# Microbial Fingerprinting of Potential Biodegrading Organisms

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**Abstract**
The world is witnessing various pollutants in the environment since the last few decades that threaten human life. The biological responses to various pollutants show variations as the living system behaves differently in their sensitivities to the same types of pollutants. The relative response and activity depend upon the duration of exposure to the specific pollutant. It is impossible to stop various activities leading to environmental pollution; however, pollutants can be eliminated from the environment using the microorganisms. Application of biological processes can be executed in order to get rid of toxic pollutants through their biodegradation. The pollutants like hydrocarbons, heavy metals, chlorinated hydrocarbons, nitro-aromatic compounds, non-chlorinated herbicides and pesticides, organophosphates, radionuclides can lead to serious health and environmental problems. The main objective of this paper is to evaluate the effects of pollutants on the living beings and environment, microbial responses to pollution, and distribution of various biodegrading microorganisms in the environment. Profiling of biodegrading microor-ganisms, microbial biosensor to detect environmental pollution, and strain improvement through genetic manipulation to enhance the biodegradation process have been discussed in detail.

**Keywords** Biodegradation · Pollutant · Microbial diversity · Genetic manipulation · Biosensor

## Introduction

The ever-growing industrialization and population are the main cause for disturbing the sustainability of our planet [56, 69]. Various types of mining industries, power plants, and petroleum refineries are the primary sources of hazardous and toxic materials that lead to pollution of air, water, and land ecosystem [51, 69, 156, 187]. The enormous release of wastewaters, slurries, solid wastes, and industrial effluents are affecting water and soil quality. As the natural ecosystem is deteriorating, the intrinsic remediation power of the earth is also getting reduced [163]. Most of the pollutants can be degraded and metabolized by natural activities of microorganisms. Microorganisms can initiate several types of reactions through metabolism that include oxidation-reduction, substitution, hydrolysis, cleavage, dehydrohalogenation, dechlorination, and dehydrogenation [106, 139, 153]. Although pollutants and toxicants affect microorganisms, they can enhance the rates of their degradation. The scientific knowledge upon microbial interactions with pollutants has helped to address the environmental pollution in the last decades [161].

Land disturbance, pollution, overpopulation, landfill, and deforestation are the major causes of ecosystem destruction. The researchers are now exploring restoration of ecosystem by using the communities such as biocrusts (communities of mosses, lichens, cyanobacteria, and other microorganisms) living on the soil surface of the drylands. Biocrusts strongly affect key processes in the ecosystem such as soil erosion, nitrogen and carbon cycling, and nutritional status [173]. Successful restoration of the

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drylands from China, Israel, and the USA has been established by using this biocrust [173].

Microbial pathways associated with degradation of different groups of hazardous chemicals and pollutants have been thoroughly investigated for decades [56]. Scientists have developed a unique methodology to remediate organic pollutants in aerobic or anaerobic condition. Some important enzymes (peroxidases, oxygenases, hydroxylases, reductases, and dehydrogenases) commonly catalyze the biodegradation of major pollutants in both aerobic and anaerobic conditions [98].

In the aerobic conditions, oxygen is the final electron acceptor. In some of the catabolic process, oxygen can also act as a co-substrate [34, 38, 46, 49, 186]. The biodegradation of hydrocarbons like petroleum pollutants in the aerobic process is mediated with the intracellular attack of organic hydrocarbons initially. This is an oxidation process, in which activation and incorporation of molecular oxygen is the key reaction catalyzed by the enzymes like peroxidases and oxygenases [1, 155]. Aromatic groups like benzene are cleaved or degraded by the microorganism through the activity of two enzymatic systems viz. dioxygenases and monooxygenases [90, 163]. Anaerobic degradation of the pollutants has been reported under reducing conditions. In this methodology, four enzymatic reactions are involved (a) addition of fumarate (catalyzed by a glycyl radical enzyme), (b) methylation of unsubstituted aromatics, (c) alkyl substituent hydroxylation (catalyzed by a dehydrogenase), and (d) direct carboxylation [163].

Biodegradation is a sustainable and eco-friendly process, which can remove the organic pollutants from the environment more efficiently with the help of microorganisms [103, 162, 163]. The main objective of biodegradation is to remove pollutants present from the ecosystem without creating problems in the biological processes associated with it. As compared to other methodologies, the biodegradation is considered universally as it provides the best results with cost-effective inputs [157].

## Types of Pollutants in the Environment

The pollutants are diversified in different places. Some of them are persistent by nature in the environmental degradation (biological, chemical, and photolytic reactions) process and stay for a long time. Several alicyclic, aliphatic, and aromatic compounds are the major groups of pollutants produced from pharmaceutical and chemical industries. They reach the soil or aquatic environment through different routes [23, 56, 104, 161].

### Organic Pollutants

Organic pollutants are unstable thermodynamically. They can be converted to harmless and non-toxic products through the physicochemical action (volatilization, photodecomposition, leaching, and partitioning) and biological action like microbial metabolism and phytoremediation. Sometimes, the concentration of these organic pollutants can be elevated to a higher extent if they are applied over short periods at high concentrations repeatedly [46, 157]. Various recalcitrant organic compounds create serious environmental hazards that are found to be persistent in soil and water environments [56, 156]. The major pollutants like polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCB), dioxins, and dibenzofurans are the major groups of persistent organic pollutant [73, 165]. The degradation patterns of these organic pollutants are also different with respect to chemical nature. Degradation of OCPs is mediated through the non-oxidative pathway/hydrolytic pathway and for polychlorinated biphenyls, generally, anaerobic degradation takes place starting with dehalogenase enzyme. Similarly, the degradation of dibenzofurans is mediated through two main pathways (angular and lateral deoxygenation) and dioxins through reductive dehalogenation.

These kinds of compounds are non-biodegradable in nature. Hence, they remain intact in the ecosystem for a long duration of time. They are also resistant to chemical, photolytic, and biological degradation. They can easily get accumulated in the adipose tissue of the human body through the food chain. Hence, they cause harmful effects to the living system as well as the environment [23, 166]. Various groups of organic pollutants have been highlighted in Fig. 1.

### Inorganic Pollutants

Inorganic pollutants are found to be non-biodegradable by nature. They can be transformed from one state to another. Various groups of heavy metals and metalloids are examples of inorganic pollutants. These can be deposited in the soil and may leach to groundwater. Various groups of inorganic pollutants have been highlighted in Fig. 2.

Any metallic element having high density than water and toxic in nature at very low concentrations are known as "heavy metal" [78]. Various groups of heavy metals like mercury (Hg), chromium (Cr), cadmium (Cd), arsenic (As), and lead (Pb) are found to be non-biodegradable, toxic, and persistence by nature [52].

### Mixture of Inorganic and Organic Pollutants

The pollutants from organic and inorganic sources and their combinations can be obtained as anthropogenic or in a natural way. For example, mollusk shells, crustacean carapaces, and teeth and bone tissues in vertebrates are present as organic-inorganic composites in nature. Some of them are found to be organometallic derivatives. They also include rodent repellants, fungicides, molluscicides, ovicides, miticides, nematocides,

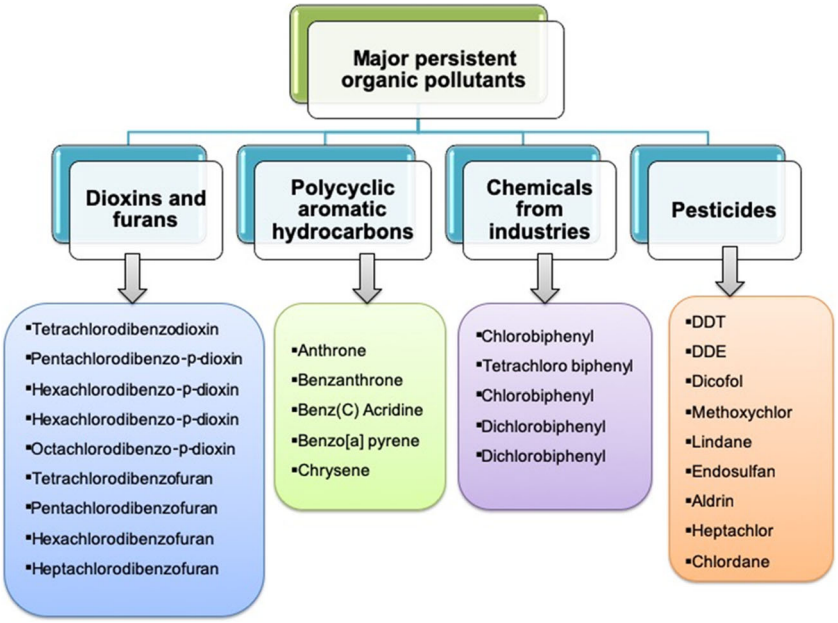


**Fig. 1** Categories of organic pollutants

antifouling paints, and wood preservatives. The waste materials from the industries (petroleum refining or mixed effluents) that release such agents reaching out the soil ecosystem. Most of the coloring agents used commercially are composed of inorganic pigments suspended in organic mixtures. Those are widely used as organic-inorganic industrial agents. Many hybrid organic-inorganic nano-composites have also developed for its use in the new catalysts, sensors, and smart membranes. These hybrid organic-inorganic nano-composites are being used to remediate volatile organic compounds (VOCs), such as ethanol and isopropyl alcohol into harmless products. The authors have executed the photocatalysis in order to recover pure water from the pollutant sample. The degradation kinetics for isopropyl alcohol was noted 0.0859 $min^{-1}$ when PVDF/P25/CuxOy inorganic-organic hybrid membranes were used [72]. The hybrid of polypyrrole/titanium (IV) was successfully synthesized and was applied for remediation of Cr (VI) from wastewater [66]. Javadian [67] fabricated a hybrid polymer of polyaniline/polypyrrole to remove the heavy metals like Co (II) from the water sample. It has been reported that *Pseudomonas aeruginosa*, *Sphingomonas* sp., *Pseudomonas putida*, *Aspergillus niger*, *Bacillus cereus*, *Arthrobacter* sp., etc. can degrade the compounds composed of both organics and inorganics [178].

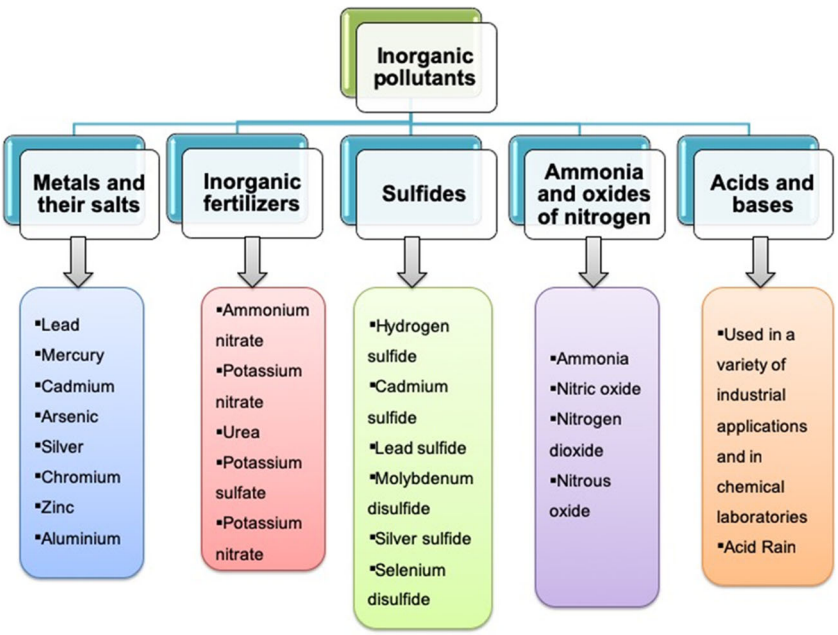


**Fig. 2** Categories of inorganic pollutants

## Microbial Responses to Pollution

The behavior of microorganisms and their response to pollutants has been investigated elaborately. The pollution that occurred by the human made the microorganisms to response initially. Modifications in the structure of a microbial community and their changes in genetic composition have been largely noticed subjected to the addition of organic pollutant [144, 163] and inorganic pollutant [118]. In accordance with these investigations, different communities of microorganisms have been described in polluted environments in accordance with the characteristics of the pollutant [42, 101, 154]. The above observations in the microbial community level are relevant to provide information with respect to the exposure of pollutants on the behavior of microbial communities.

Recently, different studies have been executed to assess the microbial activities and metabolic capacity during the course of biodegradation of the pollutants [13, 39]. The metabolism of pollutants in the microbial community is related to genetic adaptation techniques which include horizontal gene transfer and mutations [115, 159]. Various genotoxicity methodologies have been assayed using microorganisms for the evaluation of their toxicity in the polluted sites [93]. The genotoxicity assays have been also executed with different plant species for various groups of pollutants released with industrial effluents to monitor the toxicity. The plant like *Allium cepa*, *Vicia faba*, and *Vigna radiate* was used to study the effluents of pulp and paper mill, Tannery effluent, textile industry wastewater, and industrial dye effluents [77, 128, 181]. Generally, root tip cells were observed with the chromosomal aberrations (delayed anaphase, C-mitosis, stickiness, chromosome break, chromosome bridge) upon exposure with the pollutants. The physiological and metabolic characteristics have been studied for better understanding the microbial behavior and their capacities to react with pollutants. The various approaches with respect to pollutants that show responses starting from the molecular level to ecosystem level, their process of adaptation, detection tools, and culture-independent approaches have been outlined in Fig. 3.

Resistance to the various groups of pollutants may be due to overexpression of the genes responsible for tolerating the pollutants. This can be effective through mutation and horizontal gene transfer that lead to acquiring new pollutant-tolerant microorganisms. The history of pollution at a particular site also determine promptly mobilized in subsequent exposure. The rate of biodegradation of the particular pollutants also found to be faster as compared to previously exposed [11]. Hence, memory effect also increases the tolerance for the microbial community to the pollutants [81, 97].

## Biodegrading Microorganisms

### Distribution of Organic Pollutants Degrading Microorganisms

In general, every microorganism can remediate the pollutants, but few engineered microorganisms are also capable of remediating pollutants effectively. Some of the microorganisms like *Staphylococcus*, *Streptococcus*, *Bacillus*, *Escherichia*, *Klebsiella*, *Corynebacterium*, *Shigella*, *Alcaligenes*, *Pseudomonas, Acinetobacter*, and *Enterobacter* have been extensively applied for the biodegradation of persistent organic pollutants (POPs) [78, 95, 158, 160]. Among these microorganisms, *Bacillus* sp. has been extensively employed in the removal of organic pollutants [82]. The microorganisms upon action either change the functional groups present in the pollutant or change the structure of the compound into a lesser toxic form. This leads to the formation of inorganic salts, water, and $CO_2$. Among the microbial community, bacteria, fungi, and algae have the ability to transform the POPs into simpler non-toxic metabolites [2, 73, 116, 154].

Successful biodegradation of hydrocarbons, dioxins, furans, PAH, PCB, and DDT by the action of microorganisms has been reported previously. Some of bacterial strains like *Alcaligenes* sp. SSK1B, *Microbacterium* sp. BPW, and *Achromobacter* sp. SSK4 are able to remediate PAHs [167, 174]. In some studies, a bacterial consortium of *Bacillus* sp., *Pseudomonas aeruginosa*, *Pseudomonas putida*, Acinetobacter sp., *Acinetobacter* sp., *Proteus* sp. *Citrobacter freundii*, *Stenotrophomonas* sp., *Flavobacterium* sp., and *Proteus vulgaris* could able to degrade pesticides successfully [68, 109, 156, 165]. The *Streptomyces* strains could degrade chlordane and 56% through reduction of ϒ-chlordane. Degradation of organochlorine pesticides by *Alcaligenes faecalis* JBW4 was studied by some researchers and it was concluded that the isolate of JBW4 strain possessed efficient capacity to degrade the endosulfan residue [31, 76].

Fungi are known to be the potential biodegrading agent. They can destroy and deteriorate various kinds of materials like leather, plastic, textile, wood, and paper. It was reported that PAH can be metabolized by microorganisms. Some researchers had investigated the white-rot fungal cultures are potential to degrade PAH [20, 147]. Various groups of enzymes (manganese peroxidase, laccases, and lignin peroxidises) are biosynthesized by different groups of fungus, which can enhance the degradation rate of dyes, pesticides, polychlorinated biphenyls, chlorinated and phenolic compounds, hydrocarbons, etc. [103, 147]. Some fungal species like *Fusarium oxysporum*, *Mucor alternans*, *Phanerochaete chrysosporium*, and *Tricoderma viride* can degrade DDT efficiently. The degradation of benzo-(α)pyrene by *Pleurotus ostreatus* for the synthesis of ligninolytic enzyme was also investigated. Oxidation of various pollutants like pyrene,

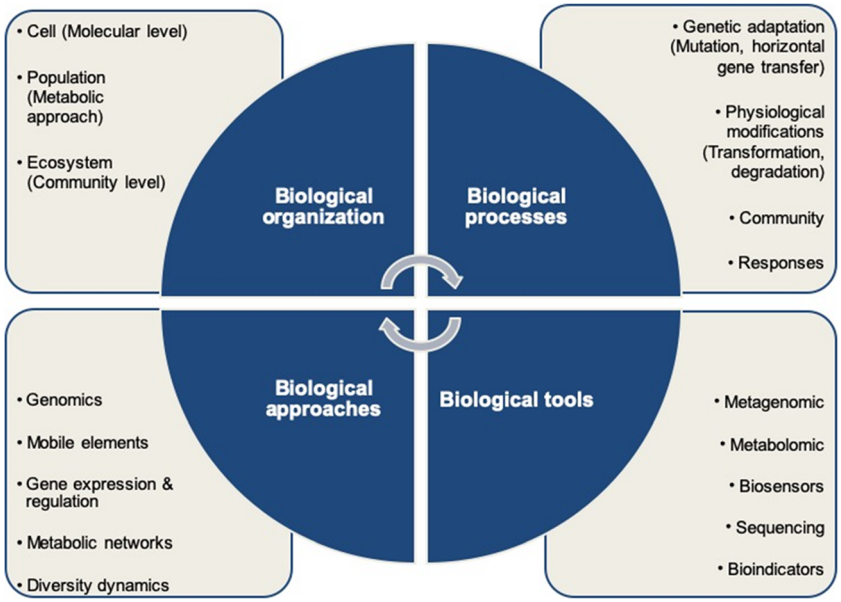


**Fig. 3** Microbial responses to the pollution at different biological organization levels

sulfamethoxazole, anthracene, and fluorine to its simpler form by *Phanerochaete chrysosporium* was also studied [5, 176]. Some of the microorganisms like *Streptomyces albogriseolus*, *Pseudomonas* sp., *Bacillus subtilis* TL-171, and *Rhodococcus erythropolis* djl-11 could able to metabolize and degrade fungicide "carbendazim" [9, 127, 190].

Algae also showed their potential in degrading organic pollutants. In a well-researched article, it was found that *Scenedesmus obliquus* GH2 can degrade both aromatic and aliphatic hydrocarbons with an artificial microalgae-bacterial consortium [145, 168]. Moreover, many algal species like *Desmarestia* sp. and *Caepidium antarticum* are capable of degrading hydrocarbons [26]. Some algae like *Scenedesmus obliquus* and *Scenedesmus quadricauda* can remove dimethomorph and pyrimethanil from their environment efficiently [40, 124, 136].

## Distribution of Inorganic Pollutants Degrading Microorganisms

Microbial remediation of Cr (VI) by the implementing mixed cultures of *Pseudomonas* sp. was investigated by some researchers [8]. Few other fungal species like *Aspergillus niger* [143], *Fusarium oxysporum* NCBT-156 [7], *Pichia anomala* M10 [96], and *Saccharomyces cerevisiae* [94] could be able to remove Cr (VI). Some of heavy metals like mercury (Hg) by *Pseudomonas aeruginosa* [185], lead (Pb) by *Pseudomonas* sp. [86], copper (Cu) by *Eichhornia* sp. [35, 129], zinc (Zn) by *Rhodobacter capsulatus* [134, 146], and cadmium (Cd) by *Bacillus cereus* [64, 105] were found to be bio-degraded successfully. The degradation pathway for inorganic pollutants is mediated through the co-production intermediates. Generally, hexavalent chromium Cr (VI) gets into the cell of the microorganism through sulfate transporters. After that, it reduces to Cr (III) through the formation of unstable intermediates, i.e., Cr (V/IV) [125].

## Profiling of Biodegrading Microorganisms

The culture-dependent method generally recovers a small portion of the diversity from environments [151]. However, culture-independent approaches (transcriptomic, metabolomic, proteogenomic, and metagenomic studies) lead to specific degradation pathways [153]. Pollution history at a specific site also matters a lot. This is due to exposure of the microbial community to the pollutant previously able to act promptly in the subsequent exposure [81]. This increased level of tolerance of the microorganisms to the pollutants is due to community shifts or physiological adaptations [11, 97]. Recently, some researchers have reported regarding the shifting of microbial community structure and changes in abundance of some species with respect to high concentrations of pollutants in the ecosystem [70, 101]. The microbial resistance and adaptation to specific pollutants may operate through overexpression or higher frequency of genes of new genetic tolerance-related capabilities horizontal gene transfer and mutation [171].

The culture-based methodology is applicable in studying microbial ecosystem of contaminated and natural environments; however, they are not providing solutions to analyze microbial genetic diversity [164]. It was found > 99% of prokaryotes present totally in any given environmental sample can be calculated through molecular analysis in non-culturable fraction [65]. Our biosphere is diversified with microorganisms which constitute 60% of the Earth's biomass

approximately [140]. The microbial catabolic diversity along with functional aspect is very important to understand biodegradation process of environmental pollutants. Traditional techniques for culturing the microorganisms deal with commercially available growth mediums (nutrient agar medium, LB Agar medium, etc.) for characterization of microorganisms obtained from the environmental sample [120]. The culture-dependent methodologies are useful to characterize the very small population and diversity (< 1%) of microorganisms in any polluted sites. The microorganisms which are viable in the natural environment and they are not cultivable in the laboratory conditions are known as viable but non-culturable (VBNC) organisms [108]. In general, VBNC organisms possess novel group and are found to be more active in the bioremediation process [120]. In culture-independent methods, the primary source of information can easily be obtained that lies in nucleic acids, lipids and proteins.

Broadly, the functional and compositional diversity of microorganisms are analyzed by various techniques viz. clone libraries, DNA microarray, isotope array, genetic fingerprinting, in situ hybridization, etc. The post-genomic approaches like metaproteomics, metatranscriptomics, and proteogenomics help researchers to assess the composition of microbial diversity in the contaminated site [164]. However, the post-genomic approaches have their own limitations. Sometimes, very low level or un-expressible environmental genes present in the microorganisms. It can be improved by transforming metagenomic DNA into several additional surrogate hosts such as *Pseudomonas*, *Bacillus*, *Streptomyces*, and *Agrobacterium*. In many cases, isolation of genomic DNA from pollutant sites (sludge, wastewater, sediment or soil) is challenging as per its quality, molecular size, and representation of all microbial genomes. Sometimes, inhibitory contaminants are frequently found, and DNA is found to be shared with low-size fragments. Heavily pollutant-affected sites often harbor very low cell densities. Therefore, metagenomic DNA cannot be extracted directly and unable to produce enough genomic material for the construction of the library. Profiling pattern with respect to various methodologies has been illustrated in Fig. 4.

## Clone Library

In the clone library method, PCR-based method is usually followed. In this method, the total DNA/RNA of environmental sample is used to identify diversity of microbial community present in the pollutant site. The product obtained from PCR is a mixture of genes obtained from the microbial community. It is a signature of from all microorganisms along with VBNC. Amplification with the PCR for the conserved genes of 16S rRNA is found in all prokaryotes. They are conserved functionally in the microbial community [65]. Some of the conserved genes such as gyrase beta subunit (*gyrB*), RNA polymerase beta subunit (*rpoB*), heat shock protein (*hsp60*), and recombinase A (*recA*) are applied to find the differentiation between the subpopulation of bacteria [54]. A marker sequence from the DNA of environmental samples is cloned and subjected to sequencing of gene fragments [120]. A sticky end (30-A) is added to the PCR product which can make ligation of plasmid vectors with an overhanging 30-T efficiently. The sequences obtained are compared with the known sequences available in databases (GenBank, Ribosomal Database Project, Green-genes, second genome) [24].

The cloning method was executed to investigate the dynamics of microbial populations in the deep subsurface of mining impacted soil in Homestake gold mine. In this work, the authors had analyzed 230 clone sequences that reveal only phylogenetic breadth inhabit particularly in the soil samples [121]. A combination of RT-PCR, Q-PCR, and clone library methods could analyze the microbial diversity found in the subsurface sediments of Hanford nuclear waste pollution site [87]. The authors have found 13 novel phylogenetic orders in 8000 sequences within δ-proteobacteria, capable of metabolizing of heavy metals and radionuclides.

## Genetic Fingerprinting Technique

The genetic fingerprinting technique is a high-throughput method dealing with random amplified polymorphic DNA (RAPD), denaturing or temperature gradient gel electrophoresis (DGGE/TGGE), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP), and rRNA intergenic spacer analysis (RISA).

DGGE methodology has implemented to the water and soil samples to assess the structure of the microbial ecology in the pollutant sites. Some researchers have used the DGGE technique in metal contaminated site to assess the soil microbial diversity. Based on their analysis, they concluded that pollutant sites are less abundant with respect to bacterial populations [32, 117]. Sulfate-reducing bacteria from contaminated petroleum hydrocarbons aquifer was examined by fluorescence in situ hybridization (FISH) technology [75, 117]. Recently, some research groups have isolated hydrocarbon degrading bacteria and diatoms from hydrocarbon-polluted sediment in coastal mudflats by reverse-transcribed bacterial 16S rRNA technology [30]. The degradation of 2,4,6-trinitritoluene by some microorganisms (γ and β *Proteobacteria*, and *Clostridia* species) was studied recently using DGGE technology [21, 45].

The microbial diversity was assessed by RAPD technique in the soil sample contaminated with pesticides (triazolone) and chemical fertilizers (ammonium bicarbonate) [183]. ARDRA technique provides very less information about the groups of microorganisms which are found in the polluted site. However, this method is very rapid and also suitable for assessing the microbial diversity with respect to changing

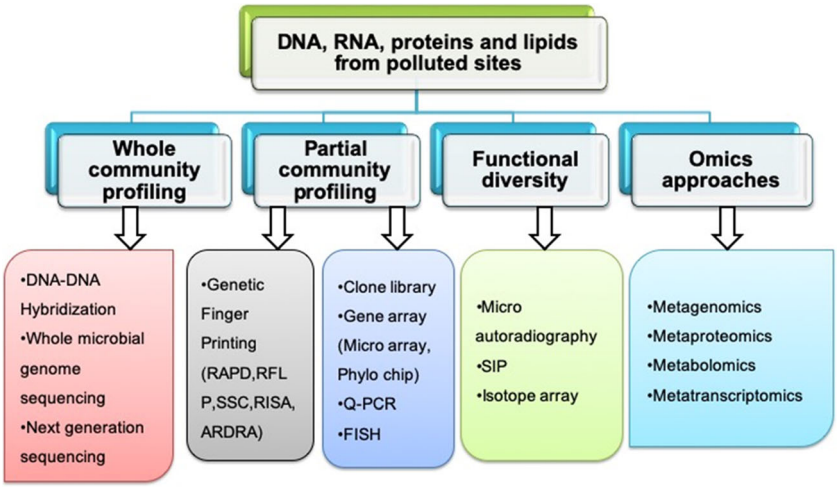


**Fig. 4** Profiling perspective for non-cultured microbial population in polluted sites

conditions of the environment. Based on the restriction profiles of clones, this technique can identify the unique clones and operated taxonomic units in environment-based clone libraries [21]. Several papers have been published in biodegradation of trichloroethane in a contaminated aquifer with the help of ARDRA technique [117, 172].

T-RFLP was developed to analyze the microbial communities with help of the clone library in a cost-effective manner [88]. An automated sequencer is used which can produce highly reproducible results for repeated samples. T-RFLP along with amplicon library was used by Allen et al. [6], to investigate the microbial community of methanotrophs inhabiting at the site of underground petroleum plume. Detection of a biodegrading hot spot was studied successfully by some researchers through T-RFLP technique in the site of tar oil-contaminated aquifer. The authors had also detected some of the toluene-degrading organisms from tar oil-contaminated aquifer [80]. T-RFLP technique was also helpful to analyze the diversity of microbial populations from soils contaminated with metals [150]. Microbial profiling of heavy metal contaminated soils was investigated using the same technique [37]. The degradation kinetics of polyaromatic hydrocarbons at low temperature was investigated by some microbial populations (α, β, and γ-proteobacteria) using RISA methodology [44].

## Functional Microbial Diversity

Functional diversity approach is currently being used to investigate the composition of microbial community and activity during the biodegradation process in a pollutant site. In this context, various metagenomics approaches like DNA microarrays, Q-PCR, microbial lipid analysis, and fluorescence in situ hybridization (FISH) have been explored to investigate the microbial degradation processes. Some of the practical applications in microbial degradation of pollutants based upon the functional gene have been summarized in Table 1.

**DNA Microarrays** Microarray is otherwise called as "microchip." It is an emerging technology in the area of genetic research. It can provide a comprehensive view of microbial dynamics and their populations in pollutant samples [53]. The pollutants to be analyzed subjected to incubate in the presence of a radioactively labeled substrate before the hybridization methodology. This will be helpful to identify the basic character of microorganisms during the degradation of a specific pollutant.

Presently, "GeoChip" microarrays have come to picture which contain 83,99,250 metric sequences covering approximately 1,52,414 genes responsible for enzymes required for resistance to heavy metals and pollutants degradation [18]. Another kind of microarray is also known as "PhyloChip" and is useful in the environmental biotechnology in the process of biodegradation for the phylogenetic analyses of bacterial communities efficiently and high-throughput manner [36].

**Microbial Lipid Analysis** Fatty acids present in the cell biomass with a constant proportion with cell biomass. Therefore, signature fatty acids exist in each microbial cell. This property helps the microbial community to be differentiated from the other groups.

The fatty acids were obtained by saponification and subjected to its derivatization producing an acid methyl ester (FAME). The signature obtained from the lipids has to compare with a reference FAME database. Banowetz et al. [12] had detected fatty acids with respect to microbial signatures with the help of multivariate statistical analyses. Analysis of soil microbial community structure by FAME profiles extracted from soils has found to be rapid and inexpensive nature. FAME analysis could successfully classify sediment soil and water samples with respect to microbial load [29, 132].

**Quantitative Polymerase Chain Reaction** This is an advanced technique used for quantification and detection of specific genes from DNA mixtures of environmental sample [58].

**Table 1** Applications of various genome-based approaches

| Sr. No | Genome-based approach | Applications | Reference |
|---|---|---|---|
| 1 | SOLiD | • Phylogenetic diversity as well as metabolic activities of communities of microorganisms in the Sargasso Sea<br>• Analysis of microbial population and dynamics from anammox wastewater treatment plant | • [189]<br>• [126] |
| 2 | Metaproteomics | Gene function and metabolic activity of acid mine drainage in microbial biofilm | [137] |
| 3 | Metabolomics | Detection and quantification of small molecules released into the environment | [4] |
| 4 | Pyrosequencing | • Detecting microbial communities along with functional genes from tannery-based wastewater treatment plant<br>• Bacterial diversity from oil-contaminated sites<br>• Detection of microbial population from the wastewater treatment plants in China | • [170]<br>• [113]<br>• [63] |
| 5 | Nanopore Sequencing | • Plasmids coding for carbapenemase was characterized in enterobacteria which was isolated from the wastewater treatment plant | [91] |
| 6 | (NGS) next-generation sequencing | • Dynamics of microbial populations were evaluated in the wastewater treatment plants<br>• Assessment of microbial communities from oil-contaminated sites in the process of bioremediation of pollutants | • [93]<br>• [113] |

Some of the researchers have determined microbial diversity from wastewater samples by using quantitative polymerase chain reaction (Q-PCR) along with T-RFLP techniques [188]. In order to quantify and characterize the active microbial community found in naphthalene, degradation process was studied by some scientists implementing this technique. In order to quantify the soil microbes, several sets of primers were also designed through rapid Q-PCR technology [47]. The Q-PCR technique was also used by some researchers in order to quantify the bacterial community and its activity of uranium-contaminated sample through in situ bioremediation process [60].

Some of the key achievements like identification of potential bacterial species like methane oxidizers, sulfate reducers, and ammonia oxidizers were characterized successfully with the help of Q-PCR technique [48]. In addition to this, bioaugmentation of atrazine-contaminated soil was also investigated by some researchers by using Q-PCR methodology [182]. Some of the functional genes namely *trzN*, *atzB*, and *atzC* responsible for degradation of atrazine were detected by the Q-PCR technology. A continuous increase in abundance of these genes during bioaugmentation process was also observed [182].

**Fluorescence In situ Hybridization** This methodology has been applied to explore bacterial communities and dynamics in the cultivated soils treated with s-Triazine [22]. In addition to this, it has also applied to investigate the simazine remediating microorganism in soil treated with s-Triazine [111].

The major limitations in fluorescence in situ hybridization (FISH) include background fluorescence, low signal intensity, and inaccessibility for the target. These limitations can be avoided by using various techniques like (a) brighter fluorochromes application, (b) treatment of chloramphenicol, and (c) amplification of signal with reporter enzymes [149]. A full-scale anaerobic sludge digester was experimented by some researchers to analyze functional community structures of bacteria and archaea with the help of FISH technology [83].

## Microbial Biosensor and Indicator—How They Respond to Pollution?

Biosensors are integrated devices that detect and quantify presence of a given chemical substance. A typical biosensor recognizes a chemical or biological reaction and gives out a signal that is proportional to the quantity of the analyte present [19]. The analyte is biological in nature and the biochemical signal generated in the biosensor is converted to a readable format using a transducer. Microbial biosensors contain immobilized microorganisms (bio element) together with a transducer (electrical element). Microorganisms show the ability to detect a wide range of signals that result from proton concentration, gaseous uptake/release, emission or absorption of light, and so on that occur due to the interaction between the organism and the analyte [112].

Hasselbach et al. [59] had utilized *Hylocomium splendens* to investigate its potential as a natural indicator for the pollution of heavy metals in northwestern Alaska. It was found that bacterium *Vogesella indigofera* reacts to heavy metals imitatively. In the absence of metal, it produces blue pigmentation which marks the morphological change that observed. Under hexavalent Cr, the production of pigment is stopped. This pigment synthesis is due to the relationship between the quantification of Cr and the blue pigment generated by the bacterium [33, 112]. Recently, a recombinant *E.coli* strain has been

developed based on the fluorescent protein expression. When the pollutants like heavy metals were exposed to these recombinant cells, the fluorescent proteins were expressed that symbolized the amount of heavy metals in wastewater samples [123]. Using silver nanoparticles, simultaneous quantitative analysis of multiple heavy metals in water samples was analyzed with multidimensional apta-sensors [141]. Recently, microbial fuel cell (MFC) was applied to detect the heavy metals present in the wastewater. In this approach, changes in voltage signal were measured through the activity of immobilized bacteria. These biosensors were introduced as a quick sensor to detect the low concentration of $Cu^{2+}$, $Zn^{2+}$, and $Cr^{6+}$ in industrial wastewater [89]. Recently, some researchers have developed an MFC-based biosensor for toxicity monitoring of the heavy metals on microbial community for a prolonged time in mining rock drainage [3]. Several reports have also introduced microfluidic-based devices which are utilizing a field-effect transistor (FET) as a signal-transducer [92, 169].

An effective system with bacterial biosensors transformed with pLUX plasmids (LuxCDABE) that is having the ability for detection of COD in pollutant water by measuring changes in the activity of bioluminescence [107]. Microorganisms usually consist of reporter genes that are constitutively upregulated by a promoter, resulting in the production of reporter proteins which act as the signal. In a recent study, a multiplex Cd biosensor has been developed by research for detection of *Psedomonas putida* 06909 [79].

There are some limitations which have been noticed in case of biosensors. The response time is found to be prolonged sometimes. In some cases, it is difficult to maintain cell viability and activity. When engineered microbes are used, the genetic stability may be found to be less durable. The diffusion rate of substrates and products across the cell membrane into cells is found slow. Environmental factors such as temperature and pH, as well as nutrient availability, also reduce the functionality.

## Role of Microbial Enzymes in the Remediation of Pollutants

Various groups of microbial enzymes viz. oxidoreductases (oxygenases, monooxygenases, and dioxygenases), laccases, peroxidases, and lipases are responsible for the degradation of the major pollutants. Some of the reported microbial enzymes and their action to remediate the target pollutants have been summarized in Table 2. Oxidoreductases catalyze the oxidation of pollutants like aliphatic olefins and chlorinated biphenyls adding the molecules of oxygen [25]. Laccase breaks the ring of aromatic compounds and does the reduction of molecular oxygen in water [135]. Wastewater treatment and polyaromatic hydrocarbon degradation are mediated by the catalytic activity of lipases [99]. Hydrolysis of phosphotriesters groups of organophosphorus pesticides is catalyzed by phosphotriesterases [130]. Carboxylesterases hydrolyzes carboxyl ester bond present in synthetic pesticides such as organophosphates [138]. The degradation kinetics for selected pollutants obtained from reported literature has been summarized in Table 3.

## Improvement of Microbial Strains by Genetic Manipulation for Enhanced Biodegradation

Various genes with the ability for degrading different groups of pollutants have been discovered from different microorganisms (as summarized in Table 4). They have provided the possibility of constructing genetically engineered microorganisms for efficient pollutants removal from the environment [159]. The genes obtained from microorganisms have been tailored to create new metabolic pathways. This leads to enhance the biodegradation mechanisms for the specific pollutants. Two pesticides degrading genes *linA* for organochlorine and *mpd* for organophosphates were integrated with the plasmid of E. *coli* to make an engineered strain which simultaneously degrades these pesticides [184]. In order to enhance the biodegradation of fenpropathrin, *pytH* gene (responsible for the synthesis of pyrethroid hydrolase) was isolated from *Sphingobium* sp. JZ-2. Later, this gene *pytH* was overexpressed in *Sphingobium* sp. BA3 helps in the construction of a recombinant strain having more biodegrading potential [41].

The enzyme azobenzene reductase or azoreductase is responsible for the removal of azo dyes from the wastewater released as industrial effluents. The azoreductase has been isolated from various microorganisms viz. *Xenophilus azovogarls* KF46F, *Enterobacter agglomerans*, and *Enterococcus faecalis* [133]. Recently, some researchers have isolated gene *fdh* (coded for formate dehydrogenase) and *azoA* (coded for azoreductase) from *Mycobacterium vaccae* and *Enterococcus* sp. L2 respectively in order to decolorize the industrial colored effluents [122].

Heavy metals can cause some physiological and genotoxic effects on different groups of microorganisms if it is exceeding a certain concentration. In order to enhance biodegradation of cadmium ($Cd^{2+}$), metallothionein gene was isolated from yeast and expressed in *E. coli* to develop a recombinant strain. This resulted strain possessed 15–20 times higher cadmium ion ($Cd^{2+}$)-enrichment capacity as compared to the parent strain [119].

A gene (*alkB*) coding for alkane monooxygenase was manipulated to the non-alkane degrading strain of *Streptomyces coelicolor* M145 strain, which could enhance the degradation ability [50]. The *xylE* is a gene which codes for catechol 2, 3-dioxygenase and essential for the biodegradation of aromatic hydrocarbon compounds. This particular gene was cloned

**Table 2** Microbial enzymes and their action to remediate the target pollutants

| S.L No | Name of the enzymes | Source organisms | Target pollutants | Reference |
|---|---|---|---|---|
| 1 | Urease | *Bacillus* megaterium strain SZK-5 | Hydrolyzed polyacrylamide (HPAM) | [142] |
| 2 | Alkane hydroxylase, lipase, esterase | *Alcanivorax borkumensis* | Hexadecane, motor oil BTEX | [148] |
| 3 | PVAase | *Bacillus niacini* | Polyvinyl alcohol (PVA) | [61] |
| 4 | Lipase, laccase, and peroxidases | *Mucor circinelloides* | Hydrocarbons | [100] |
| 5 | Laccase | *Rhodococcus ruber* | Polyethylene | [131] |
| 6 | Phthalate dioxygenase | *Variovorax* sp. BS1 | Dimethyl phthalate ester | [114] |
| 7 | Crude enzyme extract | *Actinomadura keratinilytica* strain T16–1 | Poly (DL-lactic acid) | [110] |
| 8 | Crude enzyme extract | *Bacillus cereus* | Malachite Green dye | [175] |
| 9 | Acrylamidase | *Cupriavidus oxalaticus* ICTDB921 | Acrylamide from Industrial waste water | [16] |
| 10 | Monooxygenase Diooxygenase | *Pseudomonas* sp. ASP-53 | Pyrene | [10] |
| 11 | Crude enzyme extract | *Fusarium* sp. | 4-chlorophenol | [84] |

from plasmid DNA of *Pseudomonas putida* BNF1 and inserted to the alkanes degrading strain *Acinetobacter* sp. BS3 in order to enhance its efficiency in the process of bioremediation [177]. The 26 number of bacterial strains belonging to the genera *Sphingobium* and *Sphingomonas* were sequenced completely to investigate the biodegradation of

**Table 3** Summary of degradation kinetics for selected pollutants obtained from reported literatures

| S.L No. | Microorganism(s) used | Target Pollutant | Kinetic model implementation and parameters | Specific remarks | Reference |
|---|---|---|---|---|---|
| 1 | Immobilized cells of *Halomonas* and *Aneurinibacillus* | Diesel | Monod model was fitted.<br>• $V_{max} = 1.84\ d^{-1}$<br>• $K_s = 3.23$ g/L | • The rate of degradation for the immobilized cells in straw-alginate beads was found to be 68.68%. | [179] |
| 2 | *Citrobacter* sp. NVK-2, *Providencia* sp. NVK-2A, *Citrobacter* sp. NVK-6 | Selenite contaminated water | NVK-2 strain:<br>• $V_{max} = 58.82\ \mu Mh^{-1}$<br>• $K_m = 3737.12\ \mu M$<br>NVK-2A strain:<br>• $V_{max} = 9.26\ \mu Mh^{-1}$<br>• $K_m = 3044.73\ \mu M$<br>NVK-6 strain:<br>• $V_{max} = 19.23 \mu Mh^{-1}$<br>• $K_m = 1300.17\ \mu M$ | • *Citrobacter* sp. NVK-2 was found to be potential bacterium for biodegradation of high Se (IV) concentration as having highest $V_{max}$ and $K_m$ values | [152] |
| 3 | *Pseudomonas putida* strain G3 | Butachlor | Haldane model was fitted<br>• $\mu_m = 2.74$ mg/L$h^{-1}$<br>• $K_s = 66.393$ mg/L<br>• $K_i = 1214.33$ mg/L | • The *Pseudomonas putida* G3 can degrade 100% of 700 mg/L - butachlor in 15 days | [102] |
| 4 | Mixed cultures of *Pseudomonas* and *Rhodococcus* | Atrazine, 2,4-dichlorophenoxy acetic acid (2,4-D) | Monod model was fitted.<br>For Atrazine:<br>• $\mu_m = 0.011$ L/d<br>• Y = 0.53 g/g<br>For 2,4-D:<br>• $\mu_m = 0.071$ L/d<br>• Y = 0.44 g/g | • The 2,4-D biodegradation was 6 times higher than that of atrazine | [17] |
| 5 | *Phomopsis* sp. | Anthraquinone dye | Lineweaver-Burk model was fitted<br>• $V_{max} = 8.06\ mgL^{-1}\ h^{-1}$<br>• $K_m = 62.43\ mgL^{-1}$ | • The degradation of the dye was due to production of laccase enzyme which can decolorized 200 mg $L^{-1}$ of dye within 20 min | [74] |
| 6 | *Cupriavidus oxalaticus* ICTDB921 | Acrylamide from industrial waste | Haldane model was fitted<br>• $V_{max} = 2990$ mM/min<br>• $K_m = 62.02$ mM | • The degradation of the dye was due to production of acrylamidase enzyme synthesized by *Cupriavidus oxalaticus* | [15] |
| 7 | *Fusarium* sp. HJ01 | 4-chlorophenol | The Michaelis–Menten equation was fitted<br>• $V_{max} = 11.507\ \mu Mh^{-1}$<br>• $K_m = 0.772\ \mu M$ | • The biosynthesis of chlorocatechol 1,2-dioxygenase enzyme could able to degrade the 4-chlorophenol | [84] |

**Table 4** Genes, microorganisms, and their biodegradation ability for different groups of pollutants

| Sr. No. | Source organism | Name of the gene | Targeted pollutant | Reference |
|---|---|---|---|---|
| 1 | *Sphingobium wenxiniae JZ-1* | *pbaA1A2BC* | 3-Phenoxybenzoate | [28] |
| 2 | *Acinetobacter* sp. *BS3-C23O* | *xylE* | n-Alkanes/aromatic hydrocarbons | [177] |
| 3 | *Sphingomonas* sp. *DC-6* | *CndA* | Acetochlor | [27] |
| 4 | *Pseudomonas pseudoalcaligenes* | *ophc2* | Methyl parathion | [55] |
| 5 | *Sphingobium quisquiliarum DC-2* | *cmeH* | Acetochlor | [85] |
| 6 | *Pseudomonas* sp. *CGMCC2953-pK* | *xylE* | PAHs | [192] |
| 7 | *Hansschlegelia zhihuaiae S113* | *SulE* | Sulfonylurea herbicide | [57] |
| 8 | *Streptomyces coelicolor M145-AH* | *alkB* | n-Alkanes | [50] |
| 9 | *Burkholderia cepacia* | *mph* | Organophosphorus | [43] |
| 10 | *Rhodococcus* sp. *T1* | *feh* | Fenoxaprop-ethyl | [62] |
| 11 | *Sphingobium* sp. *JZ-2* | *pytH* | Fenpropathrin | [41] |
| 12 | *Comamonas testosteroni SB3* | *dsRed* | 3-Chloroaniline | [14] |
| 13 | *Escherichia coli* JM109 | Azo reductase gene | Azo dyes, C.I. Direct Blue 71 | [71] |

PAH. They have characterized the gene cluster of *a xyl* gene in six PAHs-degrading strains [191]. The examples of various genes, source organisms, and their ability towards degrading pollutants have been summarized in Table 4.

## Future Perspectives and Concluding Remarks

The environment-friendly and low-cost bioremediation approach is considered as one of the best ways for cleaning up the polluted area. Moreover, the pollutant's degradation rate, reaction model, degradation pathways, degradation mechanisms, and physiological factors affecting the degradation should be considered for the effective bioremediation process. The degradation strategy of many pollutants along with causative genes and enzymes is also not known for many industrial pollutants. In addition to this, degraded pollutant may be found to be more persistent and hazardous than the initial pollutants. This is a major challenge and the researchers are working to overcome these problems. The concept of "nanobioremediation" has just emerged, for removing heavy metals and organic contaminants from wastewater and soil using nanoparticles synthesized by particular plants, bacteria, algae, and fungi under controlled conditions [180].

The genetic flexibility along with metabolic versatility is the key asset that opens a way for microorganisms to withstand the presence of pollutants. Microbial eco-toxicological tools not only enable to determine the concentration of pollutants but also assess the toxic effects of these pollutants at different biological levels such as genomic, metabolic, and community levels. This can be concluded that biosensors derived from microorganisms have achieved the attention of researchers because of its high specificity towards target pollutants and non-invasive nature. The degradation of environmental pollutants by the application of microorganisms is an emerging technology; however, various genetic approaches to optimize growth conditions, metabolic pathways, and enzyme production are highly useful to fulfill the demand.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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