants largely include hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2,4,6-trinitrotoluene (TNT), etc., and their toxic and mutagenic effects are well established (Berthe-Corti et al., 1998;

Rosen & Lotufo, 2007). Production of many compounds like 2,6-dinitrotoluene (2,6-DNT) and 2,4-dinitrotoluene has been declared as the plausible carcinogens in humans by IARC (International Agency of Research on Cancer) in 1996. However, information on the carcinogenic potential of TNT is still scarce, fragmentary, and yet to be witnessed. Nevertheless, significant confirmations for the contamination of groundwater reservoirs with explosives have been established in propinquity to unstable mechanized facilities (Funk et al., 1993; Spain et al., 2000; Bernstein et al., 2008).

Presently, various techniques are available to detect and quantify different trace explosives in soil and groundwater. Most of these methods employ gas or liquid chromatography coupled with mass spectrometry. Notwithstanding the highly precise and exceptionally sensitive, such analytical methodologies face certain disadvantages. They are particularly confined to specialized laboratories owing to expensive equipment and thus require precise expertise. Alternatively, an impending balancing approach offers complete information on the bioavailability and lethality of the target compounds and lays its basis on the usage of live cell sensors (Van der Meer & Belkin, 2010). Not only this, these bioreporters (Burlage, 2003; Garmendia et al., 2008; Yagur-Kroll et al., 2014) also find their application in the detection of buried remote landmines. The most common explosive material filled in these mines is TNT, either singly or sometimes in combination with RDX (Jenkins et al., 2001). Two volatile impurities viz. 2,4-DNT and more prominently 1,3-dinitrobenzene (1,3-DNB) accompany TNT (Mac Donald et al., 2003). The vapors leaked out of explosives hoard in the soil around bioreporters (Jenkins et al., 2001).

6.1 Bioreporters for the Detection of Explosives

The preceding two decades have evidenced noteworthy advancement in the plan and erection of bioreporters for the recognition and scrutinizing of miscellaneous environmental contaminants. Given below are some whole-cell bioreporters used in the detection of explosives.

6.1.1 Bacteria

The basic principle of structuring bacteria as bioreporters is based on the activation of a gene promoter in the presence of the target compounds. In the absence of the anticipated gene, however, substitute approaches are demanded for the genetic modulation. In this strategy, a gene encoding a protein is manipulated to bind similar molecules, thus amending its binding site to distinguish a new target. Based on this, the first report of recombinant bacteria to be used as bioreporters was made by Burlage et al. (1999). In this process, two bacteria *Pseudomonas putida* and *Bacillus subtilis* were genetically engineered to fluoresce as they come in contact with TNT and 2,4-DNT. Such bacteria are spewed out in the vicinity, beleaguered by landmine sanitation. After a respite of 120 minutes, the bacteria in the propinquity of concealed explosives get open to the element of TNT vapors. Consequently, the reporter gene therein gets activated. This way, upon scrutinization of the area using a UV source, the buried explosives are spotted. To bring amplification in the vapor concentration, a placid irradiation of the area by way of electromagnetic energy has also been recommended. However, a drawback of this methodology was that unswerving scattering of the bacteria on parched soils came up with an instantaneous soaking of the bacteria to the soil, leading to speedy signal loss (Burlage, 2003). However, this restriction was moderately circumvented by entering the bacteria in a matrix having water and high nutrient retaining polymer (Bjerketorp et al., 2006). Different appropriate polymers like alginate (Zohar-Perez et al., 2002), agar-agar (Kar et al., 2009), or gelatine (Heras and Lorenzo, 2011) are available.

There have been a lot of other microbial strains on record that could sense explosive-related chemicals. Galvao and De Lorenzo (2006) employed *P. putida* XylR protein. This protein degrades xylene and toluene by interacting amenably with toluene and further managing the action under the control of σ 54-dependent *Pu* promoter of TOL plasmid. An alteration in protein by changing its DNA sequence with DmpR, a comparable domain of the homologous protein, resulted in the formation of XylR mutant that retains the capacity to bind 2,4-DNT (Garmendia et al., 2001). XylR mutant was over-expressed in a DNA plasmid and interleaved into *Pseudomonas putida* strain to produce Pu:GFP. Under the control of Pu promoter, GFP got expressed. *P. putida*, an oil bacterium, makes it approved as a bioreporter host, and when introduced in a model soil setup, the gene got activated in the presence of 2,4-DNT, thus aiding in landmine detection (Garmendia et al., 2008).

Another report illustrating *Escherichia coli* as a bioreporter for detecting TNT, DNT, and DNB came out in 2014 by Yagur-Kroll et al. A clone collection comprising nearly 2000 genetically modified *E. coli* was curtained for response to 2,4-DNT. Each clone encompassed a plasmid with the *GFPmut2* gene complexed with an altered gene promoter. Of all such gene promoters, two viz. *yqjF* and *ybiJ* expressed the strongest response. While the former encodes a quinol oxidase subunit, the latter programs a protein of mysterious function. Both such promoter genes were cloned into a low-copy plasmid that expresses the *P. luminescens lux-CDABE* genes. However, these strains had a dose-dependent response to TNT, 2,4-DNT and 1,3-DNB. Captivatingly, the reporter strain docking the promoter for the *yqjF* gene as the sensing element was unable to get persuaded straightway by 2,4-DNT or TNT, but moderately by metabolites of these compounds.

Another similar study by Kim et al. (2008) made use of nitrate and nitrite sensitivity of flagellar motor of *E. coli*. In this, the KAF95 strain was used owing to the fact that it clutches a *cheY* gene deletion which imparts to it the capability of counterclockwise flagellar rotation. Therefore, in the presence of nitrate and nitrite, the flagella instantly discontinue their rotation. The motion was visualized using a microscope fitted with a camera, and exposure confines were accounted to be 2.5 mM and 12 mM for nitrate and nitrite, respectively.

This approach gained further threshold when a bioreporter was constructed to detect nitro aromatic explosives (Tan et al., 2015). However, this time the authors fused five gene promoters instead of one as the sensing element to GFP, and all responded to TNT, DNT, and DNB were fused. This approach helped in getting a detection threshold of 20.9 μM for TNT.

Most crucial application of the *Aliivibrio fischeri*, a luminous bacterium, is in the detection of toxicity of TNT in various soil samples (Frische, 2002). This approach was further accomplished by coupling various chemical analyses for the degradation of products. *A. fischeri* "lights off" assay is used to produce the details on the toxic effects of elements. With this, the author was competent enough to resolve whether TNT establishes itself as the chief toxicant in a soil sample.

The idea of bioreporter design to identify DNT was first developed by Davidson et al. (2012). They emphasized riboswitch engineering and designed the bioreporter outside the promoter–reporter fusion. An element addressing the 5′-end untranslated region of some RNAs, riboswitch, is highly specific in binding its target molecule. It essentially comprises two components: an aptamer serving to bind the target and the expression/sensing component. The latter comprises a TNT binding aptamer (Ehrentreich-Förster et al., 2008) together with a PCR-generated expression platform. This component remained positioned upstream of the gene encoding tobacco etch virus protease. Upon binding of the target DNT to the aptamer, the secondary structure of the RNA gets disfigured, which causes the related gene and hence the gene expression to change. Consequently, the expressed protease cleaves the bond between a GFP molecule and a yellow fluorescence protein. The ensuing green fluorescence endorsed recognition of 0.5-m MDNT when expressed in *E. coli*.

6.2 Yeast

The role of yeast as a tense bioreporters is incredibly inadequate despite the fact that literature is flooded with studies based on yeast strains acting as bioreporters for environmental contaminants (Sanseverino et al., 2005; Xu et al., 2013). Even dilapidation methods of assorted explosives by yeast strains have also been categorized (Zaripov et al., 2002). One noteworthy endeavor showing yeast as a bioreporter was detailed out when the principal elements of the rat olfactory system were interleaved to *Saccharomyces cerevisiae* (Radhika et al., 2007). In mammals, the olfactory sensors are stimulated by many specific odorants. Following a cascade, this acts as a signal to stimulate G protein Golf (Jones & Reed, 1989). In due course, cyclic AMP (cAMP) is synthesized. This in turn kindles Ca^{2+} channel, which amplifies Na^+ and Ca^{2+} influx. Incursion of a more positive charge generates an action potential that ultimately gets in touch with the CNS (Central Nervous System). This is eventually decoded in an odor sensation. Vetting of assorted olfactory receptors and their corresponding G-proteins for retorting toward 2,4-DNT makes Olfr226OR identification as its outcome. The yeast strain WIF-1α was exploited for the synchronization of olfactory GFP expression and cAMP synthesis (Radhika et al., 2007). Under such circumstances, stimulation of Olfr225OR and GPCR is experienced on exposure to 25 μM 2,4-DNT. Expectedly, cAMP is synthesized and GFP is expressed. All these events eventually produce dose-dependent fluorescent signals with no detection limit observed.

6.3 Microalgae

The photosynthetic activity can largely be gauged from the fluorescence capacity of chlorophyll *a*. This, in turn, is largely concurrent to the organism's well-being. Therefore, any variation in a photosynthetic cell reflects the corresponding change in its photosynthetic activity. Mostly, a stressed cell leads to the inhibition of chlorophyll *a* fluorescence (Schreiber et al., 1995). Nonetheless, this retort is quite vague; *Chlorella vulgaris* when immobilized demonstrates the evaluation of the virtual fluorescence of the cells, making it possible to detect airborne chemical warfare agents (Sanders et al., 2001). This phenomenon gained a boon in a more precise manner when Altamirano et al. (2004) contrasted the restrained chlorophyll *a* fluorescence in the WT and a TNT-resistant *Dictyosphaerium chlorelloides*, a strain of green microalga. The purported apparent difference in the TNT-specific inflorescence between the two strains endorsed the exposure of TNT concentrations as low as 0.5 mg/L.

7 Disadvantages Accrued by GEMs in the Bioremediation Process

Endorsed with increased substrate degradation and high catalytic aptitude with a mere cell mass to enclose a safe and disinfecting environment by neutralizing the harmful substances, the process of bioremediation owns numerous advantages. However, it is usually thought that GEMs showed diminishing levels of suitability due to the extra energy requirements forced via the introduction of foreign genetic material and hence are predictable to be noncompetent under real-world conditions (Lenski, 1993; Giddings, 1998). However, *Pseudomonas fluorescens* HK44 contradicts the same as these microbes survived during field release study, and no such extra load was ever observed in them (Ripp et al., 2000; Sayler et al., 1999). While several comparable studies are in favor of GEM survival being nonproblematic, an equivalent figure has not. This, in turn, points toward the idea that the survival of such genetically engineered microbes in the environment is naturally impulsive (Lenski, 1993; Morra, 1996). Nevertheless, the chief shortcomings are never carried out in traditional procedure; in certain cases, the cell's fate becomes fatal. This challenges the release of GEM as bio-remediators in the environment. Sometimes, for no reason, GEMs become sluggish and show delayed growth and substrate degradation. Apparently, seasonal variation and fluctuation in various other abiotic factors act and hamper microbial activity. Understandably, the introduced strain becomes unreactive and negatively impacts the natural, structural, and functional composition and occurrence of the microorganism community (Sayler & Ripp, 2000). Therefore, allowing for the cosmic assortment of environmental factors that persuade GEMs like competition and predation, temperature, pH, moisture, and adsorption, it is explicable that gaining a proficient representative scheme for GEM endurance will be an overwhelming endeavor.

8 Bioprocess Monitoring and Control

Bioremediation, though a potential means to clean up the environment, faces several criticisms. Notwithstanding the most inexpensively effectual treatment expertise on hand, the process accounts for no less than one-third of the conformist ignition or landfill schemes (Swannell, 1998; Zechendorf, 1999). However, one denigration is its inability to certify the efficacy of such a potentially lengthy process that bypasses the use of pricey chemical analysis methods like mass spectrometry and/or gas chromatography. Contrarily, on pooling the expense of continuous monitoring, bioremediation has become an alternative means owing to its much less cost-effective approach. As an analogy, *P. fluorescens* HK44 was used in lysimeter studies. In a biodegradation process, this physiologically active microbe was engineered to bioluminesce. During the process, GEM acts on naphthalene, degrades it, and turns out a bioluminescent signal in tandem. This signal is detected using fiber optics and photon counting modules. Thus, to improve continuous online monitoring for bioremediation and online process control, GEM *P. fluorescens* acts as the best tool. Bioluminescence has been effectively distinguished from HK44 cells dwelling straight inside the polyaromatic hydrocarbon (PAH)-polluted lysimeter soil matrix as well as from those HK44 cells restricted within biosensor devices that have been particularly scrutinized for volatile PAHs (Heitzer et al., 1994; Ripp et al., 2000). Likewise, these biosensors were used at Mississippi and Columbus Air Force Base to examine the curl dynamics controlled via groundwater aquifer tumble-down with a mock jet fuel. Similarly, the use of the *lux*-based system embraces quite a few advantages for supervising various bioremediation processes. For instance, bioluminescence is effortlessly perceived and does not entail any considerable participation of luxurious or ambiguous inspective devices. Second, the fabrication of bioluminescence due to HK44 is absolutely autonomous, and there is no requirement for supplementary accumulation of chemicals or co-factors. Furthermore, bioluminescence can be openly witnessed online and hence provides an unremitting, near real-time sketch of the bioremediation process. The ultimate use of integral microbes as chemical sensors sets aside to monitor the bioavailability of noxious waste rather than their presence. Obstinately, the analytical techniques are mostly destined to establish the occurrence of contaminants in an environmental matrix exclusive of offering any suitable report based on the biological effect of the contaminant. Expectedly, such data happen to become awfully imperative while considering the disadvantageous and hazardous health effects of chemical pollutants on living creatures.

9 Conclusion and Future Perspectives

In conclusion, there is a dire need for genetically modified organisms to remove toxic and nondegradable compounds from the soil. Genetically altered organisms have improved the utilization and elimination capacity of hazardous/unwanted wastes and laboratory products. Using biotechnologically specified microorganisms, it is possible to recycle the wastes into another form at a low cost. Moreover, bioremediation must be effective via using enzymes of different microorganisms, which have remarkable properties of degradation of toxic compounds.

In the future, using bioremediation strategies to clean up the polluted environment is highly recommendable and less harmful. Such systems can utilize different species of microbes, fungi, bacteria, and plants for metabolizing toxic compounds from the environment. In the future, using highly advanced metabolic engineering tools can produce genetically modulated organisms having the application in the remediation of a wide range of toxic environmental pollutants.

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Innovative Biofilms Mediated as Empiricist of Bioremediation for Sustainable Development

Neha Sharma, Manmeet Kaur, Manmeet Kaur, and Parampal Sahota

1 Introduction

Due to anthropogenic activities, contamination of the Earth's environment with toxic waste effluents and recalcitrant compounds has become the most pressing environmental issue in recent decades. The presence of two or more aromatic ring structures in the nature of many organic and inorganic pollutants that cause mutagenic and cancerous effects in humans is well documented and can be found in the environment (Seo et al., 2009). Polluted wastewaters containing organic and inorganic pollutants from multiple industries are discharged into the atmosphere, affecting biomes. Because conventional wastewater treatment facilities have a lot of shortcomings (cost, start-up, operation and efficiency), bioremediation has been chosen as an environmentally safe and sustainable process for the detoxification of toxic pollutants (Prasad & Prasad, 2012). Many organic and inorganic pollutants have been neutralized, degraded, mineralized and eliminated from contaminated environments using the bioremediation process (Sfaelou et al., 2016).

Ecologic clean-ups utilizing biofilm-mediated remediation have long been touted as both competitively priced and environmentally benign. When it comes to bioremediation, bacteria that form biofilms and are adapted to survive, such as those that compete for nutrients and oxygen, as well as those that tolerate pollutants, have been tested. There are biofilms of indigenous bacteria that can survive and adapt to harsh environmental conditions, such as pH, high and variable temperatures, nutrients, salinity, predation, and extensive ultraviolet exposure and high pollutant concentrations (de Carvalho, 2018). Extracellular polysaccharide (EPS) is a weird mix of polymers excreted by microorganisms that plays a critical role in the rhythm of nutrients within a biofilm matrix. Biofilms can consist of a single bacterium, fungus,

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_7

algae or archaea, or they can consist of multiple species. Structure and content of biofilms and EPS can change based on the surrounding scenarios (Yin et al., 2019).

In addition, biofilm enzymes can be used to monitor the quality of heavy metal-contaminated stream water (Pool et al., 2013). For the removal of organic and inorganic contaminants from wastewater, microbial biofilms have produced EPS (Flemming et al., 2016). Decontamination of pollutants from the environment can be achieved using microbial biofilms (Turky et al., 2017). A hypothesis is made that microorganisms in the biofilm will ingest (and thus remove) harmful organic materials from the contaminated water when it passes through. Toxic effluents can be removed from the surroundings via using biofilms. They absorb, immobilize and degrade a variety of contaminants.

2 Microbial Biofilms and Bioremediation

2.1 Biofilms

Cells are embedded in self-produced exo-polysaccharides and form a biofilm on the surface of a surface. This means that the microorganisms living in the biofilm work together to create different ecological niches within the biofilm. Their characteristics are influenced in part by their structure and diffusion of nutrients as well as by their physiological activity (Costerton et al., 1995). When microbes live in nature, they tend to live in biofilms, which are thick and sticky. Due to their physical barriers and the physiological dormant state of their cells, biofilms are highly resistant to chemical, physical and biological stresses. This makes biofilms the most tenacious microorganism on the planet because they can withstand so many different environmental stresses.

2.2 Biofilm Development

Determinants of biofilm formation include cell, surface and environmental factors. The formation of biofilms is influenced by the physiological states of the organisms involved (Costerton et al., 1995) (Fig. 1).

To begin with, microbes themselves secrete inorganic and organic molecules, which condition the substratum and create a favourable environment for cell settlement. The planktonic cells then interact with the surface, resulting in the conditioning of the surface. After that, microbial cells attach to the surface reversibly and then permanently. This is followed by a third step that involves irreversible attachment and cell multiplication. Aside from that, they secrete an extracellular matrix (EPS) that binds the cells to the surface and to one another. Protein, lipid and nucleic acid molecules make up the EPS matrix, which gives biofilms mechanical stability. It

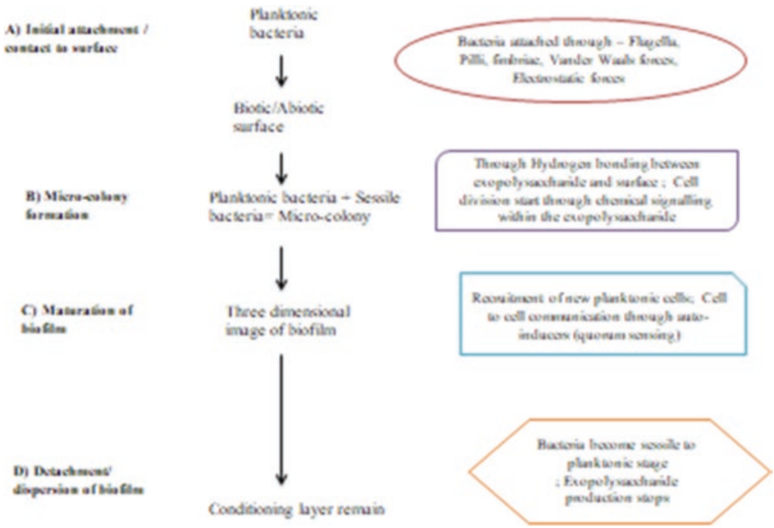

Fig. 1 Different development stages of bacterium biofilm lifecycle

also helps bacteria adhere to interfaces and forms a three-dimensional polymeric communication system that intermixes and immobilizes biofilm cells in an unusual manner (Flemming & Wingender, 2010).

2.3 *Components of Biofilms*

Inert biomass, soluble microbial products and exopolysaccharides are the three microbial products that make up the biofilm's structure. This allows for a clear distinction between biomass-associated biomolecules that are biodegradable and associated biomolecules that are utilized by the resident bacteria. A diversity of water-soluble microbial products are released by autotrophic microorganisms during regular biomass metabolism. There are many heterotrophic organisms in the biofilm that are controlled and supported by soluble organic compounds. There is a summary of the major biofilm matrix components and their respective functions (Table 1). The constituents of the biofilm matrix are outlined in the subcategories that follow:

(a) *Exopolysaccharides:* On account of their wide range of matrix polymers, EPSs are also known as the dark matter of biofilms (Wingender et al., 1999). Biofilm matrix also contains nucleic acids, proteins and lipids, so the term EPSs was coined to refer to the extracellular polysaccharides (Flemming et al., 2007). Variables from the biological and physical factors influence the quantity of extracellular polysaccharides in a cell. When EPS is present, it is necessary to have Ca21 as well as other multifarious cations for biofilm formation and

Table 1 Critical elements of exopolysaccharides (EPS) and their performance in bacterial biofilms

S. No.	EPS components	Function w.r.t. biofilms
1	Polysaccharides	Adhesion Accumulation of bacterial cells Biofilm cohesion Protective barrier Water retention Sorption of inorganic and organic compounds
2	Proteins	Enzymatic activity Electron acceptor or donor Adhesion Assemblage of bacterial cells Protective barrier Biofilm cohesion
3	DNA	Exchange of genetic information
4	Divalent ions	Mechanical stability Regulation of biofilm-associated proteins and exo-polysaccharide production
5	Lipids and surfactants	Bacterial detachment and attachment Hydrophobicity
6	Water	Provides moist environment Allows nutrients to circulate

stability (Shukla & Rao, 2013a). When it comes to exopolysaccharides, it's hard to beat them. Because they are linear, long or branched, their molecular weights range from 500 to 2000 daltons. Exopolysaccharides have been widely characterized and secluded from diversified species of bacteria and surroundings. These polysaccharides are usually hetero-polysaccharides, which contain both neutral and charged sugar molecules. The sucrose-derived glucan, fructans and cellulose on the other hand are homopolysaccharides (Wingender et al., 2001). These polysaccharides comprise organic or inorganic constituents that also impact their physical (poly-anionic or poly-cationic) characteristics to ascertain their biological function. Throughout a single bacterial species or strain, exo-polysaccharides can be incredibly diverse in composition.

There are three types of exopolysaccharide produced by *Pseudomonas aeruginosa*: alginate, pel and ps l37. For example, alginate is essential for micro-colony formation at the beginning of biofilm formation, and it also aids in biofilm maturation by providing mechanical stability to develop biofilms.

- *Extracellular proteins:* But their involvement in intercellular adhesion as well as in preserving the integrity of the biofilm matrix has indeed been omitted. Proteins in the biofilm matrix play a critical role in biofilm architecture, which has been established over the past century (Shukla & Rao, 2013a, b). Most of these proteins can form biofilms without exo-polysaccharides. A mature biofilm is therefore not dependent on exopolysaccharides. Two types of proteins are enzymes and structural proteins.

- *Enzymes:* Biofilms have been found to contain a wide range of extracellular enzymes. These enzymes are known as EPS-modifying enzymes because they participate in the degeneration of exo-polysaccharide. These extracellular enzymes digest the EPS constituents, which act as a nutrient store. But they're also beneficial for spreading biofilms, allowing bacteria to be released from their host and settle down on new substrates.

(b) *Structural proteins:* Also included in this category are biofilm-associated proteins (BAP) and homologous BAPs (Latasa et al., 2006). Proteins in this family have a high molecular mass, a core domain of tandem repeats and an attachment to the cell surface that facilitates biofilm formation. They are followed by amyloids, a proteinaceous component of the biofilm matrix that occurs in second place (Otzen & Nielsen, 2008).

(c) *Extracellular DNA:* Formerly thought to be the by-product of lysed cells, extracellular DNA (eDNA) is now understood to be an essential component of the biofilm matrix (Molin & Tolker-Nielsen, 2003). Exogenous DNA and the DNase effect on biofilm cells have shown a link between eDNA and increased antibiotic resistance in biofilms (Tetz et al., 2009). Bacterial aggregates are more stable when eDNA is present to aid in genetic information exchange. In bacterial biofilms, eDNA also facilitates cell self-organization (Gloag et al., 2013).

(d) *Lipids and biosurfactants:* Polysaccharides, proteins and DNA have hydrophobic properties. Hydrophilic properties of EPS are due to lipids and alkyl-group-linked polysaccharides, such as methyl and acetyl groups (Busalmen et al., 2002). So, *Thiobacillus ferrooxidans* secretes lipopolysaccharides (Sand & Gehrke, 2006), while *Serratia marcescens* produces extracellular lipids with surface active lipids (Matsuyama & Nakagawa, 1996). Surfactants are surface-active molecules that affect the air–water interface's surface tension by modifying the surface tension. To exchange gases, they play a crucial role in altering surface tension (Leck & Bigg, 2005).

2.4 Biofilm's Physiological State

It is during the sessile phase of growth that bacterial cells demonstrate distinct phenotypic characteristics from those that are displayed during planktonic growth (Stoodley et al., 2004). Genome transcription and cell growth rates differ from their planktonic counterpart. Planktonic cells and biofilm cells appear to differ physiologically. Biological biofilms and planktonic lifestyles have different metabolic requirements. The metabolic activities of biofilm cells are governed by local variations in the biofilm milieu. Gradients of niches are formed as a result of variations in nutrient concentrations in the matrix, as well as signalling molecules. By changing their gene expression patterns or physiological functions, seasonal bacterial cells adapt to their local environment. Reduced respiration and metabolic activity, as well as a latent state, all contribute to biofilms' physiological resistance to antimicrobials (Cogan et al., 2005).

2.5 Quorum Sensing

Signalling molecules called autoinducers (AIs) mediate the quorum sensing (QS) phenomenon. Bacteria have been extensively studied for three different types of AI: N-acyl homo-serine lactones, auto-inducing peptides (AIP) and AI-2. Quorum sensing is defined by the binding of transcription factors or cell surface receptors and the initiation of gene expression. Auto-inducers are highly specific to bacterial groups. Gene regulation in Gram-positive bacteria is mediated by AHLs, whereas AIP is the quorum sensing autoinducer in Gram-negative bacteria (Mangwani et al., 2012). Virulence, biofilm formation and sporulation are just some of the vital functions that quorum sensing regulates in bacteria via AIs (Li & Tian, 2012).

3 Biofilm Mechanisms Involved in Bioremediation

Cost-effective and green techniques are essential for the detoxification of hazardous waste containing wastewaters in order to minimize the environmental hazards caused by organic complex waste. As a result of its complexity and inability to be considered by standard processes, the majority of the methods such as activated-sludge process, trickle process, chemical oxidation, reduction precipitation or sludge separation, ion exchange treatment, reverse osmosis, cementation, flocculation, electrochemical treatment and evaporation are majorly ineffective or prohibitively expensive. Mineralization, detoxification and low operating costs are all perks of biofilm-mediated bioremediation. Table 2 shows an insight into biofilms and their significance in bioremediation.

Cometabolisms play an essential role in the degradation of complex pollutants when there are a variety of microbial species with different metabolisms present (Gieg et al., 2014). Biofilm-mediated bioremediation is characterized by adaptation to stress conditions, cometabolisms, oxygen and nutrient competition. In heavily polluted sites, the microbial populations are mostly found in biofilms, which

Table 2 Major characteristics of biofilms and their importance for bioremediation

S. No.	Properties	Characteristics
1	Quorum sensing (communication)	Biofilm formation at a threshold density
2	Intolerant of environmental stresses	Chelation, precipitation, enzymatic degradation, volatilization
3	Symbiosis and metabolic diversity	Accumulating waste products, transferring horizontal gene
4	Surfactants	Dissolving hydrophobic substrates
5	Hydrologically porous physical structure with water channels	Waste products and electron acceptors are transported
6	Microcolony and gradient formation	Redox potential and nutrient cycling due to the combination of aerobic and anaerobic processes

provide better protection, persistence and survival, as well as the ability to deal with stressful conditions. This leads to the deterioration of complex nonlinear pollutants via multiple pathways. Biofilm median is the middle mode of bacterial persistence under various environmental conditions, covered in an exo-polysaccharide matrix, which is a desirable configuration for bioremediation (Jung et al., 2013). Toxic effluents and protection from predators can be controlled effectively by the biofilm matrix than planktonic cells under stress conditions (Mah & O'Toole, 2001).

EPS in the biofilm enables nutrient exchange and waste disposal. Constrained nutrients have been shown to augment EPS production as well as expanded metal and other pollutant absorption. It is composed of microorganisms that can accumulate phosphorus and remove it from wastewater. If there are several microorganisms living together in close proximity, they will interact with each other, leading to the complete metabolization of complex pollutants (Ferris et al., 1989). Multiple biological metal and organic perilous contaminant expulsion approaches have been investigated, including biosorption (microbial or plant cell) and biotransformation (enzymes and metabolites). One utilizes a chemiosmotic gradient across the cell membrane, which is fast but non-specific, and the other uses a slower but more specific system. Because of the gradient, the site is free of heavy metals. However, the second kind of mechanism is static and precise and needs energy from adenosine triphosphate hydrolysis (Fig. 2). In microbes, electrostatic interaction, surface complexation, redox processes, precipitation and ion exchange are some of the physiochemical processes involved in biosorption.

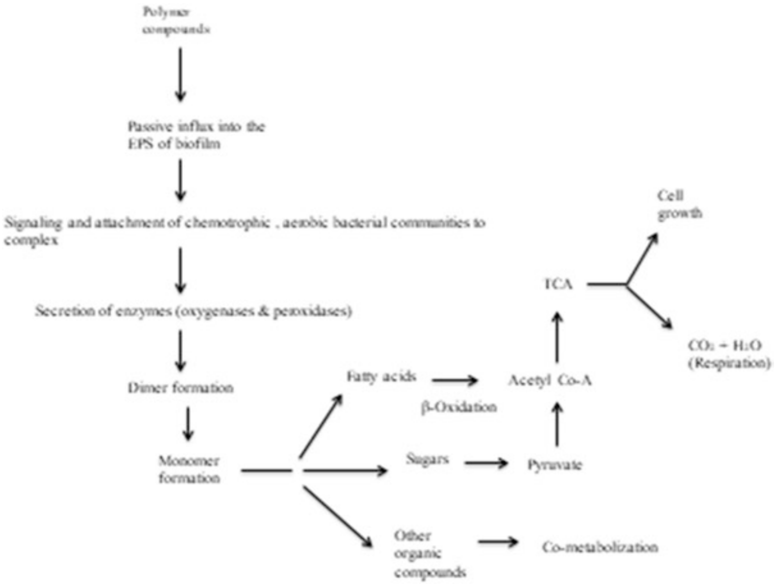

Fig. 2 Mechanism of bioremediation of complex polymers by biofilm

4 Miscellaneous Pollutants Remediated by Biofilms

Diverse pollutants such as oil spills, pesticides, other xenobiotics, persistent organic pollutants and heavy metals are being cleaned up using microbial biofilm mediated bioremediation. Remediation by biofilm has primarily been used to treat groundwater and soil contamination with heavy metals like chromium, cadmium and copper (Valls & de Lorenzo, 2002). Quite often, carbon sources are incorporated to pollute groundwater in an attempt to stimulate biofilm formation and create a barrier from the source of the pollutants. Heavy metals like Zn, Ni, Co, Cd and Cu have been removed with a biofilm reactor. Sulphate-reducing bacteria found in mine biofilms are particularly useful since they are able to turn metals from contaminated water into metal-sulphide precipitates (Muyzer & Stams, 2008).

Bacterial biofilm plays a critical role in the elimination of toxic metals from wastewater. Mosharaf et al. (2018) isolated and characterized a range of biofilm-producing bacteria from wastewater samples. They also looked at the exopolysaccharide produced by each bacterium in the biofilm matrix. There have been countless oil spills where the bacterial community has been affected. The hydrocarbon-degrading communities are represented by *Alcanivorax borkumensis*. It's unclear what physicochemical circumstances encourage bacterial adherence to oil/water interfaces. Interfacial tension was reduced as a result of the release of *A. borkumensis* cells rather than a synthetic biosurfactant. The physicochemical features of *A. borkumensis* that allow it to adhere to oil/water interfaces are investigated (Godfrin et al., 2018). OxiTop Control was used to determine the amount and persistence of biofilms that grow on the exterior of polylactide (PLA) when it was biodegraded in a variety of circumstances, including soil, compost and lake water. PLA was shown to be particularly susceptible to biodegradation in compost. Biofilms were formed by a variety of bacterial species with varying levels of abundance and hydrolytic activity (Walczak et al., 2015). *Pseudomonas* species are well known for their ability to create a variety of secondary metabolites while developing in the late exponential and stationary phases of growth. Phenazine 1,6-di-carboxylic acid (PDC) is one such metabolite that has been connected to *Pseudomonas* species (Dasgupta et al., 2015).

5 Environmental Pollutants and Bioremediation

The microbial removal of hazardous and recalcitrant substances provides more efficient and cost-effective options (Kumar et al., 2006). Because of bacteria's enormous metabolic potential, research into this potential for environmental restoration is moving at a breakneck speed (Dash & Das, 2012). All of the degradation mechanisms and detoxification procedures that bacteria use to break down a wide range of toxins are yet unknown (Shukla et al., 2014). Because of their chemical inertness, toxic substances provide a hurdle. The overuse of manmade and natural chemicals such as heavy metals, chlorinated hydrocarbons, organophosphorus, pesticides and nitro-aromatic compounds and pesticides has resulted in massive environmental degradation as a result of rapid industrialization (Seo et al., 2009). Some substances are uncertain to be destroyed in a predefined timeframe by culturable bacteria (Singh et al., 2006). In recent years, the search for feasible xenobiotic bioremediation strategies has accelerated. There are two primary techniques for bioremediation: in-situ and ex-situ. In-situ bioremediation is the process of naturally eliminating contaminants from the environment. Ex-situ bioremediation, on the other hand, relies on bacteria to break down pollutants in excavated materials (Pandey et al., 2009).

Bioremediation is based on the ability of bacteria to break down and change contaminants. Bacteria's ability to break down and detoxify a wide range of toxins can improve as they evolve genetically. Based on the bioactive metabolites of bacteria, it is feasible to bioremediate pollutants by detoxifying them, either by converting them into less harmful compounds, mineralizing, or mobilizing them (Parales & Haddock, 2004). It's necessary to boost solubilization, cell entrance, expression of degrading enzymes and catalytic breakdown. Bacterial populations can adapt in a number of ways as a result of extended exposure to xenobiotics and heavy metals in the natural surroundings.

Bacteria detoxify metals by adsorbing or synthesizing metallothionein or other proteins to trap them (Dash et al., 2013). Degradation of organic molecules is aided by a variety of intracellular and extracellular enzymes. Organic aromatic pollutants are degraded by various genes including nid, bph, atzA, nah, cphA and ntd R, and enzymes such as dehydrogenases, hydrolases, decarboxylases, transferases and dioxygenases are all involved in the breakdown of organic aromatic pollutants (Seo et al., 2009).

6 Application of Biofilm in Bioremediation

Bioremediation is the use of biological processes to reduce or eliminate hazardous environmental contaminants. Bioremediation has been considered superior to chemical and physical approaches in terms of efficiency and economic viability (Paul et al., 2005). It's a revolutionary in-situ technology for cleaning up environmental pollution that uses microorganisms. To break down the pollutant and remove it from the environment, most bioremediation procedures use biocatalysts, such as enzymes or whole live cells. Because of their catalytic stability, self-generation of pricey cofactors and capacity to carry out processes that involve multicomponent assembly, entire cells as a biocatalyst do not require enzyme purification (Woodley, 2006). Cells must be preserved and reused because the entire process can be expanded and implemented at a grander scale (Avnir et al., 2006). To immobilize cells in the body, either chemically attaching cells to a carrier material or physically trapping or encapsulating cells are utilized. As a result of immobilization (Buchholz et al., 2012),

- Biocatalyst activity and viability are reduced.
- Significant expenses are incurred.
- Insufficient immobilization concepts are to be used universally.
- The limits of substrate diffusion mass-transfer and oxygen-reducing reaction speeds are accentuated.

Biofilm communities, as opposed to entire cells utilized as biocatalysts in bioremediation processes, have a myriad of benefits. Naturally immobilized microbial cells in biofilms do not require cell immobilization because they are already embedded in self-produced EPS. Planktonic cell-mediated bioremediation, in particular, appears to be a promising approach. Biofilm mode cells are resistant and tolerant to harmful elements found in the trash because they are entrenched in a biofilm matrix, which functions as a physical barrier. This makes them desirable living catalysts for organic syntheses with technological applications (Halan et al., 2012).

6.1 Chemotaxis Significance in Biofilm Formation and Biodegradation

Chemotaxis is the movement caused by a chemical nutrient or gradient. It helps bacteria to discover the ideal circumstances for growth and survival as part of biodegradation (Paul et al., 2005). Under conditions of limited carbon and energy sources, chemotaxis, along with the ability to digest xenobiotics, may be chosen as an advantageous trait in bacteria. In many circumstances, bacterial breakdown of contaminants has proven to be successful in bioremediation. The utilization of microorganisms that exhibit chemotaxis toward contaminants has received little consideration.

The first stage in bioremediation is to assess whether a substance is bioavailable to bacterial cells. Chemotactic bacteria can be employed to enhance the bioavailability of organic pollutants, which has been proven to be a major limiting factor in the bioremediation of polluted environments. Chemotaxis allows cells to detect chemicals, such as those adhered to soil particles, and swim towards them, overcoming mass-transfer restrictions that inhibit bioremediation. As soon as cells come into contact with a surface, biofilm formation and surfactant synthesis begin, resulting in enhanced bioavailability and breakdown.

6.2 Potential of Biofilms in Remediation of Hydrocarbons

Chlorinated aromatic compounds that are chemically recalcitrant can be found in a range of chemical industry effluents and can migrate fast through soil. They're one of the most common pollutants in soil and groundwater, even at very low quantities (Kargi & Eker, 2005). To extract 2,4-dichlorophenol from synthetic wastewater, they used a rotating perforated tube biofilm reactor with mixed microbial biomass comprising activated sludge culture and DCP-degrading *Pseudomonas putida*; about 100% of the DCP was removed (Kargi & Eker, 2005). PAH breakdown is aided by bacteria that specialize in adherence to polyaromatic hydrocarbons (PAHs).

In the case of diclofop-methyl, a two-ring chlorinated herbicide that accumulates in biofilms by adsorption on microbial exopolymers has been proven to do so. The diclofop-methyl accumulated in the biofilm community during hunger (Wolfaardt, 1995). Another class of molecules known as nitroaromatics has been employed in the creation of foams, pesticides and pharmaceuticals. This is because these molecules have a nitro group, which renders them resistant to biodegradation (Lendenmann & Spain, 1998). Engineering metabolic pathways and enzymes involved in degradation, as well as boosting the copy number of degradative genes, could improve biofilm-mediated bioremediation. Biofilm production could potentially benefit from chemotactic and degradation-oriented strains. A chemotactic strain with catabolic genes could be particularly adept at biodegradation.

7 Current Status of Use of Biofilm in Bioremediation

Microbial biofilms can be programmed to form living materials with self-healing and evolvable properties. Due to processing and protein secretion capability constraints, non-natural biofilms are still not commonly used. Huang et al. (2019) established a live functional material platform that is highly flexible and customizable using *Bacillus subtilis Tas A* amyloid machinery. These unique tuneable platforms offer previously unattainable features for a number of diverse functional materials in biotechnology, biomaterials and biomedicine. Meanwhile, injection of activated sludge cells (R1), mixed culture of eight powerful phenol-degrading bacteria comprising *Pseudomonas* and *Acinetobacter* species (R2), and a blend of the above techniques boosted the effectiveness of three moving bed biofilm reactors (R3). A variety of procedures and media were employed to assess the capacity of eight bacteria to form biofilms (Irankhah et al., 2019). Biofilm-forming and contaminant-degrading bacteria can trap other sludge cells in the biofilm structure and assist in pollutant breakdown, all of which enhance treatment effectiveness. A variety of bacteria create siderophores, which are iron chelators. Bacteria are able to meet their iron requirements because of this characteristic. Certain siderophores, on the other hand, use catecholate groups to chelate iron. Under iron-deficient conditions, this enzyme also protects cells from intoxication by endogenous bacillibratin-derived catechol metabolites, as well as detoxifying ambient catechols (Pi & Helmann, 2018). Engineered silver nanoparticles (AgNPs) are regularly released into aquatic habitats. As a result, the AgNP will come into interface with natural biofilms, which are the most common bacteria found in practically every habitat. Despite the fact that nanoparticle research is expanding at an exponential rate around the world, determining the toxicity of nanoparticles in natural environments remains difficult (Grun et al., 2018). There are worries regarding the widespread usage of AgNPs and their possible harmful impact on the environmental function of lotic biofilms, such as biodegradation or biostabilization, because of this quantitative shift in the population, even after a small dose of AgNP treatment.

8 Conclusion and Future Perspective

Biofilm activities are appropriate for the treatment of intractable substances due to their high microbial biomass and propensity to immobilize molecules. Some of the ways to successful bioremediation productivity include genetic engineering to improve strains and chemotactic capabilities, the use of different population biofilms, and the optimization of physicochemical conditions. Biofilm-based bioremediation has some drawbacks. Bioremediation is a sluggish procedure compared to chemical treatment for pollutant breakdown. Bioremediation's dependability is a major limitation, limiting its application in specific scenarios with high pollution. It's vital to remember that some chemicals are resistant to biodegradation, such as plastics and some halogenated aromatic compounds. Another limitation of bioremediation is that some metabolic hazardous compounds may be created following microbial breakdown. When pollution resources are limited, immediate renovation is not necessary, or when chemical treatment is not the best option, the bioremediation strategy is preferred. Bioremediation may be slow or unsuccessful if critical nutrients for microbial development are few, notably when the amount of pollution is large. The bioavailability of a pollutant to microorganisms affects bioremediation efficiency, and toxins that aren't coated in clay are more biodegradable. Bioremediation is a relatively new idea, as biodegradation has existed from the beginning of time.

Enhanced strains that prefer contaminants over other accessible substances in terms of energy preference as substrates are required for effective bioremediation. DNA containing catabolic genes is taken up by biofilms in a novel technique to enhance bioremediation, promoting the biodegradation of certain contaminants. These microorganisms have been genetically modified to digest a variety of contaminants, including chlorinated aromatic compounds. Horizontal gene transfer of biodegradation-capable genes from genetically modified microbes to biofilm populations can also assist speed up the degradation rate. In specific circumstances of micro-pollutants, phytoremediation or chemical treatments, as well as biofilm-mediated bioremediation, could be applied. Bacterial–fungal consortia can also be employed to degrade xenobiotic substances in some situations. Even if the technique has some drawbacks, biofilm-mediated remediation can help reduce pollution in the environment.

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Major Groups of Microorganisms Employed in Bioremediation

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1 Introduction

Bioremediation is a biological process that transforms trash into a form that other species may utilize and reuse. Currently, the world is dealing with a variety of environmental pollution issues. Microbes are considered alternate methods to tackle challenges like environmental pollution (Abiala et al., 2013). Microorganisms might exist anywhere in the biosphere for their metabolic activities are unusual; hence, they can thrive in any environment. Microorganisms have quite variable

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_8

nutritional abilities; hence they are employed for bioremediation of environmental contaminants (Priya et al., 2021). Through the combined action of microorganisms, bioremediation is the strategy which triggers degradation process, eradication, fixation, or detoxify the various chemical and physical harmful wastes in the ecosystem. The main contaminants or pollutants such as heavy metals, pesticides, hydrocarbons, petroleum, and dyes are fixed by degradation and transformation via bioremediation. Even though it is dealt out enzymatically through metabolism, it contributes significantly to the solution of many environmental issues (Pal et al., 2020). Biological and abiotic variables play a role in determining the pace of deterioration of pollutants. For this purpose, currently many countries have adopted various tactics and strategies in this subject. Bio-stimulation, bio-enhancement, bio-ventilation, bio-pile, and bio-attenuation, for example, are all frequent terms. The reason behind this philosophy is that each bioremediation technique has its negative and positive points, and has specific set of applications (Phinikarides et al., 2014).

Microorganisms are spread widely across the environment due to having fast metabolism with the ability to flourish in a variety of environments. Microorganisms' nutritional diversity can potentially be employed for pollution biodegradation. This is referred to as bioremediation (Gupta et al., 2017). It is a process that focuses on the ability of some microorganisms to alter, adapt, and use hazardous contaminants in order to generate energy and biomass. Bioremediation is more than just collecting and storing pollutants; it is a well-structured microbiologically organized program activity that decomposes or converts pollutants into non-toxic elements or less harmful. Microbes employed in bioremediation to decontaminate the polluted environment are known as bioremediation agents. The most common bioremediation agents are bacteria, archaea, and fungi. Bioremediation is a process in which microorganisms are used to resolve and remove the risks of numerous contaminants from the environment through biodegradation; biodegradation and bioremediation are considered interchangeably (Rahman & Singh, 2020). Microorganisms are a valuable contamination removal technique in soil, water, and sediment, owing to their superiority over other cleanup methods. Microbes are helping to restore the natural environment and avoid pollution. The main aim of this review is to reflect current developments in the involvement or contribution of microorganisms in the bioremediation process, offer important information, and highlight gaps in this field (Haferburg & Kothe, 2007). Microorganisms are currently a trendy research subject due to their eco-friendliness and valuable genetic material that is expected to tackle environmental issues.

2 Bioremediation Management Technique and Bioremediation Agents

2.1 Bioremediation Management Technique

Bioremediation is an approach in which biological organisms are adequately applied to eliminate or minimize or decontaminate the pollutants from a polluted environment (Jagdale et al., 2018). In this process, naturally existing microorganisms are used to break down toxic elements into less or non-toxic forms (Sharma, 2012). Bioremediation technique is an easy, simple, and economical approach that has gotten attention in the past recent decade. The employment of organisms (bacteria, fungi, algae, and plants) to detoxify contaminated soil and wastewater is referred to as "bioremediation" (Kumar et al., 2011). Organic and inorganic hazardous pollutants may be decomposed, accumulated, or fixed throughout the bioremediation process, resulting in significant pollution reduction (Semple et al., 2001).

Biological organisms utilized in bioremediation to clean up contaminated sites are known as bioremediation agents. The primary bioremediation agents are biology, archaea, and fungi (Coelho et al., 2015). As discussed before, bioremediation entails the use of diverse microorganisms to break down and lessen the toxicity of environmental pollutants. As a result, the interaction of microorganisms with the pollutant environment is critical to determine the success of bioremediation process or technique (Pandey et al., 2009). These microorganisms could be found naturally in bioremediation sites or isolated from other locations and artificially injected. Microbial metabolism frequently includes biodegradation. Microbes can get carbon and energy directly from pollution in some instances (Bonaventura & Johnson, 1997). The bacteria and fungus (often utilized in bioremediation) as well as archaea which are recently discovered groups of organisms with unique potential in bioremediation will be discussed in the following sections.

There are two types of bioremediation at in-situ level, first one is intrinsic and second one is engineered bioremediation (Hart, 1996). Intrinsic bioremediation is also known as natural reduction under in situ conditions. This bioremediation technique involves the passive repairing of contaminated places deprived of human intervention and the use of external force (Macdonald & Rittmann, 1993).

2.2 Bioremediation Agents

2.2.1 Fungi Used in Bioremediation

Arbuscular mycorrhizal fungi (AMF) are the most prevalent symbiotic interaction between fungi and plants, in which the fungal partner enhances the removal of pollutants through the provision of large surface area and absorbs pollutants using its hyphae and spores, thus helping in pollutant mobilization and binding to the root (Deshmukh et al., 2016). Micro and macro fungi are used for the bioremediation of soil pollution. The main categories of soil pollutants in Europe are heavy metals, polycyclic aromatic hydrocarbons (PAH), phenols, mineral oils, nonaromatic hydrocarbons, and chlorinated hydrocarbons (CHC). Moreover, there are some new pollutants such as pharmaceutical personal care products (PPCP) and endocrine-disrupting chemicals (EDC) reported (Jasu et al., 2021). Biodegradable external (laccase and class II peroxidase) and intracellular (cytochrome P450 monooxygenase and glutathione transferase) enzymes are described for fungal species (saprophytic and biotrophic basidiomycetes) (Morel et al., 2013). Biostimulation and bioaugmentation procedures are also outlined, as well as the chemical and physical elements that affect the biodegradation process. Laboratory-scale micro-studies, which are the most fundamental way for assessing the viability of biodegradation processes, are given special emphasis (Xu & Lu, 2010).

2.2.2 Mycoremediation

Mycoremediation is a bioremediation technology that uses fungus to remove harmful substances. It can be done with molds (filamentous) and mushrooms (macrofungi). Both classes have enzymes that can degrade many contaminants. The role of fungi in bioremediation was becoming more widely understood throughout the previous century (Hassan et al., 2020).

2.2.3 Fungus (Mycoremediation)

Current bioremediation applications mainly use bacteria, and rarely fungi are used in this field. Fungi have an extremely important role because they participate in the element cycle by decomposing and transforming inorganic and organic materials (Garbisu et al., 2002). These properties can be transformed into bioremediation applications, which can reduce the risk of metals by the decomposition of organic compounds. Fungi not only have importance over bacteria in terms of their metabolic diversity, but they also have good ability of adapting to the environment (Zhang et al., 2020). They can oxidize a variety of chemical substances and can tolerate severe conditions of environment, i.e., less humidity and high pollutants concentration. Hence, for soil bioremediation, they can serve as extremely powerful tool and a few multifunctional species, e.g., white rot fungi, have become an emerging research topic (Amit Kumar et al., 2021). In this regard, many researchers mentioned that fungi, mainly saprophytic and biologically nutritious basidiomycetes, have the ability of degrading and transforming toxic compounds. Filamentous and macrofungi used for bioremediation have enzymes that degrade multiple pollutants (Purohit et al., 2018).

It has been established that micro and macro fungi have great degradation of organic pollutants and reduction of heavy metals in soil. Compared with other microorganisms, the growth morphology in the soil (i.e., the expanded hypha), the lower specificity of extracellular enzyme complexes, and the use of toxic compounds as growth substrates enhance the efficacy of filamentous fungi for the bioremediation process (Ozimek & Hanaka, 2021). However, when designing the soil fungal remediation process, certain important factors such as selection of suitable fungal strains and evaluation of their possible interactions with the contaminated microbes must be considered. For this reason, microscopic research findings showed easy and useful method to assess the viability of biodegradation processes (Khan, 2005).

2.2.4 Biodegradability of White Rot Fungi

Potential treatment methods using fungi as pollutants began in 1985, when it was discovered that the white rot fungus *Phanerochaete chrysosporium* can metabolize a variety of key environmental pollutants (Aust, 1990). These fungi have important enzymatic ability of metabolizing complex chemicals such as lignin. Later on, same ability was also found in other species of white rot fungus. White rot fungi usually degrade the lignin outside the cell by extending their hyphae which make them more advantageous (C. A. Reddy & Mathew, 2001). This helps them reaching the contaminants of soil that are not accessible by other organisms. Moreover, they also maximize the surface area for enzymatic reactions. These cheap fungi also have the ability of tolerating harsh conditions of environment such as temperature, pH and moisture content (Saxena & Misra, 2010). Although many microorganisms used for bioremediation require environmental pretreatment for survival, direct application of white rot fungi to most systems is possible because they degrade due to nutrient deficiency (Sardrood et al., 2013).

3 Factors Affecting Microbial Bioremediation

Bioremediation involves the degradation, removal, modification, fixation, or detoxification of numerous chemical compounds and physical wastes from the environment through the action of bacteria, fungi, and plants. Microorganisms function as biocatalysts by promoting the progress of biochemical reactions that break down targeted contaminants through their enzymatic pathways (Harms et al., 2011). Microorganism multiplies after contacting with various compounds to produce energy and nutrition and act on pollution. The chemical composition and concentration of contaminants, as well as the physical and chemical properties of the environment and their availability to microorganisms, all influence bioremediation efficacy (V. Kumar et al., 2018; Naz et al., 2021). This efficacy could be low due to less contact between microbes (bacteria) and contaminants, and as a result the rate of degradation is slowed down. Furthermore, the distribution of microbes and contaminants in the environment is irregular. Controlling and optimizing the bioremediation process is a complicated system due to a variety of factors (Megharaj et al., 2011). Therefore, high microbial population availability of pollutants or contaminants, and environmental conditions such as pH, soil type, temperature, the presence of O_2 or another electron acceptor, and nutrient availability play key role in the degradation process (Prasad et al., 2012).

3.1 Biological Factors

Organic compound decomposition is influenced by biological processes such as competition among bacteria for limited carbon sources, antagonistic interactions between microbes, and predation of microbes by protozoa and phages. The pace of pollutant degradation is usually determined by the amount of "catalyst" present as well as the concentration of pollutants (Joutey et al., 2013). The number of "catalysts" in this situation refers to the microbial population having the capability of metabolizing contaminants and enzyme concentration produced by each cell. The pace of pollutant breakdown can be accelerated or slowed by the production of certain enzymes by cells. Furthermore, the extent to which individual enzymes must engage in the metabolism of pollutants, and to a significant extent, their "affinity" for pollutants, as well as the availability of pollutants, are important considerations (Eskander & Saleh, 2017). In this regard, there are many biological factors which play critical role in the bioremediation process, i.e., interaction (competition, inheritance, and predation), enzyme activity, self-microbial growth, microbial population, mutation, horizontal gene transfer, and composition (Thomas & Nielsen, 2005).

3.2 Environmental Factors

The possible interactions in this process are determined by the metabolic features of microbes and the physical and chemical traits of targeted contaminants (Fan et al., 2017). The two actually interacted satisfactorily; nevertheless, it is dependent on the interaction site's environmental parameters (Gavrilescu, 2005). Environmental factors soil type, solubility, site specification, pH level, temperature, humidity, nutrients, redox potential, and oxygen level, as well as the bioavailability of contaminants, all have an impact on microbial growth and activity of pollutants concentration, type of pollutants, solubility, chemical structure, and their toxicity (L. Kumar & Bharadvaja, 2020). The kinetics of degradation is determined by the factors described above. Biodegradation could take place over a broad range of pH levels, but in most of the terrestrial and aquatic ecosystems, the optimal pH is 6.5–8.5. The types and quantities of soluble chemicals present, along with the pH and osmotic pressure of aquatic and land ecosystems, all affect the metabolic rate of contaminants. The majority of environmental influences are enumerated below (Leahy & Colwell, 1990).

3.2.1 Temperature

Temperature is the most important physical element that impacts the endurance of microbes and the composition of hydrocarbons among the physical parameter soil deterioration by natural processes is very sluggish in freezing places like the Arctic, thus microorganisms are under more strain to clean up the spilled oil (Leahy & Colwell, 1990). The water temperature in this area is below zero, which causes the transportation channels in microbial cells to close and possibly can even inactivate the metabolism of lipophilic bacteria through freezing the entire cytoplasm. The biological enzymes participating in the breakdown pathway have a preferred temperature and metabolic turnover will differ depending on the temperature (Konings, 2006). Furthermore, the breakdown of particular chemicals necessitates a specific temperature, which has a significant impact on the physiological features of microorganisms; therefore, it can either accelerate or reduce the speed of the process of bioremediation. Microbial activity increases as temperature rises, peaking at the ideal temperature (Si-Zhong et al., 2009).

3.2.2 Oxygen Concentration

Oxygen is required by some creatures, but it is not required by others. Biodegradation can be accelerated more effectively if it is tailored to their demands (Ali, 2010). Because most organisms require oxygen, biodegradation happens both in aerobic and anaerobic environments in most circumstances, the presence of oxygen can help hydrocarbon metabolism (Nzila, 2018).

3.2.3 pH

The acidity, alkalinity, and alkalinity of a chemical are represented by its pH value. It has its own effect on microorganism metabolic activities, as well as increasing and decreasing the elimination process (Adams et al., 2015). The potential for microbial growth can be determined by measuring pH in soil; the effects of higher or lower pH levels are poor; metabolic systems are extremely sensitive to tiny pH changes (Garcia et al., 1994).

3.2.4 Moisture Content

To finish their growth, microorganisms require enough water. Biodegrades are negatively impacted by soil moisture content (Kyrikou & Briassoulis, 2007).

3.2.5 Metal Ion

A modest amount of metal is essential for bacteria and fungi, but a high amount can interfere with cell metabolism (Ayangbenro & Babalola, 2017). The degradation rate is affected by metal compounds in both direct and indirect ways (Grenni et al., 2018).

3.2.6 Nutrient Availability

Nutrient availability contributes a lot in the effectiveness of biodegradation by regulating the balance of nutrients required by microbes for their growth and reproduction (Manzoni et al., 2012). By adjusting the C:N:P ratio by bacteria activity, nutritional balance, particularly the provision of critical nutrients, e.g., nitrogen (N_2) and phosphorus (P) can boost the efficiency of biodegradation. Microbes require various nutrients (carbon, nitrogen, and phosphorus) to survive and continue their activity (Jefferson et al., 2001). The degree of hydrocarbon breakdown is similarly limited at low concentrations. Addition of right amount of nutrients enhances the metabolism of microbes, which is a good way to boost biodegradation rates in cold conditions (Singh et al., 2021).

3.2.7 Toxic Compound

When certain pollutants are toxic at high concentrations, they will have toxic effects on microbes and reduce the purification rate (Abatenh et al., 2017). Toxicity varies in severity and mechanism based on the poison, its concentration, and the microorganisms exposed. Target life types are hazardous to some organic and inorganic chemicals (Karnawat et al., 2020) (Fig. 1).

4 Environmental Applications

Despite having significant biochemical and ecological properties, fungi are rarely used in the field of biotechnology (Thatoi et al., 2013). On the contrary, there is an extensive use of bacteria in this field, as excellent results are produced by them. They show several advantages such as highly specific biochemical reactions and effective decomposition of pollutants (Vangronsveld et al., 2009). The main reason why the fungus is underutilized is the cost of providing oxygen to the fungus in a polluted environment. However, filamentous fungi can be useful for situations where bacteria are usually not found to function (Goyal et al., 2016). For instance, fungi can be helpful for physically blocked contaminants which cannot be reached by bacteria, or in extreme environments such as dryness or high acidity where bacteria cannot perform well (Vijayaraghavan & Yun, 2008).

4.1 Bacteria

Bacteria are a wide variety of organisms, so they play an excellent role in biodegradation and bioremediation (Nair & Padmavathy, 2014) (Fig. 2). Bacteria have almost no universal toxins, so the right environmental conditions (aerobic,

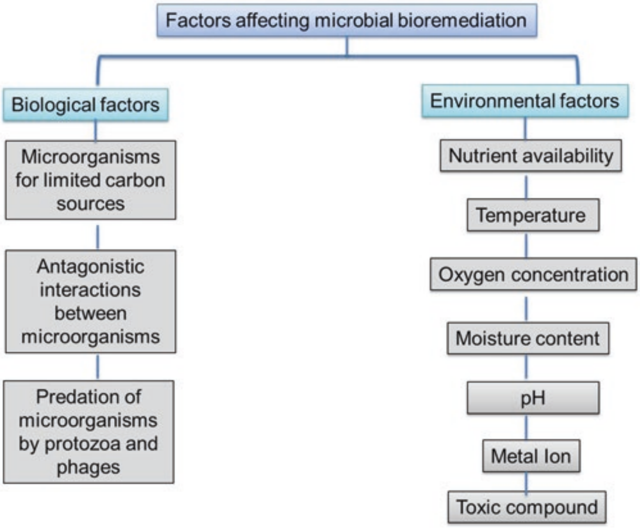

Fig. 1 Factors affecting microbial bioremediation

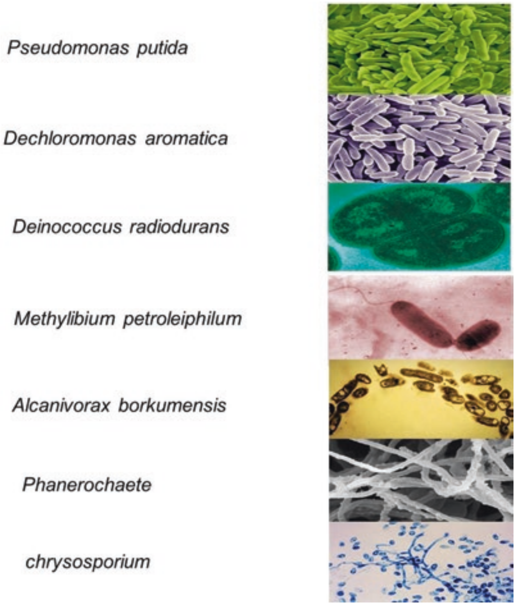

Fig. 2 Major groups of microorganisms employed in bioremediation

anaerobic, electron donors or acceptors, etc.) when provided (Nealson & Stahl, 2018), the organism is likely to be able to decompose any given substrate (Chowdhury et al., 2008).

4.1.1 *Pseudomonas putida*

Among the gram-negative soil bacteria, "*Pseudomonas putida*" has a critical role in the bioremediation of toluene, as paint thinner (Ramos et al., 2015). It can also degrade the petroleum-refined product naphthalene in contaminated soil (Kosaric, 2001). The widespread use of acids in various manufacturing units resulted in an increase in acidic waste streams discharged into the environment (Nleya et al., 2016). This co-contamination of acids and other organic pollutants confines the biodegradability of neutrophil degradants. It will be useful to increase viability and capability in bioremediation of acid waste and biodegradable activity to have GAD system or IrrE modulator in bacteria degrading pollutants to tolerate acid stress (Zhou et al. 2019).

4.1.2 *Dechlormonas*

Dechlormonas is a rod-shaped bacterium that helps in oxidizing aromatic hydrocarbons such as benzoic acid, chlorobenzoic acid, and toluene through the reduction of oxygen, nitrate, or chloric acid (N. Jiang et al., 2021). It is the only organism capable of anaerobic oxidation of benzene. This strain is particularly useful for the bioremediation of benzene, which has a high tendency of polluting ground and surface water (Philp et al., 2005). The following are the particular bacterial species known to be involved in bioremediation (Karigar & Rao, 2011).

4.1.3 *Dechloromonas aromatica*

RCB strain of *Dechloromonas aromatica* has the ability to degrade benzene anaerobically. In addition, it can also reduce percholate and oxidize toluene, xylene, and chlorobenzoate. So, all these properties created interest in this organism for use in bioremediation (Salinero et al., 2009).

4.1.4 *Deinococcus radiodurans*

Genetic engineering promotes the creation of organisms designed for bioremediation. *Deinococcus radiodurans* (the organism well known for its radiation resistance) has been designed to digest toluene and ionized mercury in high-level nuclear waste (R. Kumar et al., 2007).

4.2 Fungi

4.2.1 *Methylibium petroleiphilum*

PM1 strain of the Mthylibium petroleiphilum fungi species has the ability to grow on the fuel additive *methyl tert-butyl ether* (MTBE) and can completely degrade (Hanson et al., 1999). Pilot and field studies prove the effectiveness of PM1 for MTBE aerobic bioremediation (Bruns et al., 2001).

4.2.2 *Alcanivorax borkumensis*

Addition of inorganic nutrients that help bacteria to grow may assist in enhancing bioremediation. Some species like *Alcanivorax borkumensis* dissolve the oil through surfactant production, whereas other bacteria have the ability to degrade oil into CO_2 (Santisi et al., 2015).

4.2.3 *Phanerochaete chrysosporium*

White rot fungus has been reported for bioremediating various harmful compounds. Research by the scientists for the soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and related elements has become the focus of case studies. The use to degrade harmful compounds will be beneficial for the purification of the environment, and this technology can be effective for treating hazardous wastes (Fulekar et al., 2013).

4.3 Archaea

The role of archaea as a bioremediation agent is not as common for research studies or researchers (Naitam & Kaushik, 2021). Nevertheless, many studies have reported the demonstration of their ability to degrade various pollutants, and scientists are in search of their potential to participate in bioremediation (Pletsch et al., 1999). The biodegradation of extreme halophilic archaea has not been broadly recognized in the past, but now scientists found that they have greater catabolic diversity than expected. Pollution of hydrocarbons has been observed under severe conditions such as high salt and low or high pH or temperature (Al-Mailem et al., 2010). Archaea can adapt to various extreme environments, which helps them participate in various biodegradation and bioremediation activities under these severe conditions; in fact, it is eminent that microorganisms that naturally adapt to cold environments are important for hydrocarbon degradations in these environments. Extremely *halophilic archaea* can effectively biodegrade pollutants under high salt environment, in which bioremedial bacteria usually cannot survive or function properly (Verma et al., 2017). In addition, some archaea are also resistant to a variety of antibiotics, so they can easily participate in the bioremediation of antibiotics (Shah & Jain, 2020).

Four strains of extremely *halophilic archaea* belonging to *Halobacterium, Haloferax*, and *Halococcus* were explored to assess their potential for the biodegradation of hydrocarbons and crude oil. All these strains can use different hydrocarbons energy source (Le Borgne et al., 2008). The two Haloferax strains were grown on benzene, naphthalene, toluene, biphenyl, and n-alkane. Studies have proved that archaea can perform degradation at high temperature ranging from 40 to 45 °C and hydrocarbons are well biodegraded at these higher temperatures (Chen et al., 2019). The four strains tested are also resistant to various antibiotics, which makes it possible for them to undergo biodegradation under conditions that are not conducive to bacteria. Studies have shown that other archaea also have the ability to biodegrade at high-salt environments (Thakur et al., 2020).

5 Advantage and Disadvantage of Current Bioremediation

5.1 Advantage of Current Bioremediation

1. Bioremediation involving natural attenuation or biological stimulation is a recognized method of treating contaminated soil because it is based on natural processes (A. Kumar et al., 2011). When pollutants are present, the number of microorganisms that metabolize the pollutants usually increases, so the biodegradation rate may increase over time until it reaches a certain level. After the completion of biodegradation, the resulting products such as cellular biomass, CO_2, and water are usually harmless (Hou et al., 2018).
2. In-situ bioremediation can completely degrade pollutants into harmless products on site. This eliminates the risks involved in handling and eliminating contaminated materials (Sharma, 2012).
3. Bioremediation is a cheaper technology compared to other technologies being used to lessen the pollution (A. Kumar et al., 2011).

5.2 Disadvantage of Current Bioremediation

1. Only biodegradable compounds can undergo bioremediation. Not every compound degrades quickly and completely (Gavrilescu, 2005).
2. Biodegradation products could be highly toxic or more persistent than the previous one (original) pollutant (Docherty et al., 2010).

Table 1 List of microorganisms involved in bioremediation

Microorganisms	Microorganisms involved in bioremediation	References
Algae	*Cladophora fascicularis*, *Spirogyra* spp. and *Cladophora* spp., and *Spirogyra* spp. and *Spirullina* spp.	Deng et al. (2007); Lee and Chang (2011); Mane and Bhosle (2012)
Bacteria	*Arthrobacter* spp., *Pseudomonas veronii, Burkholderia* spp., *Kocuriaflava, Bacillus cereus,* and *Sporosarcina ginsengisoli*	Achal et al. (2012); Achal et al. (2011); Jiang et al. (2008); Kanmani et al. (2012); Roane et al. (2001); Vullo et al. (2008)
Fungi	*Penicillium canescens, Aspergillus versicolor,* and *Aspergillus fumigatus*	Kumar Ramasamy et al. (2011); Say et al. (2003); Taştan et al. (2010)
Yeast	*Saccharomyces cerevisiae* and *Candida utilis*	Kujan et al. (2006); Machado et al. (2010)

3. Usually their biological functions are very special and the existence of microorganisms able to metabolize pollutants is required. For the existence of right organisms, it is necessary to meet the appropriate environmental conditions, nutrient levels, and pollutants (V. Kumar et al., 2018).
4. It is difficult to expand the scale of research from initial small-scale research to field operations at a commercial scale. In the actual environment unevenly distributed, mixed and various phase (solid, liquid, gas) pollutants are usually found. Further research is needed for the creation of adaptable technologies (K. R. Reddy & Cameselle, 2009).
5. For the ample contact between microbes and the contaminants, more time is required for bioremediation technique as compared to other treatment technologies. The priority path and soil structure will bring uncertainty to the remediation spread (Caliman et al., 2011) (Table 1).

6 Conclusion and Remarks

It is necessary to better understand the role of microbes and their limitations to bioremediation in order to use them more effectively. The methodology could be improved by applying the principle of microbial ecology. Enhancing degradation through microbes as a means to clean up contaminated soil in situ has stimulated a lot of research. In particular, the rhizosphere is an area where microbial activity increases, which may promote the transformation and degradation of pollutants. Provision of oxygen and inorganic nutrients are the common methods for promoting degradation rate, but the use of enzymes, degrading microbes, and plants (phytoremediation) must also be considered.

1. Biodegradation is a very productive and striking choice for remediation, cleaning, management, and restoration of polluted environments through microbial activities.
2. The rate of degradation of hazardous waste depends on competition with biological agents, insufficient supply nutrients, abiotic conditions (ventilation, humidity, pH, and temperature), and less bioavailability of pollutants.
3. Due to these factors, the unsuccessful biodegradation under natural conditions leads to unfavorable results.
4. Environmental conditions must be suitable for the effectiveness of bioremediation, and the growth and activity of microbes.
5. Bioremediation is being used globally with varying degrees of success. The main advantage is that the disadvantages outweigh the disadvantages.

This can be seen at various sites that choose to use the technology and its increasing popularity over time. Usually, exploring various microbial species from various locations is effective in controlling bioremediation mechanisms.

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Microbial Exploration and Their Metabolic Capacity for Detoxification and Restoration of Natural Ecosystems

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1 Introduction

The microbial communities are distributed and allocated widely on the earth, due to their inbuilt metabolic capacity and ability, which is remarkable and they can comfortably grow in a wide range of environmental situations. The beneficial and nutritional versatility of microbial population can be used for remediation and biodegradation of contaminants and different contaminated ecosystems. Such type of phenomenon is known as bioremediation. Microbes utilize energy from the toxic contaminants and convert them to other less toxic substances and are therefore called bioremediators. The bioremediation efficient steps and processes not only collect the contaminant and utilize it, but the process of bioremediation is a microbiological well-organized procedural action that is applied to break down or transform contaminants and polluted ecosystems into less toxic or non-toxic elemental and compound forms with the help of microbial population including fungi and bacteria. These bioremediators are efficient biological agents used for bioremediation to clean up polluted sites. Microbial population like fungi, archaea, and bacteria are typical primary bioremediators. The efficient bioremediation used as a biotechnological process includes microbial population for solving, detoxifying, and removing toxicity of many contaminants through effective biodegradation from the contaminated systems. The effective bioremediation and biodegradation terms are more interchangeable processes and words. These microbial communities act as significant contaminant removal tools in water, sediments, and soil. These microbial

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_9

communities are restoring and detoxifying the original natural ecosystems and restricting further degradation and pollution.

The efficient and effective process of bioremediation converts organic pollutants mainly to carbon dioxide, water, and biomass. The pollutants can be immobilized by attaching to the humic substance fraction. The degradation process may occur under aerobic and anaerobic conditions. The primarily aerobic process is used for bioremediation, and classified as ex situ and in situ. An appropriate selection of technology among the variety of bioremediation technologies is developed to treat pollutants based on basic three principles: an amenability of the contaminant to biological transformation, the accessibility of the contaminant to microorganisms, and the opportunity for optimum biological actions. With the appropriate selection of technologies and conditioning, the process of degradation is enhanced and the efficacy of degradation is improved which primarily reduces the cost of treatment (Mohapatra, 2008). The ex-situ bioremediation methods are best used to clean pumped-out contaminated groundwater and excavated polluted soils. The bioremediation through in situ methods are defined as those which are used to soil and groundwater at the contamination site with minimal disturbance. These techniques of different types are among the most efficient and desirable options due to least disturbances and lower cost since microbial communities ensure action in place avoiding pollutant transportation and excavation. However, the depth of the soil that can be effectively treated limits in situ treatment. Almost in soils, effective oxygen diffusion for desirable rates of bioremediation extends to a range of only some centimeters around 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in many cases (Vidali, 2001). The process of bioremediation has been observed as an alternative to traditional physico-chemical methods to restore contaminated sites. Being a cost-effective, less labor-intensive, safe, and environment-friendly technique rapid development and advances are happening in this field for the past decades. Bioremediation observed effective for a wide range of soil pollutants including PAH, PCB, CAH, pesticides, explosives, even heavy metals, and radionuclides. However, in many cases, the bioremediation method combines several treatment techniques and can last for a long time (years and decades) and are often applied in combination with other techniques; therefore, it is difficult to estimate the efficacy of the same. In this context, more interdisciplinary research should be carried out with process optimization, validation, its impact on the eco-system and the effectiveness and predictability should be demonstrated to make it generalized.

The term bioremediation comprises of two parts: “bios” means life and refers to living organisms and “to remediate” means to solve a problem. The term “bioremediate” means to use microorganisms to solve an environmental issue such as contaminated soil or groundwater. The term bioremediation is the use of microorganisms to degrade environmental contamination or to prevent pollution. Especially, it is a method for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution (Table 1).

The earth has many bioremediants available that can be used against a broad range of pollutants. The process of bioremediation is a useful technique for the

Table 1 Microorganisms that remediate heavy metal pollution

S. No	Elements	Symbol	Microorganisms
01	Copper	Cu	*Bacillus* sp., *Pseudomonas aeruginosa, Chlorella vulgaris, Pleurotus ostreatus, Phormidium valderium, Volvariella volvacea, Daedalea quercina*
02	Nickel	Ni	*Pseudomonas aeruginosa, Zooglea* sp., *Chlorella vulgaris, Phormidium valderium*
03	Zinc	Zn	*Bacillus* sp., *Chlorella vulgaris, Aspergillus niger, Pleurotus ostreatus, Daedalea quercina*
04	Uranium	U	*Pseudomonas aeruginosa, Citrobacter* sp., *Chlorella vulgaris, Aspergillus niger*
05	Cobalt	Co	*Zooglea* sp., *Phormidium valderium*
06	Cadmium	Cd	*Ganoderma applantus, Zooglea* sp., *Citrobacter* sp., *Aspergillus niger, Pleurotus ostreatus, Stereum hirsutum, Phormidium valderium*
07	Lead	Pb	*Stereum hirsutum, Citrobacter* sp., *Chlorella vulgaris, Ganoderma applantus, Volvariella volvacea, Daedalea quercina*
08	Mercury	Hg	*Chlorella vulgaris, Rhizopus arrhizus, Volvariella volvacea, G. metallireducens*
09	Gold	Au	*Chlorella vulgaris, G. metallireducens*
10	Silver	Ag	*Aspergillus niger, Rhizopus arrhizus, G. metallireducens*
11	Chromium	Cr	*D. vulgaris, D. acetoxidans, D. fructosovorans, D. norvegicium*

degradation and detoxification of a wide range of contaminants present in different ecosystems. There are many compounds available within ecosystems that are detrimental and harmful, can be biotransformed or converted to less toxic or harmless products with the help of these microorganisms. Bioremediation restricts the bioaccumulation of highly toxic and detrimental pollutants from lower to higher trophic levels and movement from one ecosystem to another. Bioremediation eliminates or reduces the need to transport quantities of waste from the contaminated and can often be completed on-site, often without causing a major disruption of normal activities. Bioremediation has proven to be effective and cost-effective than other technologies for the cleanup of contaminated sites. Microorganisms used for such processes should be healthy and active, i.e., must be present in their active or exponential phase so that they can perform their work efficiently. Oxygen quantity will decide the efficiency of microbes; if sufficient amount of oxygen is present then contaminants and toxins can easily be converted into water and carbon. Xenobiotics such as nitroglycerine which is an explosive can also be cleaned up through bioremediation. Bioremediation is a natural attenuation with no or little human efforts. Furthermore, adding natural or engineered microorganisms can enhance the ideal catalytic abilities.

In bioremediation, microorganisms (bacteria, fungi, and algae) or plants are used to degrade and detoxify the hazardous pollutants present in different ecosystems and convert them into CO_2, H_2O, microbial biomass, and other less toxic metabolites. Bacteria is considered as one of the most efficient agents of biological degradation. In 1974, Williams and Murray made the first report of bacterial degradation of benzene derivatives by *Pseudomonas putida* which has a special enzymatic route to degrade these compounds and use them as a carbon source. A large number of bacterial species have been identified as efficient degraders of various xenobiotic compounds. The maximum members were from the genera of *Mycobacterium, Alcanivorax, Burkholderia, Cellulomonas, Sphingomonas, Micrococcus, Streptomyces, Bacillus, Haemophilus, Enterobacter Pseudomonas*, etc. These microorganisms can degrade polycyclic aromatic hydrocarbons (PAHs), pesticides, and azo dyes and remove or change the redox state of certain heavy metals. The degradation by xenobiotics via oxidases has been better studied in fungi as compared to bacteria although ligninolytic–like enzymes have been also found in bacteria. Enzymes identified as yellow laccases have low redox potential than fungal laccases. The normal peroxidase activity of bacteria is limited although another kind of peroxidase activity known as dye-decolorizing peroxidases has been extensively studied. Throughout the large umbrella of these enzymes, several oxidases can activate many xenobiotic compounds through the production of free radicals for their further mineralization and polymerization, rendering them non-bioavailable. Enzymes such as catalases and superoxide dismutase have been involved in PAH degradation. Pesticide compounds such as organophosphate group and carbamates can also be oxidized using bacteria with heavy metal being an exception. The bioremediation consists primarily of adsorption of these compounds into the cell wall, compartmentalization on vacuole or other organelles in eukaryotes, or changing their redox state into a less soluble form and thus making them less bioavailable.

Microorganism like fungi is among the top priorities and most promising for bioremediation since they produce a surfeit of oxidative and hydrolytic enzymes which are very effective in the process of bioremediation. One of the most important advantages of using fungi in situ is the presence of hyphae which covers a wide surface area in a single instant. Fungi can decompose lignin, cellulose, and hemicellulose, which have recalcitrant structures. The most widely studied fungal species for bioremediation are *Basidiomycota, Trametes, Phanerochaete, Pleurotus, Bjerkandera, Coriollopsis, Aspergilli, Trichoderma*, and *Fusarium*. The most important fungal-derived oxidases are laccases, peroxidases, lytic polysaccharides, and monooxygenases, which can degrade different compounds. Fungal enzymes involved in the mineralization of xenobiotic compounds are glucose oxidase, aryl alcohol oxidase, quinone oxidoreductase, and cellobiose dehydrogenase. These organisms can be indigenous to the site or can be isolated and transferred to a contaminated site. The bioremediation of pollutant compounds requires the action of several microbes, therefore sometimes potential microbes are used from other contaminated sites for the effective degradation process, and this process is called bioaugmentation (Table 2).

Biodegradation depends on favorable environmental conditions, the pollutant type, solubility of the pollutant compounds, and the bioavailability of the pollutant to the microbes. Environmental conditions are controlled to allow sufficient microbial growth for fast and effective biodegradation. Microbes with degradation potential have been isolated from contaminated environments, such as heavy metal-polluted sites, landfills, petroleum-contaminated sites, pesticide-contaminated sites, and

Table 2 Microorganisms having potential to degrade different organic pollutants

S. No	Pollutant	Microorganisms
01	Benzene, anthracene, hydrocarbons, PCBs	*Pseudomonas* spp.
02	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	*Alcaligenes* spp.
03	Benzene, hydrocarbons, pentachlorophe-Nol, phenoxyacetate, polycyclic aromatic	*Arthrobacter* spp.
04	Aromatics, long-chain alkanes, phenol, cresol	*Bacillus* spp.
05	Halogenated hydrocarbons, phenoxyacetates	*Corynebacterium* spp.
06	Aromatics	*Flavobacterium* spp.
07	Naphthalene, biphenyl	*Rhodococcus* spp.
08	Aromatics, branched hydrocarbons benzene, cycloparaffins	*Mycobacterium* spp.
09	Hydrocarbons	*Nocardia* spp.
10	Hydrocarbons, polycyclic hydrocarbons	*Xanthomonas* spp.
11	Phenoxyacetate, halogenated hydrocarbon diazinon	*Streptomyces* spp.
12	PCBs, polycyclic aromatics, biphenyls	*Cunniughamela elegans*

wastewater treatment plants. These microbes use hazardous contaminants as their source of energy and carbon source in aerobic and anaerobic conditions in other words metabolic activity can reduce or convert the pollutant to less or nontoxic metabolites. These soil microbes and pollutants should be in close contact for an effective degradation of the pollutant and it can be done by the application of surfactants. Aerobic bacterial species such as *Mycobacterium, Alcaligens, Sphingomonas,* and *Pseudomonas* are known for their aerobic degradation of hydrocarbons (alkanes and polycyclic aromatic hydrocarbons) and pesticides. A few of the aerobic methylotrophs are also recognized for the degradation of dichloroethane and trichloroethylene. There are many anaerobic bacterial species that are known for the degradation of PCBs, chloroform, and trichloroethylene (chlorinated solvent). Along the bacterial species, a few of the fungal species, such as *Phanerochaete chrysosporium*, are also reported to be efficient in the remediation of a variety of toxic and persistent toxic contaminants.

The metabolic characteristics of the selected microorganisms and physicochemical properties of the targeted contaminants determine to a large extent possible interaction during the process of bioremediation. The actual successful interaction between the microbes and pollutant however depends on the environmental conditions of the site of the interaction. Growth and activity of microbes are largely affected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site features, oxygen content, redox potential, and physico-chemical bioavailability of pollutants. These factors determine the kinetics of the degradation process. The process of biodegradation can occur under a wide range of pH. A pH of 6.5–8.5 is mainly optimum for biodegradation in most aquatic and terrestrial ecosystems. Moisture influences the rate of contaminant metabolism because it influences the kind and amount of soluble content that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems. The natural process of bioremediation is a slow and time-consuming process. Microbes degrade the contaminant and increase their population when the contaminant is still present. When the pollutant is degraded, the microbial population declines. Many of the hazardous compounds can be transformed into less toxic products, and this feature eliminates the chance of future liability associated with treatment and disposal of contaminated media. Bioremediation process does not use any synthetic and toxic chemicals. The nutrients especially biofertilizers added to make active and fast microbial growth are easily biodegraded. The natural technique of remediation is eco-friendly and sustainable. The contaminants are destroyed, or sometimes simply transferred to less toxic forms.

Bioremediation process is either in situ or ex situ. The basic bioremediation methods are biostimulation, attenuation, augmentation, venting, and piles.

2 Biostimulation

This type of strategy is operated through the injection of specific nutrients at the site of contamination to stimulate the activity of indigenous microbes. Naturally existing microbial communities are stimulated primarily by supplying fertilizers, growth supplements, and trace minerals for growth and active metabolism. Other environmental requirements like pH, temperature, and oxygen also need to be kept at optimum to speed up their metabolism rate and pathway.

3 Bioattenuation

Bioattenuation or natural attenuation is the eradication of pollutant concentrations from the contaminated site. It is carried out biologically (aerobic and anaerobic biodegradation), physically (dispersion, diffusion, advection, volatilization, dilution, sorption/desorption), and chemically (complexation, abiotic transformation, ion exchange).

The contaminants moves through soil and groundwater, they often can mix with water which reduces or dilutes the pollution. Many chemicals, like oil and solvents, can evaporate, they change from liquids to gases within the soil. Meanwhile, if these gases escape to the air at the ground surface, sunlight may destroy them. If the natural attenuation is not quick enough or complete enough, bioremediation will be enhanced by either biostimulation or bioaugmentation.

4 Bioaugmentation

The addition of additional contaminant degrading microorganisms (natural/exotic/engineered) to augment the biodegradative capacity of indigenous microbial populations on the contaminated area is known as bioaugmentation. The microorganisms are first collected from the remediation site, then separately cultured, sometimes genetically modified and returned to the contaminated site. Bioaugmentation is also referred to as the process of adding engineered microbes to a system, which act as bioremediation to quickly eliminate complex pollutants. The natural species must be genetically modified through DNA manipulation to facilitate genetically engineered microbes; as naturally they are not efficient enough to break down certain compounds. Genetically modified microbes act much faster than the naturally existing species and are highly competitive with the indigenous species, predators, and also various ecological factors. The genetically engineered microbes have shown potential for bioremediation of soil, groundwater, activated sludge, and oil spills in oceans.

5 Bioslurping

In this process, combination of vacuum-enriched pumping, soil vapor extraction along with bioventing is used for remediation of soil and groundwater providing indirect oxygen supply and stimulating the biodegradation of contaminants. This technique can also be used for remediation of semi-volatile and volatile organic compounds from contaminated soils. This method is not appropriate for remediation of soil having little permeability.

6 Genetically Engineered Microorganisms (GEMS)

Genetically engineered microorganism is a microorganism whose genetic material has been changed by applying genetic engineering techniques inspired by the natural otherwise artificial genetic exchange between microorganisms. These kinds of artistic work and scientific procedures are mainly termed recombinant DNA technology. The recent genetic engineering has improved the utilization and elimination of hazardous unwanted wastes under laboratory conditions by creating genetically modified microorganisms. Recombinant living organisms can be obtained by recombinant DNA techniques or by the natural genetic material exchange between microorganisms. Currently, development is required in gene production having the potential to degrade complex toxic substances into eco-friendly substances. The genetically engineered microorganisms (GEMs) are efficient for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of synthetic contaminants. Recently, many opportunities forward for improving degradative performance using genetic engineering actions for rate-limiting steps in known metabolic pathways, which can be genetically manipulated to yield increased degradation rates. In GEMs, four activities/strategies to be done are modification of enzyme specificity and affinity, pathway construction and regulation, bioprocess development, monitoring, and control, bioaffinity bioreporter sensor applications for chemical sensing, toxicity reduction, and endpoint analysis. The primary genes of bacteria are carried on a single chromosome but genes specifying enzymes essential for the catabolism of some of these unusual substrates may be carried on plasmids. Plasmids have been implicated in catabolism. Therefore, GEMs can be used effectively for biodegradation purposes and leads to represent/indicate a research frontier with broad implications in the future time. The major function is to speed up the recovery of waste-polluted sites, increase substrate degradation, displays a high catalytic or utilization capacity with a small amount of cell mass, crate safe and purified environmental conditions by decontamination or neutralizing any harmful contaminant.

7 Bioventing

Bioventing helps with in situ bioremediation of pollutants present in soil by providing enough supply of oxygen to microorganisms involved in converting pollutants into a harmless product. Bioventing requires low airflow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil using wells. Although rate of airflow and air interval are the most important factors of bioventing, still accomplishment depends on the number of air injection points for uniform distribution of air. The adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil. Effective bioremediation of petroleum-contaminated soil using venting has been proved by many researchers.

8 Biopiles

This is a complete treatment technology in which excavated soils are mixed with nutrients to enhance microbial activities and placed on a treatment bed with main components such as irrigation, aeration, leachate, and nutrient collection systems. Various environmental and physico-chemical parameters viz. heat, moisture, nutrients, pH, and oxygen can be controlled for further enhancement of biodegradation. Filtering and ventilation of polluted soil, addition of bulking agents like saw dust, straw, wood chips, or any other organic materials can further help in enhancement of efficiency. Biopiling can be effectively used to control volatilization of low molecular weight pollutants and can work even under extreme cold environments. Biopile can be used for treatment of huge quantity of contaminated soil in less space in comparison to different ex situ bioremediation methods comprising land farming. Biopiles are also known as biocells, bioheaps, biomounds, and compost piles. In this process, the air is supplied to the biopile system during a system of piping and pumps that forces air into the pile under positive pressure or draws air through the pile under negative pressure. The microbial activity is enhanced through microbial respiration then the result in the degradation of adsorbed petroleum pollutants became high.

The bioremediation process is broadly categorized into in-situ remediation and ex-situ bioremediation, based on the origin, transportation, and removal of pollutants from contaminated sites, as shown in Fig. 1.

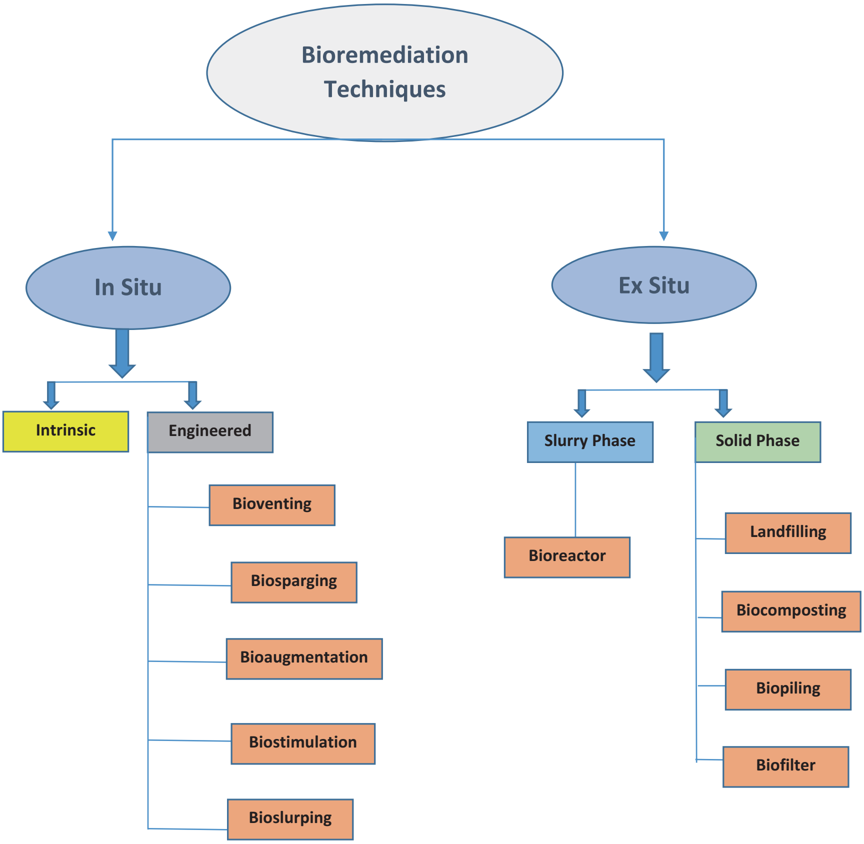

Fig. 1 Types of bioremediation

The process of biodegradation occurs under aerobic and anaerobic conditions; majority of bioremediation systems are designed to operate and degrade contaminants aerobically. Organic compounds are degraded aerobically, undergo oxidation to form less toxic compounds such as CO_2 and water. Throughout the anaerobic degradation, persistent intermediate compounds may be formed. Meanwhile, the anaerobic biodegradation of chlorinated aliphatic solvents can produce lower substituted chlorinated hydrocarbons, such as chloroethane or vinyl chloride. There are some compounds that are not readily degraded under anaerobic conditions and maybe more toxic than the original contaminant. The biodegradation of contaminants occurs as direct or co-metabolic processes. Direct bioremediation processes include the microbes that use the contaminants as a source of food or energy. When contaminants cannot be used as a food source, biodegradation may occur through co-metabolism in which the pollutant is degraded by an enzyme or cofactor produced during microbial metabolism of another compound.

Bioremediation is an emerging technology that can be simultaneously used with other physical and chemical treatment methods for the complete management of a diverse group of environmental pollutants. It seems like a sustainable approach for environmental pollution management, and hence, there is a need for more research in this area. Efforts need to be made to generate a synergistic interaction between the environmental impact on the fate and behavior of environmental contaminants and the assortment and performance of the most suitable bioremediation technique that can sustain the effective and successful operation and monitoring of a bioremediation action required. The current efforts of research and development will direct future regulations, dealing with bioremediation targets, contaminant availability, and their potential threat to the human and natural ecosystems.

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Microbes in Restoration of Polluted Ecosystems

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1 Introduction

1.1 Why Polluted Ecosystems Are Considered a Threat?

Global climate change and water scarcity are examples of problems humanity is facing as harmful after-effects of human actions over the environment ignoring sustainability principles (Santhakumari & Sagar, 2020). Pollution is an important consequence of these actions as it is a serious threat to the ecosystem, including the living beings present. Humans, for example, can be highly affected by pollutants in a negative manner not only at cellular level but also at the level of organs and systems (Fig. 1). A large array of organic and inorganic pollutants possesses the capacity to be persistent contaminants, accumulating in polluted areas for long periods of time and also entering the food chain (becoming a threat to food security) (Ojuederie & Babalola, 2017). This kind of pollution is especially difficult to deal with once the effects of legislative control inducing a reduction in new pollutant emissions take a long time to be noticed on the environment. The concentrations, for example, of these substances in freshwater predators still exceeded the limits considered safe for reproduction/survival decades after measures to reduce new emissions of persistent contaminants in the water (Kean et al., 2021).

Pesticides, for example, are ubiquitous environmental pollutants that present a risk to 64% of global agricultural land and a high risk to 31% of agricultural land worldwide. These organic persistent pollutants negatively impact biodiversity, water quality, and human health (Tang et al., 2021). In human organism this kind of

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_10

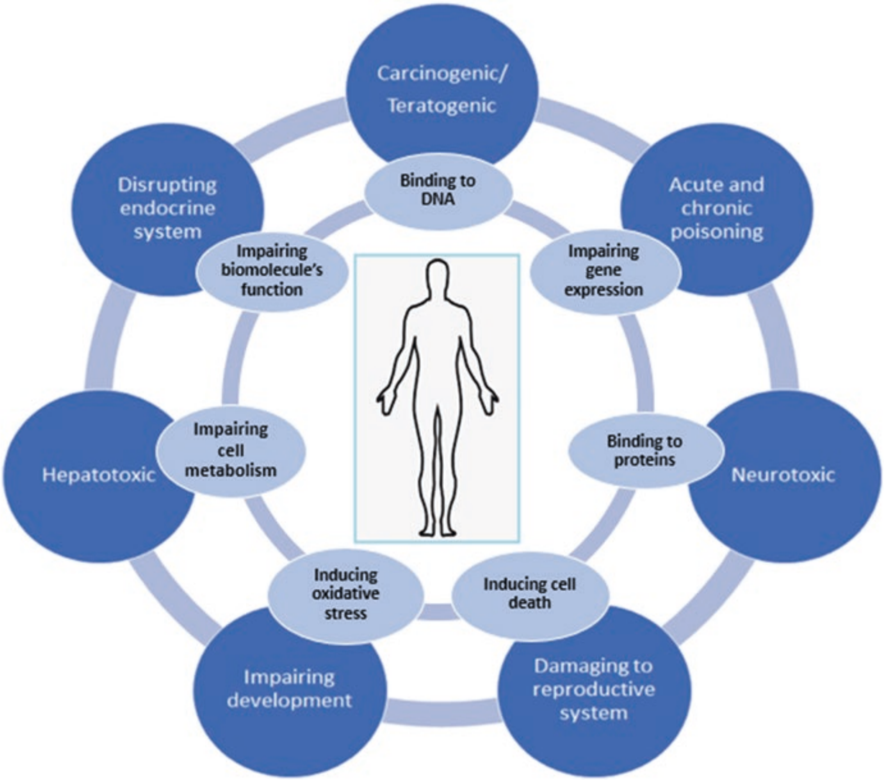

Fig. 1 Pollutants as threats to humans' health – main negative effects pollutants can cause on the human body at cellular level (inner circle) and also at the level of organs and systems (outer circle)

substance can impair the functioning of different organs and tissues through cytotoxicity and DNA damage, being neurotoxic, hepatotoxic, carcinogenic, disrupting endocrine system, and being also teratogenic (Kalyabina et al., 2021). Carbofuran [2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methyl carbamate] is widely used in agricultural practices and after inhalation, ingestion, or dermal absorption can do severe damage to different living beings causing also their deaths (Mishra et al., 2020).

When it comes to textile industry effluents, for example, the large spectrum of chemicals used during the process reflects on the polluted wastewater generated. Heavy metals and textile dyes present toxic effects on living organisms especially from the aquatic biota and to people who will drink the water even after treatment. It is common that treatments fail to remove metals on only disperse dyes (Methneni et al., 2021).

Heavy metals, for example, can enter human body through inhalation, gastrointestinal tract, or skin and cause not only membrane but also DNA damage. By binding to functional groups in proteins (such as thiol) they can disturb protein/enzyme function (Witkowska et al., 2021). Mercury, for example, is a heavy metal that can accumulate in the human's body as same as in animals' bodies causing severe problems. Fish consumption can expose humans to the neurotoxicant methylmercury (Moriarity et al., 2020; Novo et al., 2021) poisoning them. This substance is also highly toxic to animals (Davis et al., 2021). Activities such as gold mining are also risky (Achatz et al., 2021) to allow mercury intoxication. These heavy metals also damage plants negatively impacting the photosynthesis rate and the metabolism as a whole (Hu et al., 2020).

1.2 Living Beings Performing Remediation: Bioremediation

Physicochemical methods of remediation, such as soil washing, soil flushing, electrokinetic remediation, solvent extraction, incineration, and chemical reduction in the gas phase, can be applied to deal with environmental contaminants (Ajiboye et al., 2020; Baldissarelli et al., 2019; Cameselle & Gouveia, 2019). However, commonly some disadvantages are faced especially when they are applied on a large scale. High cost and generation of additional pollution are examples. Bioremediation, however, can present interesting advantages regarding the costs and it is also a process eco-friendly (Gaur et al., 2018; Gong et al., 2018; Fernando et al., 2019).

Bioremediation can be performed by a large variety of living beings: bacteria, fungi, yeasts, microalgae, and plants that can degrade contaminants in a harmless state or provide mechanisms to reduce their concentration to levels considered safe (Estrada & Quijano, 2020; Ojhaa et al., 2021). These living systems present the ability to modify and/or decompose pollutants and this ability can be naturally found on the species or added through genetic engineering strategies (Zhu et al., 2012; Ye et al., 2017).

Strategies of bioremediation can also be applied together with physicochemical strategies. The inoculation of microbes such as bacteria can contribute to enhance the efficiency of pollutants removal and restoration of ecosystems by reestablishing water and/or soil biological function and also in the treatment of contaminated air (Chen et al., 2016). Bioactive coatings, for example, allow using microorganisms immobilized in bedding nanomaterials to improve air quality (Estrada & Quijano, 2020).

Restoration of contaminated areas through bioremediation can be performed ex situ (removing samples of the polluted environment, treating and returning it to its prior localization – more easily performed when the intention is to remediate soil) or in situ (treating the polluted area directly where it is) (Ortiz-Hernández et al., 2018; Parween et al., 2018).

The efficiency of bioremediation is influenced by various aspects related to the living being employed, environmental factors of the contaminated areas, number and amount of contaminants as same as their chemical nature, and also by the protocol of remediation applied (Azubuike et al., 2016). It is common to have a redox process involved in remediation promoted by living organisms and consequently addition of organic and inorganic amendments to regulate medium physicochemical properties can favor environmental decontamination/restoration (Beiyuan et al., 2017). For example, to remediate oily contaminated soil, protocols of bioremediation can have their efficiency improved by adding biosurfactants and lipases (Kreling et al., 2021). Biochar can be used to immobilize metals and organic pollutants enhancing the bioremediation success and this type of strategy has been reported by many researchers (Rizwan et al., 2016; Yuan et al., 2017).

2 Microbes Restoring Polluted Ecosystems

There are microbes that can naturally deal well with some types of environmental pollutants, metabolizing or sequestering them from contaminated areas (which is a process known as natural attenuation). However, it is generally a time-consuming strategy to be applied (Cui et al., 2020). In order to improve process' efficiency and speed it, microbes can be submitted to genetic engineering or receive stimulus: from substances added to the polluted spot (biostimulation), from aeration of the polluted area to increase biodegradation pollutants (bioventing) or from microbial taxa with useful biodegradation/detoxification capacity (bioaugmentation) (Gaur et al., 2018; Dell' Anno et al., 2021a).

Among microbes (bacteria, fungi, yeasts, microalgae, and protozoa) bacterium is the most applied on bioremediation protocols (Jain & Bajpai, 2012) since the 1980s (Delfino & Miles, 1985; Karns et al., 1986; van der Hoek et al., 1989) with a deserved highlight being directed to genera such as *Corynebacterium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella, Enterobacter, Flavobacterium, Pseuodmonas, Bacillus, Alcanivorax, Thallassolituus, Cycloclasticus, Oleispira; Vibrio, Pseudoalteromonas* and *Marinobacter* specially when it comes to organic pollutants (Haritash & Kaushik, 2009; Kafilzadeh et al., 2011; Dell' Anno et al., 2021a, b) (Table 1).

Species naturally able to remediate can use pollutants as nutrient source, surviving in contaminated areas such as *Pseudomonas stutzeri* OX1 dealing with tetrachlorethylene (Ryoo et al., 2000) and *Pseudomonas nitroreducens* and *Pseudomonas putida* metabolizing p-coumaric acid and p-hydroxybenzoic acid (Zhang et al., 2010).

Microalgae are also very useful to be used in bioremediation protocols, especially to remediate environs contaminated by polycyclic aromatic hydrocarbons, but also to deal with heavy metals as contaminants. The genera *Chlorella*, *Selenastrum,* and *Scenedemus* deserve a highlight. *Chlorella pyrenoidosa* could efficiently remediate heavy metals (Cr, Cu, Pb, Zn, Cd, Mn, and Ni) from wastewater collected from a common effluent treatment plant (Kothari et al., 2021). *Chlorella sorokiniana* could remediate wastewater contributing to the assimilation of Zn and Ni but also nitrogen and phosphorous (Lugo et al., 2020). The main mechanism involved in this process is related to reduction in bioavailability (and consequently toxicity) due to the exopolysaccharides that make it possible pollutants' immobilization and/or internalization (Dell' Anno et al., 2021a). However, some species can use

Table 1 Examples of bacteria that can be used to bioremediate environmental pollutants

Bacterium species	Pollutant	Reference
Pseudomonas stutzeri OX1	Tetrachlorethylene	Ryoo et al. (2000)
Pseudomonas nitroreducens, *Pseudomonas putida*, and *Rhodotorula glutinis*	p-coumaric acid and p-hydroxybenzoic acid	Zhang et al. (2010)
Pseudomonas sp. strain ADP	Atrazine and cyanuric acid	Neumann et al. (2004)
Thalassolituus oleivorans	Aliphatic hydrocarbons from C7 to C20 carbons	Yakimov et al. (2004)
Flavobacterium sp.	Organophosphate pesticides	Ortiz-Hernandez et al. (2004)
Cycloclasticus spp.	Polycyclic aromatic hydrocarbons	Niepceron et al. (2009)
Achromobacter sp. WM111, *Rhodococcus* TE1, *Pseudomonas* sp. 50,432, *Sphingomonas* sp. strain SB5, *Enterobacter* sp., *Burkholderia* sp. PLC3, *Bacillus* sp., and *Cupriavidus* sp. ISTL7	Carbofuran	Karns et al. (1986), Behki et al. (1994), Chaudhry et al. (2002), Kim et al. (2004); Park et al. (2006), Mohanta et al. (2012), Plangklang and Reungsang (2013), Onunga et al. (2015), Gupta et al. (2019)
Staphylococcus succinus HLJ-10	D-cyphenothrin	Huang et al. (2020)
Novosphingobium sp. PCY, *Microbacterium* sp. BPW, *Ralstonia* sp. BPH, *Alcaligenes* sp. SSK1B, and *Achromobacter* sp. SSK4, PCY	Polycyclic aromatic hydrocarbons (PAHs)	Wongwongsee et al. (2013)
Species from *Streptomyces* gender	Chlordane	Cuozzo et al. (2012)
Alcaligenes faecalis	Endosulfan	Kong et al. (2013)
Sphingobium wenxiniae strain JZ-1	3-phenoxybenzoate	Cheng et al. (2015)
Corynebacterium variabilis Sh42	2-hydroxybiphenyl (2-HBP), catechol, and benzoic acid	Younis et al. (2020)
Staphylococcus aureus V329	Uranium (VI)	Shukla et al. (2020)
Bacillus cereus WHX-1	Chromium (VI)	Chen et al. (2021)
Escherichia coli, Streptococcus pyogenes, and Streptococcus pneumoniae	Zoxamide	Ahmad et al. (2020)
Shigella flexneri FB5	Fomesafen	Yang et al. (2020)
Species from the genus *Alcaligenes*	Cu^{2+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, and Zn^{2+}	Sodhi et al. (2020)
Oleispira antarctica RB-8	Hydrocarbons	Gregson et al. (2020)
Acinetobacter sp.	Fluoride	Shanker et al. (2020)
Klebsiella variicola	Chromium VI	Yu et al. (2021)
Enterobacter sp. MN17	Petroleum hydrocarbons	Ali et al. (2020)
Alcanivorax borkumensis	Oil hydrocarbons	Shaikhulova et al. (2021)
Vibrio fluvialis	Mercury	Saranya et al. (2017)
Pseudoalteromonas sp. SCSE709-6	Cadmium	Zhou et al. (2013)
Marinobacter sp.	Hydrocarbons	Al-Wahaib et al. (2016)

non-chlorinated hydrocarbons as carbon source degrading petroleum hydrocarbons (Chekroun et al., 2014). *Selenastrum capricornutum* and *Scenedesmus acutus* could efficiently promote the biodegradation of benzo(a)pyrene (de Llasera et al., 2016).

Fungi are capable of degrading environmental pollutants, especially organic ones (such as pesticides, dyes, and hydrocarbons) through mycodegradation, bioremediating environs (Bhattacharya et al., 2012). Important genera when it comes to this activity are *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Rhizopus,* and *Trichoderma* (Dell' Anno et al., 2021a). *Aspergillus niger* could efficiently deal with environmental contaminant 2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide, as same as the bacterium *Xanthomonas axonopodis* (Ahmad et al., 2020). The dead *Aspergillus niger* O-5 biomass could also remediate Pt^{4+} from polluted samples (Lombana-Fraguela et al., 2020). *Aspergillus* sp. A31 and *Curvularia geniculata* P1 favored the growth and development of *Oryza sativa* L. under mercury stress by sequestrating the heavy metal (de Siqueira et al., 2021). *Penicilium chrysogenum,* as same as *Alternaria alternata,* efficiently promoted polyaromatic hydrocarbons' degradation, bioremediating contaminated samples (Hamad et al., 2021). *Drechslera* sp. strain 678 proved to be an interesting option to the remediation of methyl tertiary-butyl ether, a common additive of gasoline (d'Errico et al., 2021). *Fusarium solani* exhibited high tolerance to Zn^{2+} ions and was capable of promoting their biotransformation (El Sayed, 2020); the capacity of this species to remediate metal-contaminated waste could be enhanced by the presence of the gram-negative bacterium *Comamonas aquatica* (Qurbani & Hamzah, 2020). *Lasiodiplodia theobromae* could remediate polluted samples containing benzo[a]pyrene by using enzymes such as lignin peroxidase and laccase (Cao et al., 2020). *Mucor irregularis* strain bpo1 proved to be able to promote the biodegradation of fluorene (Bankole et al., 2020) and *Mucor hiemalis* could deal well with acetaminophen, especially after pH adjustment (Esterhuizen et al., 2021). *Rhizopus stolonifer* could remediate samples polluted with Cd in an efficient manner, and when associated with the bacterium *Bacillus megaterium* also proved to be highly efficient to deal with Pb pollution (Njoku et al., 2020). 2,4,6-trinitrotoluene could be degraded by *Trichoderma viride* eradicating the toxicity associated with the pollutant (Alothman et al., 2020).

Yeasts are particularly relevant when it comes to remediating pollution caused by heavy metals (Sun et al., 2020). For example, *Diutina rugosa* standed out among 213 strains by its capacity to remediate Zn pollution (García-Béjar et al., 2020). However, organic pollutants can also be metabolized by yeasts, such as aflatoxin B_1 by *Rhodotrorula mucilaginosa* (García-Béjar et al., 2020) and azodyes by *Sterigmatomyces halophilus* SSA-1575 (Al-Tohamy et al., 2020).

The mechanisms applied by microbes to remediate (that depends on the pool of genes and consequently proteins/enzymes available) can influence the process' efficiency. However, accessibility and bioavailability of the contaminants as same as environment characteristics (salinity, temperature, pH, and redox potential) can also interfere on this capacity (Gaur et al., 2018; Fernando et al., 2019).

3 Microbes Assisting Bioremediation Promoted by Plants

Phytoremediation involves the use of plants to restore environs polluted by environmental contaminants. However, there are some researchers that also consider the microbes associated with plant roots as part of the process. That is due to the importance that plant growth-promoting rhizobacteria and plant endophytes possess in improving the remediations' efficiency making it easier for plants to deal with complex scenarios such as dealing with a large array of different types of contaminants in the same area (He et al., 2020).

Phytostabilization and rhizodegradation are examples of phytoremediation mechanisms in which the participation of microbes is crucial. For example, *Funneliformis mosseae* (a fungus species) could improve the capacity of the plant species *Robinia pseudoacacia* to remediate Pb contamination through phytostabilization. The microbe promoted Pb's immobilization, consequently reducing this heavy metal's toxicity to the vegetal species phytoremediating it (Huang et al., 2019). *Alcanivorax* and *Bacteroidetes* are microbes that can live well in stressful situation regarding salt level and also present the capacity to metabolize some organic contaminants. They proved to be important tools to favor remediation of petroleum hydrocarbons by plant species *Hylotelephium spectabile* (Cheng et al., 2019). In fact, a large array of petrochemical pollutants, and hydrocarbon in general, contaminating water and soil environment could be remediated by phytoremediation assisted by microbes through different mechanisms/strategies (Asemoloye et al., 2019; Singh et al., 2021).

It is well known, for example, that microbes can favor the removal of heavy metals and radionuclides (that generally come from industrial and municipal solid waste) performed by plants. And in situation in which removal is difficult, they can favor neutralization or conversion into less toxic substances by biotransforming, biosorbing, and biomineralizing (Thakare et al., 2021). *Enterobacter cloacae* ATCC 13047, an endophytic bacteria isolated from *Ficus septica*, for example, could remediate soil contaminated with Cr (VI) reducing the pollutant to Cr^{3+} and contributing to the survival of the vegetal species (Rohmah et al., 2020). *Streptomyces pactum* and *Bacillus* sp. co-application could improve *Brassica juncea*'s growth and also favored phytoextraction of Cd, Cu, Pb, and Zn promoted by the plant (Jeyasundar et al., 2021).

It is also interesting to mention that microbes associated with plants can also favor the vegetal's development besides improving remediation potential. For example, *Klebsiella pneumoniae* AWD5 not only enhanced the capacity of *Jatropha curcas* to deal with aromatic hydrocarbon's pollution, but also favored plant's growth in pyrene-contaminated soil (Rajkumari et al., 2018).

Not only plants are influenced by the microbes associated with them, but they can also stimulate the growth and development of microorganisms present in the rhizosphere through chemical substances such as growth factors (Dominguez et al., 2019).

It is also possible to genetically modify plants, using sequences of DNA originally present in microbes' DNA, or in other organisms, to improve the efficiency of phytoremediation (Ozyigit et al., 2021). *Arabidopsis thaliana* could have its capacity to promote mercury phytoextraction improved after genetically engineering the plant to express the bacterial mercury transporter MerC fused with SYP121 (a plant SNARE that favors protein transportation to cell membrane) under the control of a root epidermis-specific promoter. Mercury accumulation was enhanced in shoots and phytoremediation's efficiency was successfully improved (Uraguchi et al., 2019).

4 Engineered Microbes Restoring Polluted Ecosystems

The development of fields related to genomics, metagenomics, metabolomics, transcriptomics, proteomics, and genome editing technologies is crucial to the advancement of bioremediation techniques (Jaiswal et al., 2019; Marco & Abram, 2019). Synthetic biology, for example, presents strategies applicable for bioremediation that involve cell-mediated detection of pollutants and remediation by genetic circuit and microbial biosensor (Jaiswal & Shukla, 2020). Metabolic reconstruction, for example, can allow the generation of microorganisms with improved catabolic activities by genetic engineering, offering elegant strategies for the remediation of contaminated ecosystems (Janssen & Stucki, 2020).

Various examples of protocols to generate genetically modified bacteria (GMB) to perform bioremediation are available in the literature and new ones are still being proposed nowadays. Phytochelatin synthase from *Pyrus calleryan,* when overexpressed in *Escherichia coli,* allowed remediation of Cd, Cu, and Hg and also increased tolerance to the heavy metals' presence (Li et al., 2015). The expression of the azoreductase from *Enterococcus* sp. L2 (product of *azoA* gene) in *E. coli* DH5α and *Pseudomonas fluorescens* PfO- allowed decolorization of recalcitrant azo dyes. This process has its efficiency enhanced by coexpression of *azoA* with *fdh* from *Mycobacterium vaccae* N10 (Rathod et al., 2017). *Deinococcus radiodurans* (a radiation-resistant bacterium) was recently engineered to overexpress the smtA gene from *Synechococcus elongatus* fused to sequences from the surface layer proteins Hpi and SlpA. The gene is responsible to encode the metal-binding metallothionein protein that is naturally located in the cell's cytoplasm but fusion proteins took it to cell surface. This strategy offered a extraction of cadmium 1.5–3 times higher when compared to the one performed by organisms expressing only the cytosolic version of the metal binding metallothionein protein and cell-free preparations presented a potential for uranium remediation (Misra et al., 2021). Recombinant *Rhodococcus erythropolis* expressing ammonia monooxygenase and hydroxylamine oxidase offered optimized results on the remediation of pollution associated with landfill leachate (Bai & Tian, 2021).

Fungi and yeast can also be genetically modified to offer optimizations in the results of bioremediation protocols. However, yeasts are more easily genetically modified than fungi, being more applied in remediation protocols. They can deal, for example, with heavy metals' pollution promoting their accumulation, precipitation, and changing their redox state (Ayangbenro and Babalola, 2017). The gene *EpNramp* from *Exophiala pisciphila* encodes a metal transporter; yeasts expressing this protein could enhance their natural capacity to accumulate Cd^{2+} (Wei et al., 2016). The gene lac I that encodes a laccase from the fungus *Phlebia brevispora* BAFC 633 could be successfully expressed in *Pichia pastoris*, and the enzyme exhibited high tolerance to diverse solvents and NaCl, being also capable of degrading recalcitrant synthetic dyes (Fonseca et al., 2018). When the dye-decolorizing peroxidase from *Pleurotus ostreatus* (a white rot basidiomycete) was expressed in the filamentous fungus *Trichoderma atroviride* it allowed decolorization of mono-azo, di-azo, anthraquinone, and anthracenedione dyes (Cuamatzi-Flores et al., 2019).

Microalgae can also be modified to enhance bioremediation potential. Overexpression of *CrMTP4* gene in *Chlamydomonas reinhardtii* increased the potential of the organism to remediate Cd pollution. The gene encodes for a member of the Mn-CDF clade of the cation diffusion facilitator family of metal transporters (Ibuot et al., 2017). The potential to promote remediation of Cd^{2+} and Zn^{2+} ions could be enhanced in this species after recombinant expression of a protein from *Arabidopsis thaliana*: the AtHMA4 C-terminal domain protein (Ibuot et al., 2020). Cd^{2+} bioremediation could also be optimized through the expression by *C. reinhardtii* of a synthetic gene (*gshA*) encoding for a *gamma*-glutamylcysteine synthetase (Piña-Olavide et al., 2020).

5 Recovering Soil Microbial Community to Promote Ecosystems Restoration

It is also possible to apply microbes in other types of protocols aiming restoration of polluted ecosystems. For example, improving soil aggregation by influencing beneficial communities of microorganisms. The functional recovery of the soil microbial community (SMC) is essential for ecological restoration. Incorporating SMC measurement and monitoring into the study designs is a challenge, once there is still not available a metric that represents the diverse functional and compositional complexity inherent in the SMC. Focus must change from trying to compositionally recreate the "reference" SMC for the creation of functionally robust SMCs that provide ecosystem functioning and provide ongoing ecological resilience in restored ecosystems (Hart et al., 2020). Soil inoculation is a common form of microbial reforestation, which consists of moving soil from target sites to restoration sites (Wubs et al., 2016). This practice is known as "the whole community" rewilding, and although it is evident in soil inoculation studies, is very little researched outside of soil transplants and therefore rarely considered during restoration. The desired sites are on the practitioner's premises criterion; so they can adapt the community built on the base of any site they choose. However, a summary of community-wide reforestation for restoration purposes highlights that nearby remaining sites are chosen more often, which conforms to conventional restoration paradigms (Contos et al., 2021; Mcdonald et al., 2016).

6 Conclusions

Physicochemical methods to restore polluted areas may present some disadvantages (such as the high cost to be performed on large scale) that can be surpassed by bioremediation strategies. Among bioremediation strategies is a large array of protocols applying microbes as tools to remove environmental pollutants and contribute to the restoration of ecosystems. There are strategies that use only microbes on their wild form, protocols applying genetically modified versions of these organisms to optimize results, and strategies associating microbes and phytoremediation, among other types of protocols. Microorganisms proved to be efficient in performing remediation of contaminants from diverse chemical nature in different environments.

7 Future Perspectives

In order to enhance the opportunity of innovative protocols using microbes to promote remediation, it is essential that the metabolism of microbes be known in a deep way. So, advancements in the field of molecular biology and in omics platforms are highly relevant to the proposal of new rapid, eco-friendly, safe, and cost-effective technologies of bioremediation of polluted ecosystems by microbes. The improvements on the possibility of efficiently engineering the DNA of these organisms, for example, are directly dependent on these advancements. Biosafety related to the field use of microbes in bioremediation also needs to receive special attention considering also the impact of microbe-assisted bioremediation on the ecosystem as a whole.

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Microbial Biotechnology: Energy Generation Approach from the Environmental Waste

Charles Rashama, Grace N. Ijoma, and Tonderayi S. Matambo

1 Introduction

Landfills that form part of the sanitation supply chain in the terminal stages of handling municipal solid wastes (MSW) are a commonplace infrastructure across the globe. While providing this crucial service, landfills unfortunately generate significant greenhouse gas (GHG) emissions, mainly in the form of methane and carbon dioxide, arising from the biological degradation of organic components of the landfilled waste. Landfills are ranked the third largest source of anthropogenic methane (CH_4) contributing about 11% of the global CH_4 generation (Singh et al., 2018). Landfill gas (LFG) can fortunately be recovered and used as a fuel. In a time when Oil Importing Developing Countries (OIDCs) continue struggling to meet their energy demands with many of them still strongly relying on the polluting and fast depleting fossil derived fuels. Landfill gas recovery and its utilization provides an opportunity to abate pollution while addressing energy deficiency. Overreliance on fossil fuels is counterproductive toward reaching the sustainable development goals (SDGs) such as climate change, clean energy, sanitation, health, etc. As such, pivotal toward sustainability is a consideration that provides for appropriate design and management of LFG recovery systems, which will ensure a holistic approach in addressing several SDGs. The sustainability concept is premised on three pillars namely the environment, economics, and social aspects (Purvis et al., 2019). This chapter reviews key considerations in sustainably developing LFG-to-energy projects. The considerations to be discussed will cover project stages and plant areas such as gas generation predictions, gas extraction and collection systems, gas upgrading and utilization technologies, and project decommissioning decisions.

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_11

2 Landfill Gas: Production, Properties, and Sustainability

2.1 Microbial Production of Biogas

The approach that utilizes landfill for waste management encourages the proliferation and abundance of anaerobic microorganisms and their favored metabolic pathways. Landfills are designed to hold MSW and in most cases the waste is covered with soil (sanitary landfill) as the waste accumulate. Surface waste partially covered by soil may harbor microorganisms that can thrive on oxygen, thereby allowing some aspects of aerobic decomposition. However, as more soil is piled on the MSW, over time during the active lifecycle of the landfill for waste dumping, it is often the case that anaerobic conditions overtake with the creation of anoxic conditions. This arises as a consequence of the complete sealing through the burial of the MSW. The autochthonous soil microorganisms that have adapted to anoxic conditions (microaerophilic bacteria and obligate anaerobes) proliferate and dominate the degradation process. In the soil, microorganisms degrade organic compounds through consortia activities. These consortia-based interspecific activities encourage the production of an array of enzymes. It is beneficial to microorganisms in the degradation process as one species may not necessarily possess all enzymes for the degradation of certain compounds but in the community, concerted decomposition is more efficiently achieved with the collective effort by several species of microorganisms mostly comprising of bacteria, rhizospheric organisms including streptomyces and fungi. Some of the commonly existing soil microroorganisms are (then list the microorganisms given in the text) *Serratia marcescens*, *Bacillus subtilis*, *B. cereus*, *B. thuringiensis*, *B. anthracis, Glutamicibacter arilaitensis, Xanthomonas*, *Erwinia, Pseudomonas, Proteus, Ralstonia, Escherichia, Staphylococcus, Caulobacter, Neisseria Nocardia, Actinobiospora, Nocardiopsis, Streptomyces, Streptoverticillium, Streptosporangium*, and *Microbiospora* as well as *Rhizoctonia solani, Fusarium oxysporum, Sclerotium rolfsii, Pythium ultimum* (Budi et al., 2000; Felse & Panda, 2000; Someya et al., 2000; Aarti et al., 2020). In this process of anaerobic digestion (AD), the first set of metabolic activities is hydrolytic involving the production of enzymes that primarily function for organic matter decomposition and nutrient cycling (Rehman et al., 2019). Examples of such enzymes include beta-glucosidase which degrades carbon compounds to glucose that is used as an energy source by microorganisms. Similarly, different enzymes including lipases, proteases, and esterases collectively involved in fluorescein diacetate hydrolysis and degradation of all organic matter to achieve nutrient recycling also function in hydrolysis (Prosser et al., 2011).

Although hydrolysis is an important step of AD, it is a rather rate-limiting step in the overall process as the complex compounds that are being degraded usually produce toxic by-products in the form of heterocyclic compounds and some non-desirable volatile fatty acids (VFAs) which require further degradation steps

(Buffiere et al., 2018; Wang et al., 2018; Yap et al., 2018). As a result, these monomeric compounds and volatile fatty acids including lactic acid, pyruvic acid, formic acid, and acetic acid undergo the second step of acidogenesis in which organisms such as *Clostridium* sp., *B. subtilis, Pseudomonas stutzeri*, *Streptococcus* sp., *Lysinibacillus fusiformis*, and *Butyribacterium* sp. (Wainana et al., 2019; Steven et al., 2020) achieve acidogenesis via oxidation; metabolites are then taken through to acetogenesis where microorganisms such as *Acetobacterium, Acetoanaerobium, Acetogenium, Butyribacterium, Clostridium, Eubacterium,* and *Pelobacter* participate in this third step. However, in situations where varied metabolites have to be degraded to acetates, then organisms such as *Syntrophobacter wolinii* degrades propionate, *Syntrophomonas wolfei* degrades butyrate, and *Syntrophus buswellii* degrades benzoate, depending on the type of organic acids that are present (Borja, 2011; Świątek et al., 2019). This process converts the acids to acetate and H_2, which are the necessary substrates for the last step, methanogenesis, where organisms such as *Methanoculleus* sp., *Methanobacterium* sp., *Methanobrevibacter* sp., and *Methanosarcina* sp. are responsible for the production of methane and CO_2 (Serrano-Silva et al., 2014; Laiq Ur Rehman et al., 2019). As time progresses, landfill gas production subsides due to substrate depletion, without new MSW inputs on the site. But with most buried organic wastes, biogas production can span slightly over a time period averaging 40 years from landfill closure date. A typical landfill facility layout demonstrating the involvement of microorganisms in biomass degradation to produce landfill gas is shown in Fig. 1.

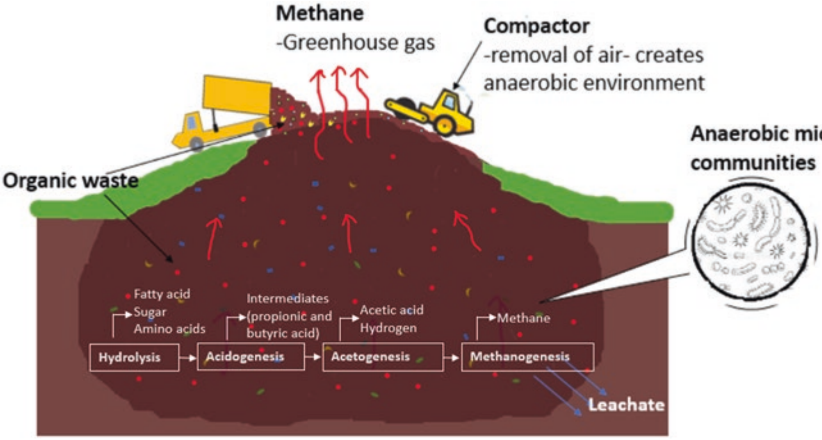


Fig. 1 Organic waste landfill with microbes degrading biomass to gas

2.2 Biogas and Landfill Gas Energy Properties

Biogas is an energy carrier due to its methane component which is combustible to produce heat or light. The carbon dioxide which makes the other fraction in biogas is a nuisance in biogas energy applications as it is principally a suppressant for fuel combustion. Pure methane has an energy density of 37.78 MJm^3 (Jingura & Kamusoko, 2017). Biogas can also be combusted in an internal engine to provide motive power for vehicles or electrical generator sets. In the case of electrical generator sets, the biogas can be combusted to enable the conversion of the engine mechanical power to electricity. The higher the proportion of methane versus the carbon dioxide in the gas, the higher will be the energy density of the biogas. Carbon dioxide and other gases in the biogas dilute the methane fraction and this is therefore undesirable. Landfill gas as an energy carrier is considered inferior to biogas, due to low methane content and higher impurity levels. Other impurities, although normally in small quantities, that can be detected in these gases include hydrogen sulfide, siloxanes, nitrogen, hydrogen, and water vapor. Commercial technologies are available on the market for biogas upgrading to remove contaminants and increase the methane content though this comes at a cost to biogas end users (Sun et al., 2015; Singhal, 2017). Table 1 outlines a few property variations between landfill and anaerobic digester gas (biogas). This information illustrates that LFG may be used in similar energy applications as biogas though the upgrading requirements in cases where this is required for the two bioenergy carriers may slightly vary depending on source, substrate, and pollutant limits acceptable for each specific application.

Table 1 Comparison of biogas and landfill gas characteristics

Characteristic	Biogas operations	LFG operations	Implication on landfill project sustainability	References
Feedstock variability	Nearly constant and can be manipulated	Composition changes more often and difficult to control	Unreliable gas output prediction in landfills results in gas wastage (flaring) or costs related to equipment oversizing	Levis and Barlaz (2011)
Methane composition (%)	55–70	40–70	Higher concentrations infer high calorific value which improves project economics	Rajaram et al. (2012); Sitorus and Panjaitan (2013)
Carbon dioxide component (%)	30–45	30–60	Reduces calorific value of the gas. High content increases purification costs	Rajaram et al. (2012); Sitorus and Panjaitan (2013)
Hydrogen sulfide (ppm)	200–4000	800	Corrodes heat exchange equipment or engines. Generates GHGs on combustion	Rajaram et al. (2012); Sitorus and Panjaitan (2013)
Metal components ($\mu g/m^3$)	n.d	Vary across landfills	Toxicity to human beings and clogging engine nozzles	Cullen and Feldmann (1997)

2.3 *The Sustainability Aspects of Recovering Landfill Gas for Energy Use*

While developing LFG recovery systems, it is important to address sustainability aspects. The concept of sustainability emanated from the desire to protect the environment in the wake of rapid economic developments that took place in many countries during the first and second industrial revolutions peaking in the late nineteenth century. Generally, sustainability evaluates any development on the economic merits, environmental harm that may affect future generations' potential to benefit from current resources as well as the current ethical and social acceptability of the process or project (United Nations, 2020). This concept gained widespread attention around 1900 prompting the United Nations (UN) to develop a set of seventeen (17) time-bound goals referred to as the Sustainable Development Goals (SDGs). These goals commenced in 2015 and are targeted to reach maturity by 2030. These SDGs are aimed at fostering a focused approach among countries for addressing the subject of sustainability. Moreover, SDGs involve all economies and encompass a broader scope of development covering poverty, economy, environment,and social dimensions as opposed to the narrower predecessor goals (Millennium Development Goals (MDGs)). The MDGs focused more on ending poverty in developing nations with very limited scope to involve developed economies. The eight (8) MDGs were formulated in 2000 and expired in 2015 when SDGs came into effect. The use of LFG as fuel contributes both directly or indirectly, in addressing some of the SDGs as depicted by the connecting lines between LFG activities and the SDGs affected by each activity in a hierarchy form of Fig. 2.

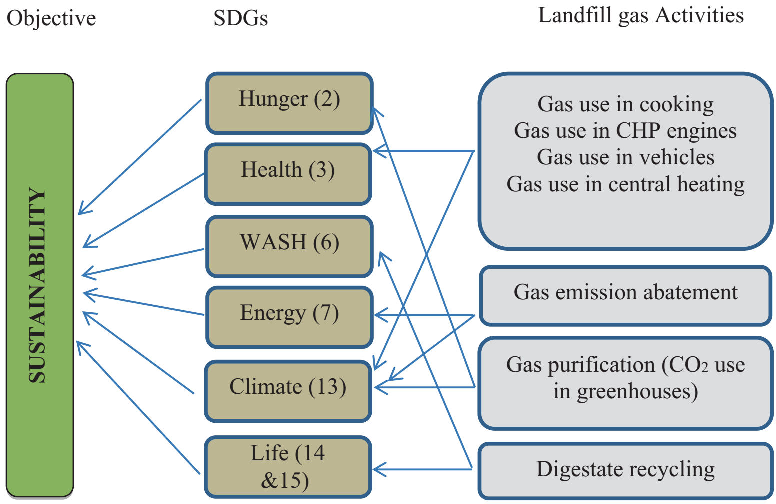


Fig. 2 Contribution of landfill gas recovery activities into the sustainability space. WASH stands for water, sanitation, and health, CHP stands for combined heat and power. The number in brackets denotes the SDG number allocated by the United Nations

There are many approaches and tools used to evaluate a development project's sustainability. These approaches were reviewed by Ness et al. (2007) who pointed out three broad classifications of these methods as the indicators, product assessment, and the integrated assessment (Ness et al., 2007). The integrated assessment which covers both qualitative and quantitative attributes of a project appears to be more suitable for the LFG biogas development context and will therefore be adopted in discussing sustainability issues in this chapter. Concisely, the social, economic, and environmental dimensions of the key activities in LFG development for energy use will be the central theme of discussion in this chapter.

3 Landfill Gas Capturing and Usage: General Process Description

Although there are a few variants to the process flow diagrams (PFD) for LFG extraction and processing, the basic one is described by Liu et al. (2017) as depicted in Fig. 3. Briefly explained, gas extraction involves sucking out the gas from a landfill, by creating a vacuum using a blower that will be connected to perforated pipes. Along the gas conveyance pipes, water vapor condenses and is knocked out. The gas is then directed to the processing units where pollutants like hydrogen sulfide, carbon dioxide, and sometimes siloxanes as well as volatile metals are removed. The

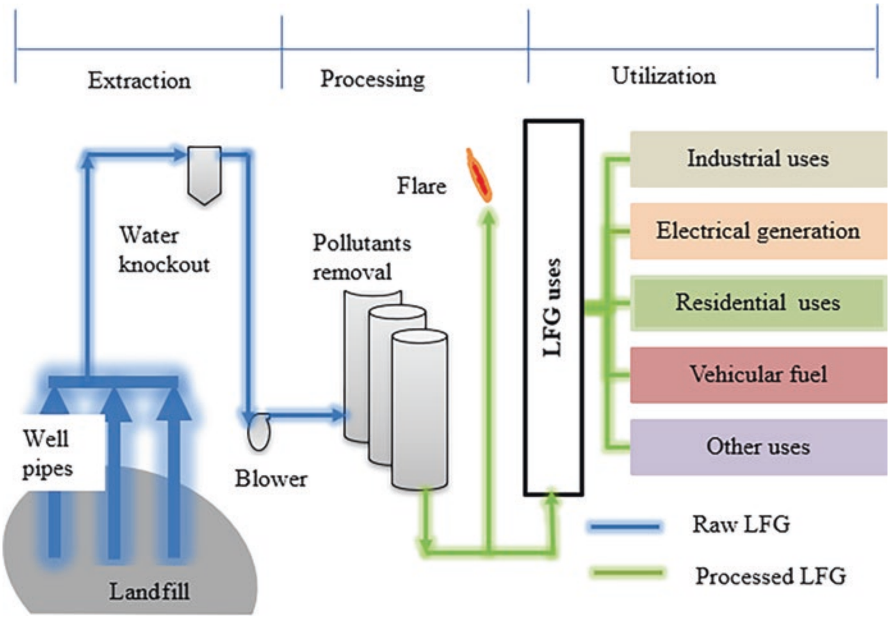


Fig. 3 Schematic diagram depicting landfill gas recovery for energy use

extent of gas processing depends on the intended final gas utilization. Processed gas is directed to various end uses. If there is excess gas supply than consumption, flaring facilities are incorporated on the gas delivery piping to cater for system overpressure (Liu et al., 2017).

4 Landfill Gas Quantification Models

To design and operate a sustainable LFG recovery system the potential amount of LFG that is likely to be generated and collected from a landfill must be estimated carefully since this is an important input for the feasibility study (Majdinasab et al., 2017). Test wells can be drilled on a landfill to gather and estimate LFG production potential as well as collection efficiency data but this process is expensive and time-consuming. Gas output predictions are therefore achieved through empirical, mathematical, and numerical modeling approaches. There are more than twenty (20) LFG generation projection models and modeling approaches in existence that have been developed and applied across the globe with additional eight (8) models that are country-specific (Rajaram et al., 2012).

The abundance of LFG generation prediction models is dictated by variability in the composition of wastes landfilled, climate conditions, differences in landfill management practices such as leachate recirculation ratios, cover designs, etc. The most popular models however are the LandGEM, IPCC, and the country-specific models (Gollapalli & Kota, 2018). The LandGEM model was developed for American conditions though it can be modified and adapted for use in other countries across the globe, most likely with limited adjustments. The model is therefore widely accepted in America and the Latin American region. The IPCC model which is more flexible in terms of accommodating variable conditions is currently the most preferred model by international development agents for clean development mechanism (CDM) funding requirements under the Kyoto Protocol arrangements. The eight country-specific models that were developed by the Global Methane Initiative (GMI) have the combined attributes of the LandGEM and the IPCC. These country-specific LFG output prediction models factored regional local conditions and are therefore popular in the regions or countries for which they were specifically designed for. Table 2 highlights common aspects which affect accuracies in most LFG models. Aspects of sustainability in LFG development project for energy recovery are also highlighted in the same table. It is not the intention of this chapter to discuss detailed technical differences, assumptions held, and application suitability of the various LFG projection models. Majdinasab et al. (2017) and Rajaram et al. (2012) reviewed the development of the various LFG models, comparing their relative differences, advantages, and disadvantages so these sources can be consulted to better understand this subject (Rajaram et al., 2012; Majdinasab et al., 2017).

Table 2 Landfill gas modeling variables and considerations that affect sustainability

Modeling variable	Influence on modeling and key considerations	Sustainability aspect affected	Reference
Amount of waste	Available waste quantities change with lifestyle and changing demographics. Securing accurate data on waste quantities is a problem particularly in developing countries. Consider these challenges to make reasonable adjustments on LFG model forecasts.	Cost – Overestimating gas output affects plant sizing with possibilities of a big underutilized plant. Underestimations of gas outputs will also imply inadequate exploitation of the resources with possible excess methane escaping into the atmosphere as GHG or flared.	Scarlat et al. (2015)
Composition of waste	Waste compositions change inconsistently in most cases. Foodstuff waste degrades fast shortening the landfill lifespan. Lignocellulosic behave otherwise while inorganics do not degrade. Make informed estimates of these site-specific compositional issues in modeling and it would be better to be conservative on high methane-yielding components.	Cost – Incomplete usage of gas generated because of undersized units or vice versa. Environmental/social – Compliance relies on understanding the complete degradation of waste components.	Mou et al. (2015); Chakma and Mathur (2016)
Moisture content	Moisture affects LFG generation rates. Local rainfall patterns, runoff seepage control into the landfill and leachates recirculation rates must be considered during LFG modeling.	Degradation rates affect project lifespan hence costing. Leachates come with environmental litigation costs and social unrest from affected communities.	Chakma and Mathur (2016); Gupta and Paulraj (2016)
Temperature	Anaerobic digestion proceeds reasonably well at temperatures between 30 and 40 °C. If fluctuations in local temperatures are anticipated, then this should be factored in modeling LFG model output. Site-specific rather than default values in the model are always recommended.	Inaccurate projections due to non-consideration of temperature will affect the project economics and potentially if output is underestimated the excess generation will be released as GHG into atmosphere	Chakma and Mathur (2016); Vu et al. (2017)
Age of the landfill	Landfill gas production rates and cumulative amount is time dependent so it is important to factor in landfill age-related considerations in the interpretation of the model outputs.	Poorly timed record inputs will affect anticipated gas volumes leading to mismatched and unnecessarily costly designs.	Rada et al. (2015)

(continued)

Table 2 (continued)

Modeling variable	Influence on modeling and key considerations	Sustainability aspect affected	Reference
Oxygen ingress into landfill	Consider air infiltration caused by aggressive operation of collection (vacuum) systems and uncovered landfills. Air reduces methanogenesis hence the projected methane gas outputs. Some of the organics will also be degraded aerobically without methane generation if air infiltration is high while some methane is oxidized by methanotrophs.	If oxygen effects are not accounted for especially in shallow-depth landfills, the risk of overestimating methane gas generation is high and will affect project financials negatively	Rachor and Gebert (2013)

5 Landfill Gas Extraction and Collection

Major components of a gas extraction system include the extraction wells, header and distribution piping, moisture removal equipment, and blowers for vacuum creation. Different options for each piece or components of equipment and the ultimate plant design exist on the market. The selection criteria for these units ought to consider the fitness for purpose, cost-effectiveness, and improvement of the energy project sustainability attributes. Some of the recommended key considerations are briefly discussed in Table 3 with emphasis placed on the sustainability impact of each consideration.

6 Landfill Gas Purification Technologies

The gas processing section objectives include removal of pollutants such as hydrogen sulfide, siloxanes, and other non-methane organic compounds (NMOCs) which are detrimental to the end-user equipment or the health of gas users (Kaparaju & Rintala, 2013). Processing also improves the calorific value of the LFG gas by removing the non-combustible components such as carbon dioxide. The choice of equipment and technologies applicable to LFG gas upgrading depends on the intended final utilization of the gas. It may not be economically sustainable to incur high purification costs for low Btu applications (boilers, furnaces, micro-turbines) where energy efficiency may be poor or minimal equipment damage is expected from gas contaminants (Kaparaju & Rintala, 2013). Some of the gas purification technologies and considerations to make as well as their implications on project sustainability are briefly discussed below.

Table 3 Considerations in the design and operation of landfill gas collection systems and their sustainability implication

Extraction item	Key considerations	Design criteria	Sustainability implication	References
Gas collection system design and installation				
Facility review	*Climate* Precipitation seepage (dependent on covers and liners) into landfill waste as well as leachate management affects methanogenesis. *Operational goals* Thermal, electricity, or compressed natural gas (CNG) use affect N_2 and O_2 tolerance levels in LFG. *Operational practices* Shallow site require shallow wells which are less efficient, non-compacted waste generate less LFG, poorly covered sites emit gas to the atmosphere while increasing air filtration into LFG on extraction	Assess local rainfall patterns then in low precipitation areas recirculate leachates while landfills in high precipitation areas require reliable covering. Tighter controls and monitoring of air filtration in CNG and electricity than thermal use. Reducing well spacing, increasing bentonite plugs around extraction well and putting air quality sensors on wellhead are some of the measures employed. Aim for well-managed landfills in terms of waste compacting, landfill covering and deeper wells in deep sites for improved collection and LFG quality.	Cost and gas supply reliability throughout project lifespan depends on digestion rates. Leachates management also affects the environmental sustainability Tighter control mechanisms increase capex but save on Opex High Opex and environmental emissions	Li et al. (2013); Abushammala et al. (2016)

LFG collectors	*Vertical extraction wells* Continuing or terminated waste reception, borehole diameter, casing material, well depth, and packing materials affect collection efficiencies *Horizontal extraction wells* Amount of accumulated waste, flooding risks, Spacing of holes, casing material's standard diameter ratios (SDR). *Wellheads* Process parameter monitoring and adjustments	Ideal for sites where waste placement has stopped though can still be implemented where placement is ongoing but with increasing costs. Larger borehole diameters (>24 inches) are recommended for easier gravel backfilling, structural integrity, and more LFG extraction. PVC or steel depending on cost and availability better suits the casing material because of their strength. Backfilling material must have good draining properties and must be compatible with leachates (avoid high carbonate materials). Care must be taken on borehole depth to prevent dam liner damages (>5 m from liner surface) A minimum of initial 10 m waste depth must accumulate before installing collectors to prevent air ingress. Water flooding is a common problem hence trenches for collectors must be set at a small elevation to the horizontal to facilitate draining. Sumps to help in water collection and pumping out may also be incorporated. HDPE material having SDRs less or equal to 17 is recommended. Key parameters measuring instruments in wellhead must always be regularly calibrated to ensure correct data recording and process control of each well.	Larger borehole diameters are costly at installation. However, the improved LFG collection efficiency cuts on the operating cost in the long run. Vertical well installation while waste placement is still going on requires regular extension of the well casings as well as replacements caused by cloggings from settling wastes. An alternative well design to curb this is the Caisson well. Risk of damage to collectors from overburden is high which may cost the business in downtimes while potentially emitting the unrecovered methane to the atmosphere. Maintenance of instruments in good working condition can be costly but necessary for smooth and safe operations.	Wang and Achari (2012); Zheng et al. (2019)

(continued)

Table 3 (continued)

Extraction item	Key considerations	Design criteria	Sustainability implication	References
Lateral and header piping	*Placement* Pipe slope and layout *Materials* Flexibility/rigidity. Thermal and sunlight exposure. *Size* Gas production rates, velocity, and condensate flow	Take advantage of topography to achieve slopes of 2–4% that help in draining of condensate. To avoid possible low points on the piping network due to excessive waste settlement, layouts are recommended to follow natural roadways and berms. HDPE is more flexible than PVC during unavoidable landfill differential movements. HDPE also resists heat and UV degradation better than PVC. Pipe size must accommodate maximum expected production with gas flow velocities limited to maximum of 6 m/s for counterflow and 12 m/s for concurrent flow of gas and condensate to allow free flow of condensate in pipes	The ergonomic designs may influence safety and social dimensions of the pipe network. Depending on availability and local market conditions HDPE can be more costly than PVC. Undersizing results in poor LFG collection efficiency (pollution). Oversizing is an unnecessary cost.	Zhang and Sarica (2011)
Handling surface runoff and condensate	System airtightness and capacity	Condensate drains with airtight mechanisms must be well maintained to avoid flooding in pipes which negatively affect capacity utilization of the gas collection system as well as leachate composition	Safety and capacity utilization can be compromised by poorly maintained condensate handling systems	Baziene et al. (2013); Townsend et al. (2015)
Blowers	Collaboration with manufacturers	Factors that affect blowers functionality are supplier dependent and working with the manufacturer during selection, sizing, installation and operation may save a lot of headache	Custom-designed equipment are normally expensive to run (through maintenance and backup service plans) but cheaper to install	Townsend et al. (2015)

6.1 Moisture and Particulates Removal

Moisture cause rusting and reduce LFG calorific value. Adverse effects also arise from particulates that may block gas nozzles in end-use equipment. Knockout pots, moisture separators, mist eliminators, direct cooling as well as compression followed by cooling are equipment and techniques that have been traditionally used to remove moisture and at times concurrently entrapping the particulates as well. More advanced and efficient but possibly expensive absorption and adsorption technologies for moisture removal also exist with filters also employed for particulates removal where high particulate loads cannot be adequately addressed by basic entrapment in condensate.

6.2 Hydrogen Sulfide Removal

Combustion of H_2S in internal engines impacts on engine components' corrosion as well as the safety of people when acidic sulfur oxides are produced. Arising from this are engine repair costs, environmental litigation claims from acid rain-related damages, and social issues such as workers' moral, health, and safety which are all project sustainability components to consider when developing LFG projects. Iron sponge and use of microorganisms that consume H_2S have been employed in managing its removal from LFG (Sun et al., 2015).

6.3 Siloxanes Removal

Siloxanes in LFG emanate from household and industrial wastes and they endanger the combustion engine components by forming silicon dioxide (SiO_2). This SiO_2 reduces equipment efficiencies and is difficult to reverse once formed. The SiO_2 formation is more detrimental when LFG is used in engines and turbines than in thermal applications such as boiler furnaces. Therefore, the decision to undertake the expensive adsorptive, absorptive, or cryogenic condensation to mention a few treatments (Ruiling et al., 2017) for siloxane removal must be based on a cost-benefit analysis to be performed before project implementation.

6.4 Carbon Dioxide Removal

High-grade fuel applications like compressed natural gas and microbial cells require high methane content and ultrapure gases so CO_2 removal becomes paramount. Most gas cleaning technologies however remove a number of contaminants simultaneously, using principles such as surface adsorption (molecular sieves), membrane separation, or a solvent treatment system (Sun et al., 2015). Technology choice for solvents used to depend on solvent's selective affinity for either CO_2, H_2O, or H_2S matched with considerations for the level of contaminants in the gas as well as the end user contaminant acceptable limits. Solvents commonly employed are organic amine-based, such as methyl diethanolamine (MDEA), methyl ethanolamine-diethanolamine adsorption (MEA-DEA), and diglycolamine (DGA). Alternatively, hot potassium carbonate, propylene carbonate, and selexol have also been used. These technologies are generally expensive and must be used for high market value product applications.

7 Potential Landfill Gas Utilization

Landfill gas utilization depends on a number of factors including the quality of the LFG, the amounts of LFG generated, cost of the utilization technology as well as competition from other resources used for similar application. However, the most dominant factor is the quality or purity of LFG with the broad LFG quality grades being low, medium, and high. The LFG applications compatible with each specific LFG grade are depicted in Fig. 4.

Low-grade LFG is used after moisture removal only from the raw landfill gas and no extra processing steps. However medium-grade LFG is produced by removing sulfur, siloxanes, volatile organic compounds as well as mercaptines and moisture

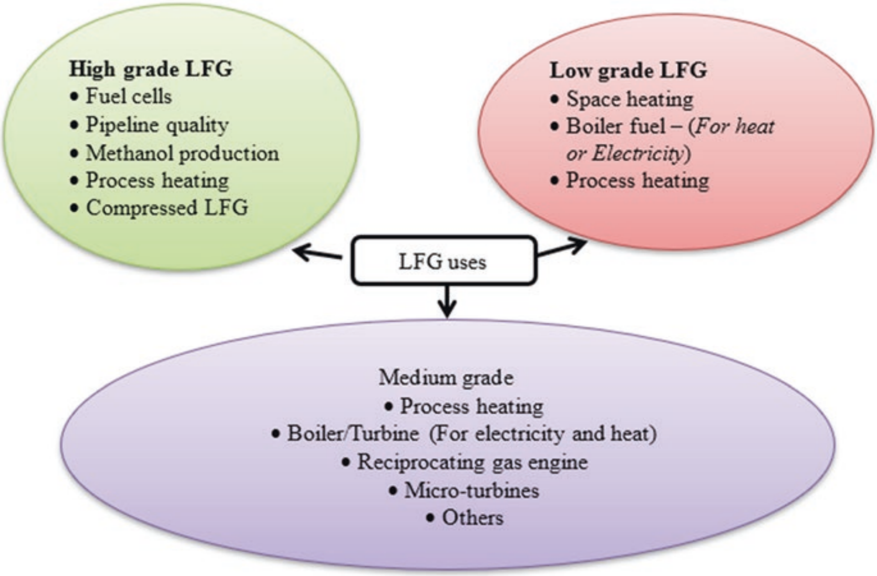
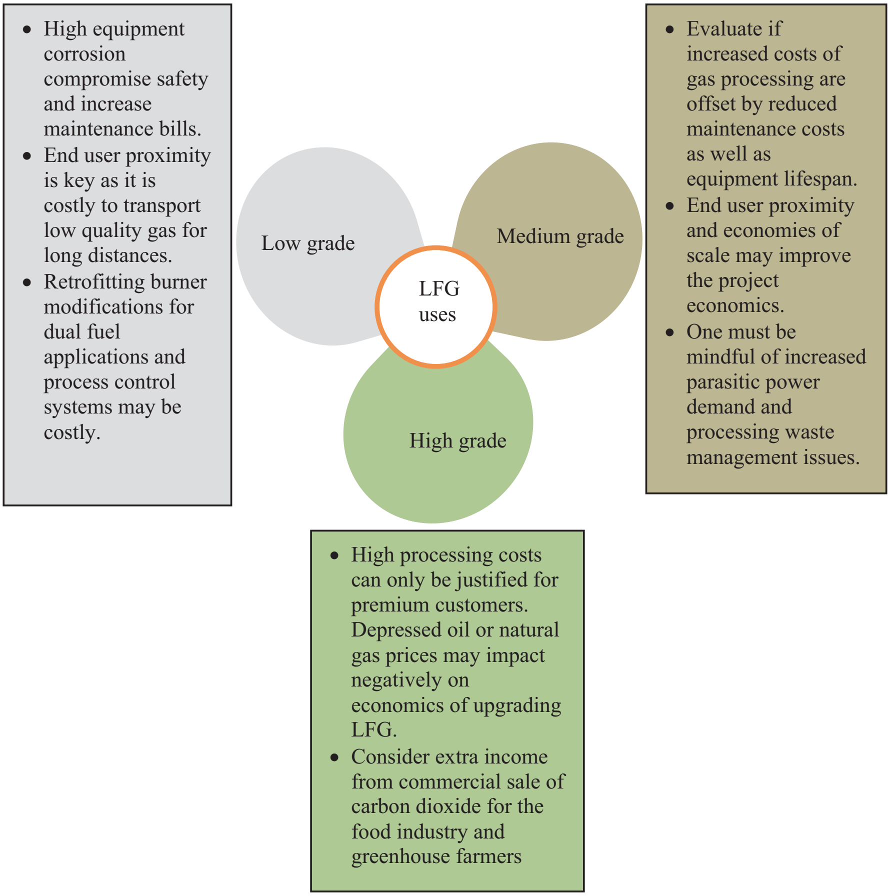


Fig. 4 Different landfill gas utilizations based on quality of the gas

from the raw landfill gas. Common low-grade LFG applications involve thermal units such as boilers, furnaces, driers, and kilns where the damaging LFG is not in direct contact with the major equipment. Infrared heating, leachate evaporation, and microturbines have also been reported to employ low-grade LFG. Medium-grade LFG finds applications in thermal equipment just like the low-grade LFG, and can also be used for electricity generation in gas turbines, reciprocating engine, and combined heat as well as power (CHP) units. High-grade LFG is distinguished from low and medium grades LFG by less pollutant load and a higher calorific value. High-grade LFG is produced by removing carbon dioxide from the medium-grade LFG. Key considerations in LFG utilizations that may impact on sustainability are depicted in Fig. 5 (bulleted points in textbox). One major consideration for any LFG grade utilization is proximity to the gas generation site since pipeline transportation and the subsequent pressures required are a costly that increases with distance (Hoo et al., 2018).



- High equipment corrosion compromise safety and increase maintenance bills.
- End user proximity is key as it is costly to transport low quality gas for long distances.
- Retrofitting burner modifications for dual fuel applications and process control systems may be costly.

- Evaluate if increased costs of gas processing are offset by reduced maintenance costs as well as equipment lifespan.
- End user proximity and economies of scale may improve the project economics.
- One must be mindful of increased parasitic power demand and processing waste management issues.

- High processing costs can only be justified for premium customers. Depressed oil or natural gas prices may impact negatively on economics of upgrading LFG.
- Consider extra income from commercial sale of carbon dioxide for the food industry and greenhouse farmers

Fig. 5 Sustainability considerations for different landfill gas utilization options

8 Costing and Financing of Landfill Gas Recovery Projects

The economic evaluation of a landfill project follows a 5-step process involving costing (capital and operating), revenue calculations, economic feasibility calculations, selection of best design and finally choosing a suitable financing option. The Landfill Gas Energy Cost Model (LFG*cost-Web*) is a web-based tool useful in performing LFG economic feasibility studies which was developed by the Landfill Methane Outreach Program (LMOP) of the U.S. Environmental Protection Agency. At each stage of the economic evaluation process, some key considerations with a few of them highlighted below affect the overall project sustainability.

8.1 Capital and Operating Costs

Capex and Opex values for the project are collected at this stage. Various equipment and end-use technologies must be considered with actual suppliers' quotations to ensure that the most advantageous option is finally selected. Preliminary evaluation can be based on typical costs while the detailed feasibility study must apply project-specific values. Each technology must be evaluated considering also its non-monetary merits and demerits with possibilities of economies of scale also explored.

8.2 Revenue Calculations

All revenue streams including sales of gas, electricity, and utilities (in the case of steam and hot water from CHP) engines must be considered. Other revenues from various schemes like premium pricing, tax credits, GHG credit trading, incentive payments, or Kyoto protocol mechanisms (Clean Development Mechanism (CDM) and Joint Implementation (JI)) can also be incorporated in the evaluations where applicable.

8.3 Economic Feasibility Study

This phase of the project must consider the cost of capital, economic inflation, risk sensitivities, and price uncertainties. These including the revenue and capital cost components collected above are used to deduce internal rates of return (IRR), net present values (NPV), payback periods (PBP), and annual cash flows that form the basis of economic feasibility of the project.

8.4 Selecting the Best Design

At this stage, the successful projects based on economic feasibility are further subjected to sensitivity analysis and non-price factors to test their robustness against these factors. Risk factors are weighed at this stage and the most advantageous option is chosen. The use of experienced developers in factoring these non-price factors especially around the risk of poor gas prediction modeling may be helpful at this stage. Other factors to consider include political risk, equipment reliabilities, availability of electricity or utilities off-takers and construction materials availability. The United Nations Convention for Climate Change (UNFCCC) provides guidelines and tools for investment analysis and economic feasibility studies respectively for projects targeting CDM certification.

8.5 Choosing the Financing Option

There are many financing mechanisms and instruments for LFG projects around the globe and a choice of the best available one or mix of them must be chosen for each specific project. The various funding options have conditions and requirements to be met therefore expert advice and thorough searches for the best option may be required. Some of the funding options to consider are the Kyoto protocol mechanisms, Bank finance (Multilateral, sub-regional, national and local) then private investors, and leasing arrangements (Sell and lease back, lease pass-through). Grant opportunities and internal resources can also be used to fund LFG development projects. The detailed advantages and disadvantages of each of these funding opportunities should be explored with the help of experienced Finance practitioners to make the best selection.

9 Decisions on Decommissioning

When LFG generation dwindles with the lifespan of a landfill, a period is reached when it would not be best economic practice to extract the gas for energy uses. Issues of reliability and quality of gas arise, and the project must be decommissioned. Hsieh et al. (2008) described what they called a sustainable landfill whereby on decommissioning the landfill is first subjected to aerobic conditions by pumping air into the wells to facilitate rapid composting of residual organics prior to mining the landfill (Hsieh et al., 2008). The mining is done to recover resources (compost is used for soil conditioning and undecomposed materials are recycled) and also to reuse the land space. If the landfill is not composted and mined, then landfill aftercare strategies must be put in place until the landfill is deemed safe for human health and the environment (Laner et al., 2012). The different landfill aftercare options and considerations that may affect sustainability are reported in Table 4.

Table 4 Landfill aftercare options and their sustainability. (Laner et al., 2012)

Aftercare alternative	Specific description	Sustainability strengths or shortcomings	References
Timeframe based	Regulations will stipulate when a developer/owner can abandon the landfill	The developer/owner knows what is expected of them so can budget beforehand but society is vulnerable to any chemical, biological, or physical landfill status that may threaten human health and the environment after the developer abandons project. Landfill post-closure challenges such as settlement can last 20–30 years which is too long a time to plan for.	Laner et al. (2012); Chu (2016)
Perpetual care	Owner's responsibility to monitor and maintain landfill never ends	Offers maximum protection from human health and environmental threats but at a cost which is indirectly borne by citizens and manufacturers as disposal fees	Zomeren et al. (2011)
Target values for different streams	*Leachate quality* COD: <200 mg/l BOD_5/COD ratio: <0.01 NH_4: <300 mg/l *Landfill gas production* Gas generation rate: <25 m^3/h Area-specific methane generation rate: <0.001 $m^3\ CH_4/(m^2\ h)$ CH_4/N_2 ratio: <0.01 CO_2/N_2 ratio: <0.01 *Waste quality* Biodegradability: respiratory index (RI4):62.5 mg O_2/g DM Methane generation potential in 21 days:0.01 m^3CH_4/kg DM	The metrics do not consider site-specific conditions like precipitation which might trigger different requirements for long-term post-closure landfill management. Inaccuracies in measurement due to non-homogeneity in landfill waste streams from different locations of the landfill may result in erroneous inferences. Wrong prescriptions will be recommended at cost and possible human health and environmental risks.	Pivato (2004); Cossu et al. (2007)
Complete waste stabilization	Physical, chemical, and biological stability must be achieved before landfill abandonment.	Heterogeneity in landfills makes sampling for analysis challenging, expensive and unreliable. Dealing with ammonia accumulation in leachates, persistent chemicals, slow degradables, etc., makes complete stabilization an imagination that may be difficult to achieve.	Laner et al. (2011); Weber et al. (2011)

(continued)

Table 4 (continued)

Aftercare alternative	Specific description	Sustainability strengths or shortcomings	References
Performance-based	Current landfill performance and data trends are used to forecast the future possible status and this is matched with the targeted end use of the landfill site to determine aftercare interventions.	Site specificity and adaptation to local conditions allow these methods to be more suitable in addressing human health and environmental threats. One of these methods called the evaluation of post-closure care (EPCC) methodology enables cost reductions because issues of aftercare management are treated in a modular manner. If one issue is resolved, interventions for that issue are stopped then resources are spared for other modules. Overall this cuts short the aftercare period.	O'Donnell et al. (2018)

10 Concluding Remarks and Future Considerations

Landfill gas extraction, collection, processing, and use is a technically cumbersome process with considerations that impact on cost, safety, health, environment, and community acceptance. Emissions of GHGs and other obnoxious gases to the atmosphere are never eliminated completely in a landfill despite LFG extraction for energy or other uses. Landfill gas generation projections are never accurate and contingencies in equipment sizing are unavoidable. Management of leachates presents challenges to landfill operators. Landfill gas recovery plant designs and operational challenges mentioned in this review are not exhaustive but serve to highlight some of the difficulties experienced towards achieving sustainability in LFG operations. While there are LFG recovery attributes that resemble the biogas industry, a properly managed industrial biogas system is by far a more sustainable waste handling and renewable energy recovery option than landfilling for the degradable fraction of the municipal solid waste. Biodigesters in biogas systems are completely sealed hence almost all the generated biogas is captured for productive use and consequently, the generation can be accurately predicted. In this case, end-user equipment can be sized accordingly. Digesters limit methane gas losses to the atmosphere and air ingress into the gas lines as experienced in LFG systems. A bioreactor landfill tries to mimic industrial anaerobic digester operations but the digestion efficiency in a landfill bioreactor never matches that of its industrial counterparts. Many municipal jurisdictions are therefore now moving away from the waste landfilling practices towards waste sorting at source followed by diverting degradable organics towards industrial biodigesters. The remaining waste after the removal of degradable organics can be managed through gasification, pyrolysis (Jenkins, 2020), incineration, and recycling. In this chapter, we recommend this latter approach to solid waste management route going into the future as opposed to landfilling. Proper selection of processing plant is required at landfills to separate biodegradables from non-biodegradables. Meanwhile, it may also be beneficial to bioprospect for microorganisms adapted to landfill conditions, preserve them then use this as starter cultures in future anaerobic digesters that will be handling degradable organics of municipal solid wastes or related substrates. This reduces the lag phase of microbial activities and increases the efficiency of degradation. Landfill gas collection from old landfills should be continued mainly for low-grade thermal applications that are less expensive to install and can easily be switched over to other fuels once the LFG is exhausted. Low-grade LFG applications also imply minimal equipment redundancy on project mothballing since minimal gas purification investment is made in these applications. To improve the sustainability of these LFG operations some of the considerations discussed in this chapter will be helpful and therefore should be borne in mind by developers and operators.

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Microbial Degradation of Industrial Pollutants from Different Environments

Ambalika Sharma, Richa, Anu, and Asha Kumari

1 Introduction

Anthropogenic activities are putting immense strain on the terrestrial ecosystem and the global environment. We have reached many milestones in our lives as a result of the rapid and global growth of industries, and our lifestyles have gotten more efficient and advanced. World economic expansion has paralleled industrialization and has had a significant impact on our lives in every sector. However, progress and standards have a flip side, since industrial pollution is the greatest pressing concern facing modern society (Kaushik et al., 2012). Industrial effluents contain dangerous inorganic and organic contaminants that pollute water streams and the surrounding soil ecosystem, affecting the health of all living species (Maszenan et al., 2011; McIlroy et al., 2015). Effluent water and solid waste discharge account for nearly a third of overall water pollution in India, and 3.4 million people globally suffer and die as a result of increasing industrialization (Rajaram & Das, 2008). Natural water ecosystems may be harmed as a result (Kansal et al., 2011). Currently, environmental sustainability is a major source of concern, and research experts are paying close attention to it.

Microbes have a critical role in the environment, society and economy. For millennia, they have been used to produce a wide range of products, such as probiotics,

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_12

enzymes, and biofuels such as bioethanol and hydrogen gas. In industries, these microorganisms are currently playing a significant role in the cleanup of toxic waste. The breakdown of organic compounds in wastewater is primarily carried out by microbes and the enzymes that they produce. Microbes play an important role in the regulation of every ecological process as primary engineers. They act as an all-purpose catalyzer.

Bioremediation is a technologically advanced technique which uses microbes to break down contaminants in wastewater and the soil environment. It is a method for converting biodegradable, complex harmful chemicals into safe, acceptable end products. The colloid that is suspended and does not settle is collected and integrated into biological floc and biofilm. Important nutrients, valuable metals, and specific organic elements can all be extracted and recovered. It is a less expensive technology that produces no waste as a byproduct. Apart from being a cost-effective and environmentally benign technology, bioremediation is the ideal alternative to traditional wastewater treatment and management methods for protecting our environment (Maszenan et al., 2011).

Bio-remediators are microbes that can degrade almost all organic contaminants (Hiraishi, 2008; Fenchel et al., 2012). Co-metabolism is a mechanism in which microbes break down organic contaminants. Microbes present in the rhizosphere of terrestrial and aquatic plants decompose complicated carbon substances to get electron acceptors and simple organic carbon through this process (Stottmeister et al., 2003). The rate of biodegradation in natural water is determined by the microbial population and xenobiotics present (Paris et al., 1981). Macrophyte species have a significant impact on microbial counts (Calheiros et al., 2009). Plants provide organic carbon to rhizosphere microorganisms, which help them breakdown complex organic molecules (Mori et al., 2005), hydrocarbons, and aromatic hydrocarbons, for example (Mordukhova et al., 2000). IAA is a significant phytohormone that has the power to regulate plant development in both positive and negative ways. Many bacteria, both phytopathogens and those that stimulate plant growth, have the capacity to manufacture IAA (https://doi.org/10.1007/s10482-013-0095-y). Rhizobacteria's ability to produce indole-3-acetic acid (IAA) is a crucial instrument for promoting and facilitating plant growth. Plant roots naturally excrete organic substances, such as L-Trp, which rhizobacteria can utilise to synthesize IAA, enabling non-native plant species to withstand biotic and abiotic stress conditions (https://doi.org/10.1186/s43141-020-00035-9). Pollutant degradation and plant growth-promoting properties were also demonstrated by many bacteria isolated from aquatic plants (Golubev et al., 2009; Huang et al., 2004).

2 Application of Microbes in Treating Wastewater from Various Industries

Microbes play a crucial part in the waste recycling process; they are the basic agents that are responsible for biodegrading inorganic and organic wastes, as well as nutrient recycling in the natural environment. Microbes play an important part in waste recycling and wastewater treatment during the fermentation process, as well as the

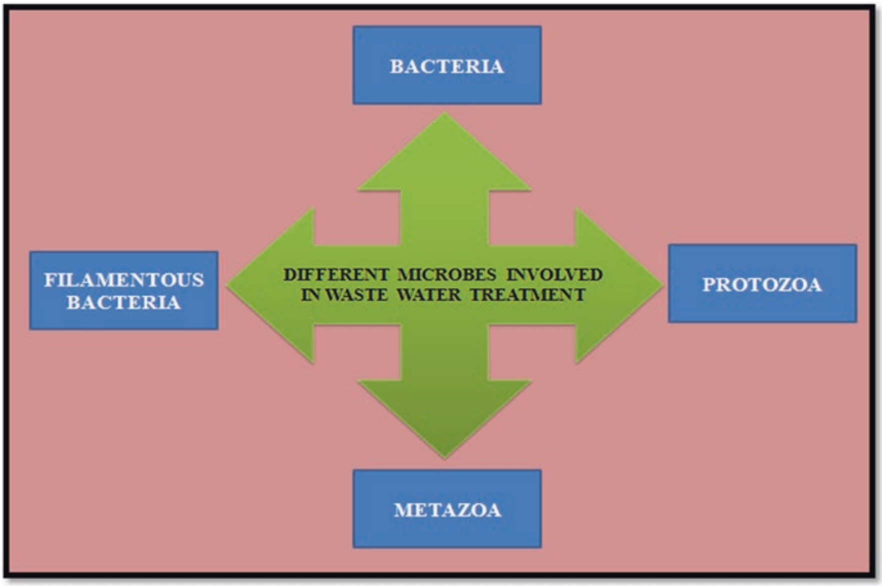


Fig. 1 Different types of microbes involved in wastewater treatment

production of alternative energy. The following microorganisms are commonly found in contaminated water treatment processes: Microorganisms play an important part in waste recycling and wastewater treatment during the fermentation process, as well as the production of alternative energy. The following microorganisms are commonly found in the wastewater treatment process. Figure 1 represents the different microbes involved in wastewater treatment:

2.1 Bacteria

Bacteria have the potential to convert organic materials found in wastewater treatment systems to less complicated molecules. They are responsible for eliminating and converting these organic molecules in effluent treatment and play an important role in wastewater treatment. As a result, these bacteria are critical for the proper operation and preservation of microbial treatment systems. The size of bacteria ranges from 0.2 to 2.0 m in diameter and they are responsible for the majority of the wastewater treatment in septic tanks (Stevik et al., 2004). Although not all bacteria are hazardous, a few of them cause diseases in humans and animals when they come into contact with water. Cholera, dysentery, typhoid fever, salmonella, and gastroenteritis are some of these disorders (Jenkins et al., 2003). The susceptible agent is bacteria, and waterborne gastroenteritis of unclear source is frequently reported. This condition could be caused by certain strains of *Pseudomonas* and *Escherichia coli* that affect newborns. These microorganism strains have also been linked to gastrointestinal illness epidemics (Metcalf et al., 2003).

2.1.1 Types of Bacteria

(A) Anaerobic Bacteria

Anaerobic organisms or anaerobes are bacteria that do not require oxygen to grow (Upcroft & Upcroft, 2001). If free oxygen is present, it may respond negatively or possibly perish (Levinson, 2014). An aerobic organism (aerobe), on the other hand, is one that requires oxygen to survive. Anaerobes can be either unicellular (protozoan bacteria) or multicellular (Danovaro et al., 2010).

(B) Aerobic Bacteria

Bacteria that live in an aerobic environment require oxygen to survive. They can be found in damp, aerated soil with organic carbon sources. Aerobic bacteria are divided into two categories.

(C) Facultative Bacteria

Facultative bacteria can behave both aerobically and anaerobically depending on the circumstances. They obtain energy through anaerobic pathways in decreased conditions and aerobic pathways in oxidative situations. Figure 2 represents the types of bacteria with their examples:

Examples of bacteria involved in wastewater treatment: Fig. 3 represents the examples of bacteria in wastewater treatment:

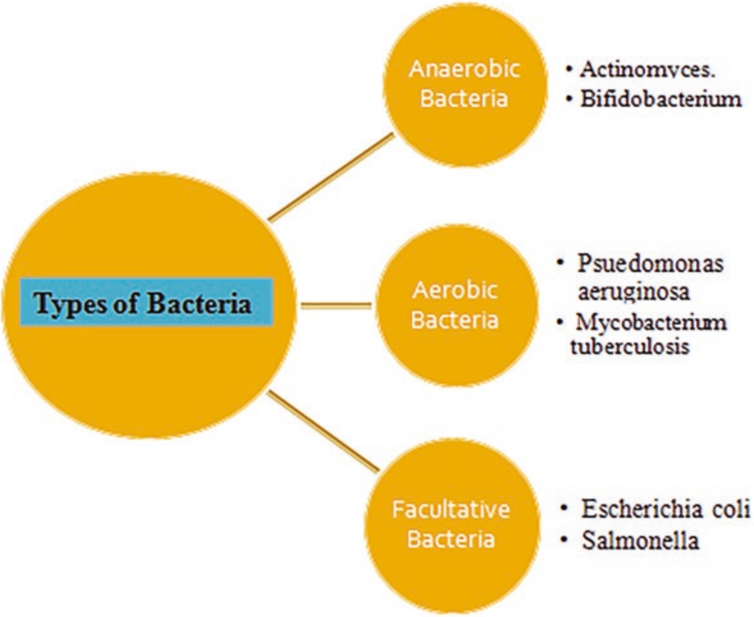


Fig. 2 Types of bacteria with examples

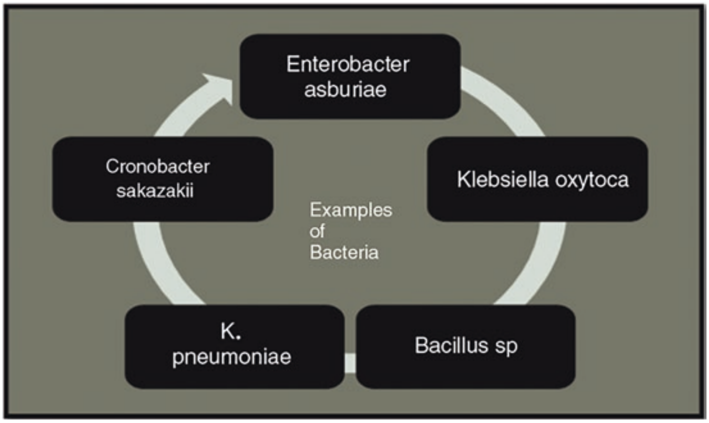


Fig. 3 Examples of bacteria involved in wastewater treatment

2.2 Filamentous Bacteria

Filamentous bacteria are long strands of bacteria which grow end to end and interlock to form a net, similar to strands of hair or spaghetti. These bacteria form lengthy filaments as they grow. Filamentous bacteria are generally found in the biomass of activated sludge. They are a common component of sludge biomass, and the presence of these bacteria is crucial and beneficial for optimal floc development. Their population is influenced by nutritional conditions in the wastewater. Activated sludge has been shown to have up to 25 different species of filamentous bacteria. One of the principal filamentous bacteria is *Nocardia* spp., which causes foaming. Figure 4 represents examples of filamentous bacteria in wastewater treatment.

2.3 Metazoa

In effluent that has been maintained for a long period, especially in lagoons, there is a predominance of multicellular eukaryotic organisms with a size greater than most protozoa, and their concentration is quite low. Metazoans found in activated sludge are known to play a variety of roles. They eat other microorganisms and help to clear the flux (Fig. 5).

2.4 Protozoa

Initially, the activated sludge process was considered to be harmed by protozoa (Fairbrother & Renshaw, 1923). Yet, some writers are able to estimate the size of this type of species' effect on effluent quality and assess its involvement (Curds,

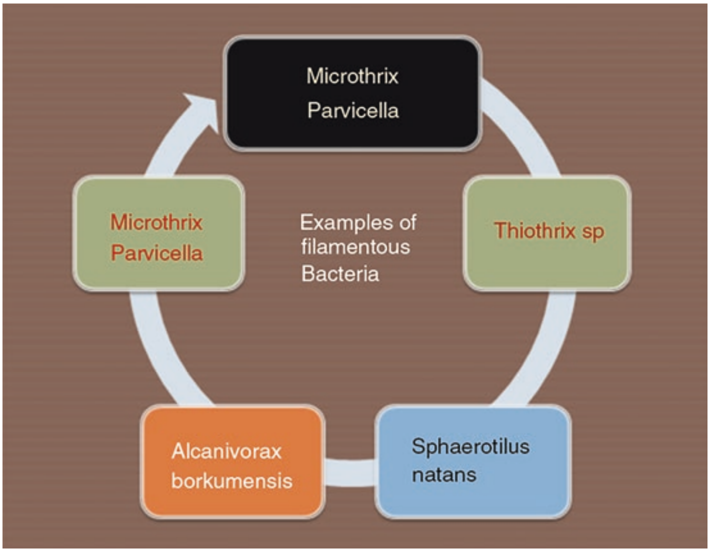
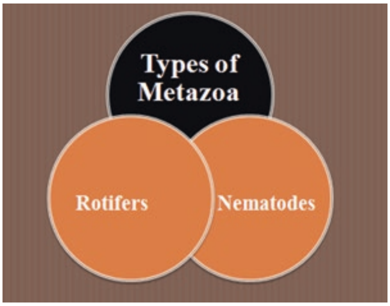


Fig. 4 Examples of filamentous bacteria involved in wastewater treatment

Fig. 5 Examples of types of metazoan involved in wastewater treatment



1968). Actually, protozoa are thought to be the extremely important bacterivorous grazers. Several protozoa that live in activated sludge have clearance rates ranging from 4×10^{-7} to 1×10^{-6} ml medium protozoa^{-1} h^{-1} (Bloem et al., 1988). Protozoa of many sorts are found in wastewater treatment systems and play various roles (Fig. 6).

3 Wastewater Treatment Techniques

Biological wastewater treatment technologies rely on microbial communities' natural ability to purify themselves (Misal et al., 2011). Microbes are used to remediate wastewater in an environmentally friendly manner. Bioremediation employs techniques that take advantage of microbial metabolism's inherent ability to digest

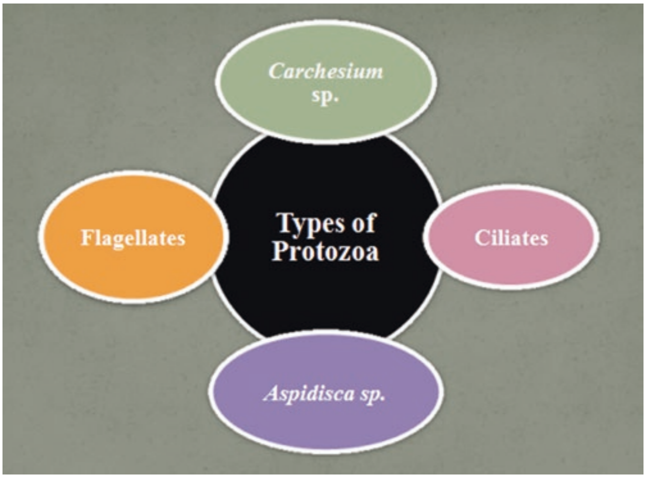


Fig. 6 Examples of types of protozoa involved in wastewater treatment

poisonous and complex xenobiotic chemicals that are harmful to most life forms and the environment. Toxic macromolecules are broken down into simpler forms, such as water, carbon dioxide, certain sugars, acids, and microbial biomass, which are harmless to the atmosphere and living beings.

3.1 Factors That Affect Bioremediation

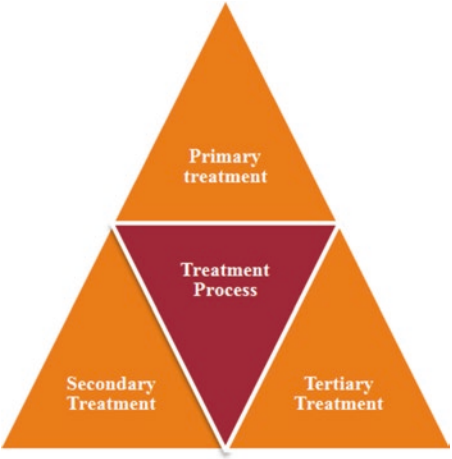
The microbial group is critical for both remediation and maintaining an effective equilibrium in the concentration of a variety of substances found in water and soil (Xu et al., 2018). A number of physiochemical parameters are required for microbial bioremediation of effluents containing various toxicants, as follows:

- Waste retention time during the treatment process.
- The presence and concentration of additional wastewater components.
- Temperature, pH, and nutrition availability.
- Waste produced in terms of stereochemistry and toxicity.
- The biological treatment's and strain's effectiveness.

3.2 The Treatment Methodology

- Primary Treatment (Fig. 7)
  In initial treatment, there is removal of oil, suspended solids, and gravel.
- Secondary Treatment

Fig. 7 Steps of wastewater management treatment process



The application of microbes in anaerobic as well as aerobic conditions to reduce biological oxygen demand, remove color, phenol, and oil.

- Tertiary Treatment
  In this remedy, the utilization of ion exchange for final wastewater treatment, treatment, electrodialysis, and reverse osmosis.

4 Role of Microbes to Degrade Petroleum Hydrocarbons Contaminant

Priority contaminants are stubborn petroleum hydrocarbon pollutants. Cleaning these poisons out of the atmosphere is a major issue. Bioremediation, that uses natural microbial biodegradation activity, has become a popular strategy for restoring petroleum hydrocarbon-damaged settings. Microorganisms used to produce petroleum hydrocarbons are found all over the world. They remove toxins from the environment by naturally biodegrading them. Using oleophilic microorganisms (individual isolates/consortium of microorganisms) to remove petroleum hydrocarbon contaminants from the environment is both environmentally and economically beneficial. Petroleum oil is a vital resource for which every nation struggles ferociously. Without a doubt, the petrochemical sector prospers while human activity relies on oil to meet its energy needs. These facts undoubtedly have a factor in the petrochemical sector's success. On the other side, using petroleum has a detrimental effect on the ecosystem. Petroleum use, on the other hand, has a negative impact on the environment (Chen et al., 2015). Blowouts during oilfield development, leakage from storage tanks and oil pipelines, oil well waxing, oil tanker and tanker leakage accidents, overhauls of refineries and petrochemical production equipment are all usual causes of release of petroleum hydrocarbons during petroleum production, refining and processing storage and transportation, as well as spills and discharges of petroleum hydrocarbons (Das & Chandran, 2011).

Petroleum-based products are the primary energy source for industry and everyday life. During the exploration, production, refining, transportation, and storage of petroleum and petroleum products, leaks and accidental spills are common. Natural refined oil seepage is estimated at 600,000 metric tons per year, with a range of uncertainty of 200,000 metric tons per year (Holliger et al., 1997). The unintentional or intentional release of hydrocarbons into the atmosphere is a chief source of soil and water contamination (O'Brien et al., 2017). Physical and chemical methods, a combination of the two ways, have been employed to address petroleum hydrocarbon pollution. However, these procedures frequently fail to entirely remove emulsified and decompose oil from soil and water, resulting in increased remedy costs. At the same time, it will pollute the environment repeatedly, thus physical or chemical measures can only be employed in emergency situations (Sakthipriya et al., 2015).

Some oil-loving bacteria in the environment were employed to use biological ways to break down petroleum hydrocarbon contaminants. This approach has a minimal processing cost and does not result in recurring contamination. Microorganisms can break down petroleum hydrocarbon contaminants in nature in a variety of ways. Some toxic and damaging petroleum hydrocarbon pollutants can be degraded into innocuous chemicals with the use of appropriate petroleum-degrading bacteria, and even some hydrocarbon substances can be completely degraded (Hommel, 1997). To improve the efficiency with which petroleum-degrading bacteria degrade petroleum hydrocarbon pollutants, we must first gain a thorough understanding of the mechanism by which petroleum-degrading bacteria degrade petroleum hydrocarbon pollutants, which will serve as a solid foundation for future research and application.

4.1 Degradation of Petroleum Pollutants by Microbial Mechanisms

Microorganisms degrade petroleum hydrocarbons primarily through the catalysis of intracellular enzymes. The process of microbial breakdown of petroleum hydrocarbons is divided into four stages: First, petroleum pollutants are emulsified by surfactants generated by microorganisms; second, the emulsified petroleum hydrocarbon is absorbed by the microorganism's surface and thirdly, the petroleum hydrocarbon adsorbed on the cell membrane's surface enters the cell membrane via active or passive transport, a process known as endocytosis. Finally, the petroleum hydrocarbon that enters the cell performs an enzymatic reaction with the appropriate enzyme in order to fulfill the goal of pollutant degradation. Figure 8 represents the schematic diagram of the four main phases of microbial degradation of petroleum hydrocarbons.

The enzymatic important response in the early intracellular assault of organic pollutants is oxygen activation and incorporation, which is catalyzed by oxygenases and peroxidases. In a step-by-step process, peripheral degradation pathways convert organic pollutants into intermediates of central intermediary metabolism, such as the tricarboxylic acid cycle. In the production of cell biomass, the primary precursor

Fig. 8 Schematic diagram of four main phases of microbial degradation of petroleum hydrocarbons

metabolites acetyl-CoA, succinate, and pyruvate are employed. Gluconeogenesis generates the sugars required for various biosynthetic pathways and growth (Das & Chandran, 2011). The breakdown of petroleum hydrocarbons can be mediated by a particular enzyme system. Another mechanism is microbial cell adhesion to substrates and biosurfactant synthesis (Bonomo et al., 2001; Atlas, 1986).

4.2 Main Principles of Microbial Degression of Petroleum Hydrocarbons in the Atmosphere

Abiotic mechanisms that influence degradation, such as oil biodegradation, have a key role in determining the fate of petroleum hydrocarbons in the environment. The petroleum hydrocarbon biodegradation rates are affected by factors that influence microbial growth and enzymatic activity. The quantity and quality of the hydrocarbon mixture, as well as the features of the impacted environment, determine the stubbornness of petroleum contaminants. Petroleum hydrocarbons can exist indefinitely in one environment, but can be entirely biodegraded in another in just a few hours or a week. The microbe’s activity can be influenced by the following factors: pH, oxygen, temperature, nutrients, etc., as shown in Fig. 9. Many of the following

Fig. 9 Factors that affect the biodegradation of petroleum hydrocarbons

limiting factors have been recognized to treat the biodegradation of petroleum hydrocarbons:

- Oxygen
- Temperature
- Nutrients
- Activity of water
- Bioavailability
- pH
- Salinity
- Microbial community
- Toxicity

4.2.1 pH

The pH of a solution can vary greatly, and this must be taken into account while developing new biotic treatment procedures. The pH of the surrounding atmosphere has an impact on processes inclusive cell membrane transport and catalytic reaction balance (Atlas, 1986). In most aquatic environments, heterotrophic bacteria choose a neutral to alkaline pH, and acidity of soil varies greatly, ranging from 2.5 to 11 pH in alkaline deserts (Hambrick III et al., 1980). Heterotrophic fungus and bacteria prefer a pH that is close to neutral, while fungi may tolerate acidic circumstances. The researchers investigated microbial mineralization of both naphthalene and octadecane (Dibble & Bartha, 1979). When the pH is raised from 6.5 to 8.0, then the rate of octadecane mineralization increases dramatically, while the rate of naphthalene mineralization remains steady. The highest degradation of unrefined oil was by *Pseudomonas aeruginosa* in water pH 8.0. In oil sludge samples, the greatest degradation rate was at pH 7.8 (Pawar, 2015). Some scientist discovered that a soil pH of 7.5 was best for petroleum degradation hydrocarbons (Atlas, 1981).

4.2.2 Temperature

Temperature influences petroleum hydrocarbon biodegradation by altering the chemical and physical components of petroleum hydrocarbons. Heterotrophic fungus and bacteria prefer a virtually neutral pH (Bisht et al., 2015). The degradation rate generally lessens at low temperatures, which are assumed to be due to low enzymatic activity (Colwell et al., 1978). The rate of hydrocarbon metabolism reaches its maximum at high temperatures ranging from 30 to 40 °C. Despite the fact that biodegradation of hydrocarbons can occur at a broad range of temperatures, the ability of degradation decreases as the temperature drops (Pawar, 2015). Figure 10 shows the maximum rates of deterioration in soil, marine, and freshwater habitats, respectively, at temperatures of 30–40 °C, 20–30 °C, and 15–20 °C (Bisht et al., 2015). They demonstrated that Metula crude oil degradation may be achieved using mixed cultures of marine bacteria (Von Wedel et al., 1988).

4.2.3 Oxygen

The oxygen concentration has been identified as the rate-limiting variable in the breakdown of petroleum hydrocarbons in the environment (Haritash & Kaushik, 2009). The amount of oxygen in the soil is determined by microbial activities, soil

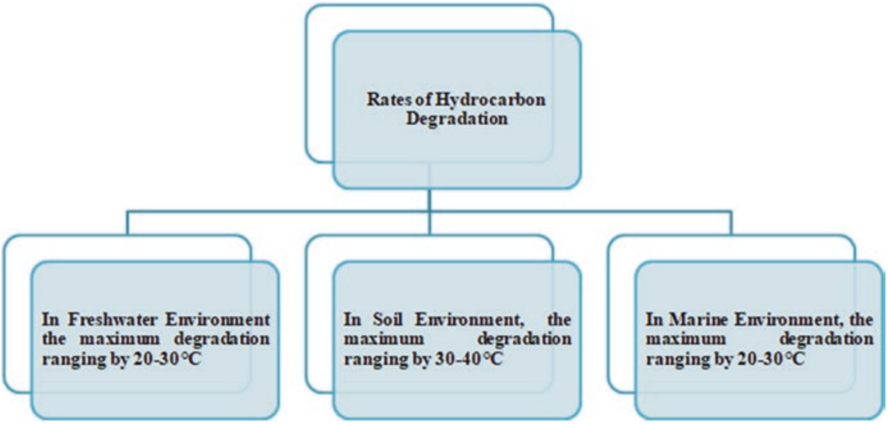


Fig. 10 Hydrocarbon degradation rates in fresh water, soil, and marine environments

type (wet or dry), and the existence of usable substrates, the entire of these lead to oxygen exhaustion. According to some research, anaerobic petroleum hydrocarbon degradation by microbes can occur at especially low rates (Grbic-Galic & Vogel, 1987).

Therefore, the nonexistence of molecular oxygen, sludge, and soil microbial consortia have been shown to be proficient in metabolized alkyl-substituted aromatics, acenaphthene, naphthalene, benzene, 1,3-dimethylbenzene xylene and toluene (Meng et al., 2017). Within anaerobic settings, PH biodegradation was slower than in aerobic conditions. The catabolism of every cyclic, aliphatic, and fragrant molecule by microbial oxygenases is well thought-out as a fundamental stage in the biodegradation process (Atlas, 1985).

4.2.4 Nutrients

In some situations, the critical nutrient components for effective biodegradation pollutants are such as nitrogen, iron, and phosphorus (Kalantary et al., 2014). A quantity of these nutrients may happen to a restraining feature, disturbing biodegradation processes. Carbon is obtained from organic sources (petroleum hydrocarbons), while oxygen and hydrogen are obtained from water (Hesnawi & Adbeib, 2013). The spills of oil generate a huge enhancement in levels of carbon and fall in phosphorus and nitrogen levels in marine and freshwater habitats.

The quantities of nitrogen and phosphorus in marine environments are short due to swampland and are not capable of offering nutrients due to severe plant demands. As a result, nutrients were required to aid in the biodegradation of pollutants (Kerr & Capone, 1988). Excess nutrients, on the other hand, can stifle the activity of biodegradation (Kalantary et al., 2014).

4.2.5 Salinity

Salinity and petroleum hydrocarbon mineralization rates in estuary sediments have a favorable association (Minai-Tehrani et al., 2009). Researchers noted that the evaporation of salt ponds and also the hydrocarbon metabolism rates were significantly decreased when salinity increased in the range of 3.3--28.4% because of a broad-ranging decline in the rate of metabolic microbial activity (Qin et al., 2012). Salinity has a noteworthy contact lying on bioremediation and biodegradation processes, appropriate microbial diversity, and microbial growth (Ebadi et al., 2017). It has a negative impact happening on the movement of a quantity of important enzymes involved in the hydrocarbon breakdown process (Semple et al., 2003).

4.2.6 Activity of Water

Water is accessible for microbial growth and microbial metabolism as well as biodegradation of hydrocarbons. In other words, water plays an important role in the growth of many types of microorganisms. Biodegradation was best in oil sludge with 30–90% water saturation (Pawar, 2015). According to Atlas (1981), this could be another example of microbial decomposition being limited to hydrocarbons. The movement and proliferation of microorganisms are directly influenced by water availability (Atlas, 1981).

4.2.7 Microbial Community

Petroleum hydrocarbons can be degraded by bacteria, yeast, fungus, and certain algae. The availability of microorganisms that can catabolize pollutants is one of the most important factors influencing the degradation of petroleum hydrocarbons. In addition, microbes and fungi play a role in the decomposition of hydrocarbons in the soil (Kalantary et al., 2014). Petroleum hydrocarbon can be used by microbes as a source of food. They can be found in large quantities around oil-contaminated areas such as shipping lanes, ports, oil fields, crude oil seeps, and petrol stations, in addition to further alike locations.

4.2.8 Bioavailability

The chemical portion of soil that can be in use or changed by livelihood pathogens is referred to as bioavailability. Influence of physical, chemical, and biological elements as well as rate of biodegradation is also known as bioavailability. Restrictions in hydrocarbon bioavailability can have a considerable impact on pH, microbial population, moreover the level of decomposition of the hydrocarbon. The bioavailable portion of the hydrocarbons is the portion that bacteria can access. Petroleum hydrocarbons are classified as hydrophobic organic pollutants because of their limited bioavailability. These compounds have low water solubility, making them photolytically resistant (Lundstedt et al., 2003).

4.2.9 Toxicity

The basic idea behind biological action is to use microorganisms to eliminate poisons and contaminants in a confined area. The augmentation and amputation of oxy-polycyclic aromatic hydrocarbons, such as coumarins, quinones, and PAH-ketones, were studied utilizing a bioreactor to treat impure PAH gas-work soil recently. These chemicals are created via microbial metabolism of PAHs, as well as photo-transformation and chemical oxidation of PAHs (Van Beilen & Funhoff, 2007).

5 Influence of Enzymes in Petroleum Hydrocarbon Degradation

Microorganisms contain enzyme systems that digest and exploit various hydrocarbons as the source of energy and carbon, making this conceivable. The microbe's breakdown of petroleum hydrocarbons, chlorinated oil, and other chemicals is aided by cytochrome P450 hydroxylases (Scheller et al., 1998). *Candida* species such as *Candida maltose*, *Candida tropicalis*, and *Candida apicola* had their cytochrome P450 enzymes isolated (Chakrabarty, 1996). Alkane oxygenases, such as integral membrane di-iron alkane hydroxylases, copper-containing methane monooxygenases, cytochrome P450 enzymes, and soluble di-iron methane monooxygenases, are found in eukaryotes and prokaryotes are involved in degradation of alkanes due to aerobic conditions (Scheller et al., 1998). Fungi are effective petroleum hydrocarbon degraders. Fungi have various benefits over bacteria, including the capacity to develop on a spacious variety of substrates. Extracellular enzymes are also produced, which can penetrate polluted soil and eliminate contaminants. Growth parameters such as oxygen, nutrient accessibility, and ideal enzymic surroundings depend on temperature, pH and substance arrangement, compound partitioning in augmentation medium, and cellular transport qualities, all influence on effectiveness and amount of pollution biodegradation by fungal enzymes. For the reason that asymmetrical configuration of lignin, fungi have developed to improve their ability toward breakdown and to impregnate various organic pollutants (Atlas, 1985). These technologies are significant, cost-effective, and easy to use and also have accomplished concentrated efforts on oil spills and another infected circumstance. More research might be done to evaluate the routine or effectiveness of the isolated strains. It is also possible to investigate how this technique might be used to degrade hydrocarbon-polluted water supplies. As a result of this analysis, it is possible to infer that pathogens degradation can be well thought-out as a crucial constituent in petroleum hydrocarbon remediation concentrated efforts.

6 Role of Microbial Degradation for Chemical Release

A huge amount of natural and artificial compounds are liberated into the atmosphere due to industrial and agricultural advances. The chemical industries are continuously developing the new herbicides, insecticides, refrigerants, solvents, and flame retardant. The chemical industries enhance the pollution load by releassing various kinds of contamination in the environment as well as the lifestyle is due to the pollution arising from the industrial activities, resulting in gas emissions, wastewater polluting the natural ponds and underground water, solid and slurry waste, polluting the soil and underground water. Because microorganisms lack the necessary degradative enzymes, mainly human-made compounds are vastly determined in the environment (Aislabie et al., 1997). Massive volumes of chlorinated compounds have been released into the environment as herbicides/pesticides, solvents, and degreasers, along with other industrially useful compounds, resulting in serious pollution issues. Bioremediation is a process wherein bacteria or their products are used to speed up the decomposition and detoxification of pollutants, which is a new way to battle pollution. Unfortunately, the majority of chlorinated chemicals are prepared and break down quickly in nature. Individual cause in favor of the sluggish rate of degradation is short of suitable genes for complete breakdown of such chemicals in natural microflora.

7 Role of DDT Microbial Degradation and Their Residues

Biodegradation of DDT basically depends upon co-metabolism. Co-metabolism is the transformation of a substance by a microorganism utilizing into another substance. The co-metabolized substance is not incorporated into an organism's biomass and the organism does not derive energy from the transformation of that substance (Atlas, 1988). The mechanisms of DDT microbial degradation is appropriate. They include the addition of DDT-metabolizing microbes to contaminated soils and the manipulation of environmental conditions to enhance the activity of these microbes. Flooding of soil and the addition of organic compounds can enhance DDT degradation. As biodegradation may be inhibited by lack of access of the microbe to the contaminant, the soil may need to be pretreated with a surfactant (Anju et al., 2010). One route for DDT deletion from soil is microbial degradation of DDT residues. The biodegradation of DDT, DDD, and DDE by bacteria and fungus is covered. However, the residues of DDT preserve biodegradation in dust at a time-consuming rate. A number of solutions are offered to improve in-situ deterioration. They involve adding DDT-metabolizing microorganisms to polluted soils and/or manipulating ecological surroundings to boost these bacteria movement. Ligninolytic fungi and bacteria that degrade chlorobiphenyl are both interesting alternatives for cleanup. Soil flooding and the accumulation of organic material can boost DDT degradation.

8 Role of Microbial Degradation of Pesticide Residues

They are extensively used today to prevent and organize crop diseases and vermin, but residues of pesticide included caused considerable destruction to human being strength and the atmosphere. According to worldwide ecological restitution, science and technology were studying the degradation of microbiological bacteria and pesticides that present in soil environments. Nowadays, pesticides are widely used in preventing and controlling the diseases and pests of crop, but at the same time pesticide residues have brought serious harm to human's health and the environment.

Pesticides are a class of chemicals that include insecticides, herbicides, fungicides molluscicides, rodenticides, nematicides, and plant enlargement regulators. They are used to direct pests, weeds, and crops diseases, as well as for human and animal strength. Pesticide use has the advantage of increasing crop/food efficiency and reducing vector-borne disease transmission (Zhang et al., 2011). A pesticide is a product or assortment of compounds that are used to kill pests, including insects, rodents, fungi, and unwanted plants (Singh & Walker, 2006). Pesticide definitions change over time and among countries. Pesticides are, nevertheless, essentially the same: they are a (diverse) compound to facilitate harmful and effective against intended organisms while being harmless for non-target organisms and ecosystems.

Microorganisms that can degrade pesticides might come from a variety of places. They are mostly useful to rural crops, therefore earth, along with pesticide industrial bilge water, manure mud, activated slush, wastewater, unusual waters, sediments, areas nearby pesticide manufacturing, and even some living organisms, is the primary source of these chemicals. Microbes that have been discovered as insect killer degraders have been inaccessible as of a spacious range of places that have been polluted with pesticides in general. Nowadays, in various laboratories around the world there are collections of microorganisms characterized by their growth, identification and degradation of pesticides. The isolation and characterization of microorganisms are able to degrade pesticides and also have given the possibility to count with new tools to restore polluted environments before the final disposition. The isolation and characterization of pesticide-degrading microbes open up the option of using novel techniques to rehabilitate contaminated habitats or treat wastes before final disposal (Kaur et al., 2016).

8.1 Types of Pesticides

There are two types of pesticides.

- The Key Types of Pesticides in Agriculture

  Different types of pesticides are used in agricultural production such as organic nitrogen, organic phosphorus, organic chlorine, carbamate, and insect growth regulators. Several types of organic pesticides used in agriculture are shown in Table 1.

- Types of Pesticide-Degrading Microorganism

  Various studies suggested that a wide range of microorganisms are capable of degrading pesticides. A variety of degrading microorganisms have been isolated and identified. These microbes included that *Bacillus, Mycobacterium, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas Aspergillus, Mycobacterium,* white rot fungus, and additional fungi were established. Algae include marine chlorella, among other things. Table 2 shows a list of the most prevalent degrading-pesticide bacteria.

Table 1 Types of pesticides in agriculture

Pesticide types	Pesticide names
Organic nitrogen	Chlordimeform, benzoylphenyl ureas
Organic chlorine	Aldrin, chlordane
Pyrethroid	Cypermethrin, flumethrin
Insect growth regulators	Azadirachtin, spinosad

Table 2 List of microbes and their potential role in degrading different types of pesticides

Microorganism types	Species	chemical name/chemical pesticides
Bacteria	*Pseudomonas*	Aldrin, Endrin
Actinomycetes	*Nocardia*	Aldrin, Diuron
Fungus	White rot fungi	Aldicarb, Altrazine
Algae	*Chlamydomonas*	Phorate

8.2 Mechanism of Microbial Degradation of Pesticide Residues

Pesticide use is still quite low in most developing nations, with India's input of 330 g/ha compared to 1490 g/ha in a developed country like Japan (Anonymous, 1979A). Conversely, some places where pesticides are used extensively, such as tea, cotton, and cocoa, as well as rice farming, can lead to environmental contamination. It may be simply noticed that environmental parameters present that are limiting the biodegradability of these so-called non-degradable compounds. Pesticides removal from the environment required favourable conditions such as temperature, pH, redox potential, nutrient balance, substrate concentration, bioavailability, and also insolubility, absorption, competing for polymerization, complexing, and binding reaction. These parameters depends upon the chlorinated pesticides which are coupled to the presence of the suitable/appropriate microorganisms (Tang, 2018). Pesticides have been employed as a primary microbial nutrition in recent years, and they eventually degrade into tiny molecules. The process involved the compound entering the body of the bacteria in a specific way, referred to as an enzymatic reaction, and it was followed by a number of biochemical and physiological reactions involving a variety of enzymes. Finally, the pesticide was completely broken down into slightly smaller molecular compounds with no or low toxicity, (Huang et al., 2018).

8.3 Factors Affecting the Microbial Degradation of Pesticide Residues

Many factors, including internal and external ecological conditions as well as the structure of the pesticide and the microorganisms, prevented pathogens from breaking down pesticide residues. Following are some factors responsible for the microbial degradation of pesticides:

- Microbial genus, metabolic activity, and adaptability directly influenced pesticide degradation as well as transformation (Baxter & Cummings, 2006)
- Effect of pesticide structure
- Environmental factors

8.4 Microbial Species, Metabolic Activity, and Adaptability Directly Influence Pesticide Degradation and Transformation

Many researchers' investigations have revealed that diverse classes of microorganisms or various strains of the equivalent species react differently to the identical macrobiotic substrate or hazardous metal and those microorganisms have well-built capacity to adjust their surroundings and become domestic. The novel substances could cause microorganisms to develop or create an innovative system of enzyme to break down these using the adapted mechanism. The most significant criteria were functional features and degradation changes (Chrzanowski et al., 2012).

8.5 Pesticide Structure

The molecular mass, spatial arrangement, amount, variety of substituents, and placement of pesticides all influenced the effectiveness of microbial degradation (Bhattacharya et al., 2006). Many synthetic polymers have been developed mainly from petroleum and coal products and are not good for the environment's health perspective. Herbicide use has become an unavoidable part of agricultural production, and as a result, a slew of new environmental issues have emerged, including the threat to human health and extreme pesticide substance in rural and ancillary yield.

Even if some harmful substances are slowly destroyed in nature by naturally produced microbial communities, this was still a novel dispute for the microbial world. Microbial deterioration is a deliberate process that might require several structural changes. Thus, compared to now commonly use prepared bioheterologous compounds, the expected evolutionary procedure of microorganisms be plainly not capable to assemble the needs of pesticide degradation. As a result, after a long-term influence, the ecosystem's balance will be ruined (Singh & Walker, 2006; Baxter & Cummings, 2006). As a result, it was critical and necessary to investigate a number of strategies to help microbial plants accomplish the greatest pesticide breakdown in a small period of time.

8.6 Environmental Factors

pH, temperature, oxygen, nutrition, carbon dioxide, humidity, substrate concentration, surfactant, salinity, and other factors have been shown to influence degradation. Temperature, pH, and substrate concentration are all required by bacteria or their enzymes of their proper functioning as well as for their activity, 2010). Nutrient insufficiency was a significant preventive feature for the growth of microorganisms along with population maintenance. The most critical elements that influenced bacterial growth and reproduction were temperature and humidity. Pesticides used in agriculture during the time included phosphorus, chlorine, pyrethroid, chloronicotinyl insecticide, and various fungicides, among others. Many pesticide-degrading microorganisms, including bacteria, fungus, algae, and other microbial strains, could be screened from expected manure or soil. *Bacillus subtilis*, *Pseudomonas*, *Klebsiella* sp., and other bacteria were found. *Aspergillus* spp., *Trichoderma* spp., and white rot fungi, etc., were found. Also, algae had marine chlorella and so on (Hommel, 1990).

9 Conclusion

To clean up the atmosphere is a real-world issue. The deeper knowledge of the biodegradation pathway is important for the environment because it relies on native bacteria to convert organic pollutants. The degrading method of pathogens entails the elimination of diverse degradable materials from the environment after the necessary removal of considerable numbers of various physical and chemical ways. This is conceivable because microbes comprise enzyme systems that can take down and employ various hydrocarbons such as carbon and energy sources. Future rules will be guided by current investigations and enlargement initiatives, which will concentrate on bioremediation targets, pollutant accessibility, and their potential danger to innate ecosystems and individual strength. Furthermore, transdisciplinary technologies would make it simple to predict the accessibility and biodegradation of contaminants in some natural or manmade systems, as well as the degree of harm to human health caused by various environmental pollutants. As a result of this analysis, it is possible to infer that microbial degradation can be well thought-out as a crucial constituent of a hydrocarbon remediation cleanup plan.

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Microbial Enzymes and Their Importance in the Environmental Decontamination

Prem Lata, Younis Ahmad Hajam, and Shweta Singh

1 Introduction

Enzymes are playing a significant role in a metabolic reaction as a biocatalyst. Enzymes are of particular interest because they are used as natural catalysts in a lot of procedures on a manufacturing scale. Enzymes that are obtained from microbes are known to be better-quality ones. Numerous microbial enzymes are now used in a variety of industrial processes. Microbes comprising fungi, yeast, and bacteria are utilized worldwide for the synthesis of various enzymes for industrial purposes (Pandey et al., 1999). A variety of enzymes have been designed and engineered according to the requirements with the input of metagenomics, biochemical reaction, and protein engineering.

Numerous molecular techniques also are implemented on microbial enzymes to advance the superiority and performance at the commercial level (Chirumamilla et al., 2001). Therefore, several goods are being manufactured in the large-scale market with the use of bioprocess technology. Human activities (e.g., modern agricultural practices, industrialization, increasing population, and detrimental competition for primacy) have negatively impacted the planet. Such activities have resulted in the increased accumulation of pollutants such as "phenols, azo dyes, polyaromatic hydrocarbons (PAHs), polychlorinated compounds, pesticides, amd heavy metals." Therefore, they remain in the environment for prolonged periods, resulting in critical and long-lasting effects that have detrimental effects on the biotic

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_13

components of ecosystems (Vidali, 2001). These pollutants have "teratogenic, carcinogenic, mutagenic, and harmful effects" on humans and/or organisms and are affecting every inch of the earth's area (Leung, 2004).

Therefore, the elimination of organic waste from the environment should be a greater concern. The conventional methods for removal of xenobiotic contaminants involve dumping the garbage in a pit and treating it by high-temperature incineration and breakdown through ultraviolet (UV) rays and chemical methods. Lack of room, elevated cost, complex procedures, laborious regulatory requirements put on decontamination by various countries, and refusal by the global public, however, has decreased the use of physical and chemical tactics. The elevated production of sludge by several techniques brings various constraints—that is, it needs to be safely discarded or the sludge could result in the generation of harmful secondary pollutants (Bernhard-Reversat & Schwartz, 1997). Consequently, there is a great need to follow biologically favorable methods than unfavorable physical and chemical methods.

Bioremediation is a biotechnological process facilitated by biological organisms and alteration in pollutants responsible for bringing back the "recalcinated environment and handling pollutants by detoxification and mineralization. The treatment of persistent organic pollutants (POPs)" with the help of microbial enzymes, which are "environment-friendly, cost-effective, innovative," and promising (Dua et al., 2002), are ongoing. It is a slow process, and until now only a few species are generating specific enzymes capable of destroying pollutants. Thus, for bioremediation, genetically engineered microorganisms are considered a better option than conventional methods to produce the desired enzymes under enhanced conditions.

With this process, scientists use the microorganisms or genetically engineered microorganism's ability to generate specific enzymes capable of catalyzing and metabolizing the xenobiotic pollutants to attain energy and biomass, along with agrochemical, microplastic, polyhalogenated compounds, and hydrocarbons. This process changes the detrimental form into nondetrimental form and in some cases novel products (Dana & Bauder, 2011). Bacteria are ubiquitous because of their metabolic activity and can grow in any environmental conditions and produce enzymes. Several aerobic bacteria (e.g., "*Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium*") have enzymes that have been found to degrade pesticides and hydrocarbons (Fig. 1). Whereas, anaerobic bacteria are effective for the bioremediation of "polychlorinated biphenyls (PCBs), dichlorination of trichloroethylene (TCE), and chloroform" (Hammel, 1997). The chief enzymes obtained from microorganisms and genetically engineered microorganisms are often used for the degradation of the different classes of pollutants involved in the "bioremediation processes that consist of cytochrome P450, laccase, hydrolases, dehalogenases, dehydrogenases, proteases, and lipases."

Fig. 1 Microbial action for the decontamination of the planet

2 Enzymes

Enzymes act as a catalyst and help to complete the process or increase the rate of a reaction under favorable conditions. An enzyme can be a protein, but all proteins are not enzymes. They must involve at least one "polypeptide moiety." There are some active sites, and those noted directly involve or participate in the process; some groups are attached to the active sites. These groups are important for catalytic activity. They are attached either with covalent or noncovalent bonds. The moiety among proteins or glycoproteins in these enzymes is known as an apoenzyme. On the other hand, the moiety in nonproteins is called a prosthetic group, and the combined association of both the "apoenzyme and prosthetic group forms holoenzymes."

2.1 Enzyme Nomenclature

A single catalytic entity is named an enzyme. Various researchers have reported that the enzyme names relate to their functions (Fig. 2) in a specific manner to catalyze the reaction (Lehninger et al., 2004).

2.2 Enzyme Classification

The specific enzyme is identified by an "enzyme commission (E.C.) number" according to the International Union of Biochemistry (IUB) guidelines. There are six divisions: "oxidoreductase, transferases, hydrolases, lyases, isomerases, and

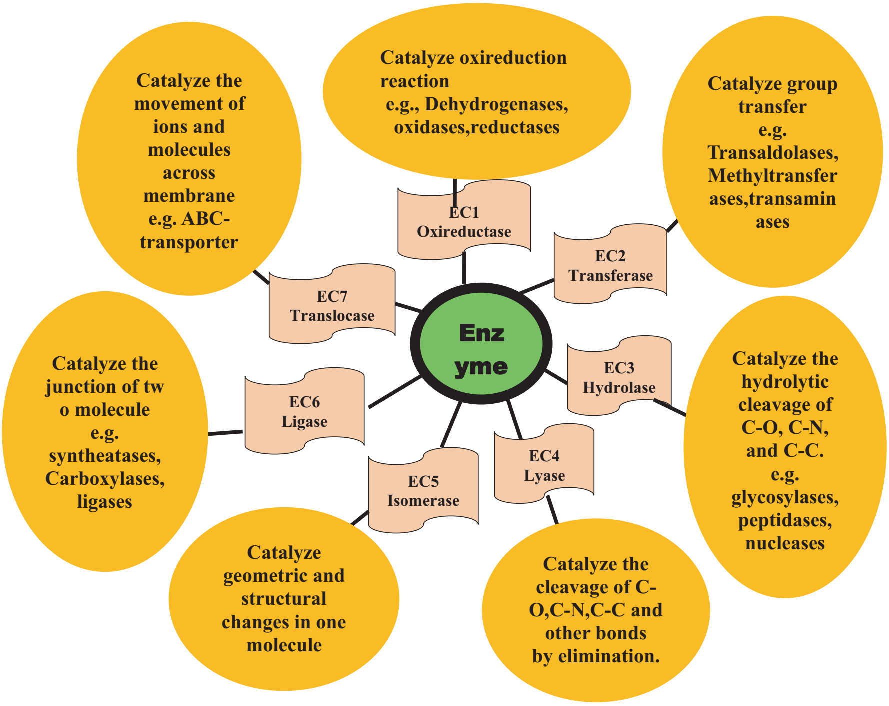

Fig. 2 Classification and function of enzymes

ligases.” Among all these, oxidoreductase helps to mediate electron and proton transfer, Transferases mediates functional group transfer (from donor to an acceptor). Hydrolases and lyases catalyze and enables cleaving of C-C, C-O, and C-N and removal of double bonds, respectively, by facilitating the formation of groups (i.e., around a double bond). Isomerases mediate the isomerization and ligases help to facilitate the molecule fusion reported by various researchers (Lehninger et al., 2004).

3 Microbial Enzymes in Environmental Decontamination

3.1 Microbial Oxidoreductases

Various “authors have reported that purification of toxic organic compounds is facilitated by oxidoreductase (by bacteria, fungi, and higher plants) because of oxidative coupling (Gianfreda et al., 1999; Bollag & Dec, 1998). Energy is obtained by microbes through biochemical reactions that produce energy.” It is facilitated through enzymes to break bonds. It also mediates the electron transfer (from donor to acceptor). The oxidization of contaminants occurs during oxi-reduction reactions. It resulted in nontoxic compounds. Oxidoreductase also occurs in some phenolic compound humification. This happens when lignin breakdown is formed in the soil environment.

Several workers have reported that it also is used for decontamination of "toxic xenobiotics (e.g., phenolic compounds through polymerization, binding to humic substances, etc.)" (Park et al., 2006). The decolorization and breakdown of "azo dyes" form because of microbial enzymes (Vidali, 2001; Williams, 1977; Husain, 2006), and there are so many bacteria that can change radioactive metals from an oxidized soluble form to an insoluble (reduced) form. Radioactive metal acts as an electron receptor when bacterium is found in some electrons from organic compounds at the time of energy generation; in some cases, when an intermediate donor is used then reduction in bacterial species can be found that reduces "radioactive metals" in an indirect manner. Leung (2004) reported that in metal-reducing bacteria the precipitants gave redox reactions results.

Some industries, mainly paper and pulp, generate chlorinated phenolic compounds (one of the most "profuse recalcitrant" waste), and in the process of pulp bleaching, all these compounds are formed (i.e., breakdown of lignin). There are many species of fungi that perform the elimination of "chlorinated phenolic" compounds (from the polluted environment). The fungi activity forms "extracellular oxidoreductase enzyme" actions such as laccase, manganese peroxidase, and lignin peroxidase (Rubilar et al., 2008). Some enzymes are released from the roots of a plant, which help to purify contaminated water, have phenolic compounds. The Fabaceae, Gramineae, and Solanaceae families are known to be "secrete oxidoreductases"; it helps in the oxidative breakdown of some soil particles. Various researchers have reported that, generally, there are three classes of compounds focused on the phytoremediation process of contaminants such as "chlorinated solvents, explosives, and petroleum hydrocarbons" (Dur'an & Esposito, 2000; Newman et al., 1998).

3.1.1 Microbial Oxygenase

Oxygenases belong to an enzyme group (i.e., oxidoreductase). The decrease of reduced substrates occurs because of them; this occurs when the oxygen atom is removed from molecular oxygen "(by FAD/NADH/NADPH)." Oxygenases have two categories based on the number of oxygen atoms in the oxygenation process (i.e., mono- and dioxygenase). In organic compound metabolism, they play a significant role. They elevate their water solubility, or cleavage formation, in an aromatic ring. Mainly, oxygenase acts contrary to some chlorinated aliphatic compounds because, when oxygen is added to the organic molecules, it breaks the aromatic rings.

In enzyme history, bacterial monooxygenase and dioxygenase are used mostly in bioremediation and various researchers have studied its importance in that process (Arora et al., 2009; Fetzner & Lingens, 1994; Fetzner, 2003). Many environmental pollutants are formed by halogenated organic compounds, which are used as "herbicides, insecticides, fungicides, hydraulic and heat-transfer fluids, plasticizers, and intermediates for the synthesis of chemicals." The breakdown of pollutants occurs because of oxygenases specifically, and it also facilitates “dehalogenation reactions” of methane, ethane, and ethylene through versatile enzymes” (Fetzner & Lingens, 1994).

3.1.2 Monooxygenases

Monooxygenase adds one atom oxygen molecule in the substrate and, based on cofactor monooxygenase presence, they are divided into two subclasses (i.e., “flavin-dependent monooxygenases and P450 monooxygenases”). Flavin is comprised of a prosthetic group in flavin-dependent monooxygenases. It uses NADP or NADPH as a coenzyme. On the other hand, P450 is comprised of oxygenases that have a heme group (present in prokaryote and eukaryote organisms). Monooxygenases contain multiple enzyme families. They mediate the oxidative reactions of the substrate, and this is different from steroids and fatty acids. Monooxygenases act as biocatalysts in the process of bioremediation; and they have enormous selective regions and “stereoselectivity in a high substrate range.” Usually a monooxygenase requires a cofactor for their proper functioning; however, some “monooxygenases do not require any cofactors.” They function properly without a cofactor.

Various studies have reported that only molecular oxygen is required for their proper functioning and a substrate is used as a reducing agent (Arora et al., 2010; Cirino & Arnold, 2002). It regulates “desulphurization, dehalogenation, denitrification, ammonification, biotransformation, and biodegradation” of aromatic compounds reported by several researchers (Arora et al., 2010). Among methane, ethylene, and ethane monooxygenase, methane has various suitable features, and the enzymes most suitable for hydrocarbons (e.g., methanes, alkenes, cycloalkanes, alkenes, ether, aromatic compounds, etc.) breakdown (Fox et al., 1990; Grosse et al., 1999). In a condition in which oxygen levels become high, then monooxygenase regulates “oxidative dehalogenation” reactions. A number of researchers have reported that substrate oxidation forms dehalogenation. This occurs because of the synthesis of labile products (Fetzner & Lingens, 1994; Fetzner, 1998; Jones et al., 2001).

3.1.3 Microbial Dioxygenases

A microbial dioxygenase is a multidimensional enzyme system; it adds molecular oxygen to the substrate. Aromatic hydrocarbon dioxygenases belong to the Rieske nonheme iron oxygenase family. Mainly, it regulates aromatic compound oxidation. After that, it is used for environmental remediation. In this, before the formation of oxygenase components, a family member requires at least 1–2 electron transport proteins. Authors have gotten results with both 2Fe-2S, and mono-iron is present in every alpha subunit of “naphthalene dioxygenase” (i.e., a crystalized structure) (Dua et al., 2002). The natural breakdown of aromatic compounds occurs because of catechol dioxygenases in nature; generally, they are present in soil bacteria. Usually, enzymes are used to make Fe (III), but in some cases, they form Fe (II) and Mn (II) as well.

3.2 *Microbial Laccases*

Microbial laccase is form a multicopper oxidase family that is manufactured by plants, fungi, insects, and so on. It helps to regulate the oxidation in a large amount of reduced phenolic substrate and aromatic as well, through reduction of O_2 into water (Gianfreda et al., 1999). It is present in various isoenzyme forms and is encoded by a specific gene. In some cases, the gene expression varies according to the inducer's nature (Rezende et al., 2005).

In certain cases, several organisms can synthesize intralaccases and extracellular laccases. It forms to mediate ortho, paradophenols, aminophenols, polyphenols, lignins, and so forth oxidation with a few inorganic ions (Ullah et al., 2000; Couto & Herrera, 2006). As scientists know, laccases are responsible for oxidation of phenolic and methoxyphenyl acids. It also forms decarboxylation and demethylation (attack on the methoxy group). These also help to regulate lignin depolymerization and form various phenols. Several researchers have reported that these compounds are used as nutrients for microorganisms to depolymerize into humic materials (Kim et al., 2002).

Among the biological agents, it forms special groups of ubiquitous ones. The enzyme oxidoreductase has an ability to be used in several applications such as biotechnological and bioremediation (Gianfreda et al., 1999). Because of the unstability of pH, the laccase's specificity ability may change and be inhibited by reagents (e.g., halides, azide, hydroxide, etc.) (Xu, 1996). In every laccase, the inhibitory tolerance power by halides is different. In fungi, the synthesis of laccase becomes sensitive toward the concentration of nitrogen; usually, for good nitrogen concentration, laccase is required in massive amounts. Various workers have reported that the synthesis of recombinant laccase can be through "homologous or either heterologous means" (Gianfreda et al., 1999).

3.3 *Microbial Peroxidases*

Microbial peroxidase is a universal enzyme. In this hydrogen peroxide, oxidoreductase is a donor, and it mediates lignin oxidation of some phenolic compounds. In mammals, peroxidases help to regulate biological processes (e.g., immune system and hormones), and in plants, they regulate lignin, suberin synthesis, cross-linking of the cell wall, anti-pathogen, and so on metabolism (Hiner et al., 2002; Koua et al., 2009). "Heme peroxidase" is subdivided into two parts. Among them, one was found in animals, and the second one was found in plants, fungi, and prokaryotes. Based on sequence, peroxidase is further divided into three classes; Class-I is intracellular enzymes "(consists of yeast cytochrome C peroxidase, ascorbate peroxidase, and bacterial gene duplicated catalase peroxidases)." Class-II involves "secretory fungal peroxidases" (i.e., lignin peroxidase and manganese peroxide) from phanerochaete crysosporium and Corprinus cinereus peroxidase or ARP. Class II functions mainly to break down the wood lignin.

"Class-III consists of plant peroxidases" (from HRP, soybean, and barley). It further acts as biosynthetic enzymes that help to form the cell wall, and its lignification has beem reported by various researchers (Hiner et al., 2002; Koua et al., 2009); however, the non-heme peroxidase does not possess the evolutionary linkage, so they are put into five different families (e.g., "thiol peroxidases, alkyl-hydro peroxidases, non-haem haloperzoxidation, manganese catalase, and NADH peroxidases"). Among them, Thiol peroxidase is the largest, which is further divided into two subfamilies (i.e., glutathione peroxidases and peroxiredoxins) (Koua et al., 2009).

4 Classification of Peroxidase Enzymes

Based on source and activity, they are further categorized into three types—that is, lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and versatile peroxidase (VP); they are the most studied among all the peroxidases. This is because of the enormous ability to break down the contaminated or toxic substances in the environment.

4.1 Microbial Lignin Peroxidases

They are heme proteins, secreted by the "white-rot fungus during secondary metabolisms." Lignin peroxidases are responsible for lignin breakdown and some phenolic compounds. It is because of the cosubstrate H_2O_2 and mediators (e.g., veratryl alcohol) and reduced to H_2O by acquiring electrons from lignin peroxidases. "Further reaction becomes reversible by acquiring an electron from veratryl alcohol and veratryl aldehyde forms versatile alcohol by taking electrons from the substrate." Various researchers have reported that the reaction causes oxidation in several compounds (e.g., halogenated phenolic compounds, polycyclic aromatic compounds, and other aromatic compounds) that precede nonenzymatic reactions (Yoshida, 1998; Ten Have & Teunissen, 2001). Like other enzymes, lignin peroxidase also plays a significant role in lignin biodegradation, which is an essential part of the plant cell wall, and it also can oxidize aromatic compounds (in which redox potential is more than 1.4 V). Nevertheless, the proper redox mechanism is still not clarified (Piontek et al., 2001).

4.2 Microbial Manganese Peroxidases

Microbial manganese peroxidases are a heme enzyme (i.e., extracellular). It is extracted from basidiomycetes fungus (lignin-degrading). It helps in Mn^{2+}–Mn^{3+} oxidation in long reaction steps. In this, Mn^{2+} starts synthesis of MnP and acts as a substrate for MnP. Mn^{3+} synthesized through MnP regulates some phenolic compounds' oxidation. Ten Have and Teunissen (2001) have reported that Mn^{3+} chelate oxalate is very small to spread into areas inaccessible (even to the enzyme—for example, the lignin structure (xenobiotic pollutants) is present in the soil and cannot be recognized readily.

4.3 Microbial Versatile Peroxidases

It is an enzyme that helps to regulate Mn^{2+} methoxybenzenes, phenolic aromatic substrate (MnP, LiP, and HP) oxidation. It generally shows the specificity of the substrate increasingly. Also, it has the ability of substrate oxidization without manganese than other peroxidases. However, versatile peroxidases can oxidize phenolic and non-phenolic lignin model dimer reported by different researchers (Ruiz-Due˜nas et al., 2007). So, for best biotechnological applications well-organized versatile peroxidases overproduction is required (Tsukihara et al., 2006).

4.4 Microbial Hydrolytic Enzymes

"Most soil pollution and water pollution are produced by chemical industries and petroleum hydrocarbons, which are the major problem throughout the world." It is majorly present in the aquatic and terrestrial ecosystem because of its vast utilization. To avoid contamination in the environment, bioremediation is the best technology through physicochemical treatment. Also, with the "organic pollutants," hydrolysis occurs because of bacterial activity, in which extracellular enzyme activity plays a significant role in degradation (in that it has < 600 dalton mm (molecular mass) (Vasileva-Tonkova & Galabova, 2003).

This enzyme can cleave the bond between the toxic molecules (resulting in reduced toxicity of the chemicals) and showed good results for oil spills' biodegradation and "organophosphate and carbamate insecticides." DDT is an example of organochlorine insecticides found in the soil in a well-organized manner. Hydrolytic enzymes further undergo rapid degradation in anaerobic environments reported by various researchers (Williams, 1977; Vasileva-Tonkova & Galabova, 2003; Lal & Saxena, 1982). It also regulates some reactions (e.g., condensation and alcoholysis), and also forms three groups of enzyme classes based on bond type. Amylases, protease, lipase, DNases, pullulanase, and so on are extracellular hydrolytic enzymes. They have substantial potential to be used in various areas like the food and chemical industries, and so forth (S'anchez-Porro et al., 2003). "Hemicellulose, cellulose, and glycosidase" have a significant role because of their biomass degradation (Schmidt, 2006).

4.5 *Microbial Lipases*

Lipases help in the breakdown of lipids (from various animals, plants, and organisms), and recently it was reported that lipases are associated with the inhibition of organic pollutants in the soil. Various researchers have reported that it is the best area for gaining more knowledge about bioremediation of "oil spills" (Margesin et al., 1999; Riffaldi et al., 2006). The main source of lipases are bacteria, plants, animal cells, and so on, and the microbial lipases are used in multipurposes because of their utilization in industries in massive amounts. Some reactions (e.g., "hydrolysis, interesterification, esterification, alcoholysis, and aminolysis") are mediated by the enzymes (Prasad & Manjunath, 2011). Lipases are universal enzymes and mediate hydrolysis or "triacylglycerols" of glycerol and free fatty acids. In the lipid–water interface, lipolytic reactions occur. This lipolytic substrate forms in equal manners (with monomeric, micellar, and emulsified states). Microbial lipase is divided into two categories; "elevation of enzyme activity soon after the formation of emulsion by triglycerides and lipase with a ring of protein layer on the active site" has been reported by various researchers (Sharma et al., 2011).

Triglycerides are a major part of natural oil or fat that hydrolyze into "diacylglycerol, monoacylglycerol, glycerol, and fatty acids." Among these, glycerol and fatty acids are used as raw materials (e.g., monoacylglycerol is an emulsifying agent in food, cosmetics, etc.). "Triolein hydrolysis (from *Candida rugose*) shows effectiveness in the biphasic oil-water system, and it gets absorbed in this in a huge …water phase." Lipase helps in the breakdown of the ester bond of "triolein," which is synthesized in diolein, monoolein, and glycerol, and in every reaction, oleic acid is synthesized.

Several researchers have reported that the activity of lipase is the most effective indicator for the cleavage of hydrocarbons in soil (Margesin et al., 1999; Riffaldi et al., 2006). It also is very effective in regiospecific-compound synthesis. They are mainly used in the pharmaceutical industry. "Various researchers have reported that because lipase functions in bioremediation, it also is used in other products like food, detergent preparation, cosmetics, and so forth. But the production cost is very high, so its industrial utilization [has] become reduced" (Sharma et al., 2011; Joseph et al., 2006).

4.6 *Microbial Cellulases*

Cellulase is the most abundant polysaccharides; it was first discovered and isolated by Anselme Payne. "It is the main substance of plant material. It is used as soil fertilizer, fodder for cattle, [and to] ignite cellulosic material to generate heat energy."

It is cost-effective and useful in the industrial sector. Cellulase is naturally produced by bacteria fungi and actinomycetes like microorganisms. “It is composed of endo- and exoglucanases. But the enzyme’s composition is always varying from microorganism to microorganism.” *Trichoderma viride* and *T. reesei* are aerobic cellulolytic fungi.

According to recent market reports, cellulase is used in coffee processing, for wine making, for producing fruit juice and laundry detergent, and as cleaning and washing agent. Cellulase is used to fight against biofilms by pseudomonas. In this way cellulase helps with many treatments against antibiotic-resistant bacteria to overcome healthcare sector-related problems. Because of the reduction of fossils in the world which has been stimulated by enzyme hydrolysis and formation of bioethanol from cellulase. With the combination of hemicellulases and lignin, cellulase forms ligocellulase, which is a compressed structure. Textile businesses are the largest industry in the world. Demand for fashions by customers rises day-by-day, seeking uniqueness in style and color in clothes.

Since the 1990s cellulases have been used in the production of detergents. It gets rid of the cellulases microfibrils, which are formed during washing of clothes. Cellulases have an effective function for softening materials and brightening colors. *Humicola*, Trichoderma, and *Bacillus* produce neutral, acidic, and alkaline cellulases, respectively. Cellulase is used for the removal of ink in the paper industry (Leisola et al., 2006).

4.7 Microbial Proteases

Proteases are the degradative enzymes that are obtained from the plants, animals, and microbes (Barrett & McDonald, 1986). Because of the industrial applications, proteases with a microbial origin are preferred over the others. Proteases are one of the three largest groups of enzymes used in the industrial sector. Various physiological events (e.g., birth, aging and death) can be regulated with the application of proteases (Chou et al., 1997, 2000, 2003; Chou & Howe, 2002; Chou, 2004, 2006). The involvement of proteases in the pathophysiological process could lead to the formation of therapeutic agents. Such agents can be used against fatal diseases like cancer and AIDS (Rawlings et al., 2004).

A large portion of commercial proteases have a microbial origin (Beg & Gupta, 2003). Because these proteases show various qualities such as high-yield, minimum-space requirements, infinitely little time consumption, vivid genetic manipulation, and increased cost-effectiveness (Nisha & Divakaran, 2014; Ali et al., 2016). The classification of the proteases (microbial origin) is based on pH properties; functional groups, and orientation of peptide bonds (Gessesse, 1997; Panda et al., 2013). Microbial proteases also can be divided into intracellular and extracellular proteases. Intracellular proteases play a vital role in hormone regulation, protein turnover; whereas extracellular proteases lead to protein hydrolysis (Rao et al., 1998; Adrio & Demain, 2014).

Depending on the pH properties, the proteases can be further subdivided into the alkaline, acidic, and neutral proteases. The pH range for alkaline, acidic, and neutral proteases is 9–11 (Varela et al., 1997), 3.8–5.6, and 5–8, respectively. The alkaline proteases can be obtained from the Bacillus genus (Varela et al., 1997); whereas the acidic and neutral proteases can be obtained from the Aspergillus fungal species (Sielecki et al., 1991), and Bacillus stearothermophilus (Fitzgerald et al., 1990; Dawson & Kent, 2000), respectively.

Proteases are helpful in the modification of taste and the enhancement of shelf life of the proteins used in the food industry (Rao et al., 1998). As a specific example, in the meat industry, proteases are used for the tenderization of beef. Hydrolyzing of the kappa casein is done with the help of proteases, leading to the formation of cheese (Ray, 2012). Proteases play a vital role in waste management. The waste generated because of the poultry industry can be easily converted to animal feeds with the help of proteases. For this purpose, a customized process known as keratinolytic was developed (Neklyudov et al., 2000; Lasekan et al., 2013). In addition, proteases are quite useful in the leather industry. Since the beginning of the industrial era, harsh chemicals have been used for the dehairing, bating, and soaking of animal hides and skins. “But with the help of proteases these functions can be done in an ecofriendly way” (Takami et al., 1992).

Currently, proteases also are being used as the cleansing agents for laundry, contact lenses, and dentures. “Amongst the enzymes’ sale, proteases are widely purchased by the detergent industry. *B. subtilis*-derived proteases can be used for the silver recovery form the X-ray films; thus, making the process ecofriendly.” In the chemical industries, proteases are used for the synthesis of peptides (Godfrey & West, 1996). Proteases are also used in the silk industry. It has been observed that the fibres obtained are of superior quality and the final threads are very strong (Yadav et al., 2011). In the medical field, various medicine formulas (e.g., ointment composition) can be produced with the alkaline proteases derived from *B. subtilis* (Sen et al., 2011; Anbu, 2013; Awad et al., 2013). In some of the enzyme-deficiency cases, the oral prescription of alkaline protease is given.

Some proteases (e.g., fibrinolytic enzymes) have been used as anticancer drugs (Jaouadi et al., 2011, 2012). In case of therapeutic applications, proteases are used for the hydrolysis of collagen, which inhibits amino acid formation, and it can lead to the formation of low-molecular-weight peptides. Proteases (e.g., elastoterase) can be used for healing of burns and wounds (Romsomsa et al., 2010; Suwannaphan et al., 2017). The proteases, thus, have widespread advantages such as ecofriendly, ease of production, and cost-effectiveness. These enzymes also have a widespread applications in the field of medicine, waste management, chemical and detergent industries, and so on.

5 Conclusions

Remediation is a biotechnological process facilitated by biological agents and changes in the physical and chemical structure of pollutants, making them less toxic and reliable. It recalcinates the environment by detoxification and mineralization of pollutants. Therefore, bioremediation mediates microbial enzymes, and genetically engineered microorganisms are considered a better approach for comparison to the conventional methods to produce the desired enzymes under enhanced conditions. Microorganisms or genetically engineered microorganisms can catalyze and metabolize the xenobiotic pollutants to obtain energy and biomass, along with agrochemicals, microplastics, polyhalogenated compounds, and hydrocarbons.

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Structural and Functional Dynamics of Bacterial World for Sustainability

Shabana Gulzar and Ubaid Yaqoob

1 Introduction

The vast diversity in plant-associated microbes has been an area of interest and research for over a century. The fact that bacteria residing in the root nodules of leguminous plants fix the atmospheric nitrogen made scientists believe that plants are associated with an abundance of diverse microbes. Plants have evolved with a multitude of microorganisms that profoundly benefit plant growth and development in particular as well as the ecosystem in general. The study of plant-associated microorganisms is quite interesting and needful due to functional potential and remarkable structural dynamics of these microorganisms associated with plants. There is an urgent need to meet the increasing global demand of food as well as combat the severe climatic conditions for the growth of plant crops. In addition to this, the soil would not be conducive for plant growth as it will be nutritionally depleted and contaminated with inadequacy of water resources as well (Singh & Trivedi, 2017). The sustainable ecosystem functioning globally needs an innovative production system that would maintain soil fertility and adequate water resource. The use of available natural resources, such as plant-associated microbiomes, is the most suitable approach to contribute to an improved and sustainable plant crop production system (Altieri, 2018; Bender et al., 2016; Eyhorn et al., 2019). A vast diversity of microbiomes improves ecosystem functioning (Isbell et al., 2015). Due to their broad range of genetic and metabolic diversity, the bacteria are highly adapted to varied ecological niches (Toft & Andersson, 2010). Microorganisms,

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_14

particularly bacteria, have lived in association with plants since ancient times and are beneficial to their host plant. These microbes associated with plants acquire soil nutrients by regulating all biogeochemical processes in the soil resilience to abiotic stresses, and pathogen control, destruction of pollutants, hence enhancing crop productivity, overall environmental quality and hence an increase of ecological adaptations (Schlaeppi & Bulgarelli, 2015). This chapter reviews and highlights the significance, composition, structural and functional dynamics of the plant-associated bacteria.

2 Diversity and Composition of Plant-Associated Bacteria

The plant microbiome encompasses the entire microbial community of plants including bacteria as well as fungi. Microbiome can be defined as the definite number of genes that are found in association with the host plant in a particular environment (Boon et al., 2014). It is believed that the microbiome interacts with the host plant dynamically to build up a symbiotic association that has a remarkable impact on the overall functioning of the host plant (Foo et al., 2017). The most significant type of plant-associated bacteria in the microbiome, that promote ecosystem functioning and sustainability and the potential of host plants, reviewed so far include the rhizobacteria and *Actinobacteria* (Ana et al., 2020).

2.1 Plant-Associated Rhizobacteria

The rhizobacteria include the variety of bacteria that inhabit the rhizosphere of the host plant thus benefitting the surrounding environment. The common plant-associated bacterial species belong to the genera *Lactobacillus*, *Acetobacter*, *Azospirillum*, *Serratia*, *Bacillus* and *Psuedomonas* (Backer et al., 2018). The host plant develops a symbiotic association with these rhizobacteria. The host plant enables the association of these bacteria by producing various secretions like carbohydrates, organic acids, minerals and root exudates that are utilized by rhizobacteria for their nutrition and development of symbiotic association with the host plant (Hassan et al., 2019; Braga et al., 2018). The rhizobacteria may invade either the inner structures, leading to endophytic establishment or the intercellular spaces of the host plant (Abbas et al., 2019; Akram et al., 2016).

The rhizobacteria, once in an established association, favours the host plant growth and development directly or indirectly (Backer et al., 2018; Akram et al., 2016). Directly, the host plant synthesizes some important nutrients like potassium (K), zinc (Zn) and silicon (Si) that lead to the growth of host plants (Backer et al., 2018; Rijavec & Lapanje, 2016; Angulo et al., 2014). Besides, the rhizobacteria generally synthesize various phytohormones like gibberellins (gibberellic acid, GA_3), auxins (indoleacetic acid, AIA), cytokinins, ethylene and abscisic acid

(ABA), to further enhance growth, cell division and overall development of the host plant. These phytohormones can further stimulate the secretion of the enzyme 1-aminocyclopropane 1-carboxylate deaminase (ACC), which mitigates the amount of ethylene hormone in the roots of the crops, thus improving the root length and density (Backer et al., 2018; Angulo et al., 2014; Penrose & Glick, 2001). The rhizobacteria indirectly promote plant growth through the modification of the rhizosphere environment, bringing about an inner resistance and adaptation in the host plant to cope with the external environment, such as protection against herbivores, insects and pathogenic microorganisms, and stimulate the synthesis of both physical and chemical obstructions and against abiotic stress. In turn, these substances allow the rhizobacteria to be more competitive in niche invasion and inhibit the interference by other pathogenic bacteria and fungi. These changes are brought about by the bacteria through the production of peculiar organic compounds and secondary metabolites (Takishita et al., 2018; Cappellari et al., 2017; Bhattacharyya & Jha, 2012). The common example includes the secretion of terpenoids (volatile secondary metabolite) by the bacterial species associated with mint and grape plants that prevents these plants against some harmful soil-borne pathogens (Cappellari et al., 2017; Salomon et al., 2016). Certain bacteria have been proven to facilitate the availability of important nutrients like iron to plants (Liu et al., 2017). The plant-associated bacteria especially some species of *Bacillus* and *Pseudomonas* have been reported to serve as biofertilizers, pesticides and stress-resistant for many important agricultural crops (Naeem et al., 2018; Hussain et al., 2016; Turatto et al., 2017; Chatterjee et al., 2017).

2.2 Plant-Associated Actinobacteria

The *Actinobacteria* were previously known as Actinomycetes and form the largest group of prokaryotes. *Actinobacteria* can inhabit plant roots endophytically or remain attached to epidermal or subcortical root cells. The endophytic bacteria have been described to enter the host plant through root hairs, stomas, wounds and sprouting hydathodes, while in free-living bacteria, structures like flagella, fimbria, exopolysaccharides and lipopolysaccharides may enable the bacterial attachment to the plant roots (Kandel et al., 2017). *Actinobacteria* are generally Gram-positive bacteria and either may be aerobic or anaerobic displaying distinct morpho-chemical characteristics of these particular moneran taxa (Bhatti et al., 2017; Sousa & Olivares, 2016; Barka et al., 2016; Trujillo et al., 2014).

The study *Actinobacteria* has a vast scientific and commercial importance, owing to their ability to synthesize a wide variety of secondary metabolites with potential biological activities including antimicrobial, anti-inflammatory, and anti-tumour, besides the production of plant growth regulators under abiotic and biotic stress conditions (Manivasagan et al., 2014). These bacteria thus promote the growth and development of important crop plants like tomato and corn, and are also responsible for inhibiting the growth of phytopathogens, and can even facilitate the availability of nitrogen to many plants. Nitrogen-fixing bacteria include members of the *Frankia* family, etc. Some of them are used as alternative for nitrogen fertilizers (Bhatti et al., 2017). The important actinobacterial genera include *Microbacterium*, *Micrococcus*, *Streptomyces* and *Frankia*, which have been reported to provide protection to their host plants against diseases and pathogens as well as enhance the growth of these plants (Bhatti et al., 2017; Barka et al., 2016; Vurukonda et al., 2018).

3 Plant-Bacteria Association: A Boon to Plant Growth

The scientists have unveiled the vast significance of the relationship between plants and bacteria; nature has endowed the plants with the ability to promote this association with the bacteria so that these get the ultimate benefit from these minute creations of nature. Apart from these useful bacteria, there are a number of harmful microbes that act as pathogens (e.g., *Agrobacterium, Pseudomonas*, etc.), thus causing a hurdle in plant growth. The most significant benefit of plant-associated bacteria includes their antimicrobial activity against these plant pathogens, through the production of siderophores (chelating agents that chelate ferric ions), certain volatile compounds, antibiotics and enzymes besides regulating the phytohormone levels. These associated microbes restrict the plant pathogen growth also through competition for nutrition as well as microenvironments and hence overall niche (Ellis, 2017; Kumar et al., 2018). A remarkable feature of these plant-associated bacteria is that they render resistance to their host plants thus boosting their immune system (Hunter, 2016). The Actinobacteria are able to control the wilt caused by *Fusarium* sp.; some actinobacteria were able to control the pathogen that caused a lot of damage to some cereal plants; besides, these microbes also promote plant growth through biofertilization by facilitating the availability and uptake of nutrients by plants (Trivedi et al., 2017; Durán et al., 2018). Another important benefit of the microbiome is the stimulation of plant growth and development through the production of phytohormones like auxins, gibberellins and cytokinins and decreasing ethylene concentration in the host plants (Backer et al., 2018; Angulo et al., 2014; Penrose & Glick, 2001). The plant-associated bacterial species enhance the resistance of their host plants to many unfavourable and stressful conditions that are non-conducive to plant growth. The bacteria growing in the rhizosphere of the *Hibiscus* plants enhance their germination and growth under non-conducive conditions (Kumar et al., 2019; Shahzad et al., 2017; Yuan et al., 2019).

The extensive use of pesticides in agriculture has a detrimental impact on the soil, and the microorganisms associated with plants are an anecdote to it; these microbes are able to degrade the harmful contaminants in the soil. The contamination of the soil leads to changes in the composition of the microorganisms inhabiting these soils. Many scientists have proposed some models that advocate human intervention and supervision for the efficient establishment of beneficial bacteria and other microorganisms in the contaminated soils and optimized phytoremediation through the plant-microbe interaction (Thijs et al., 2016; Yergeau et al., 2015).

4 Conclusion

The plant-associated bacteria and other microorganisms are believed to be important natural resources that would serve as an ultimate alternative to face the global demand for food. In order to evolve and perpetuate the concept of sustainable agriculture besides the proper functioning of our ecosystem, there is a need to research and explore the functional potential of the microbes associated with plants and select more efficient microbial groups that would enable the mitigation of pesticide and chemical fertilizer use, enhance plant growth and accomplish this sustainability (Johns et al., 2016). There is a need to augment the research on the molecular aspects of plant microbial functioning that would include sampling, extraction, amplification of DNA, development of bioinformatics in addition to the conventional techniques for proper understanding and application of plant microbiomes in sustainable agriculture. Despite the fact that ample data and knowledge have been generated from time to time regarding the functional and structural aspects of plant-associated microorganisms, there is still more scope to unravel the structural complexities and functional intricacies involved with these minute but worthwhile creatures of the universe.

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Plant-Associated Bacteria in Ecosystems Functioning and Sustainability

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1 Introduction

Sustainable crop production will be the fundamental concern of the twenty-first century. Producing adequate food for the world's rising population, renewable energy, and basic molecules in industrial processes all demand increased output. Current agricultural production techniques, for example, inappropriate application of chemical pesticides and fertilizers, have resulted in a slew of human health and environmental issues (Gunnell et al., 2007). New, endemic or re-appearing plant diseases continue to pose a hazard to the development of plant development and health around the world (Miller et al., 2009). Agricultural approaches that are both sound and environmentally benign are becoming increasingly popular. Plant biotechnology has aided in the development of novel crop varieties that are disease resistant, drought and salt tolerant, and nutritionally valuable. Plant-associated microorganisms are usually disregarded in breeding procedures, despite the fact that they conduct critical ecosystem functions for plants and soils. Throughout the last 100 years, however, research has repeatedly proved that microorganisms have an

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_15

intimate relationship with their host plant and are capable of both stimulating plant development and suppressing plant illnesses.

The plant microbiome, which includes the rhizosphere, endosphere, leaf surfaces, and other tissue compartments, can house various bacteria. Plant-associated microbes have been shown to be beneficial in terms of plant health by altering plant physiology, growth, and environmental adaptability/tolerance (Grover et al., 2011; Mendes et al., 2011). Diversified bacterial communities can be found on the surface of seeds, roots, leaves, fruits, as well as vascular tissue, stems, and the intercellular spaces within the plant tissues. All of these bacterial groupings share a number of characteristics that are essential for the host plant's growth promotion.

2 The Phyllosphere

The phyllosphere relates to the above-ground surface of plants as a habitat for microbes. This category includes leaves, stems, blossoms, and fruits. Leaves are the most prevalent tissue for microbial colonization among them. The phyllosphere bacteria interact with the host plant and have the ability to affect the physiology of the host plants.

3 The Spermosphere

The spermosphere is the region surrounding germinating seeds in the soil. It ranges from 1 to 12 mm wide at the soil surface (Schiltz et al., 2015). A multitude of complicated interactions occurs within the spermosphere involving the germinating seed, the soil, and microbes. Many chemicals exuding from seeds impact the microbial populations that live there, either by inhibiting or stimulating their growth (Schiltz et al., 2015).

4 The Rhizosphere

The rhizosphere is the small zone of soil surrounded and impacted by plant roots. It can range in width from 2 to 80 mm from the root surface depending on the plant species. It is the most dynamic and has a substantial impact on the development and the nutritional state of plants (Jones & Hinsinger, 2008; Hinsinger et al., 2009). The presence of root exudates and microbial breakdown products (metabolites), which maintain a diversified and densely populated bacterial population, causes chemical changes in the rhizospheric soil.

Additionally, endophytic sites and vascular tissue are the internal regions of the plant, i.e., epidermis, xylem, and phloem that offer a unique habitat for numerous bacterial communities that have a significant impact on the development of the plants.

Bacteria communities that colonize different regions of plants are divided into epiphytic bacteria which live on the surface, endophytic bacteria which live within the plant tissues, phyllospheric bacteria which nurture on leaf surfaces, and rhizospheric which dwell near the soil. Epiphytic bacteria play a significant role in their host plant's water economy. Beneficial endophytes influence the growth of plants through different mechanisms including hormone synthesis, improved nutrient assimilation, and protection from abiotic/biotic stresses. Numerous studies have reported that endophytic bacteria help in the development of plants such as wheat, rice, canola, potato, tomato, and a variety of other plants (Misko & Germida, 2002; Marquez-Santacruz et al., 2010; Sturz & Nowak, 2000; Mei & Flinn, 2010). For instance, the beneficial endophyte *Paraburkholderia phytofirmans* PsJN, which was isolated from surface-sterilized onion roots and classified as *Pseudomonas* before being reclassified as *Burkholderia* (Sessitsch et al., 2005; Sawana et al., 2014) was able to promote tomato plant growth (Pillay & Nowak, 1997) and upregulated genes involved in signal transduction, protein metabolism, defense pathways, transcription, transport, and hormones metabolism (Galambos et al., 2020). Phyllospheric bacteria provide nutrients the ability to tolerate environmental stress to the host plants. Beneficial rhizospheric bacteria protect plants against pathogens while also aiding in nitrogen fixation. Bacterial communities are associated with plant roots because of the availability of resources such as amino acids, sugars, organic acids, and other small molecules from the plant exudates which can account for up to a third of the carbon fixation of the plants (Whipps, 1990; Bais et al., 2006; Badri et al., 2009; Badri & Vivanco, 2009). Overall, plant-bacterial interactions influence ecosystem functioning in natural ecosystems and agricultural systems through carbon sequestration and nutrient cycling.

Plant Growth-Promoting Bacteria (PGPB)

Soil microorganism can be classified into bacteria, fungi, actinomycetes, protozoa, algae, and nematodes. Majorly, bacterial communities are predominating among the other life forms, accounting for 95% of the total microorganism (10^8 to 10^9 cells/gram of soil). However, the reduced number of bacterial population plummets approx. 10^4 cells/gram is reported under the stressed soil condition (Schoenborn et al., 2004; Timmusk et al., 2011).

Various factors influence the number and type of bacterial load in different soils viz. temperature, moisture content, availability of salt and chemicals, as well as the quantity and varieties of different flora found in such soils (Glick et al., 1999a, 1999b). The interaction of bacteria with plants can be helpful, harmful, or neutral (Lynch, 1990).

Free-living bacteria that are actively involved in specific symbiotic relationships with plants (e.g., *Rhizobia* sp. and *Frankia* sp.), provide positive influence on promoting plant growth. PGPB can influence plant growth either directly by employing resources acquisition or indirectly by reducing the inhibitory effects of some pathogens on plant growth and development, i.e., via biological control of plant pathogenic bacteria or fungi (Glick, 1995).

Historically, *Rhizobia* spp. were extensively studied on the physiological, biochemical, and molecular biological aspects (Dixon & Wheeler, 1986; Fischer, 1994; Long et al., 1982). A vast range of mechanisms has been studied in order to better understand and acknowledge the processes utilized by PCPB (Glick et al., 1999a, 1999b; Glick, 1995; Kloepper et al., 1989). The effect of bacteria on plants may alter because of the variation in the environmental conditions or availability of certain chemicals. The IAA overproducing strain *Pseudomonas fluorescens* BSP53a was capable of stimulating the root development in blackcurrant cuttings while suppressing root development in cherry cuttings (Dubeikovsky et al., 1993).

The following are the mechanism of action:

1. *Direct Mechanism*

PGPB employs a variety of pathways to enhance plant growth and development in a variety of environmental conditions. Biofertilization, phytohormone production, root stimulation, rhizo-remediation, plant stress control, and effective absorption of particular nutrients from the environment are examples of direct mechanisms. Cumulatively, the agricultural soils lack a sufficient number of compounds that led to sub-optimal plant growth. To mitigate these problems, and to attain sufficient plant yields, farmers have extensively utilized chemicals which are the source of nitrogen and phosphorus. Thus, this makes the entire process expensive and poses human health and environmental hazards. Table 1 summarizes the details of the direct mechanism.

Hormones influence a plant's response to the environment, as well as its growth and development (Davies, 2004). During stress or development-limiting environmental conditions, plants strive to modulate their phytohormones to overcome the generated stress (Salamone et al., 2005). A variety of phytohormones promote plant development (Fig. 1).

Table 1 Several direct mechanisms and their role in plant growth development

Types of direct mechanism	Microorganisms	Role	References
Nitrogen fixation	*Azospirillum* sp., *Rhizobia* sp.	Nitrogenase(*nif*) essential genes and required for nitrogen fixation	Bashan and Levanony (1990), James and Olivares (1997)
Solubilization of phosphate	Mycohrrizae	Phosphorus solubilization and mineralization	Richardson (2001), Rodríguez and Fraga (1999)
Sequestering iron	*Pseudomonas* sp.	Low-molecular mass siderophores (approx. 400–1500 Da) and membrane Bacterial species produced receptors that aid in the update of iron	Hider and Kong (2010), Neilands (1981)

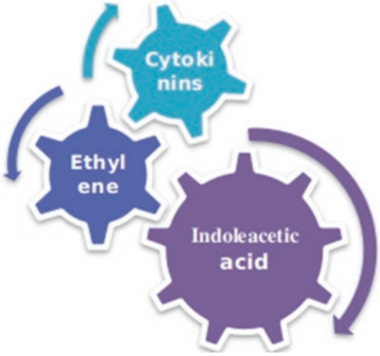

Fig. 1 Essential/several hormones produced by plants

Several strains of *Axontobacter* spp., *Rhizobium* spp., *Pantoea agglomerans, Rhidospirillum rubrum*, *Pseudomonas fluorescens, Bacillus subtilis*, and *Paenibacillus polymyxa* have been reported to produce cytokinins in cell-free medium (Joo et al., 2005; Kang et al., 2009). Indoleacetic acid (IAA) helps in division, expansion, and differentiation of plant cells; triggers the germination of seed and tuber; enhances the rate of xylem and root development; maintains the vegetative growth process; initiates lateral and adventitious root formation; and mediates light, gravity and fluorescence responses. It has an effect/impact on several essential processes, i.e., photosynthesis, pigment production, biosynthesis of different metabolites, and stress tolerance/resistance (Spaepen & Vanderleyden, 2011; Tsavkelova et al., 2006).

Nutrient Uptake

Nitrogen

Nitrogen is a macronutrient that has a fundamental role in plant growth, development, and energy metabolism. Even though the atmospheric N_2 content is 78%, developing plants cannot utilize it. Biological N_2 fixation (BNF) transforms atmospheric N_2 into a form that plant can use by nitrogen-fixing bacteria converting N_2 to NH_3 via a complex enzymatic process known as nitrogenase (Kim & Rees, 1994; Gaby & Buckley, 2012, Rubio & Ludden, 2008, Ahemad & Kibret, 2014 Gómez-Godínez et al., 2019).

Rhizobacteria that promote/enhance plant growth use symbiotic and non-symbiotic ways to fix atmospheric N_2 and make it available to plants. Symbiotic nitrogen-fixing is a mutualistic association between a microbe and a plant. The microorganism penetrated the root and subsequently develops nitrogen-fixing. Plant growth-promoting rhizobacteria, i.e., *Sinorhizobium, Mesorhizobium, Rhizobium*, and *Bradyrhizobium* are commonly found as symbionts in leguminous plants, whereas in non-leguminous shrubs and trees *Frankia* is found (Zahran, 2001).

On the other hand, non-symbiotic nitrogen-fixing bacteria offer a small percentage of the fixed nitrogen required by the bacterially associated host plants. Nitrogen-fixing rhizobial bacteria (a-proteobacteria) family invade and create a symbiotic complex with the roots of leguminous plants. The creation of nodules, in which rhizobia colonize as internal symbionts, is the consequence of a complicated interplay/invade between the host and the symbiont (Giordano & Hirsch, 2004). Diazotrophs are rhizobacteria that promote plant growth by fixing nitrogen in nonleguminous plants and can forma non-obligate association with their hosts (Glick et al., 1999a, 1999b). The nitrogenase complex, a complex enzyme (Kim & Rees, 1994), is responsible for N_2 fixation. Nitrogenase is a metalloenzyme with two components composed of dinitrogenase reductase and dinitrogenase, an iron protein. Dinitrogenase reductase produces a high reducing power electron, which dinitrogenase uses to reduce N_2 to NH_3. Three distinct N-fixing mechanisms have been identified based on the metal cofactors Mo-nitrogenase, Fe-nitrogenase, and V-notrogenase. The major part of biological fixation is completed by molybdenum nitrogenase, which is available and easily accessible in all diazotrophs (Bishop & Joerger, 1990). Nitrogen-fixing genes known as nif genes are reported in both symbiotic and free-living systems (Kim & Rees, 1994). For the formation, creation, and operation of nitrogenase (nif) structural genes, genes involved in Fe, protein activation, iron-molybdenum cofactor biosynthesis, electron donating genes, and regulatory genes all are essential. Nif genes are usually found in a 20–24 kb cluster in diazotrophs, with seven operons synthesizing 20 distinct proteins (Glick, 2012). The molybdenum nitrogenase enzyme complex is composed of two proteins encoded by the nif DK and nifH genes. The NifDK component is a heterotetrameric (a2b2) protein composed of two ab dimers with a 2-fold symmetric connection. One iron-molybdenum cofactor is found inside the active core of each a-subunit (NifD) of NifDK (FeMo-co) (Rubio & Ludden, 2008).

Fix genes, which are found in both free living and symbiotic nitrogen fixation system, governs the symbiotic interaction of nif genes in Rhizobium by requiring low-oxygen condition. (Kim & Rees, 1994). Because nitrogen fixation is an energy-intensive process, microorganisms that fix nitrogen necessitate at least 16 mol of ATP for each mole of lower nitrogen, bacterial carbon resources would be better spent on oxidative phosphorylation, which generates ATP, rather than gluconeogenesis, which generates energy storage capacity of glycogen (Glick, 2012). Treatment of legume plants with rhizobia with a deleted gene for gluconeogenesis resulted in a considerable rise in both the number of nodules and dry weight of plant when compared to the wild-type strain (Zorreguieta et al., 2001).

Phosphate Solubilization

Phosphorus (P), after nitrogen, the second most important macronutrient for plant growth, is abundant in both the forms, i.e., inorganic and organic in soils. Plants have a limited number of possible forms, despite having a vast P reservoir. The majority of soil phosphorus in soil is insoluble, and plants may only acquire it in two soluble forms, dibasic (HPO_2^{-4}) and monobasic ($H_2PO_4^{\ -}$) ions (Bhattacharyya & Jha, 2012; Alaylar et al., 2020). Insoluble P can be observed in both inorganic minerals, such as apatite, and organic forms, like phosphomonoesters, inositol phosphate (soil phytate), and phosphotriesters. To address soil P deficiency, phosphatic fertilizers are commonly applied to agricultural areas. According to Mckenzie and Roberts (1990), plants that can take less phosphatic fertilizers are immediately converted into insoluble complexes in the soil. However, using phosphate fertilizers on a regular basis is quite expensive and non-eco-friendly. As a result, researchers are seeking for an eco-friendly and cost-effective strategy to boost crop yield in low-phosphorus soils.

Phosphate solubilizing microorganisms (PSM) can provide an accessible form of phosphorus to plants, making them a feasible alternative to available chemical phosphatic fertilizers (Khan et al., 2007). Among the several PSM(s) populating the rhizosphere, phosphate-solubilizing bacteria (PSB) are considered potential biofertilizers because they may feed plants with P from sources that are otherwise inaccessible through in a variety of ways (Zaidi et al., 2009). The most important phosphate solubilizing bacteria have been identified as *Microbacterium, Azotobacter, Enterobacter, Rhizobium, Flavobacterium, Microbacterium, Bacillus, Burkholderia, Pseudomonas, Beijerinckia, Sinorhizobium* sp. *RC02, Acinetobacter* sp. *RC04,* and *Serratia* (Bhattacharyya & Jha, 2012; Zhang et al., 2018).

The solubilization of inorganic phosphorus is carried out by the action of low molecular weight organic acids like gluconic and citric acid, produced by a variety of soil bacteria (Zaidi et al., 2009). On the other hand, organic phosphorus is mineralized through the formation of phosphatases that catalyze the hydrolysis of phosphoric esters (Glick, 2012).

Phosphate solubilizing bacteria not only fulfill the requirement of P to plants, but also aid in their growth by increasing the supply of BNF production and availability of other trace minerals. (Suman et al., 2001; Ahmad et al., 2008; Zaidi et al., 2009). Plant growth-promoting rhizobacteria *Pseudomonas auricularis* (HN038) and *Bacillus aryabhattai* (JX285) increase growth, photosynthetic, nutrient uptake, and the production of tea oil (Wu et al., 2019).

Siderophore

Iron (Fe) is an essential component for practically all living things. Except *Lactobacilli* sp., all known bacteria fundamentally require iron (Neilands, 1995) to survive. In aerobic environments, where it is possible to occur insoluble oxyhydroxides and hydroxides can form, iron is mostly found as Fe^{3+}, rendering it reachable to both plants and microbes (Rajkumar et al., 2010). Bacteria obtain iron mostly by the secretion of siderophores, which are low-molecular-mass iron chelating agents. The majority of the siderophores are hydrophilic and can be differentiated into intracellular or extracellular. Rhizobacteria differ regarding the ability to use siderophores supplied by other rhizobacteria of different genera (homologous siderophores), while others can use siderophores generated by other rhizobacteria of other genera (paralogous siderophores) (heterologous siderophores).

In Gram-positive and Gram-negative rhizobacteria, iron (Fe^{3+}) in the Fe^{3+}-siderophore complexes on the bacterial cell membrane is transformed to Fe^{2+}, which is subsequently taken up by the cell from the siderophore via a gated mechanism connecting the inner and outer membranes. During the reduction step, siderophore may be destroyed or recycled (Rajkumar et al., 2010; Neilands, 1995). When there is a lack and shortage of iron, siderophores act as solubilizers for iron from minerals or organic molecules (Indiragandhi et al., 2008).

Plants usually absorb iron from bacteria acquiring different strategies, including chelation and release, direct uptake of siderophore-Fe complexes, and ligand exchange reactions (Schmidt, 1999). Crowley and Kraemer (2007) uncovered a siderophore-mediated iron transport system in oat plants and concluded that rhizosphere bacteria supply iron to oat, which has mechanisms for utilizing Fe-siderophore complexes under iron-limited situations. *Bacillus*, *Azotobacter, Azadirachta, Burkholderia, Rhizobium, Aeromonas*, *Streptomyces* sp., *Pseudomonas, Serratia,* and other plant growth-promoting rhizobacteria have been demonstrated to take up radiolabeled ferric siderophores as a sole source of iron. Similarly, *Arabidopsis thaliana* plants absorbed the Fe-pyoverdine complex produced by *Pseudomonas fluorescens* C7, due to the increase in iron in plant tissues and increased plant growth (Vansuyt et al., 2007). In *Zea mays,* the effect of the siderophore-producing *Pseudomonas* strain GRP3 was studied. After 45 days, chlorotic symptoms diminished, and iron, chlorophyll a, and chlorophyll b levels rose in strain GRP3 infected plants, compared to control plants (Sharma & Johri, 2003).

Potassium

Potassium (K) is the highly important third most macronutrient for plant growth. Soluble potassium concentrations in soil are typically low, and more than 90% of potassium in the soil is in the form of insoluble rocks and silicate minerals. As a result of unbalanced fertilizer application, potassium deficiency is becoming one of the most significant constraints to crop productivity. Plants with low potassium levels will have underdeveloped roots, continue growing, generate small seeds, and produce inferior yields. This emphasized the importance of finding an alternative native source of potassium for plant root uptake and maintaining potassium levels in soils for agricultural output sustainability.

Plant growth-promoting rhizobacteria are capable of dissolving potassium rock through the production and release of organic acids. *Paenibacillus* sp., *Burkholderia, Acidothiobacillus ferrooxidans, B. edaphicus, Pseudomonas,* and *B. mucilaginosus* have all been found to create potassium in a viable form from potassium-containing minerals in soils. As a result, adopting potassium-solubilizing plant growth-promoting rhizobacteria as a biofertilizer for agriculture development can serve to minimize the usage of agrochemicals while also encouraging sustainable crop production (Kang et al., 2017).

Phytohormone

A variety of microorganisms live in the rhizosphere, and they can produce compounds that govern plant growth and development. Plant growth-promoting rhizobacteria produce auxins, gibberellins, cytokinins, and ethylene which can impact cell proliferation in the root architecture by producing an excessive production of lateral roots and root hairs, resulting in an increase in nutrition and water intake.

IAA Production

Indole acetic acid (IAA) is the most common natural auxin present in plants and has a beneficial effect on root growth. Up to 80% of rhizobacteria colonized the seed or root surfaces can produce indole acetic acid (IAA), which is thought to work in concert with endogenous IAA in plants to increase cell growth and improve the host's absorption of minerals and nutrients from the soil. IAA stimulated plant cell elongation, division and differentiation; root development and increases xylem content, adventitious and lateral root formation, influences photosynthesis, induces seed and tuber germination; controls vegetative growth processes; regulates responses to gravity, light, and florescence; formation of both shoot and root apical meristems (Kepinski, 2006; Casimiro et al., 2001; Sachs, 2005). In bacteria, tryptophan, an amino acid typically found in root exudates, has been identified as the primary precursor molecule for IAA production (Zhao, 2010). In bacteria like *Rhizobium, Klebsiella, Bradyrhizobium, Pseudomonas, Agrobacterium*, and *Enterobacterium,* the synthesis of indole acetic acid involves the generation of indole-3-pyruvic acid and indole-3-acetic aldehyde, chemically synthesized hormones are considered less effective as they have a poor tolerance between suppressive and stimulatory levels, but microbial hormones have a higher tolerance due to their continuous slow release.

Ethylene

Ethylene is a key phytohormone that affects plant growth and development in a variety of ways, that includes lateral bud development, root initiation and elongation, promoting fruit ripening, anthocyanin synthesis, promoting lower drooping, enhanced seed germination, promoting leaf abscission, and the synthesis of volatile compounds responsible for aroma in fruits are all enhanced by ethylene. High ethylene concentrations cause defoliation and other cellular functions, which may result in decreased crop productivity (Bleecker & Kende, 2000). The 1-aminocyclopropane-1 carboxylic acid (ACC), which is a direct precursor of ethylene, is catalyzed by ACC oxidase.

ACC deaminase is an enzyme that catalysis the hydrolytic cleavage of ACC thus inhibiting ethylene production. *Pseudomonas* sp. that consists of ACC deaminase along with *R. leguminosarum* was found to enhance fresh biomass, straw yield, grain yield, nodule dry weight, nodule number, and nutrient uptake in lentil grains as a result of lowering ethylene production (Kaneko et al., 2002; Ma et al., 2003). *Burkholderia*, *Rhizobium*, *Pseudomonas*, *Agrobacterium*, *Ralstonia*, *Azospirillum*, *Acinetobacte*r, *Serratia*, *Achromobacter, Alcaligenes, Bacillus, Burkholderia,* and *Enterobacter* are among others that have ACC deaminase-producing bacteria (Table 2).

2. *Indirect Mechanism*

   The use of biocontrol bacteria that indirectly boost plant development has piqued curiosity since it uses bacteria instead of chemical pesticides. Induced systemic resistance (ISR), antifungal and antibacterial production by PGPB are examples of indirect methods which are effective in plant protection (Kloepper & Schroth, 1981; Egamberdieva & Lugtenberg, 2014). The following is a list of compounds and hormones that underlie this category (Compant et al., 2005).

   2.1 *Production of compounds with antibiotic and lytic activity:* Beneficial bacterial such as PGPB produce antibacterial and numerous other metabolites

Table 2 Different types of rhizobacteria and their functions

Rhizobacteria	Crop	Function	References
Sphingomonas	Tomato	Gibberellin synthesis	Khan et al. (2014)
Chryseobacterium	Tomato	Siderophore production (Increase soil microbial biomass)	Radzki et al. (2013)
Azotobacter	Wheat, tobacco, maize, coffee	Nitrogen fixation	Wani et al. (2013)
Phyllobacterium	Strawberry	Potassium and phosphate	Flores-Félix et al. (2018)
Pseudomonas	Mung bean	ACC deaminase synthesis	Ahmad et al. (2013)
Bacillus sp. JC03, *E. coli* DH5α	*A .thaliana*	Strigolactones production	Jiang et al. (2019)
C. zhacaiensis, B. amyloliquefaciens	Tomato	Cytokinin production	Selvakumar et al. (2018)
Stenotrophomonas maltophilia	Wheat	Nitrogenase activity, P-solubilization, IAA, ACC deaminase	Verma et al. (2014a, b)
Paenibacillus mucilaginosus	Soybean	Potassium and phosphate solubilization	Ma et al. (2018)
Bradyrhizobium diazoefficiens USDA110	Soybean	Nitrogen fixation	Sibponkrung et al. (2020)
Rahnella aquatilis (PGP30), *Pseudomonas brassicacearum* (PGP291), *Rhizobium* sp. (RhOF57A)	Faba bean	Phosphate, potassium solubilization, nitrogen fixation, EPS production	Bechtaoui et al. (2020)
Sinorhizobium sp. RC02, *Acinetobacter* sp. RC04	Safflower	Phosphorous solubilization, promote seed germination	Zhang et al. (2018)
Bacillus aryabhattai (JX285) and *Pseudomonas auricularis* (HN038)	*Camellia oleifera* Abel.	Solubilization of phosphate increases growth, photosynthesis, yield, and increases tea oil	Wu et al. (2019)
Azospirillum	Maize	Nitrogen fixation	Gómez-Godínez et al. (2019)

that play a crucial role in the protection of plant from the plant pathogen especially fungus (Haas & Keel, 2003; Mazurier et al., 2009). Also, some of the enzymes secreted by the biocontrol bacteria have the ability to lyse the cell walls of *Fusarium oxysporum, Phytophthora* spp., *Rhizoctonia solani*, and *Pythium ultimum,* all of which are considered as pathogenic fungi (Frankowski et al., 2001; Kim et al., 2008).

2.2 *Siderophores:* Some bacterial species, on the other hand can act as biocontrol agents through the development of siderophores. Siderophores from PGPB can block phytopathogens from acquiring iron, restricting their growth (Siebner-Freibach et al., 2003).

2.3 *Plant-induced systemic resistance:* Plant growth-promoting bacteria can activate the resistance in plants by a process known as induced systemic resistance (ISR), in which plants' defense system is activated against infection caused by the pathogen. The ISR-positive plants are also known as "primed" due to their tendency to react and respond quickly and strongly against the pathogenic attack (Pieterse et al., 2009).

3. *Modulating the Stress Impacts of Environmental Conditions*

Ideally, a major part of the plant growth and development would be thought to be linearly decreasing over the period of time (Glick et al., 2007). However, in the natural environment, a large number of biotic and abiotic stresses can stifle the growth of the plant. Among them are extreme temperature, intensity of light, flood, drought, toxic metals and organic pollutants, radiation, injury, insect attack, nematode infection, high salinity, metal and metalloids, hypoxia, and various pathogens such as disease-causing viruses, bacteria, and fungi (Mayak et al., 2004).

Many environmental stresses such as phytopathogenic infection may lead to the production of the inhibitory stress hormone ethylene (Glick, 2004). Studies have shown that many ACC deaminase-producing PGPB have the ability to protect the plants from abiotic stresses (Reed & Glick, 2005).

In addition, it has been reported that PGPB may help plants in mitigating abiotic stresses by synthesizing indoleacetic acid (IAA) that facilitates the growth and development of the plant in the presence of growth-inhibiting compounds (Wani et al., 2008).

According to one study, the IAA and ACC deaminase mechanisms work synergistically to enhance plant growth (Gamalero & Glick, 2010; Salamone et al., 2005). Plants roots exudate consist of an amino acid known as tryptophan. PGPB converts the tryptophan present in the soil to IAA. The IAA produced by bacteria is released and absorbed by the plant cells which results in the activation of the auxin signaling pathway, which is comprised of several auxin-responsive factors and the plants' IAA pool (Fig. 2). IAA absorption leads to cell growth and proliferation of the plant. Simultaneously, few IAAs activate the transcription machinery that leads to the transcription of the gene encoding the enzyme ACC synthase. Production of ACC synthase increases the levels of ACC and ultimately ethylene (ACC is the precursor of ethylene which is catalyzed by the enzyme ACC oxidase into ethylene).

In addition to IAA, cytokinins (compounds with an adenine-like structure) can stimulate cytokinesis, or cell division. Cytokinins are produced by several yeast strains, and by a number of soil bacteria, including PGPB (Salamone et al., 2001). Transgenic plants that are developed to overproduce the cytokinins during abiotic stress have been shown to effectively tolerate the negative impacts of environmental challenges (Stearns et al., 2012).

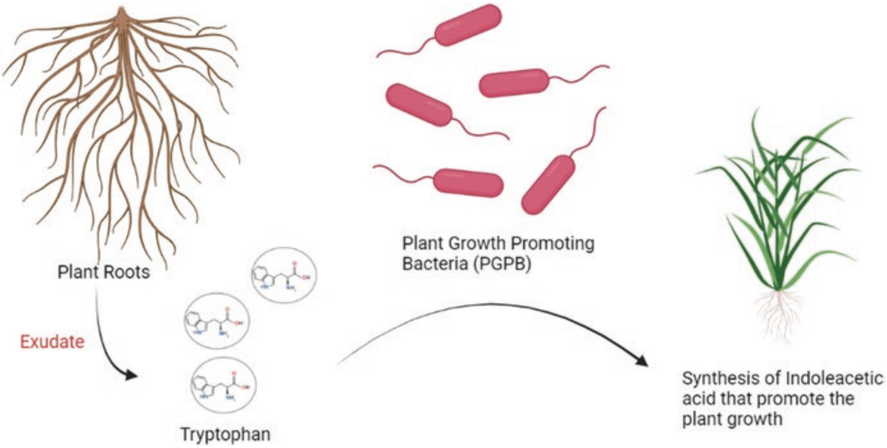

Fig. 2 Representation of PGPB role in converting the tryptophan into IAA for plant proliferation

Another compound, Trehalose, a non-reducing disaccharide, α, α-1,1-glucoside, comprises two molecules glucose and fructose that are extensively found in nature. It is found in bacteria, yeast, fungi, plants, insects, and invertebrates. Trehalose can provide plants protection against drought, excessive salt, and harsh temperatures. This compound is a highly stable metabolite that is resistant to acids and high temperatures and when cells dry up, it can form a gel phase, which replaces water and reduces drought and salt damage (Rivero et al., 2007).

Challenges
Despite the fact that bacteria are being utilized effectively in many developing countries for crop protection and production, there are still certain limitations/challenges that exist in the terms of the widespread adoption of the plant growth-promoting bacteria. For example, a number of unique methods have to be developed for bacteria growth, storage, shipping, formulation, and application while moving the studies done in laboratory and greenhouses to field trials and large-scale commercial fields. Secondly, the general public must be made aware of the widespread use of these beneficial bacteria in agricultural fields. Before the general public accepts the widespread discharge of growth-promoting bacteria into the environment, the myth must be dispelled that limits the microorganisms to their role as pathogens.

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The Science of Microbial Enzymes as Detoxification Tool for Inorganic and Organic Pollutants

Indu Bhardwaj, Richa Verma, Vijay Kumar, and Nidhi Bhardwaj

1 Introduction

Existence of life on the globe is directly connected to the overall status of the surrounding atmosphere. But due to advances in technology, industry, and science, a huge quantity of waste is drained off into the ecosystem, which in turn causes dangerous effects on mankind and ultimately onto the ecosystem of earth (Karigar & Rao, 2011). The significant use of chemicals in industries and military services, inefficient waste management, and inadvertent leaks all contribute to the contamination of land, water, and air (Eibes et al., 2015). Environmental pollution is continuously rising due to human activities like technological progress, urbanization, dangerous farming, and fast industrialization, all that harm the environment (Nematian & Kazemeini, 2013; Peinado et al., 2010). Pollutants such as azo dyes, phenols, polyaromatic hydrocarbons, insecticides, polychlorinated chemicals, and bulky metals are generated as a result of these processes (Elekwachi et al., 2014). Toxic heavy metals that are harmful to plants, like arsenic (As), cadmium (Cd), mercury (Hg), zinc (Zn), chromium (Cr), uranium (Ur), selenium (Se), silver (Ag), nickel (Ni), and lead (Pb) can inhibit plant development (Nematian & Kazemeini, 2013; Peinado et al., 2010) and cause cancer in human beings (Dixit et al., 2015). Heavy metal toxicity agitates the composition of reactive oxygen species (ROS), which reduces the antioxidant systems (superoxide dismutase, glutathione, and so on) that preserve cells. If this condition remains, the normal functioning of

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_16

organisms will be harmed, which will very ultimately result in cell death (Ojuederie & Babalola, 2017).

The mechanisms of bioremediation at which microbes act on toxic metals comprise biosorption (physicochemical metal sorption by the cell), bioleaching (heavy metal mobilization via organic acid excretion or methylation reactions), intracellular accumulation biomineralization), and enzyme-catalyzed transformation (redox reactions) (Lloyd & Lovley, 2001). Figure 1 summarizes the most important microbial activities that impact metal bioremediation.

As a result, these pollutants endanger human being and other existing organisms. In the past years, garbage was traditionally discarded by digging a pit and stowing it with worthless stuff. This method of waste disposal was tough to maintain due to the absence of a new location each time you dumped. Modern waste disposal techniques such as incineration at high temperature and chemical disruptions such as base-catalyzed dichlorination and UV oxidation have emerged. Though they may be quite successful in decreasing a broad range of pollutants, they also have a number of downsides. These approaches are difficult to understand, expensive, and unpopular with the public (Karigar & Rao, 2011). To overcome these challenges, several chemical and physical procedures for purifying pollutants, namely pollutant adsorption, oxidizing agents, electrochemical remedies, ion interchange, and membrane filtering have been utilized; numerous approaches, such as high-temperature burning and feces oxidation, have also been used (Shome, 2020). However, they did not receive much attention because of their limitations, which included high costs, non-specificity, and the possibility of secondary pollution creation; hence, eco-friendly, and biological procedures, known as bioremediation, were developed (Singh et al., 2008). This is when bioremediation comes into play (Perelo, 2010; Gao et al., 2018).

Bioremediation is the transformation or breakdown of pollutants by microorganisms into less hazardous or non-dangerous compounds (Karigar & Rao, 2011). The

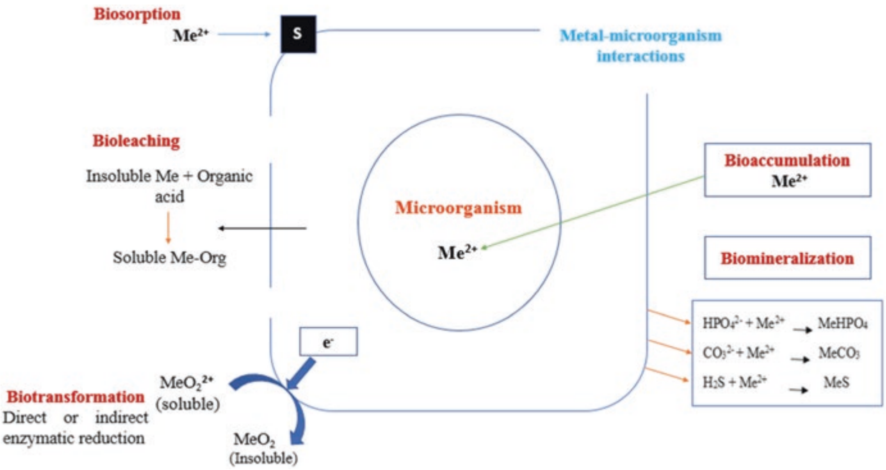


Fig. 1 Microbial activities that impact metal bioremediation

main aim of bioremediation is to encourage them to function by giving optimal quantities of nutrients and other chemicals required for their metabolism in order to detoxify pollutants that are toxic to the environment and life forms. The word "biodegradation" is frequently used in the area of waste supervision, ecology, and atmospheric remediation [bioremediation] (Joutey et al., 2013). The bioremediation method is mostly dependent on microorganisms that attack toxic compounds enzymatically and transform them to harmless compounds (Vidali, 2001; Dana & Bauder, 2011). For pollutants bioremediation, various enzymes such as monoxygenases, dioxygenases, peroxidases, halogenases, transferases phosphotriesterases, oxidoreductases, and hydrolases, from plants, bacteria, fungus, and algae have been utilized (Rao et al., 2010; Pieper et al., 2004). The term phytoremediation is used for the plants in the bioremediation of pollutants (Leung, 2004). The material is transformed by microbial organisms through metabolic or enzymatic mechanisms. It works on the basis of two processes: growth and co-metabolism. During growth, an organic pollutant serves as the only source of carbon and energy. This technique results in complete degrades (mineralizes) of organic pollutants. The metastasis of biotic substance in the existence of a growth substrate that serves as the chief source of carbon and energy is referred to as co-metabolism (Fritsche & Hofrichter, 2001). Various microbes, including bacteria, fungi, and yeasts, are participating in the degeneration process (Joutey et al., 2013). Only a few fungal and bacterial species have been shown to be effective pollutant degraders. Numerous strains have been shown to be successful as bioremediation delegates in lab situations. Microbial growth is influenced by temperature range, pH, oxygen, morphology of soil, moisture content, and an optimum quantity of nutrients, low bioavailability of pollutants, and the existence of another harmful substance. The majority of bioremediation systems work in aerobic climates, but in anaerobic conditions may allow the microbial breakdown of resistant compounds (Vidali, 2001; Dixit et al., 2015). In order to remediate the malignant, organo-pollutants and lignin both bacteria and fungi depend on the involvement of diverse intracellular and extracellular enzymes (Vidali, 2001). Although bioremediation may be well-turned when atmospheric conditions assent for microbial growth and activity, its application frequently involves in the changing atmospheric parameters to permit for quicker bacterium growth and degeneration (Karigar & Rao, 2011).

This chapter will attempt to develop an in-depth understanding of the biodegradation process by covering all sorts of microbial enzymes involved in the breakdown of toxic pollutants. Furthermore, we are also conscious of the word "biodegradation" that is frequently employed in the area of biomedicine, ecology, waste management, and the natural environment, and is presently associated with ecologically friendly items.

2 Bioremediation

Bioremediation is described as a procedure and items that are "cost-effective and practical to minimizing pollutants at the source and reducing threats to the environment and human health" (Alkorta et al., 2017). It involves the removal of contaminated water from groundwater, soils, air, surface water, and sediments (Shome, 2020). The procedure of bioremediation may be divided into three different stages. The first stage is to decrease pollutants with the help of local microorganisms via natural attenuation that did not requisite human activity. Second is biostimulation that is used to boost the systems' influence and increase the speed of biodegradation by injecting nutrients and oxygen into them. Lastly, microbes are introduced to the systems during bioaugmentation. These supplementary microbes should be much effective at degenerating the destinating contaminant than native flora (Diez, 2010). Bioremediation has the ability to convert extremely toxic chemicals into non-hazardous materials. Its primary methods of degenerating and detoxifying chemical contaminants are intracellular accumulation and enzymatic alteration (Singh et al., 2008).

Enzymes are the most effective bioremediation agents because they accelerate all chemical reactions on pollutant. Enzymes can be utilized in bioremediation in two ways: as an isolated enzyme that is introduced to the polluted region, or as a complete cell, such as fungus, bacteria, or algae (Theerachat et al., 2012; Festa et al., 2008). Bacteria are widely spread in the biosphere owing to their metabolic ability to grow under a broad range of environmental circumstances and produce enzymes. The major typical enzymes from microbes and genetically modified microbes amenable for the breakdown of many kinds of contaminants utilized in bioremediation methods comprise hydrolases, laccases, cytochrome P450, dehalogenases, proteases, lipases, and dehydrogenases (Sharma, 2012). The capacity of microbes to break down pollutants is dependent on the adequacy of atmospheric factors for their development and metabolism, which includes pH, temperature, and moisture.

Different enzymes isolated from aerobic bacteria like *Alcaligenes*, *Sphingomonas, Mycobacterium, Pseudomonas*, and *Rhodococcus* are often destroyed by pesticides and hydrocarbons. On the other hand, enzymes isolated from anaerobic bacteria, have been employed in the bioremediation of chloroform, polychlorinated biphenyls (PCBs), and the dichlorination of trichloroethylene [TCE] (Bhandari et al., 2021). The technique of bioremediation is a low-cost approach compared to other ways since it does not require a large number of resources. Microbes and plants are the most important participants in the entire process. The process of bioremediation is shown in Fig. 2.

Bioremediation can be done in-situ and ex-situ. In situ bioremediation is an on-site cleaning method for polluted atmosphere that provides contaminated soils with nutrients to boost microbes' capacity to break down toxins, as well as adding novel microbes to the atmosphere or improving existing microorganisms' ability to

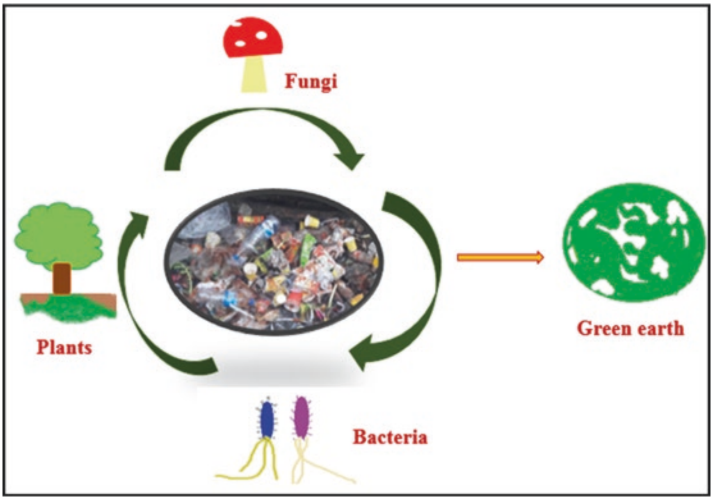


Fig. 2 Process of bioremediation

consume exclusive contaminants via genetic engineering (Mani & Kumar, 2014; Lu et al., 2014). The unavailability of sufficient nutrient in the contaminated area affects the utilization of indigenous microbes in the atmosphere for in situ bioremediation (Smith et al., 2015; Azubuike et al., 2016).

Ex situ bioremediation entails transporting contaminates from their actual place to a new area for remedy, which is determined by the amount of pollution, the kind of pollutant, the depth of contamination, and the cost of remedy, the geographical situation, and the geology of the polluted area (Ojuederie & Babalola, 2017). The efficiency of bioremediation is demarcated by a quantity of parameters, which comprises the type of microbes used, the prevailing environmental conditions at the polluted site, and the level of pollutants present (Mousavi et al., 2021).

Over the last few decades, very dangerous chemical substances have developed and liberated into the environment directly or indirectly over a lengthy period of time. These substances include fuels, polycyclic aromatic hydrocarbons, pesticides, polychlorinated biphenyls, and dyes (Diez, 2010). Other manufactured chemicals, such as radionuclides and metals, are much more resistant to biodegradation by indigenous plants than naturally occurring organic molecules, which disintegrate rapidly when introduced into the environment (Joutey et al., 2013). This is where bioremediation plays a vital part in the degradation of contaminants in the atmosphere. Figure 3 shows the different causative agents of environmental contamination.

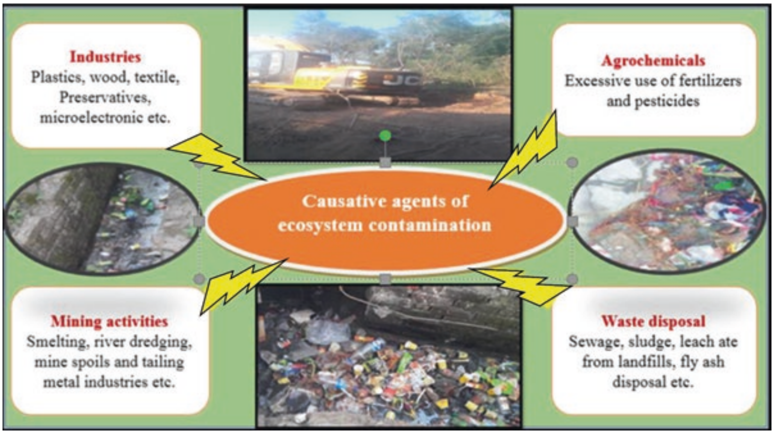


Fig. 3 Various causative agents of environmental contamination

3 Microbial Enzymes That Are Used in Bioremediation Process

Weedkillers, insecticides, dyes, pharmaceuticals, and polymers, among other toxic compounds, pollute the land, air, and water in significant quantities every year. A most hydrocarbons that must be cleared away globally are chlorinated hydrocarbon, organo-cyanide, scented compounds, aromatic compounds (PAHs), polymers, and glucocorticoids. Their toxicity is primarily due to their solid structure (Karigar & Rao, 2011). Laccases, CYP450, hydrolases, de-halogenases, de-hydrogenases, proteolytic enzymes, and lipases are the most noteworthy key enzymes from microorganisms as well as genetically modified microscopic organisms responsible for the oxidation of several types of contaminants employed in biomonitoring practices. Pesticides and hydrocarbons have frequently been destroyed by enzymatic reactions from aerobe microbes such as *Mycobacterium, Pseudomonas, Alcaligenes, Rhodococcus,* and *Sphingomonas.* Bioremediation of organochlorine pesticides (PCBs), de-chlorination of tri-chloroethylene (TCE), and chloroform have been achieved using enzymes from anaerobic bacteria (Sharma, 2012). Intracellular accumulation and enzymatic transformation are the two main ways it degrades and detoxifies contaminants (Singh et al., 2008). Bioremediation can be made more successful and environmentally sustainable with the use of microbial enzymes (Abatenh et al., 2017). The function of an enzyme is to break down complicated pollutants into simple molecules that microbes could use as a feed ingredient (Alkorta et al., 2017; Bak et al., 2011). The protein substituent, such as glycoprotein, is present in these enzymes (Abatenh et al., 2017). Various enzymes from bacterial, fungal, and algal species have been employed for bioremediation of contaminants, including mono- and dioxygenases, halogenases, peroxidases, cytochrome P450, laccases, phosphor-triesterases, proteases, lipases, hydrolases, transferases, and oxidoreductases (Rao et al., 2010; Pieper et al., 2004). We are attempting to assess the most important enzymes for pollution bioremediation as well as gain insight into their mechanisms of action.

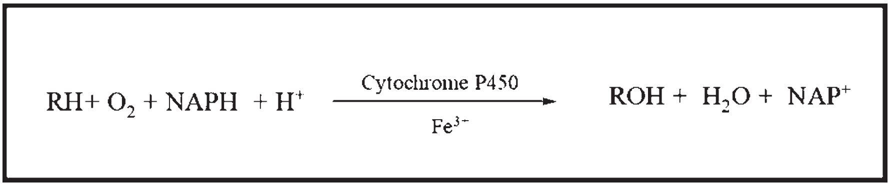
1. *Cytochrome P450 (CYP 450)*

The plant CYP450 is a large family of widespread heme enzymes present in all three different domains: Eubacteria, Bacteria, and Archaebacteria (Li et al., 2020), is responsible for a variety of tasks in living systems, including the construction of large organic ingredients and biochemical changes, as well as the bioaugmentation of hazardous substances (Anzenbacher & Anzenbacherova, 2001). These P450s have an inherent ability to degrade xenobiotics (Kumar, 2010) via chemical transformations such as aliphatic hydroxylation, ep-oxidations, de-alkylations, dehalogenation, further different processes in activations, which seems to be essential in bioremediation chemical approaches. Polycyclic aromatic hydrocarbons (PAHs) are known to be metabolized by Cytochrome P 101, Cytochrome P 102, Cytochrome A1, Cytochrome 1A2, and Cytochrome 1B1, with Cytochrome 1A1 showing strong action toward the dibenzo-p-dioxin (DD) and mono-, di-, and trichloro-DDs, and Cytochrome 1A1 mutant, F240A, displaying response for 2,3,7,8-tetra-chloro-dibenzo-p-dioxin (Guengerich, 2018).

All these compounds produce carbon substrate and oxidized products by employing O_2 and NADP/H as a non-organic substrate (Lamb et al., 2000). Engineering and non-engineering protein-based investigation on microbial cyp450s could have been undertaken for the bioremediation of organic pollutants and hydrocarbons. Kumar et al. investigated modified Cytochrome P102A1, displayed improved performance toward polycyclic aromatic hydrocarbon, polychlorinated biphenyls, which are commonly utilized in toxic chemical bioremediation, gaseous alkane detoxification, and terpenes (Chakraborty & Das, 2016). Similarly, Chakraborty and Das discovered genes that are involved in catabolism, plasmids, and DNA/RNA expressing cytochrome P450s in microbes such as *Gordonia, Mycobacterium, Rhodococcus*, and *Pseudomonas* used for the degradation and elimination of persistent organic contaminants (POPs). Figure 4 shows the enzymatic processes which is mediated by cytochrome P450.

2. *Microbial Oxidoreductases*

Various bacteria, fungi, and higher plants (Megharaj et al., 2014) use oxidoreductases to detoxify harmful chemical molecules via oxidative coupling. Microbes obtain energy through breaking bonds and are responsible for sending electrons



$$RH + O_2 + NAPH + H^+ \xrightarrow[Fe^{3-}]{\text{Cytochrome P450}} ROH + H_2O + NAP^+$$

Fig. 4 General enzymatic processes mediated by cytochrome P450 from an electron-enriched donor substrate to electron deficient molecule. The pollutants are eventually converted to less harmful molecules through oxidation and reduction reactions (Karigar & Rao, 2011). Various synthetic organics such as phenolic, aniline, and azo ring compounds, which are found in xenobiotics or the soil environment, are detoxified by oxidoreductases. Hence, bioremediation must be a required process in the paper and pulp sector (Rubilar et al., 2008). Several fungal species have been found to be effective at removing chlorinated phenolic chemicals from polluted environments. Extracellular oxido-reductase enzymes, i.e., lignin peroxidases and laccase which are excreted by fungal mycelia in the surrounding atmosphere, are primarily responsible for fungi's activity (Arora et al., 2009). Fungi like *Trametes versicolor, Phanerochaete chrysosporium, Basidiomycetes*, and *Pleurotus ostreatus* (Oyster Mushroom) are more efficient at killing bacteria in the soil (Rubilar et al., 2008).

2.1 *Microbial Oxygenases:* The oxidoreductase family includes oxygenases (Fetzner, 2002; Wang et al., 2018a, b). Based on the number of oxygens used for reactant oxygenation, oxygenases are classified into two types: monooxygenases and dioxygenases (Abatenh et al., 2017). Dehalogenase enzyme cleaves C-X bonds (Wang et al. 2018a, b) using three processes, including hydrolytic, oxygenolytic, and reductive which can accomplish dehalogenation by replacing the halogen atom with a OH from H_2O and hydrogen atom from H_2. Because these are widely used as fungicides, weedkillers, insect repellent, and intermediates in catalytic reactions, halogenated chemical compounds make up the most significant families of emerging pollutants. The degradation of these pollutants is carried out by certain oxygenases, in collaboration with multi-functional enzymes, such as oxygenases that mediate de-halogenation processes of halogenated methanes (CH_4), ethanes (C_2H_5), and ethylenes (C_2H_4) (Chauhan et al., 2017). They have also important participation in controlling the digestion of natural mixtures by enhancing their reactivity, polarity, and achieving aromatic ring cleaving activity (Abatenh et al., 2017). Figure 5 shows the monooxygenase catalyzes the degradation of aromatic compounds.

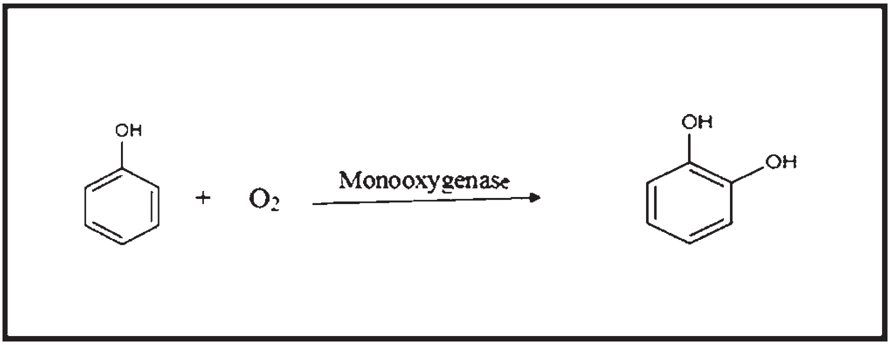


Fig. 5 Monooxygenase catalyzes the degradation of aromatic compounds

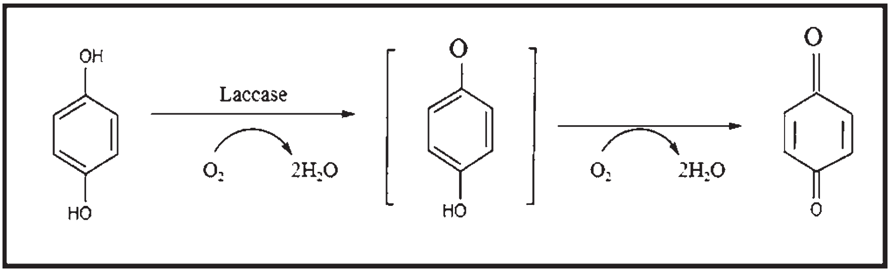


Fig. 6 The general chemical mechanism for phenol oxidation by laccase enzyme

3. *Microbial Laccases*

Laccases (p-di-phenol: dioxygen (O_2) oxidoreductase) have a large collection of multicopper oxidases in their primary structure. Isoenzymes are created when separate genes code for distinct laccase structures (Arregui et al., 2019). Ortho-diphenols, polyphenol, lignin, aminophenol, para-diphenols, polyamines, aryl diamines, and a few inorganic ions are all catalyzed by intracellular and external laccases.

Laccases, which are responsible for decarboxylation, polymerization, and demethylation to humic compounds, convert lignin to phenols. They offer a lot of biotechnological and bioremediation potential. Halides, cyanide, azide, and hydroxide are all used to inhibit activity. It is delicate to nitrogen levels (Hiner et al., 2002). Figure 6 shows the chemical mechanism for phenol oxidation by laccase enzyme.

4. *Microbial Peroxidases*

Peroxidases are highly oxidizing enzymes [donor: hydrogen peroxide oxidoreductases] (Koua et al., 2009; Bansal & Kanwar, 2013). Coal, lignins, lignocellulosic materials, and certain other polyphenols combinations are catalyzed without the use of hydrogen (Cocco et al., 2017; Hiner et al., 2002). Peroxidases are proteins that are either heme (Fe) or non-heme in nature. They also play a vital role in controlling metabolic processes performed in animals, i.e., processes that run to regulate immune system and hormonal control. It also plays a role in auxin metabolism, lignin and suberin synthesis, cell wall crosslinking, pathogen defense, and cell enlargement in plants (Koua et al., 2009; Bholay et al., 2012).

Heme peroxidases are categorized into two major groups: those only seen in animals and those observe in plant, fungus, and bacteria. Because of sequence comparison, the second group of peroxidases has been classified into three groups. Intracellular enzymes, such as yeast plant ascorbate peroxidase (APX), cytochrome c peroxidase, and bacterial gene-duplicated catalase peroxidases, are classified as Class I. Secretory fungal peroxidases such as manganese peroxidase (MnP), lignin peroxidase (LiP), and manganese peroxidase (MnP) from *Phanerochaete chrysosporium*, as well as *Coprinus cinereus* peroxidase and *Arthromyces ramosus* peroxidase, belong to Class II (ARP). The breakdown of lignin in wood appears to be the primary function of class II peroxidases. Secretory plant peroxidases, such as those from horseradish (HRP), soybean, or barley, are found in Class III. These peroxidases appear to be biosynthetic enzymes involved in the construction of plant cell walls and the lignification of wood (Koua et al., 2009; Bholay et al., 2012). Non-heme peroxidases are divided into five distinct families based on their evolutionary relationship. Thiol peroxidase, alkyl hydroperoxidase, non-heme haloperoxidase, manganese catalase, and NADH peroxidase are some of the enzymes involved (Bholay et al., 2012). Bio-pulping, bio-bleaching, and polymerization are all carried out by microbes. Multiple lignin and manganese peroxidase isoenzymes are found in many fungus species. Because of the cleavage of polymeric materials into short-chain water-dissolvable particles, these enzymes produce metabolically active and non-specific free radicals derived from peroxide, which facilitate their transportation through microbial layers for intracellular destruction (Cocco et al., 2017).

4.1 *Microbial Lignin Peroxidases*

In land ecosystems, lignocellulose breakdown is a critical phase in carbon recycling. In the process of delignification, basidiomycetes are quite stable. The heme protein of lignin's peroxidases is primarily by white rot and brown-rot basidiomycetes because of their metabolizing activity, which includes the optional digestion of lignocellulose. They degrade the woodlands quite effectively (Kumar & Chandra, 2020; Dashtban et al., 2010). The brown rot fungus is very picky. In the presence of the co-substrate H_2O_2 and the veratrole liquid LiP, lignin and other phenolic mixtures degrade. When LiP is oxidized, it gives an electron to H_2O_2 to reduce it to water and nascent oxygen. So, returning to its former condition, LiP gets one electron from veratrole liquid. So, returning to its former condition, LiP gets one electron from veratrole liquid. This produces veratraldehyde, which returns to veratrole liquid after receiving electrons from the substrate. This produces veratraldehyde, which returns to veratrole liquid after receiving electrons from the substrate (Chowdhary et al., 2019).

4.2 *Microbial Manganese Peroxidases*

The extracellular heme chemical MnP (manganese peroxidase) is found in the lignin-degrading basidiomycetes organism. Manganate oxidation to manganite is a multi-step process. The process is started by Mn2+ functioning like a precursor for MnP. The creation of Mn3+, which is important in the formation of diverse cyclic combinations, occurs as a result of this. The chelate oxalate of Mn^{3+} that results quickly diffuses into places that are inaccessible to the catalyst, such as lignin or structures that are closely mimicking xenobiotic toxins deep within the dirt that are not accessible to the proteins (Urzúa et al. 1998; Thakur et al., 2019).

4.3 *Microbial Versatile Peroxidases (V.P.)*

V.P. chemicals oxidize methoxybenzene, Mn2+, and sweet-smelling phenolic substrates like MnP and LiP, which have a high selectivity. V.P., unlike other peroxidases, can oxidize substrates without leaving any manganese residue. The great efficiency of V.P. with both phenolic and nonphenolic lignin display dimers is

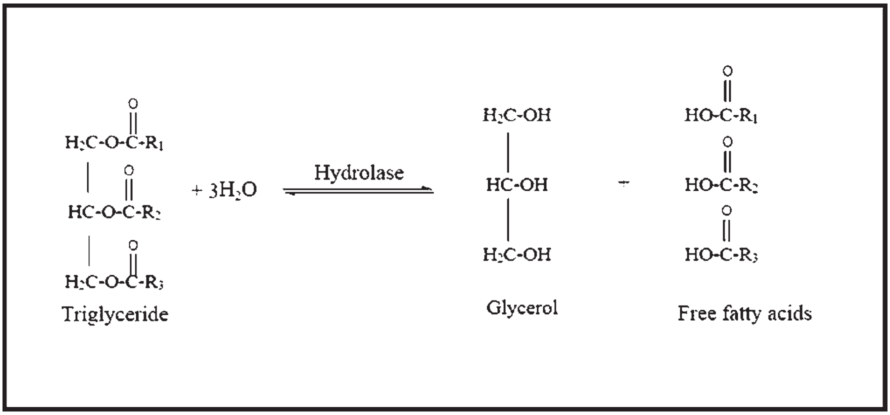


Fig. 7 General chemical mechanism for triglyceride by hydrolase enzyme

another aspect. It is frequently sought after for biotechnological businesses and bioremediation due to its high productivity (Karigar & Rao, 2011).

5. *Hydrolases*

Hydrolytic enzymes disrupt chemical connections between hazardous compounds to lessen toxicity. This technique degrades oil spills, carbamate insecticide, and organophosphate insecticides, Condensation and alcoholysis are also catalyzed by it. The availability, tolerance, and non-selectiveness of this enzyme are its key advantages. DNases, proteases, xylanases, amylase, and lipases are examples of external hydrolases that seem to have a broad array of applications in the foods, chemical, biomedical, or feed industries. Cellulases, hemicellulases, and glycosidase are particularly active in biomass decomposition (Peixoto et al., 2011; Sharma et al., 2011). Figure 7 shows the general mechanism for triglyceride by hydrolase enzyme.

5.1 *Microbial Lipases*

Triacylglycerols are converted to glycerol and long-chain fatty acids by lipases, which are enzymes that facilitate this process (Prasad & Manjunath, 2011). Lipase is a lipid-degrading enzyme found in microorganisms, plants, and animals. Microbial lipases are more adaptable due to their wide range of industrial applications. Hydrolysis, esterification, alcoholysis, interesterification, and aminolysis are among the processes that these enzymes can catalyze (Steinfeld et al., 2015). Lipase activity was found to be the more effective indication for measuring hydrocarbon degradation within soils (Joseph et al., 2006). Lipase seems to have a wide spectrum of applications in foods, chemicals, detergents, cosmetics, or paper industries, as well as diagnostic utility to bioremediation, but the cost of production has restricted their manufacturing application (Singh, 2003). Figure 8 shows the enzymatic mechanism for triacylglycerol by lipase enzyme.

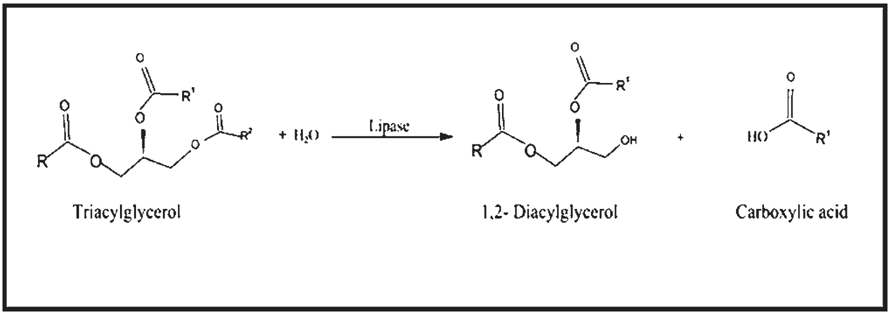


Fig. 8 The general enzymatic mechanism for triacylglycerol by lipase enzyme

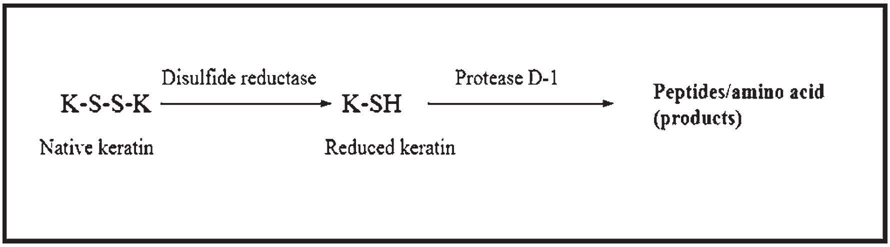


Fig. 9 The general chemical mechanism for keratin by protease enzyme

5.2 *Microbial Proteases*

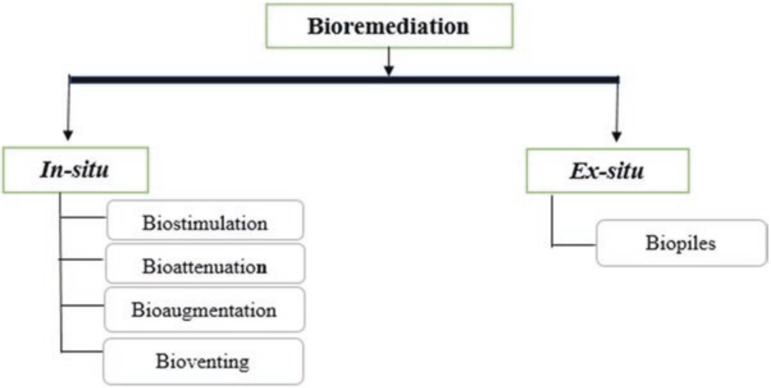
Proteases are enzymes that hydrolyze and manufacture polypeptide chains on liquid & nonliquid environments, respectively. Proteolytic enzymes are generally categorized into endopeptidases or exopeptidases based on whether they catalyze peptide chains. Proteases are used in the food, textile, detergents, and pharmaceutical industries (Beena & Geevarghese, 2010). Proteolytic enzymes are used in cheeses and detergent companies for a long time. In the leather business, alkali proteolytic enzymes are being used to eliminate hair and other debris from the skin of animals. In the pharmaceutical industry, numerous types and specificities of proteases are used to create successful therapeutic medications. Clostridial collagenase or subtilisin is often used in concert with wide antibiotics to treat burns and lesions (Adams et al., 2015). Figure 9 shows the general mechanism for keratin by protease enzyme. Table 1 shows the mechanism and functions of industrial important microorganisms that play a key role in bioremediation.

Table 1 Industrial important microorganisms that play a key role in bioremediation

S. No.	Enzymes	Mechanism	Function
1.	Cytochrome P450	By reducing or oxidizing heme iron, it performs electron transfer processes and catalysis. When pyridine nucleotides are used as an electron donor, oxidizing by-products and carbon compounds are produced.	Within the body, various chemicals and substances are synthesized and processed.
2.	Dehalogenase	It mostly occurs through three mechanisms: (a) *Oxygen-lytic methods:* Catalysis by di/monooxygenase, which integrates one/or two molecular O_2 atoms into the substrate. (b) *Hydrolytic methods:* The H_2O molecule acts as a co-factor, and the halogen substitution is changed by the OH group in the nucleophilic substitution (SN) reaction. (c) *Reductive mechanism:* In this pathway, which is connected to the urea (carbamide family), in an aerobic environment, halide is replaced by H^+, with organo-halides acting as the final electron acceptor.	Halogens are removed by breaking the carbon-halogen bond.
3.	Dehydrogenase	As an electron acceptor, use coenzymes like Nicotinamide adenine dinucleotide phosphate (NAD^+/$NADP^+$) and FLAVIN like flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) to catalyze the reactions.	Oxidation of organic molecules produces energy.
4.	Protease	Protein peptide bonds are broken down by catalysis.	Protein degradation (e.g., casein), unhairing of leather, and sewage treatment.
5.	Laccase	Several aromatic compounds are used to reduce the oxygen molecules, with one electron oxidization.	In aromatic compounds, ring breakage reduces one molecule of oxygen in H_2O, leading to the formation of free-radical.
6.	Hydrolase	In triglyceride hydrolysis, the peptide link between 1 M (mole) glycerin (G) and 3 M of saturated fats is broken.	Protein and fat decomposition
7.	Lipase	The aspartic acid, L-histidine, and L-serine residues ($C_3H_7NO_3$) of the triacylglycerol lipase transfer an electron to the carbonylic of the substrates, which is then targeted by the hydroxy (OH) residue of the $C_3H_7NO_3$ (L-serine). During the di-acylation phase, a thiol (nucleophile) attacks the enzyme, prompting it to regenerate and release the product.	The catalysis of mono and lipid (triglycerides) into fat and glyceryl is catalyzed by this enzyme. The esterification and transesterification processes should also be catalyzed.

4 Types of Bioremediation

There are several approaches used in bioremediation procedures. The primary bioremediation techniques include attenuation, biostimulation, augmentation, heaps, and venting.



1. *Biostimulation*

The technique is similar to the inoculation of adequate nutrition at the location (soils/groundwater) to enhance the ability of microbial biomass. Its primary goal is to promote the growth of native or naturally occurring bacteria and fungi communities. First, by providing manure, nutrients, and microelements. Second, through supplying additional biological needs such as hydrogen ion concentration (pH), temperatures, and O_2 to enhance its metabolic activity and pathways (Kumar et al., 2011; Naik & Duraphe, 2012).

A tiny quantity of contaminant may also function as a stimulus via trigger the operons for biodegradation enzymatic activity. Most of the time, this systematic approach would be maintained by adding nourishment and O_2 to aid native bacteria. These nourishments are the fundamental components of existence, allowing bacteria to produce essential elements like cell biomass, energy, and enzyme to break down contaminants (Mulligan & Yong, 2004).

2. *Bioattenuation*

The process of eliminating contaminant concentrations in the environment is referred to as bioattenuation or natural attenuation. It is performed in natural activities, which may comprise (aerobically and anaerobically biodegradation, flora, and fauna absorption), chemical reactions like (complex formation, abiotic transformation, and ion exchange), and physical processes like (convective heat transfer, dispersing, dilution, evaporation, and desorption). If the ecosystem is contaminated by chemicals, nature may clean it up in four ways (Abatenh et al., 2017): (1) Some chemicals are used as food by bacteria or microscopic bugs that live beneath the soil and in groundwater sources. When the compounds are entirely digested, they can be converted into noble gases [also called inert gas] and H_2O. (2) Chemical compounds may attach to the ground and bind to them, allowing them to stay in place. The pollutants are not removed, but they have prevented them from damaging surface water and leaving the area. (3) Pollutants can mix with pure water as it flows through the land and groundwater. This helps to minimize or dilute pollutants. (4) Some chemicals, such as solvents and oils, have the ability to drain, which indicates they can transform from liquid to gas in the soil. If these gases enter the atmosphere at ground level, they could be destroyed by sunlight (Sayler & Ripp, 2000).

3. *Bioaugmentation*

The addition of pollution-degrading microbes (genetically engineered, native, or foreign) to boost the bioremediation capabilities of indigenous bacterial communities in contaminated areas is known as bioaugmentation. Microbes are extracted from the remediation site, cultured separately, genetically modified, and then reintroduced to the same area to speed up the development of indigenous microbial communities and accelerate degradation, which partially feed on contaminants at the site (Thapa et al., 2012). Genetically modified microbes have shown promise for the bioremediation of land, water, and sludge, with improved degradation ability of a broad range of physicochemical pollutants (Malik & Ahmed, 2012; Gomez & Sartaj, 2014). Furthermore, genetically engineered microorganisms have demonstrated and proven that they can improve the degradative effectiveness of a variety of environmental pollutants. Because of the broad metabolic profile, fewer complicated and innocuous end products can be produced (Agarry & Latinwo, 2015).

Natural species are unable to break down some substances quickly enough, thus they should be genetically engineered (GE) by gene editing. GE microorganisms degrade contaminants considerably quicker than native species, competing with indigenous species, predators, and abiotic forces.

4. *Bioventing*

It is the process of venting oxygen through soil to encourage the growth of natural or imported bacteria and fungi in the soil by supplying oxygen to existing soil microorganisms. The most prevalent method of supplying oxygen is by direct air injection into residual pollution in soil via wells. Adsorbed fuel residues are decomposable, as are volatile substances when vapors migrate slowly through biologically active soil. Many studies have demonstrated that bioventing may effectively remediate petroleum-contaminated soil (Delille et al., 2008).

5. *Biopiles*

Biopiles are sometimes referred to as bio-cells, bio-heaps, and humus. During the biodegradation process, piles are utilized to lower the amounts of oil (petroleum) contaminants in the soil. Air is circulated to the biopile system during this phase via a pump and pipes system that either forces air into pile under positive pressure or draws air through the pile under a pressure gradient during this procedure (Sharma, 2012). When bacterial growth is increased by bacterial respiration, the outcome in the breakdown of adsorbed petroleum pollution is increased (Dell'Anno et al., 2012).

4.1 Advantages of Bioremediation

- It is a time-consuming biological process that is an appropriate disposal treatment procedure for polluting substances such as soil. When pollutants are present, microbes can decompose them and multiply proliferate. As the pollutants are destroyed, the bioremediation population reduces (Sayler & Ripp, 2000).
- Environmentally friendly and long-lasting.
- It is free of any very harmful substances.
- To encourage strong and rapid microbial growth, bio-fertilizers as well as other micronutrients are used. On lawns and gardens, it is commonly utilized. As a result of bioremediation, which turns poisonous chemicals into H_2O and non-toxic substances (Sharma, 2012).

4.2 Drawbacks of Bioremediation

- It is only applicable to biodegradable chemicals.
- Further study is needed to design and make up bioremediation methods suited for regions with complicated structure of pollutants which are not properly dispersed throughout the environment. Liquefied, solidified, or gaseous can all be contaminants (Jain et al., 2010).
- Biological processes are often extremely specialized, therefore not all substances are vulnerable to total breakdown. The presence of metabolite-active microbial bacterial diversity, adequate eco-friendly development conditions, and correct amounts of nutrition and toxicants both are essential site factors for success.

5 Conclusions

In this chapter, we present an insight into the involvement of enzymes in the bioremediation of non-chemical and inorganic contaminants. Furthermore, physico-chemical approaches to treating polluted soil and water are ineffective for contaminants, but bioremediation offers us a new way to remove all those harmful pollutants from the environment. Enzymes provide us with an important tool to treat bioremediation by using eco-friendly strategies. Enzymes found in microbes are used to remediate chemical contaminants. Different enzymes are released by microorganisms when they expose themselves to contaminated areas. The use of bioengineered microbes in present research encourages the more effective breakdown of organic and inorganic contaminants from the environment. PHA's (poly-halogenated aromatic compound) and biopolymer polycyclic aromatic hydrocarbons engage with the binding site (or called active site) of the CYP-450 enzyme and are oxidized into non-toxic compounds. Using hydrolytic reactions, protease enzymes are responsible for degrading protein polymers, disposable products, dyes, northern shrimp wastes, and O_2-biodegradable polymers. By alcoholysis reactions as well as condensation, bacterial hydrolases target cyanides, and nitrile-comprise chemicals and convert them into less harmful products. These co-polymers, such as PCL, PHA, artificial fibers, and methylparaben, were efficiently decomposed into biodegradable materials due to the enzyme substrate-specificity and full strength of microbial lipases. As a result, this data suggests that all microbial enzymes used in bioremediation for the breakdown of contaminants are environmentally valuable for restoring the various physico-chemical characteristics of land degradation. Although various techniques or strategies are used for the extraction of microbial enzymes, using them for bioremediation purposes offers a potential strategy to mitigate contaminants and produce a safer and more efficient ecosystem for all life forms.

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