Advancement of Omics: Prospects for Bioremediation of Contaminated Soils

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Abstract

The soil is a complex mixture of organic matter and minerals, supporting a discrete array of life. Severely polluted soils have been detoxified using a variety of microorganisms. Bioremediation is a process of removal of environmental contaminants utilizing microbes through a variety of enzymatic processes. In situ processing, high public acceptance, and a comparatively lower cost hasten the overall process of bioremediation. However, it is not always effective due to its relatively long time scales and the variable range of contaminants. Varying degrees of success rate have been noticed at different sites worldwide. This chapter attempts to link the traditional and cutting edge technologies such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics to numerous bioremediation techniques as they play a symbolic role in the study of the regulation of numerous mineralization pathways. Extensive data are being generated using these techniques, but their application is still in the infant stage. A stepwise organization of data is needed within the instructive databases. Microbial-assisted contaminant attenuation and in-depth analysis of the organism's metabolism will accelerate the overall process of bioremediation. Thereafter, the next decade will going to decipher the cellular mechanisms and molecular manipulations using an integrated omic tool approach.

Keywords

Soil · Contaminants · Bioremediation · Mineralization · Omic tool

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5.1 Introduction

The soil is the most common home of more than 10^{16} diverse microbes/ton due to its heterogeneity, favoring the formation of micro-niches (Olaniran et al. 2013). Soil contamination is a change in the biological and physiochemical nature of the soil which has a detrimental effect on the living organisms residing in it. The different types of soil contamination (Fig. 5.1) are agricultural waste, industrial waste, urban waste, and radioactive waste. The fertilizers, pesticides, industrial effluents, and radionuclides flow down to the nearby water bodies or any other soil location resulting in biomagnification. This creates an interruption in the biochemical pathways and leads to harmful diseases. Improper dumping of waste in landfills and public places results in erroneous disintegration of the waste and deposition of contaminants in the soil. Limitless deposition of waste results in increased bacterial growth that causes a rise in the generation of methane gas which eventually leads to global warming. Nuclear power plants and nuclear testing add wavering amount of radioactive material to the soil (Mishra et al. 2015).

Earlier, the disposal of waste was done by throwing the waste in a hole, but due to the lack of new areas, the practice was difficult. With the emerging techniques like chemical decomposition and incineration at high temperature, the disposal of waste became effective, but they came with several disadvantages like obscure methods, expensive, and others. Alternative techniques like bioremediation were hence implemented (Karigar and Rao 2011).

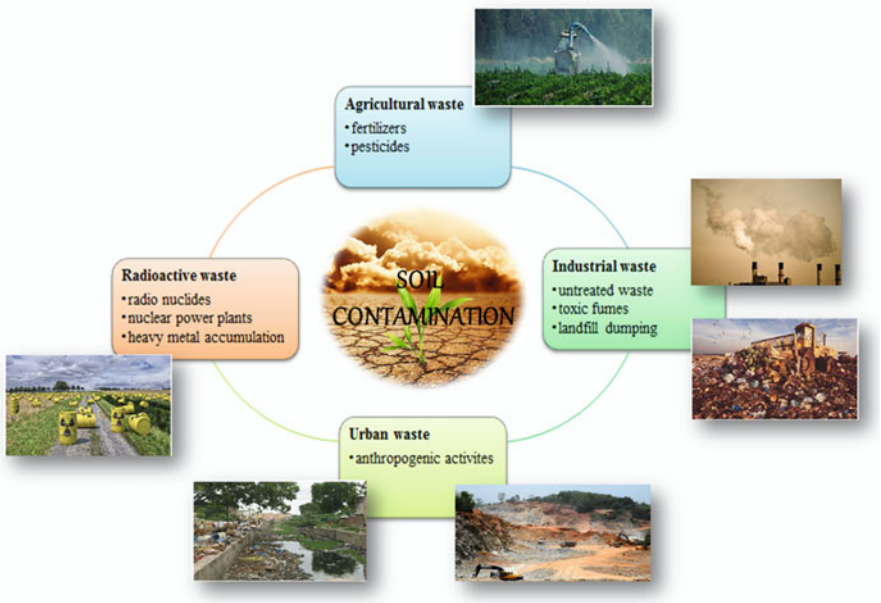

Fig. 5.1 Classification of soil contamination pertaining to their source

Bioremediation is the process of speeding up the process of natural biodegradation in the contaminated areas by the application of microbes (Calvo et al. 2009). The various strategies that are being used to remediate the soil are either removing the pollutant present in the soil or reducing its effect by stabilizing it (containment) (Cunningham and Berti 1993). In general, bioremediation strategies can be classified into the following three processes:

1. Biodegradation: Organic compounds are fragmented down into reduced inorganic or organic compounds.
2. Biotransformation: The hazardous molecules are reformed into a reduced or nonhazardous molecule.
3. Biomineralization: The organic compounds are entirely degraded into inorganic compounds like carbon dioxide or H_2O_4.

The type of contaminant determines the remediation process that will be implemented. The soil remediation cost depends on soil properties, site conditions, volume to be remediated, and type of contaminant. The increasing urbanization and industrialization lead to contamination of soil by organic and inorganic pollutants and, hence, have led to the deterioration of the environment and the human health (Dong et al. 2013).

5.2 Traditional Technologies for Soil Remediation

Along with biological, physical, and chemical concerns, remediation strategy depends upon the legitimate and economic considerations as well. Such strategies are preferred that result in minimum adulteration to the soil.

Different strategies for bioremediation of contaminated soil (Fig. 5.2) are:

5.2.1 Physical Remediation

It includes predominantly thermal desorption and soil replacement. It can be further done by replacing clean soil with contaminated soil, followed by its treatment, soil spading (the contaminated area is dug deep to spread the contaminants into deep sites and naturally degrading the pollutant), and soil importing (clean soil is added to the affected site on the surface, and mixing is done to decrease the concentration of the pollutant).

Thermal desorption is the removal of the pollutant by its volatility.

5.2.2 Chemical Remediation

1. *Chemical leaching*: Soil washing/flushing is done with reagents, surfactants, water, and chelating agents. Soil washing is a strategy where liquids like aqueous

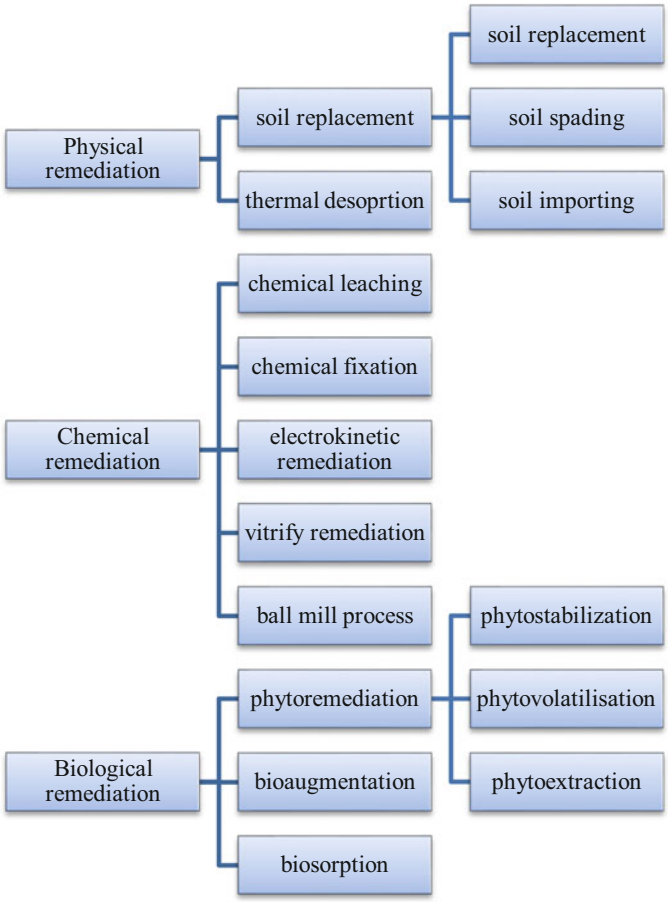

Fig. 5.2 Conventional methods for treatment of soil contamination

solutions are used to separate the pollutants from the soil. The contaminants adhere to the soil particles, but they have low water solubility. To increase the solubility, additives like surfactants and chelating agents are applied along with the process (Mao et al. 2015).

2. *Chemical fixation*: The movement of heavy metals is decreased by adding reagents. The reagents make the heavy metals insoluble in soil, thereby decreasing its toxicity (Yao et al. 2012).
3. *Electrokinetic remediation*: In this strategy, voltage is applied at the two sides of the soil to create an electric field gradient. This technique provides minimum disturbance to the topsoil and treats the lower surface contaminants (in situ) (Gan et al. 2009).
4. *Vitrify remediation*: The organic matter present in the soil is heated at 1400–2000 °C, and it is volatized. The end products (after pyrolysis and steam)

are collected and cooled to form a rock-type substance that creates hindrance in the movement of the heavy metals. This technique can be applied in situ as well as ex situ (Yao et al. 2012).

5. *Ball mill process*: Soil sample along with grinding media is added to the reactor (i.e., mill pot). In the absence of any chemical agents, the grinding process removes the contaminants and maintains the soil property as well (Shin et al. 2016).
6. *Subcritical water extraction process*: Instead of organic solvent, superheated water is used as a solvent. The water is heated at a pressure less than 22.1 MPa and temperature range of 100–374 °C. It follows the principle of pressurized liquid extraction (PLE) (Islam et al. 2013).

5.2.3 Biological Remediation

1. *Phytoremediation* is containment or removal of the contaminants by the use of green plants. Phytoremediation generally includes three processes: phytostabilization (adsorption, reduction, and precipitation of the pollutants at the roots of the plants), phytovolatilization (converting pollutant to a gaseous state), and phytoextraction (tolerant and accumulating plants are used) (Yao et al. 2012).
2. *Bio-augmentation* is the introduction of microorganisms at the contaminated site. The microbes are generally added to such areas where the microbes that can degrade the pollutants are in a low amount (autochthonous microorganisms) or the population present at the site does not have the catabolic pathway to degrade the pollutants (Yao et al. 2012).
3. *Biosorption*: Biomass (containing inactive, dead microbes) can bind to heavy metals and concentrate them. First, physical adsorption at the cell surface occurs, and then the metal ions gain access to the cytoplasm via the cell membrane. The type of microorganisms used defines the type and quantity of metal binding on them (Yao et al. 2012).

5.3 Traditional Tools of Omics

Specific genes (often 16s rRNA gene) were cloned in early environmental gene sequencing to produce a microbial diversity profile unlike microbial genome sequencing and traditional microbiology which depends upon cultivated cultures. The inherent soil microbial functions are nutrient recycling along with essential elements, the formation of organic matter, and decomposition aiding the natural process of soil bioremediation (Garbeva et al. 2004). The microbial world primarily constitutes of the organisms that are already cultured consisting of 1% of the overall soil microbial community. Using culture-based techniques, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are the most governing phyla isolated from soil (Schloss and Handelsman 2004). The viable and nonculturable

microorganisms inherently propagate in habitual environments, but then they are dormant in the laboratory or artificial surroundings. Standard culture-based approaches cannot culture these organisms, but they embrace a copious standing in the ecosystem. Hence lipids, nucleic acids, or proteins were used from the soil samples for direct assessment of their function to overcome this problem. Familiar genes such as ITS, 18S rRNA, and 16S rRNA are used as a biomarker for identification of microbial community population in the culture-independent techniques. Hence for an enhanced phylogenetic and functional categorization of the microbial community in the soil, amalgamation of specific molecular tools such as genetic fingerprinting, quantitative PCR, fluorescence in situ hybridization technique (FISH), microbial lipid analysis, stable isotope probing, microradiography, clone library method, and DNA microarray has been developed to understand the interaction of the microorganisms with various natural factors in the soil microenvironment.

Genetic fingerprinting techniques perform the direct analysis of specific molecular biomarker genes using their amplified PCR products. The relationship between diverse communities of microbes is studied using cluster-assisted analysis which compares fingerprints from various samples using software such as GelCompar. Temperature gradient gel electrophoresis and denaturing gradient gel electrophoresis (TGGE/DGGE), single-stranded conformation polymorphism (SSCP), random amplified polymeric DNA (RAPD), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), amplified ribosomal DNA restriction analysis (ARDRA), and length heterogeneity PCR (LH-PCR) are the most prominent techniques used in genetic fingerprinting. Multiple samples are evaluated at a glance through a generated community fingerprint based on sequence polymorphism.

Amplified ribosomal DNA fragments get separated using DGGE and TGGE. Identical length DNA fragments get separated based on their variable and nucleotide composition. At the 5′ end, a GC-rich primer prevents the thorough alienation of the PCR fragments. The illustration of a solo species by several bands is the constraint associated with this method (Dowd et al. 2008). The solicitation of traditional omic tools along with their sampling source is presented in Table 5.1. Using this technique, analysis of rhizospheric bacterial populations and assessment of the microbial soil community have been done in paddy agricultural soils in recent times (Srivastava et al. 2016; Schloter et al. 2018). RAPD, due to its high speed and ease of use, is considered a simpler technique for the assessment of inherently allied bacterial species, functional and structural interpretation of the microbial communities in the soil, and genetic fingerprinting. Synthetic oligonucleotide primers having random nucleotide sequences are annealed at multiple locations on the genomes at low temperature. Assessment of laboratory-scale biodegradation of fuel oil-contaminated soil has been studied using this technique (Piñón-Castillo et al. 2017). SSCP uses the principle of separation of a single-stranded DNA by electrophoresis. T-RFLP uses fluorescently labeled 5′ primers. Advanced throughput analysis and evaluation of numerous assorted samples at a lone time is the major advantage of this technique. Using bacterial 16S rRNA or 18S rRNA gene amplicon and fungal communities to breed restriction fragment contours, ARDRA serves as

Table 5.1 Application of traditional omic approaches in soil bioremediation

Traditional tools	Application	Sample	References
DGGE/TGGE	Rhizoremediation using GMO and its effect on different bacterial communities	PCB-contaminated soils	De Cárcer et al. (2007)
	Identification of compositional and structural changes in fungal and bacterial communities under different autotoxin concentrations	Cucumber seedling from agricultural soils	Zhou and Wu (2012)
	Evaluation of change in environmental conditions in carbon dioxide-rich volcanic vents	High CO_2 concentrated sites at Laacher See	Frerichs et al. (2013)
	Analysis and diversity assessment of rhizospheric bacterial population	Paddy soil	Srivastava et al. (2016)
	Assessment of soil quality using microbial indicators	Agricultural soil	Schloter et al. (2018)
	Analysis of degradation assessment of polycyclic aromatic compounds	Agricultural land around gas plants	Shahsavari et al. (2019)
RAPD	Evaluation of the soil microbial genetic structure by soil microbial community analysis	Multisampling points of rhizospheric and nonrhizospheric soils where *Panax ginseng* had been grown for 3 years	Li et al. (2010)
	To determine the genetic fidelity of micro-propagated plants	*Eclipta alba*, a medicinally important plant	Singh et al. (2012)
	Analysis of induced heavy metal *Hibiscus rosa-sinensis*	*Hibiscus rosa-sinensis* plant	Bhaduri and Fulekar (2015)
	To determine the laboratory-scale biodegradation by autochthonous bacteria	Fuel oil-contaminated soil	Piñón-Castillo et al. (2017)
	Viral and bacterial community responses to subsurface Fe (III) reduction	Subsurface soil	Liang et al. (2019)
SSCP	Distribution and diversity of polyhydroxyalkanoate-producing bacteria	Agricultural soil	Gasser et al. (2009)
	To analyze rhizosphere and fungal diversity	Canary Islands	Zachow et al. (2009)
	Phylogenetic studies of soil microbial communities	Urban storm water sedimentary layer	Badin et al. (2012)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Rapid profiling of soil microbial communities	Agricultural topsoil	Stefanis et al. (2013)
	Analysis of microbial diversity	Landslide soil	Guida et al. (2014)
	Assessment of soil microbial community and impact on agricultural land management	Agricultural soil, Mediterranean region	Bevivino and Dalmastri (2017)
	Hybridization in the *Thaumetopoea pityocampa-wilkinsoni* complex	Pine soil samples	Petrucco-Toffolo et al. (2018)
	Analysis of microbial community diversity in contaminated soil	Agricultural soil	Panigrahi et al. (2019)
T-RFLP	Evaluation of bacterial microbial diversity between two pinyon rhizosphere soils samples and two tree interspace soil samples	Pinyon rhizospheric soil and interspace soil samples	Dunbar et al. (1999)
	Compositional change in response within bacterial communities to AM extraradical mycelia in artificial conditions	Arbuscular mycorrhizal infected plants on bulk soil samples	Toljander et al. (2007)
	Yield determination of oilseed rape monocultures	Rhizospheric and bulk agricultural soils	Hilton et al. (2013)
	Myxomycetes' characterization in two different soil samples	Agricultural soil	Hoppe and Schnittler (2015)
	Comparison of microbial communities under Cacao agroforestry, Peru	Tropical soil, Peru	Buyer et al. (2017)
	Acetogenic contribution to anaerobic degradation in rice field soils	Rice field soils	Fu et al. (2018)
	Assessment of microbial diversity	Rhizospheric soil, Trindade Island, Brazil	Camacho-Montealegre et al. (2019)
ARDRA	Inoculation of PGPB strain between mother and daughter strawberry plants via stolon	Bulk soils growing strawberry plants	Guerrero-Molina et al. (2012)
	Assessment of microbial diversity in different soils	Pepper-grown field	Lee et al. (2006)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Isolation of clusters of soil and identification of isolates having antagonistic properties against *Phytophthora capsici*	Soils from glacial area	Yang et al. (2012a, b)
	Assessment, development, and evaluation of a biopile for remediation of soil	Hydrocarbon-contaminated soil	Baldan et al. (2015)
	Comparison of bacterial diversity for forensic applications	Agricultural soil	Habtom et al. (2017)
	Analysis of nitrogen-fixing bacterial community during the rice-growing season	Agricultural soil	Chakraborty and Islam (2018)
LH-PCR	Differentiation of soil bacterial community structure	Two different soil types with conventional and continuous grass plots	Ritchie et al. (2000)
	Characterization of phylotypes in soil fungal communities	Tomato-cultivated agricultural soils	Wu et al. (2008)
	Assessment of soil microbial properties by fecal detritus interaction	Pasture soil	Slade et al. (2016)
	Analysis of nitrogen cycling during decomposition by vertebrates	Forest soil	Keenan et al. (2018)
	Assessment of bioremediation potential of contaminated soil	Municipal waste-dumped soil	Awasthi et al. (2019)
RISA	To determine the effect on the structure of the rhizobacterial community in field-grown maize by PGPR *Azospirillum lipoferum* CRT1	Maize-grown agricultural topsoil	Baudoin et al. (2009)
	Estimation of species richness from multiple samples in different environments	Decatur silt loam soil	Mathew et al. (2012)
	Analysis of soil microbial communities	Agricultural soil	Navarro et al. (2015)
	Analysis of microbial community structure and diazotrophic abundance	Paddy soil	Srivastava and Mishra (2018)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Characterization of microbial communities in industrial soil	Industrial soil	Shekhar et al. (2020)
Quantitative PCR	Evaluation of bacterial diversity in an acidified soil by 16s rDNA analysis	Acid forest soil, Mt. Coot-Tha, Brisbane, Australia	Stackebrandt et al. (1993)
	Determination of abundant population of bacteria and specific methanotrophic groups	Flooded rice fields	Kolb et al. (2003)
	Evaluation of common microbial taxonomic groups using taxon-specific real-time primers	Agricultural soil	Fierer et al. (2005)
	Quantitative detection of sulfate reducers, methane oxidizers, and ammonia oxidizers by targeting dsrA, pmoA, and amoA genes	Saline and hypersaline soda lakes	Foti et al. (2007)
	Effect on the ammonia-oxidizing activity by bacterial communities in the rhizosphere of a fluvo-aquic soil by long-term fertilization	Fluvo-aquic soil	Ai et al. (2013)
	Assessment of soil acidobacterial communities	Amazon forest soil and soybean croplands	Navarrete et al. (2013)
	Analysis of soil microbial diversity and abundance of relic DNA	Agricultural soil	Carini et al. (2017)
	Quantification of *Fusarium* species in the root rot complex	Field pea soil	Zitnick-Anderson et al. (2018)
	Assessment of microbial respiration by digestate application	Weathered petroleum hydrocarbon-contaminated soil	Gielnik et al. (2019)
FISH	Evaluation of different microbial communities and its phylogenetics and diagnostics	Bulk agricultural soils	Moter and Göbel (2000)
	Detection of live bacteria in *Arabidopsis thaliana*'s root segments by CARD-FISH	*Arabidopsis thaliana* plants grown in natural soil conditions	Lundberg et al. (2012)
	Attenuation of complex tar oil in soil	Tar oil-laden soil	Ivanov et al. (2017)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Physiochemical and microbial analysis of dumpsite soil	Dumpsite soil	Oshoma et al. (2017)
	Analysis of microbial bioremediation of pollutants	Agricultural soil	Bharagava et al. (2019)
Microbial lipid analysis	Assessment of soil microbes using lipid extraction	Agricultural soil	Oates et al. (2017)
	Analysis of microbial nematodes in aggregates of soil	Red soil	Jiang et al. (2018)
Stable isotopic probing	Investigation of groundwater to characterize *Pseudomonas* species which degrade naphthalene	High pseudomonas population in groundwater microcosms	Huang et al. (2007)
	Detection of spatial variation within active microorganisms in relation to rhizospheric carbon flow	Rice microcosms in rhizospheric soils	Lu et al. (2007)
	Identification and assessment of *Planctomycetes* of a complex heteropolysaccharides	Agricultural soil	Wang et al. (2015)
	Analysis of active diazotrophs	Agricultural soil	Angel et al. (2018)
Microradiography	Characterization of autotrophic nitrifying bacteria in biofilms	Nitrogen-rich soils	Okabe et al. (2004)
Clone library	Characterization of microbial diversity in subsurface mining-affected soils	South Dakota, USA	Rastogi et al. (2009)
	Assessment of carbon fixation rates and bacterial diversity	Agricultural soil, China	Lynn et al. (2017)
	Identification of *Bacillus* community in Ararat plain, Armenia	Saline alkaline soil	Panosyan et al. (2018)
	Identification of heavy metal hyper-tolerant eukaryotic aldehyde dehydrogenase	Metal-contaminated soil	Mukherjee et al. (2019)

the most crucial tool in the process of unique clone identification obtained from the environment. Analysis of nitrogen-fixing bacterial communities in peak rice-growing season has been done using this technique (Chakraborty and Islam 2018). RISA analyzes the phylogenetic diversity of the microbes built on the intergenic length adjustment in the transcribed spacer region within the 23S and 16S genes for prokaryotes and 23S and 18S encoding rRNA genes for eukaryotes (Fuhrman et al. 2008). Quantitative PCR determines the manifestation and plethora of operative and taxonomic gene markers in the exploration of soil microbial communities (Bustin et al. 2005). SYBR green fluorescent dyes or fluorescent probes measure the accumulated amplicons in each cycle of PCR. Quantification of *Fusarium* species in root rot complex in field pea soil has been studied using quantitative PCR (Zitnick-Anderson et al. 2018). The conjugation of a fluorochrome with an oligonucleotide probe is the basic principle behind FISH. Due to its immense genetic stability and high copy number, probes of 16S rRNA are conventionally exploited in this technique. On hybridizing the homologous sequence to its fluorescent probe, the fluorescent intensity is measured using a fluorescent microscope. Lipids as opposed to nucleic acids are used in microbial lipid analysis for the assessment of soil microbial communities (Banowetz et al. 2006). The biomass of a cell has a constant amount of fatty acids which is stable in nature and gives a clear differentiating picture between the different taxonomic populations of microbes. Extraction of fatty acids is done using saponification and derivatization, generating FAMEs which are further analyzed through gas chromatography. The radiolabelled substrate is used in microradiography by metabolic active cells. Combining microradiography with FISH identifies the phylogenetic active cells which are radioactive in nature and consume the substrate (Rogers et al. 2007). The individual gene fragments are sequenced following cloning in the clone library method. The PCR-obtained sequences are compared to green gene, ribosomal data projects, and gene banks (DeSantis et al. 2007). Characterization of the microorganisms in environmental samples is also done using DNA microarrays. DNA from the environmental samples generates fluorescently labeled amplified PCR products which are directly hybridized to the microarrays having known sequence molecular probes (Gentry et al. 2006). The overall evaluation process is enhanced due to the rapid replication by the DNA microarray, aiding as a significant advantage. The intensity of the signal on the microarray is directly related to the ampleness of the target organism in the sample. Besides having enhanced responses, these conventional techniques also have their specific limitations. However, with the integration of advanced omic tools, the process of microbial community analysis has been undergoing an enhanced revolution with improved understanding and application to decipher the role of microbial communities in soil bioremediation.

5.4 Advanced Omic Tools

The DNA-based molecular techniques do not provide detailed information about gene expressions under in situ conditions. Hence sequences within the metagenomic databases from uncultured microbes provide fruitful insight into the functional microbial diversity. Therefore, post-genomic methodologies such as metagenomics, metatranscriptomics, proteogenomics, and metaproteomics provide a potential connection among the genetic and functional resemblances between numerous communities of microbes, hastening the process of soil bioremediation.

The direct collection of microbial genomes from ecological samples is known as metagenomics, community genomics, or environmental genomics (Riesenfeld et al. 2004). The communication of uncultured organisms with altered environmental factors and their biochemical role can hence be studied using metagenomics. Using functional metagenomic libraries, various functional molecules such as microbial enzymes (lipase, amylase, cellulase) and antibiotics, by companies such as Terragen are derived (Rondon et al. 2000). In the aerobic conditions, the acid sulfate soil microbial community was characterized to study the structural and functional genes, using the tools of metagenomics. Both the topsoil and parent materials underwent significant changes on incubating in aerobic conditions. The archaeal community significantly decreased, whereas the sulfur-cycling genes enhanced in the parent material (Su et al. 2017). However, at the genetic level, the relationship between community composition and taxonomic diversity remains to be determined.

Metatranscriptomics or environmental transcriptomics is the study of the variations in the microbial expression of genes under specific conditions by capturing the total mRNA (Moran 2009). Recently, the overall phylogenetic pool of the functionally and taxonomically appropriate microbial communities has been analyzed using the double-RNA method or both the rRNA and mRNA, noticing a considerable diversity among the microbial communities. Recently, through direct species electron transfer, the interaction between *Methanothrix* and *Geobacter* was studied in paddy soils under methanogenic terrestrial environments. *Methanothrix* is the prominent microbial contributor in the global methane production, but very little is known about its physiology and ecology. The transformation of methane from acetate serves as an important contribution by *Methanothrix* in terrestrial ecosystems (Holmes et al. 2017). Nitrogen-transforming reductive gene transcripts were identified through metatranscriptomics in waterlogged paddy soils. Reductive nitrogen transformation was actively induced due to the presence of anoxic environments (Masuda et al. 2017). Using rice straw as a source of carbon, the severity of seawater salinity on paddy fields was studied to observe the significant changes in mRNA expression throughout the whole community (Peng et al. 2017). The diversity of microorganisms is crucially analyzed using metatranscriptomics, elucidating the community composition and deciphering their potential in soil bioremediation.

Metaproteomics or environmental proteomics is the qualitative and quantitative study of proteins on a large scale of diverse microbial species (Wilmes and Bond 2006). Under stressful conditions, proteofingerprints are generated to indicate the functional status of microbial societies (Keller and Hettich 2009). In recent times,

metaproteomics has been exercised in various environments such as sediments, soil, freshwater, and marine systems. However incorrect metagenomic information, flush variety of microbial organisms, and soil heterogeneity have a negative influence on the process described (Wang et al. 2016). The bacterial metabolic functions correlated with a plant in serpentine soil contaminated with nickel, cobalt, and chromium were also interpreted using metaproteomic approach. (Mattarozzi et al. 2017). A bacterial protein database was constructed through the genera identified using 16S DNA profiling. A continuum of bacteria is revealed by the proteins involved in response to stimulus and transportation of nutrients. The bacterial biocatalysts play a vital part in the evolution of a post-petroleum bio-based economy, but the difficulty in analyzing the genetic information limits the biocatalyst's capability (Sukul et al. 2017). The bioactivity from the proteome of an environmental sample can hence be efficiently analyzed using functional metaproteomics. Hence biomass quantification in metaproteomics serves as an important tool to interpret the correlation between various soil microbial communities in a sustainable environment.

Metabolomics characterizes the response of soil microbial communities to specific biological factors, abiotic pressures, and its immediate environment. Organic, low molecular weight biomolecules occurring naturally in a cell, tissue, or biofluid are known as metabolites. A comprehensive study of metabolomics is the production of a range of metabolites or metabolomes in communication with a natural environmental stimulation (Miller 2007). Recently, metabolomics has had numerous prominent applications in the field of environmental sciences such as the development of biomarkers, feedback to altered levels of environmental stress, assessment of risks on toxicant exposure, and disease monitoring and diagnosis (Viant 2009). Prominent deviations were observed in the metabolic pathways of *S. meliloti.* Simultaneously this could be related to other bacteria experiencing a series of abiotic pressure. Some of the noticeable modification changes were the presence of intermediates in myo-inositol degradation and changes in the biosynthesis of exopolysaccharides and pentose phosphate pathway (PPP). In accordance to metabolic adaptation, enhanced acid tolerance phenotypes and improved competitiveness in nodulation are associated with the same in rhizobia (Draghi et al. 2017). Osmotic adjustment and osmoprotection were calculated in the classical species of cowpea (Goufo et al. 2017). The organic solutes in plants uphold an optimal turgor by enacting as osmolytes or as radical scavengers to protect the metabolic functions and hence survive the extreme drought conditions. By analyzing the salt-tolerant mechanisms and the metabolic profile of plants, enhanced sustainable crop production can be achieved worldwide, leading to the development and protection of natural plant reserves and hence aiding the bioremediation potential of the soil microbial community.

5.5 Application of Omic Tools in Bioremediation

Microorganism utilizes organic compounds as a sole carbon source and to manage their biomass and assemble suitable enzymes and cofactors for their oxidation/reduction. Hence, the organic compounds should be nontoxic or less damaging to microbial growth. The microorganisms participating in the metabolic degradation of organic compounds are heterotrophic. Molecular methods like cloning, fingerprinting, ARISA, RFLP, etc. are used to study microbial diversity (Fig. 5.3). These techniques yield information on how environmental factors change the microbial community structure. More advanced techniques like Illumina and 454 sequencing are also being used to study the microbial diversity of the polluted areas. Different approaches are used to remediate contaminated soils (Yergeau et al. 2014). The present scenario includes the implementation of various omic tools (Table 5.2) to study the microbial diversity of the contaminated soil with that of uncontaminated soil, thus providing better insight for the development of the new remediation technique or improving the already existing methods.

The uptake of heavy metals like mercury can lead to biomagnification. The heavy metals interrupt with the energy metabolism of the plants. Transcriptomics helps in the early detection at molecular levels. The changes in the genes in the presence of a low and high concentration of metals can also be studied (Villiers et al. 2012;

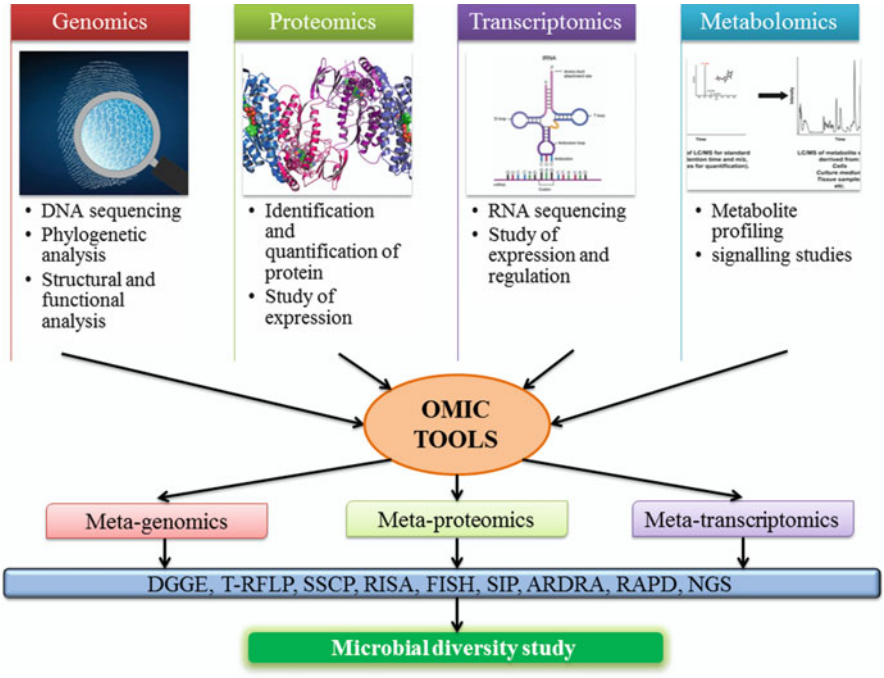

Fig. 5.3 Application of omic tools in soil bioremediation: a conceptual framework

Table 5.2 Application of advanced omic approaches in soil bioremediation

	Contaminants	Omic tools	Applications	References
Biotransformation	Mercury	Transcriptomics	Analyzing metabolic pathway and tolerance response	Beauvais-Flück et al. (2017)
	Arsenic	Transcriptomics, proteomics, metabolomics	Analysis of transport and accumulation as in plant	Tripathi et al. (2012)
	Zinc	Proteomics	Differentially expressed proteins in *Arabis paniculata*	Zeng et al. (2011)
	Cadmium	Proteomics, transcriptomics	The interaction between *Arabidopsis halleri* and selected bacterial strains	Farinati et al. (2011)
			Study the response of the plant to cadmium, viz., high-throughput techniques	Villiers et al. (2012)
		Metabolomics	Analysis of metabolic and growth profile on cadmium-contaminated tomato plants	Hediji et al. (2010)
			Study of the response of cadmium exposure by *Arabidopsis thaliana*	Sun et al. (2010)
	Nickel	Proteomics	Phenotype analysis after Ni hyperaccumulator In *Noccaea caerulescens* subsp. *caerulescens*	Visioli et al. (2012)
	Trace metals	Metagenomics	The decrease in microbial diversity of arbuscular mycorrhizal fungi found in contaminated soil	Hassan et al. (2011)
	Cadmium and zinc	NSG	Pyrosequencing revealed the interaction between *Arabidopsis halleri* and the microbial community	Muehe et al. (2015)
	Copper	Genomics	DGGE analysis to study the microbial diversity of contaminated and uncontaminated soil	Altimira et al. (2012)
	Uranium, nickel, cobalt, cadmium	Genomics	To determine the genomic sequence of *Caulobacter* sp. strain OR37	Utturkar et al. (2013)
Biodegradation	Hydrocarbon, pesticides, herbicides	Metagenomics	Transgenic plants that contain transgenes which either metabolize the xenobiotic or increase the resistance toward the pollutant	Abhilash et al. (2009)

			Analysis of anaerobic degradation of quinoline by denitrifying bacteria using metagenomics	Wang et al. (2017)
			Anaerobic degradation of hydrocarbons using metagenomic analysis	Espínola et al. (2018)
			To test the PAH removal potential by indigenous bacteria from Taean coast, Korea	Lee et al. (2018)
			Degradation of herbicides and pesticides by identifying a novel gene	Jayaraman et al. (2019)
	Fertilizers	Metagenomics	Shotgun metagenomic sequencing reveals a shift in the pathways of the microbes	Fierer et al. (2012)
			Analysis of organically fertilized zoo soil using metagenomics	Meneghine et al. (2017)
	Hydrocarbons	Bacterial modifications	Saline environment results in biodegradation	Le Borgne et al. (2008)
		Proteomics	Partially explains the changes that occur in the soil microbial diversity	Bastida et al. (2010)
			Proteomic characterization of plasmid PLA1 and its biodegradation potential of degrading polycyclic aromatic compounds	Yun et al. (2014)
			Proteomic analysis of the biodegradation of cyanide wastes	Luque-Almagro et al. (2016)
			Analysis of pyrene degradation by *Brevibacillus brevis*	Wei et al. (2017)
			Biodegradation and structural analysis of aniline degrading bacteria	Hou et al. (2018)
			Assessment of microbial function and diversity in petroleum-associated environments	Pal et al. (2019)
		Genomics	Using DGGE and 16S rRNA analysis, the microbial diversity of the forest soil was studied	Ahn et al. (2006)
			16S rRNA study and phylogenetic analysis were used to study the microbial community	Hamamura et al. (2006)

(continued)

Table 5.2 (continued)

	Contaminants	Omic tools	Applications	References
			Genomic tools were used to study the remediation effect of different plants was assayed via Phytoremediation	Phillips et al. (2006)
			DGGE and phylogenetic analysis revealed a shift in the microbial diversity	Labbé et al. (2007)
			The phylogenetic and biogeographic diversity of thermoacidophilic cyanidiales were studied	Toplin et al. 2008
			The contaminated and uncontaminated soils were assayed for the microbial differences	Vivas et al. (2008)
			DNA probe labeling and pyrosequencing were incorporated to study the pathway of hydrocarbon-degrading genes	Bell et al. (2011)
			The PCR-DGGE analysis was used to assess the total petroleum hydrocarbon (TPH)	Maqbool et al. (2012)
			Using RT-qPCR, the hydrocarbon degrading genes were studied	Yergeau et al. (2012)
			Monitoring and detection of genes that lead to aromatic and aliphatic degradation by oligonucleotide microarray method	Kim et al. (2014)
			Subtraction of cDNA revealed presence of zinc finger motifs in the high accumulators of organic pollutants	Inui et al. (2015)
			PCR-DGGE was used to assay the better of the two bioremediation techniques applied	Khudur et al. (2015)
			Genomic analysis and enrichment of root endophytic bacteria from *Populus deltoides*	Utturkar et al. (2016)
			Analysis of pyrene degradation by bacterial consortia	Wanapaisan et al. (2018)

			Analysis of biodegradation potential of *Rhodococcus* strain	Zampolli et al. (2019)
		Functional Metaproteome	Analysis of the biochemical pathways of the microbial communities	Benndorf et al. (2007)
			The analysis of PAH degradation by bacteria in terrestrial and marine habitats	Grube et al. (2015)
			Characterization of phenanthrene-degrading bacterial consortia and assignment of their ecological roles	Festa et al. (2017)
			Analysis of microbial community structure using rhizoremediation	Kotoky et al. (2018)
		Next-generation sequencing	Pyrosequencing helped to study the relationships among soil properties, pollution rate, and microbial diversity	Sutton et al. (2012)
			454 sequencing evaluates the microbial community with the oil spill	Yang et al. (2012a, b)
			Ion torrent and Illumina sequencing revealed high expression of hydrocarbon-degrading genes in the contaminated soil	Yergeau et al. (2014)
			Analysis of marine microbial communities in crude oil-contaminated water	Krolicka et al. (2017)
			Bacterial diversity analysis in heavy oil well reservoir	Shibulal et al. (2018)
			Microbial distribution analysis in PAH-contaminated landfill soil	Koshlaf et al. (2019)
		Transcriptomics	Analysis of biomass degradation by anaerobic fungal isolate	Couger et al. (2015)
			Analysis of polysaccharide-degrading potential by bacteria in arctic sea sediments	Rapp et al. (2016)
			Analysis of alkane degradation by *Pseudomonas extremaustralis*	Tribelli et al. (2018)

(continued)

Table 5.2 (continued)

	Contaminants	Omic tools	Applications	References
			Analysis of drainage effect on paddy soil microbiome	Abdallah et al. (2019)
		Metabolomics	Analysis of biodegradation potential of the bacterial population in contaminated crude oil	Bargiela et al. (2015)
			Study of biodegradation of azo dye by bacterial consortia	Shanmugam et al. (2017)
			Analysis of biodegradation of hydrocarbons anaerobically	Gieg and Toth (2018)
			Analysis of nanomaterials in agricultural soil	Zhao et al. (2019)
	Herbicide	Metabolomics	Study of alachlor biodegradation by *Paecilomyces marquandii*	Szewczyk et al. (2015)

Beauvais-Flück et al. 2017). The tools of genomics like DGGE (denaturing gradient gel electrophoresis) of 16S rRNA enhance the study of several communities of microbes in non-polluted and polluted soils and, therefore, help in the isolation of the heavy metal-resistant bacterial strains (Altimira et al. 2012; Utturkar et al. 2013). The adaptation of any organisms to the surroundings is reflected in their biological activities which can be calculated by doing transcriptomics, proteomics, and metabolomics analysis. Thus, the techniques of the bioremediation can be enhanced, and the scope of remediation technique can be improved (Hediji et al. 2010; Tripathi et al. 2012). The bacterial soil community affects the uptake of metals by plants by either stimulating the plant growth or by metabolizing the heavy metals. Pyrosequencing, a next-generation tool, of 16S rRNA provides a better picture of plant-metal-microbe interaction in the soil (Muehe et al. 2015).

Phytoremediation is one of the cost-effective remediation techniques in use for years now. The purpose of plants to attenuate the xenobiotics makes them more feasible method than the physical and chemical processes. The transgenic plants result in either degradation of the xenobiotics or increased resistance of the plant to the pollutant (Abhilash et al. 2009). The industrial effluents are estimated to be 5% saline and hypersaline. Microbial diversity is less as compared to non-extreme environments. Thus degradation of the pollutant becomes a significant problem in such regions. The halophilic microorganisms are proposed to be a favorable applicant for the remediation of the hypersaline environments. Though the metabolization of hydrocarbon reduces at high salt concentration, a lengthy exposition period has shown a significant amount of degradation and metabolized hydrocarbons (Le Borgne et al. 2008). The genomic sequence analysis discloses the genes that might be involved in the degradation. Further, proteomic analysis of the microbe in the presence of different concentrations of hydrocarbons affirms the genes involved in the degradation (Yun et al. 2014; Wei et al. 2017). Application of techniques like RT-qPCR quantifies the expression of various hydrocarbon-degrading genes and thus provides an insight into the shift in the microbial communities (Yergeau et al. 2012).

5.6 Future Prospects

There have been unprecedented changes in the field of microbial ecology with the augmentation and advancement of numerous molecular-based omic tools. The inherent functional and taxonomical diversity of the innate communities of microbes present in the soil has been investigated using diverse post-genomic approaches, revealing the superficial knowledge about the metabolic and genetic heterogeneity present in the most copious organisms in the planet, known as the prokaryotes. Implicit questions, for instance, “How the physical, chemical, and biological factors regulate the microbial communities?” and “How many bacterial species are currently present in the planet?”, and the broad knowledge about the metabolic diversity in the elementally present microorganisms still remain to be understood. As most of the classified genes have no autologous sequences in the present databases, deciphering

the utilitarian roles of uncultured organisms has become an appalling task. Numerous technical challenges still remain to be overcome although there has been immense progress in the field of identification and classification of the intrinsic microbial communities in the soil by the application of proteogenomic, transcriptomic, and metagenomic approaches. New insights into the soil microbiology can be provided using interdisciplinary omic system technologies, revealing the interaction between proteins, genes, and environmental factors. Hence the upcoming years will see a prioritized area of research in the development of consequential multi-omics approaches.

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