Chapter 10
Advances and Applications of Bioremediation: Network of Omics, System Biology, Gene Editing and Nanotechnology

Rohit Chakraborty, Sahita Karmakar, and Waliza Ansar

Abstract Environmental pollution has been on the incline in the recent decades owing to expanded human movements on energy utilizations, perilous agricultural techniques, and surge in industrialization. Heavy metals, pesticides, various nuclear wastes, greenhouse emitting gases, and hydrocarbons are the well-known pollutants that cause environmental and human health problems due to their toxicity. Bioremediation pinpoint the involvement of chemical machinery in environmental decontamination of pollutants by microbial discourse or web through in situ or ex situ outcome. For degrading the pollutants, in situ process required bioaugmentation, biosparging, and bioventing while ex situ bioremediation involves composting, bioreactors, electrodialysis, land farming, and biopiling. Microorganisms utilizing hydrocarbon as the sole resource of carbon and energy have a vital role in the biodegradation of pollutants. Due to the continuous environmental variations, the microorganisms thriving in that environment are well equipped to survive. The actinomycetes, fungus, and thermophylic bacterium like microbes in different biomes have been isolated in biodegradation. With the improvement of scientific technologies, the system biology, omics (proteomics and glycomics) nanotechnology, and gene editing tools are being used in bioremediation of heavy metal pollutants, plastics, petroleum, organic pollutants, or other hydrocarbon, acid leachate, biofilm formation, and xenobiotics. System biology approaches are very promising in decoding the existence of microbial populations under varied environmental setup. Omics such as proteomics and genomics aid in analyzing genetic or protein-level regulation for bioremediation with sequencing, MALDI-TOF, and novel functional genes' involvement in bioremediation pathways of pollutant degradation.

Keyword Bioremediation · Microbial nanotechnology · Gene editing · System biology · Proteomics · Glycomics · Genomics · Xenobiotics · Pollution

R. Chakraborty · S. Karmakar
Department of Environmental Science, Asutosh College, 92, Shyama Prasad, Mukherjee Road, Kolkata 700026, India

W. Ansar (✉)
Department of Zoology, Behala College, 32 Upen Banerjee Road, Parnasree, Behala, Kolkata, West Bengal 700060, India
e-mail: waliza_ansar@yahoo.co.in

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Abbreviations

CMC	Critical micelles concentration
CNTs	Carbon Nanotubes
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DCE	Dichloroethane
DDE	2, 2-Bi's(p-chlorophenyl)-1, 1-dichloroethylene
DDT	Dichlorodiphenyltrichloroethane
HCNTs	Hybrid carbon nanotubes
HDR	Homology directed repair
MB	Microbial bioremediation
MNPs	Magnetic nanoparticles
MWCNTs	Multi-walled carbon nanotubes
NHEJ	Non-homologous end joining
NMs	Nanomaterials
NPs	Nanoparticles
NZVI	Nanoscale zero-valent iron
PAHs	Polycyclic aromatic hydrocarbons
PAM	Protospacer adjacent motif
PAMAM	Polyamidoamine
PBS	Polybutylene succinate
PCE	Perchloroethylene
PCL	Polycaprolactone
PE	Polyethylene
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PLA	Polylactic acid or polylactide
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
SVocs	Semi-Volatile organic compounds
SWCNTs	Single-walled carbon nanotubes
TALENS	Transcription activator-like effector nucleases
TCE	Trichloroethylene
Vocs	Volatile organic compounds
WRF	White rot fungi
ZFNs	Zinc finger nucleases
ZFPs	Zinc finger proteins

10.1 Introduction

Due to extensive urbanization, industrial progress, and recent agricultural processes, an unleashed upsurge of varied contaminants or pollutants are outsourced into the nature. These pollutants can pollute medium in air, water, and soil and cause abhorrent changes in the environment. Pollution can cause biodiversity losses, huge deforestation, soil degradation, and damage to human well-being and wealth. These pollutants are heavy metals (cadmium, zinc, arsenic, nickel, chromium, mercury, lead, and copper), combustion pollutants like carbon monoxide (CO), ammonia, emissions of chlorofluorocarbons (CFCs), hydrocarbons, organic polluting compounds (dioxins, furans, and volatile organic compounds, VOCs), nitrogen and sulfur oxides and dioxides, and various particulate matter. Many of these pollutants may act as potent or suspected mutagens and carcinogens and may modify ecosystem regulations. Thus, a number of eco-friendly cleanup technologies have been advocated using phytoremediation, eradication, bioremediation, bioattenuation, physical and chemical bioremediation. For environmental remediation, age-old unsustainable methods of treatment like pump-and-treat, isolation, and disposal to landfill are gradually turning up to be redundant. Expansion of alternative sustainable treatment techniques provides effective remediation of contamination and also restoring the integrity of natural habitats and niche.

10.2 Bioremediation: The Network of Biochemical Process

Bioremediation is the outcome of different chemical reactions to degrade contaminants by creating a web of metabolic pathway at the contaminated site. Common bioremediation methods include bioleaching, land farming, bioventing, bioreactor, bioslurping, bioaugmentation, composting, natural attenuation, phytoremediation, biostimulation, and rhizofiltration [1]. Bioremediation of an on-site polluted zone mostly interplay in two dissimilar ways. For the first method, optimal conditions (like nutrients, temperature, and presence of oxygen) are utilized to its maximum to stimulate or trigger to the growth of indigenous microorganisms (pollutant-eating microbes) inhabiting the contaminated site. Genetically modified microbes can also be engineered and imposed on the contaminated site for better result. Contaminants can also be carried to a second site, processed according to granularity or contaminant nature, and then, microbes were added to continue the bioremediation process.

Bioremediation (unique eco-friendly method) is the interaction of biochemical processes inclined to decompose and expand microorganisms metabolic network at the polluted site by in situ or ex situ methods [2]. Both aerobic and anaerobic processes of degradation cascade have been shown in the mineralization and stabilization of pollutants. In diverse environmental setup, both aerobic and anaerobic methods may be applicable in single mode or in complex mode. *Pseudomonas, Sphingomonas,*

Alcaligenes, Mycobacterium, and *Rhodococcus* bacterial species degrade hydrocarbons and pesticides under aerobic conditions by utilizing carbon as the main source of energy.

Within the arena of bioremediation, phytoremediation (Fig. 10.1) offers promising benefits with the synergistic employment of plants and microbes. Moreover, various plants exhibit a diversity of additional decontamination methods in comparison with microbes' population alone. The science of system biology and multi-omics provides information about the microbial biology and inter-microbial interactions [3]. All these microbes-related operating systems require optimal conditions to respond and propagate. But in environmental stress (such as extreme temperature, availability of oxygen, and pressure) settings, depending on the type of contaminant and the dose of the inflicting pollutant, microbes respond differentially [4]. Thus, system biology with the aid of genomics, proteomics, transcriptomics, and metabolomics helps in identifying genetic level regulation, target proteins, post-translational modifications, metabolic cascade, and signal transduction pathways analysis for bioremediation. Gene level regulation can be identified by next-generation sequencing and high throughput sequencing to pinpoint the novel functional genes engrossed in bioremediation pathways of assorted relentless contaminants [5].

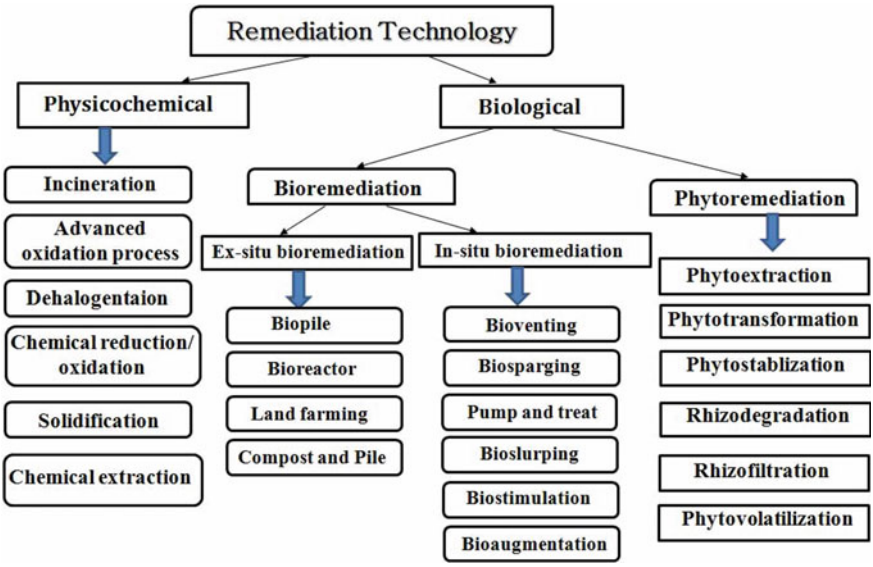

Fig. 10.1 Different techniques of bioremediation

10.3 In Situ and Ex Situ Bioremediation

In situ bioremediation (bioaugmentation, biostimulation, bioslurping, bioventing, biosparging, etc.) cleanup without elimination of soil from the polluted site of contamination and *ex situ* (biopiling, land farming, bioreactors, biofilter, electrodialysis, and composting) remediate the undigged soil at the polluted place and thereafter transporting them to another location for treatment (Fig. 10.1). Simultaneous multiple bioremediation techniques, along with bacterial dispersal networks in spatial configuration, application of genetically engineered microorganisms as designer biocatalyst, employment of efficient, and novel metabolic, extending the substrate range of accessible pathways may enhance the efficacy of bioremediation even for recalcitrant compounds.

10.3.1 Techniques of Ex Situ Bioremediation

Ex situ process of bioremediation methods are mainly evaluated depending on: kind of physical/chemical pollutant, depth of pollution spread, the expense of detoxification, geographical setting, intensity of pollution, and geological information of the contaminated pollution-loaded site [6].

Land Farming

This technique is the uncomplicated, less equipped treatment process (superficial soil of 10–35 cm) in which polluted sediment, soil, or sludge is unearth and layered on a ready bed and circularly shifted for aerobic degradation and volatilization by autochthonous microorganisms. It depends on pollutant depth, extent of tillage, and irrigation for thorough aeration and nutrient (nitrogen, phosphorous, and potassium) enrichment. It is mainly applicable for hydrocarbon decontamination mainly aromatic hydrocarbons [7–9].

Biopile

Biopile (biomounds, biocells, or bioheaps) implicates up-surface piling (stacking) of blended contaminated soil (with petroleum hydrocarbon mostly), on a treatment area/bed using remediated by forced aeration (by irrigation and tillage), leachate collection, and nutrient amendment (carbon and nitrogen) [10, 11]. It is applicable for different oils (diesel, crude, lubrication) in severe environmental conditions. The process can be enhanced by adding more pollutants degrading microbes, mechanical degradation, ambient environment, aeration, and sieving techniques of contaminated soil before actual procedure of eradication [11, 12].

Bioreactor

Bioreactor (bioremediation modes are batch, fed-batch, continuous, semi-batch and multi-stage batch, sequencing batch), as the name signifies, is a biological reactor

where the polluted materials (slurry or dry matter) were incorporated for chemical reactions in optimum growth conditions (temperature, pH, aeration velocity, agitation, substrate, and inoculum application dosage) for natural maintenance and mimicking of cells of indigenous microbes or genetically modified organisms. It is applicable for petroleum, polyaromatic hydrocarbon, linear alkyl benzene sulfonate, total nitrogen, etc [8, 12].

10.3.2 Technology of In Situ Bioremediation

In situ techniques of bioremediation is primarily low-cost technology applied for treatment of contaminations (dyes, heavy metals, hydrocarbons, chlorinated solvents, etc.) without excavation and disturbance in soil texture in suitable environmental conditions (moisture content, pH, temperature, nutrient availability, electron acceptor). But on-site installation of some equipment is required to introduce indigenous microbes for acquaintance [8, 12].

Bioaugmentation

The in situ bioaugmentation techniques increase the biodegradative capabilities of microbes (indigenous or allochthonous or genetically modified form rather than single isolates) of desired catabolic pathways to deteriorate polyaromatic hydrocarbons (effective for high molecular weight and recalcitrant compound also) in such site [13]. Bioaugmentation and biostimulation goes hand in hand for multiple remediation. The thriving of the exogenous species in competition with indigenous microbes causes a risk in this process.

Bioventing

Bioventing is an engineered bioremediation where controlled activation of airflow by providing oxygen to vadose (unsaturated) layer by indigenous aerobic microbes to degrade VOCs and semi-VOCs (SVOCs). It also restores the quality of polluted site, where air flow rate is the prime factor [14, 15].

Bioslurping

Bioslurping is an effective technique (uses a slurp which draws free products and soil gas) for groundwater and soil remediation; it involves soil vapor mining, vacuum-enhanced pumping, and bioventing to activate the biodegradation of contaminant by indirect supply of oxygen. The method is applicable for volatile organic and semi-volatile compounds and also LNAPLs (light non-aqueous phase liquids). This low-cost technique is not proper for degrading soil with excessive soil moisture and low permeability [16].

Biosparging

Biosparging disperses or injects air into subsurface soil (saturated zone) causing upward movement of pollutants to unsaturated zone. Biosparging has been widely

applied for toluene, diesel, benzene, ethylbenzene, kerosene, etc., remediation by indigenous microbes. Soil permeability, pollutant bioavailability to microbes and high airflow rate for pollutant volatilization regulate pollutant biodegradability [17].

Biostimulation

Biostimulation involves the supplement of nutrients (nitrogen, potassium, and phosphorus), substrates, electron donors or acceptors, to the contaminated sample to activate metabolic activities of autochthonous microbes. Pollutant concentration directly affects the increased metabolic activity of the microbes, although excessive stimulation of microbes leads to deteriorating bioremediation process [18].

10.4 Pollutant Degradation by Bioremediation

Microorganisms are best applied to the task of bioremediation because they secrete enzymes. Microbes being small, when comes in contact with pollutants, feed on them as their food. Thus, bioremediation with operational technology depends on indigenous microbes growing on the polluted sites, encouraging them to propagate (on site) by providing them with the best-suited nutrients and other relevant chemicals indispensable for their metabolic pathways. Scientists are presently studying ways to implant/introduce contaminated sites with exotic microorganisms including genetically modified/augmented microorganisms mainly designed to degrading the pollutants (even recalcitrant) of concern at pollution site. Microbial degeneration of organic materials mostly occurs because the microbe utilizes the pollutants for their own propagation and growth. Organic contaminants provide a resource of carbon, which is one of the essential nutrient-enriched blocks of cells, and they supply electrons, as source of energy.

Microorganisms uptake energy resources by catalyzing energy-producing oxidation–reduction reaction-like chemical reactions that first break chemical bonding and then transfer electrons away from the pollutants (oxidized). The electron donor is the contaminant, whereas the electron recipient is known as electron acceptor. Microbes release extracellular enzymes which aid in remediation of diverse types of fossil-based and bio plastics [19]. Fungi and bacteria through various enzymatic and metabolic cascades degrade these polymers into aerobic by-products of water and carbon dioxide. The nature and degradation rate of released enzyme concoction diverge based upon the type of microbial isolated species (multiple /single isolate) and even intra-species variations in a proficient and environmentally sustainable way. Thus, degradation of polymer is species-specific. For example, *Bacillus* sp. and *Brevibacillus* sp degrade polymer by proteases while fungi degrade lignin by laccase enzyme. Under stressful intolerable conditions, microbes produce exoenzymes and/or their end products for rapid detoxification of fossil- and bio-based biodegradable polymers through enzymes like proteases, cutinases and lipases [19–22]. Furthermore, enzymes like esterases and lipases, produced by *Achromobacter*

sp., *Rhizopus delemar*, *Candida cylindracea*, and *R. arrhizus,* have been reported to work on complex polymers [19, 23, 24].

Polymers (non-biodegradable and biodegradable) are degraded by bioremediation [25, 26]. The primary mechanistic process involved in plastic biodegradation involves surface colonization and enzymatic hydrolysis of plastics. Plastics polymer are compounds formed of a varied array of synthetic, mixed, semi-synthetic inorganic, and organic compounds [19, 25, 26]. Plastics are extracted mostly from petrochemical materials containing natural gas oil and coal. Various polymer products like polycaprolactone (PCL), polyethylene (PE), polyhydroxybutyrate (PHB), polyurethane (PUR), etc., are frequently applied for diverse purposes [25, 26]. Most of the bio-fossil-based plastics (like PE and PVC), used at present, are non-biodegradable and accumulate in nature causing impervious damage [22] and causing reduction in soil fertility, human health issues and ecological crisis [27]. Importantly, the surplus amount of plastic polymers hamper plankton growth, disrupting aquatic food chain [21, 28]. So their appropriate waste management, strategic control of garbage, and applicability are needed as they have complex structure and slow mineralization [29].

Degradation products (using oxygen as electron acceptor, under aerobic conditions) of polymers were subsequently low molecular weight monomers, dimers, and oligomers and thus finally to water and carbon dioxide by transformation. Under different anaerobic conditions, anaerobic bacteria utilize sulfate, nitrate, iron, etc., as electron acceptors to degrade polymers under optimum conditions [21, 30]. New microbe released enzyme pathways and strains need to be investigated under optimum state for the bioremediation of non-biodegradable polymers (bio- and fossil-based) for sustainable utilization [31] (Fig. 10.2).

Microorganisms can thrive under different habitats like ice-covered regions, rocks, water bodies, and deserts [32]. Therefore, floating/ immersed plastic trash is colonized by microorganisms and be a part of marine ecosystem and marine food chain [33–37]. Bacterial adherence may start immediately or make take time followed by biofilm production causing change in primary ecosystem [31, 38] (Fig. 10.3; Table 10.1).

In biosorption technology, heavy metal ions were removed from wastewater using mostly non-living algae and inactive biomass. Heavy metal ion accumulation by microbes mostly takes place in two phases. Firstly, the cell metabolism-dependent active sorption technique, which is actually the intracellular heavy metal ion uptake process. The other is cellular metabolism-independent inactive biosorption process which mostly occurs on the cell surface. Both these processes uptake the heavy metal ions inside the cytoplasm of the algae cells for further detoxification [39–44]. However, inadequate biosorption of heavy metal polluted ions through algae cell causes destruction and toxification of live cells [42, 44]. Live algal cell intracellular uptake is dependent of the particular growth phase (mostly growth phase), optimum environmental conditions, and the metal ion absorption potential of the live cell. The process is quite complex. On the contrary, non-living algal cells mostly uptake heavy metal ions extracellularly on its cell surface by forming biomass assemblage and binding of polymers (like cellulose, pectins, glycoproteins, etc.) through adsorption [45, 46]. Both these methods have the prospective of cost-effective effluent treatment.

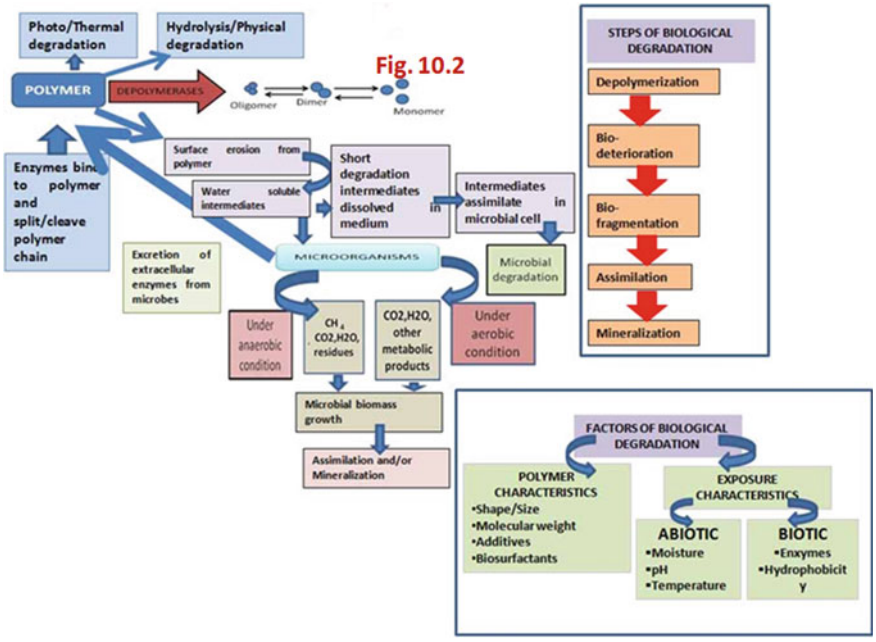

Fig. 10.2 Mechanisms and factors of biodegradation. The first step in plastic biodegradation (apart from photodegradation and physical degradation) is adherence of microbes with polymers consequent by surface microbial colonization. Microbial enzymes attach to the polymer and hydrolyze it. Under aerobic conditions, electron acceptor is oxygen, which is utilized by the bacteria forming water and CO_2 as end products. Under different anaerobic conditions, polymers are crushed down by anaerobic respiration bacteria using manganese, iron, sulfate, nitrate, and carbon dioxide as molecule of electron acceptors. Finally, assimilation and mineralization take place. Factor of abiotic and biotic conditions needs to be optimized [31].

10.5 Computational Biology of Degradation Network

System biology provides all valuable information about the target microbial population (single or mixed) for bioremediation. To characterize the metabolic functions and the elucidation of acclimatization or adjustment for a unique microbe community or unique species in normal habitat, various microorganisms cannot be propagated and the metabolism of those proliferating in single monoculture are most uncommonly to be the same as those microbes propagating in assimilated pattern. Microbial metaproteomics, employing mass spectroscopy-based categorization of amino acid substitutions, somewhat resolved the strain-specific identification of microbes. The performance of microbes selected for bioremediation varies in field as comparison to laboratory setup. Monoculture species perform less in laboratory as its synergistic partners of bioremediation in field was absent during its pure stain-specific isolation.

The main application of different researches is the application of fishing out the peptide sequences and its homology with that particular organism with known

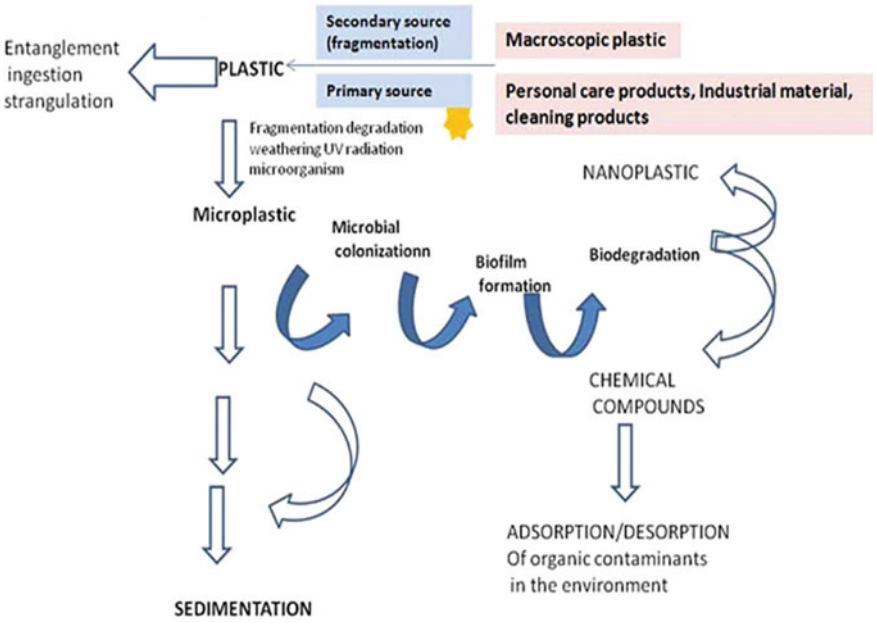

Fig. 10.3 Microplastics and interplay of marine environment. Pollutants from household sources or industry first go into the rivers and finally reach the oceans. Primary microplastics (diameter less than 5 mm) and secondary microplastics (larger diameter) accumulate in the water, loaded with biotic and different abiotic dissolved constituents, and then, biofouling (colonization of the biomass on floating plastic) takes place through bacterial degradation by primary and secondary colonization [31, 34, 35].

sequence when multiple genomic informatics were available from well-known microbes in the same contaminated community are accessible. Acidophilic microorganisms (*Leptospirillium* group II, *Bacillus acidocaldarus,* etc.) can maintain pH homeostasis, regulates proton permeation, and behaves as extremophilic organisms that can acclimatize well in harsh conditions to continue bioremediation. Furthermore, proteomics-analyzed genome typing unwinds an adaption tactic of Leptospirillum group II to intolerant environmental conditions through inter-population recombination [53]. Recently, single-cell sequencing [54] was developed to provide cell-specific genetic data from a single-cell culture of the un-cultured bacteria population, even for the less-abundant microbes. However, both difficulty of metagenomic assembly and the host sequence contamination can be easily avoided by this method [55]. Thus, unique combinations of dual metaproteomics and single-cell sequencing techniques will supply new knowledge into the role via species-specific unique protein identifications as biomarkers among a varied microbial community. Cross-strain identification by community proteomics can be greatly advanced by single-cell sequencing (despite high costs per sample) as compared

Table 10.1 Microorganisms involved in biodegradation of polymer [31]

Plastics and it derivatives	Microbes involved	Application	References
Polyhydroxyalkanoates	*Pseudomonas stutzeri*	Surgery, subsequent wound dressing, drug delivery, and bio-implant patches	[47]
Polyethylene	*Rhodococcus rubber Brevibacillus borstelensis*	Garbage and grocery bags, packaging of different film, insulation for cables and wires	[48]
Terephthalate and polyethylene	*Ideonella sakaiensis*	Packaging foods and beverages,	[47]
Polyethylene succinate	*Pseudomonas* sp.	Processed into films, bags, or boxes,	[49]
Polylactic acid	*Amycolatopsis* sp., *Bacillus brevis*, *Penicillium Roquefor*	Plastic films, bottles, and biodegradable medical devices	[21]
Polyvinyl alcohol	*Pseudomonas O-3, Pseudomonas putida*	Medicines, coating of ceramic, coating present on adhesives, reprography, and photography	[50]
Nylon	*Pseudomonas* sp. *and Flavobacterium* sp.	clothing, carpets, tire cords, conveyor belts, and brushes	[21]
Polycaprolactone	*Fusarium solani, Clostridium acetobutylicum, C. botulinum*	Long-term usage items, films on agriculture, seedling containers, Fibers and aquatic weeds	[51]
Polyester	*Phanerochaete chyrsosporium, Streptomyces* sp.	Carpets, making air filters, ropes, film making, plastic bottles, preparing fishing nets	[52]
Blends of starch/polyester/citric acid		Present in different fibers and engineering thermoplastics	
Blends of starch/citric acid ternary/poly vinyl alcohol	*Alcaligenes feacalis, Listeria monocytogenes, Escherechia coli*	Agricultural applications, different types of packaging materials	

to metagenomics. In in situ microhabitat, the biofilm formation, the signal transduction cascade, the network of cellular coordination function, and different post-translational modifications (glycosylation, phosphorylation, ubiquitination, acetylation, and glutathionylation) of bacteria can be validated by high throughput of strain-specific proteome data. The alterations in signal transduction may be linked with related environmental factors or stress in modulating important physiological processes [56]. Finally, different omic approaches, like metagenomics, metaproteomics, metatranscriptomics, and metabolomics, are rapidly developing to expand

avenues in assembled multi-omic technology in microbial toxicology. With the appreciation of computer-analyzed biology and network, a better revealing of the system biology in a microbial population in a natural habitat may set the future directives to meta-analyze and integrate multiple sets of informatics data.

The execution of microbial bioinformatics and computational tools provide a resource in a progressive technological approach toward the pesticide biodegradation [57–59]. For this, online podium of biodegradative datasets were open-access and analyzed to get back information on bioremediation pathways of xenobiotic pesticides by microbes and detoxifying network created by resistant chemicals [59, 60]. These databases encompass the Biodegradation Network-Molecular Biology database sets (Bionemo; biodegradation genes and their transcription and regulation), by University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD; http://umbbd.msi.umn.edu/predict/ reveal the data for biodegradation pathways and microbial enzyme-catalyzed biocatalytic reactions), Microbial Genome Database (MBGD, comparative investigation of microbes at the genomic level), Pesticide Target interaction database (PTID, annotation of 1347 pesticides along with pesticide target interactions, to propose novel agricultural chemical end products), BioCyc and MetaCyc, Biodegradative Oxygenases Database (OxDBase; biodegradative oxygenase database), and other databases operating in Linux and also compatible with both windows [60, 61]. UM-BBD-Pathway Prediction database is applicable for herbicide, fungicide, algaecide, rodenticide, bactericide, nematicide, etc [61, 62].

In silico process of metabolic engineering of targeted microbes has been applied in various field of microbial toxicology for biodegradation and bioremediation cellular processes employing in silico tools accessible publicly for users for desired data mining sets and dissecting the metabolic cascades of a cellular physiology [62]. Metabolic pathway analysis (MPA), metabolic flux analysis (MFA), and flux balance analysis (FBA) are most commonly used engineering tools for stoichiometric quantitative investigation of metabolic systems of web [63]. Organizing and knowing flux (flow of substance with the side edges carrying a definite value) led to modify the micro-biological cascade dynamics by metabolic engineering [61]. These in silico techniques can also be manipulated to evaluate properties of degrading bacteria [64]. QSAR (quantitative structure–activity relationship) and 3DQSAR chemical atomic models are employed to learn the toxicological level of xenobiotic pesticide molecules at diverse ecological habitats and to trace the level of biomagnification of pesticides in different food web. All these computational tools help to validate different interacting genes, widespread genomic data, and understanding genome scale models [61].

10.6 Gene Editing: Fishing the Functional Gene for Better Bioremediation

Bioremediation is a method that uses microbial population to eradicate, neutralize, or mineralize pollutants from polluted environments. According to many studies, the occurrence of a huge number of unidentified microorganisms aiding in bioremediation in contaminated environments can only be outlined using culture-independent methods [1, 65]. The analysis of 16S rRNA genes has reformed the research of microbial biodiversity in the natural habitat, both by culture-dependent and culture-independent methods [66]. For the study of microbe-related ecology, molecular biology devices have been widely used. Microbial inter-relations within the same communities are also noticed with system biology technology [67]. This method is also productive in analyzing the existence of microbes under extreme pressure and temperature conditions [68]. Omics system biology studies using genomics, transcriptomics, metabolomics, and proteomics support microbial bioremediation network analysis at the genetic level control for bioremediation (Fig. 10.4) [69]. The progressive techniques of culture-independent methods use sequencing and in silico techniques for both sequence and function-driven gene fishing for bioremediation applications [70]. Recent advancements in environmental bioremediation include molecular genetics and knowledge-based study to rationalize protein modification

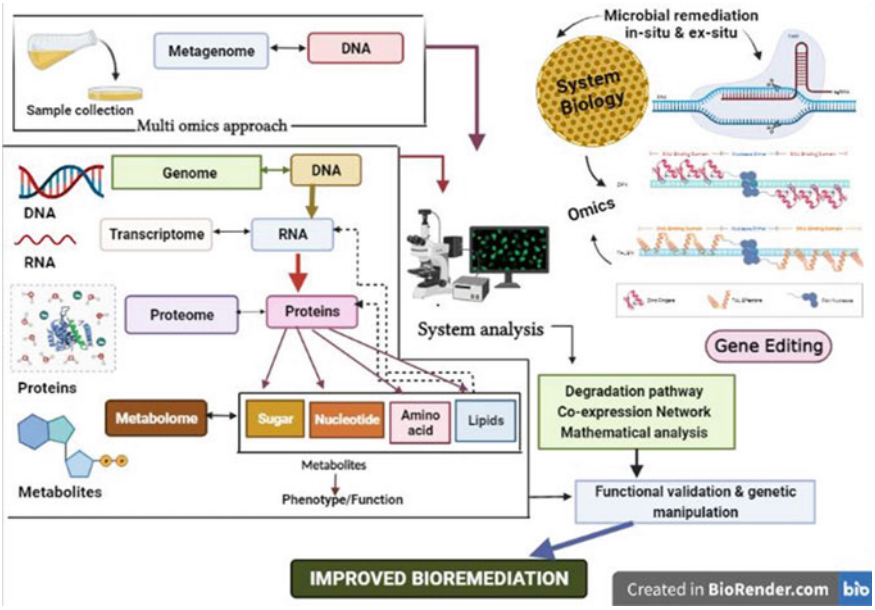

Fig. 10.4 Conjugation of multi-omics system biology and gene editing [69] [created in biorender.com]

to provide a better outcome into the development of designer enzymes/biocatalysts as per requirements [71, 72].

10.6.1 Gene Editing Tools

This is a unique technology that permits the manipulation of DNA sequence through the use of engineered nuclease enzymes employed as molecular scissors. These restriction enzymes have a wide range of functions in animal, plant, and microbial studies [73]. The editing technique encompasses triggered with a self-guided designed sequence which is complementary to the sequence of the novel gene of particular interest, helping a break at an operation site, repairing it with homologous recombination, and manipulating or moderating (deletion or insertion) a desired fragment of sequence [74]. The targeted genome engineering by definite gene editing method led to further usage of microbes in diverse fields like agriculture, food and medicine, clinics, etc [75].

The important gene editing tools are TALEN, CRISPR-Cas, and ZFN [76] for structural genetic variations. These definite gene editing tools aim to develop improved microbial populations with more complex genes and to design target-specific engineered microorganisms [77, 78]. It is the cradle for altered genetic sequence makeup that differs from native variants in order to obtain targeted new microorganisms with functional genes of interest for [79, 80].

10.6.2 Genetic Variation and CRISPR Targeting

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-associated (Cas) nuclease system is a highly utilized podium for genome mediated engineering [73, 74, 80]. CRIPSR-Cas mainly of Type I-III along with its subtypes provides an efficient gene editing method when applied on model organisms. CRISPRs, along other nuclease enzymes like zinc finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs), have supplied the platform for unprecedented functional genetic research in the laboratory setup as well as the impending probability for therapeutic applications of a diverse range of genetic diseases [76, 81, 82]. At present, the *Streptococcus pyogenes* Cas9 (SpCas9) nuclease recognizing the 5′-NGG-3′ PAM sequence is mostly used to study genome variations. CRISPR nuclease activity is based on Watson–Crick model of base pairing a guide RNA (gRNA) designed to pinpoint sequences with a cognate genomic DNA sequence upstream or downstream of a nuclease-recognized protospacer adjacent motif (PAM) (Fig. 10.5) [83]. As CRISPR systems actually mediate genomic cleavage at a cheap, simple and easy way, this technology of CRISPR targeting would logically affect the genetic variation by decreasing or increasing sequence homology at off-target and on-target sites or by moderating protospacer adjacent motifs.

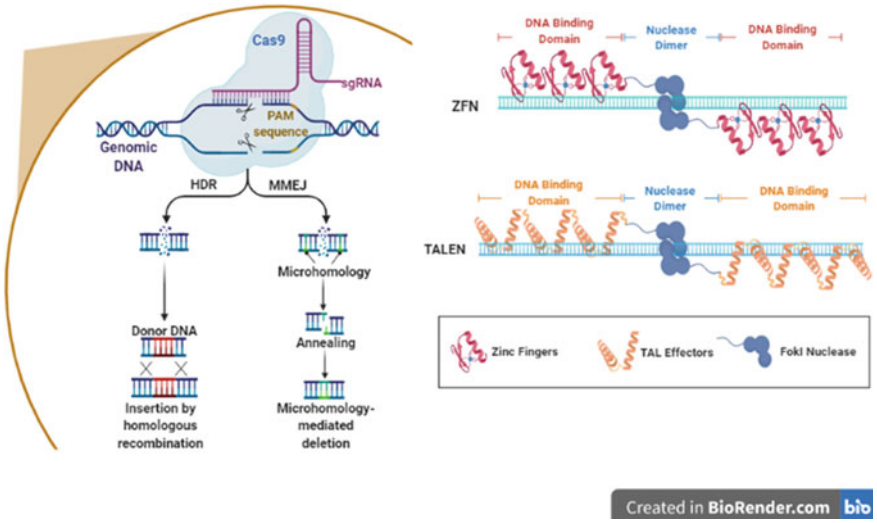

Fig. 10.5 Gene editing by ZFN, TALEN, CRISPR associated with Restriction enzymes like Cas 9. Mechanism of target DNA recognition in TALEN and ZFN depends on DNA–protein interaction whereas in CRISPR it is DNA–RNA interaction. DNA cleavage and repair in TALEN and ZFN take place in double-stranded DNA induced by FokI. In CRISPR, it cleaves both single- and double-stranded DNA induced by Cas9 enzyme (created in biorender.com) [76, 79, 82, 85]

Moreover, the sequential preferences of PAM sequence to study directed evolution and/or structure-guided mutagenesis can differ widely across Cas9 orthologs obtained from various bacteria such as *Campylobacter jejuni* (PAM: 5′-NNNNRYAC-3′), *Staphylococcus aureus* (PAM: 5′-NNGRRT-3′), *Neisseria meningitides* (PAM: 5′-NNNNGATT-3′), *Staphylococcus thermophilusST1* (PAM: 5′-NNAGAA-3′), *S. thermophilus A* (PAM: 5′-NGGNG-3′), and *Bacillus laterosporus* (PAM: 5′-NNNNCNDD-3′).

TALENs or Transcription activator-like effector nucleases, is a pioneering tool for gene editing and modification (Fig. 10.5) [84]. TALENs have TAL proteins initially secreted by the pathogenic bacteria *Xanthomonas*. TAL proteins are so powerful that they can combine to sequences as short as 1–2 nucleotides [85]. Each zinc finger domain recognizes a 3- to 4-bp DNA sequence, and tandem domains may be able to connect to a unique extended nucleotide sequence inside a cell's genome (generally with a length that is a multiple of 3, commonly 9 bp to 18 bp). TALENs structurally have 34 amino acid tandem repeats and show its nuclease activity after efficient binding. TALENs are now preferred for gene knock out (NHEJ-Non-homologous end joining) and gene knock in (HDR-Homology directed repair) of the desired gene or functional gene [72]. Two protein domains, one needed for sequence-specific cleavage and the other for binding and recognizing the specific binding site, combine to make the TALENs a powerful gene editing method. It is used on a diverse range including frogs, mammalian cells, rats, zebrafish, and chickens and other eukaryotic organisms [71].

ZFN or zinc finger nuclease is most widely used as artificial restriction endonuclease enzyme (Fig. 10.5) [76, 86]. ZFNs have inbuilt ZFPs (zinc finger proteins) as eukaryotic transcription factors which act as a DNA-binding domain. The nucleotide cleavage domain (Folk1) derived from *Flavobacterium okeanokoites* is also found in ZFNs [87]. Depending on the target site, the cleavage domain is surrounded by a large number of ZFPs (usually four to six). Each zinc finger domain recognizes a 3- to 4-bp DNA sequence, and tandem domains can possibly attach to an extended nucleotide sequence that is unique within a cell's genome (generally with a length that is a multiple of 3, commonly 9 bp to 18 bp). These ZFPs allow for precise target-specific unique gene editing with their eighteen base pair specificity. ZFPs structurally have an alpha-helix in place of two antiparallel running layer of sheets. ZFPs are mostly 30 amino acids long [88]. This gene editing technique is noted with knock in (HDR-Homology directed repair) and genes knock out (NHEJ-Non-homologous end joining) for flourishing prokaryotic and eukaryotic gene editing technology [89].

10.7 Microbial Glycoconjugates, Biofilm Formation, and Bioremediation of Organic Pollutants

The surfaces of all microbes are encoded with sugar molecules such as lipopolysaccharides, capsular polysaccharides, glycoproteins, secreted exopolysaccharides and lipo-oligosaccharides, in bacteria, and lipopolysaccharides in Gram-negative bacteria, lipophosphoglycan in Leishmania, and lipoarabinomannans in mycobacteria [90]. Glycoconjugates are mainly amphiphilic compounds produced on the cell membrane of the microbes. Release of the glycoconjugates depends on the exact strain of the microbes, nutritional requirements (nitrogen and mainly carbon), trace elements, and the optimal growth settings. They behave as biosurfactant during the microbial stationary growth phase [91, 92].

These molecules have hydrophobic and hydrophilic moieties that decrease the interfacial and surface tension. However, glycoconjugates, as mentioned in studies, can have wide range of structures. Glycoconjugates includes peptidoglycans, glycoproteins, glycopeptides, lipopolysaccharides, glycolipids, and glycosides. Different microbial strains also produce extracellular glycoconjugates like sophorolipids, rhamnolipids, and glycoproteins, exopolysaccharides, and glycollipopeptides. Microbial glycoconjugates amplify the bioavailability of organic pollutants, reduces surface tension, creates a solvent interface, and accelerates microbial metabolism, thus enhanced the detoxification of these harmful pollutants from the nature [92].

Organic pollutants (OPs) had adverse effects on biotic organisms present in the ecosystem, liable for diverse harmful consequences in humans, together with unfavorable mutagenic, teratogenic, and carcinogenic effects. The bioremediation of OPs usually uses physical and also chemical methods like aeration,

pumping, soil washing, incineration, oxidation, etc. The rich array of metabolizing enzymes of single or mixed microbial cultures participated in the bioremediation processes through simple techniques employing both aerobic and different anaerobic metabolism [31].

The most preferred anaerobic metabolism releases parent compound like trichloroethylene (TCE), and also harmful products like vinyl chlorides (VCs) and dichloroethylene (DCE). VCs and DCE have elevated environmental toxicity than their original parent compound, i.e., TCE. Studies indicate that microbial glycoconjugates (either secreted outside or present inside the cell) and other glycolipids play a vital function in the transport of OPs across microbial membranes. Therefore, aerobic metabolism employs diverse broad spectrum catabolite enzymes (like oxygenases) to degrade Ops from contaminated sites [88, 90].

Microbial glycoconjugates accelerate the bioremediation of the OPs through biofilm formation. In environment, microorganisms interact with biotic and abiotic environmental (like synergistic and antagonistic effects) factors to produce differential glycoconjugate surfactants at polluted sites. Mixed microbial population are better adapted for biofilm production and bioremediation than single microbial strain because diverse microbe communities have more number of reporting genes and a well-bound network of diverse metabolic activities to reveal finest output within the minimal period [93]. Mixed microbial populations showed the collective result on the bioremediation of the OPs.

10.7.1 Glycoconjugates and Waste Water Treatment: A Network

Microbial glycotechnology involving the stimulated sludge process is mostly relevant for wastewater bioremediation. Microbes during aerobic digestion of waste pollutants produce flocs (floc-forming microbes) by the web of different extracellular polymeric substances (EPSs). Enzymes of microorganisms hydrolyze the sludge, releases EPSs, and recognizes glycoconjugates and polysaccharides in concert with an array of lectin [94]. Glycoconjugates can effectively decrease the surface-generated and interfacial tension of water during treatment of wastewater. Rhamnolipids reduces interfacial tension and improves solubility during removal of hydrocarbons and pretreatment of waste activated sludge [95]. The bacterial isolates of Enterobacteriaceae, Aeromonadaceae, Bacillaceae, Pseudomonadaceae, and Gordoniaceae families were efficient candidate for wastewater treatment and bioremediation [96]. The wastewater treatment candidate bacterial strains showed biofilm formation at polluted sites and antibiotic resistance due to their biosurfactant property and low surface tension values [96, 97]. Sophorolipids are employed in oil biodegradation of contaminated water and oil spill management as a glycoconjugate biosurfactant [90, 98].

10.7.2 Microbial Glycoconjugates in Pesticide Degradation

Presently, pesticides of various categories belonging to organophosphates, organochlorines, and pyrethroids group are hydrophobic, showing poor bioavailability and also low water solubility. Microbial glycoconjugates help in the process of desorption of pesticide from contaminated earth granules. They behave as biosurfactant molecules, decreases surface tension, and uplifts the biodegradation by utilizing microbial metabolic pathway [99]. These surface-active amphipathic emulsifying glycoconjugate molecules increase the partitioning of aqueous phase from the hydrophobic pesticides by releasing small emulsions at and above their actual critical micellar concentration (CMC). Thus bioavailability and mobilization of pesticides enhance their uptake microbial cells during metabolic activity [100] by making them more degradable. The commonly used glycoconjugates for pesticide bioremediation are glycolipopeptides, sophorolipids, rhamnolipids, and fructose lipids. Rhamnolipids produced by *P. aeruginosa* showed enhanced solubilization and bioavailability of endosulfan isomers of pesticides [101]. Endosulfan and hexachlorocyclohexane (HCH) are highly hydrophobic pesticides which were solubilized by thermostable rhamnolipid glycoconjugate produced by *Lysinibacillus sphaericus* strain IITR51 [102, 103]. The detoxification of persistent organochlorine like Lindane in the natural habitat continues with the glycoconjugate assemblage in a minimal salt medium by *Sphingomonas* sp. NM05, *Pseudomonas aeruginosa*, *Pseudozyma* VITJzN01, *Rhodococcus* sp. strain IITR03, *Arthrobacter globiformis*, and *Bacillus subtilis* [102].

10.7.3 Biosurfactant as Glycoconjugate

With the advancement of the humanity, various industrial materials and end products, like petroleum, pesticides, medical waste, plastic, etc., have caused a havoc pollution in spheres of air, water, and soil causing unsustainable ecosystem and dampen human well-being. Persistent pollutants go through the food chain and create various perilous results on living organisms. Microbial glycoconjugates have more applications in various industries (agricultural, textile processing, pharmaceutical, personal care, cosmetics, and food industries) and environmental relevance like hydrocarbon degradation, soil bioremediation, and oil recovery.

Bioremediation has the ability to eradicate potential pollutants through biochemical mineralization resulting in eco-friendly products or no by-products in a low operational cost, low energy requirement, economic effectiveness, and permanent bio-moderation process. Pollutants like phenols, crude oils, heavy metal, petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), etc., possess high-pitched toxicity and low bioavailability to microbes, causing failure of bioremediation.

Biosurfactants, being eco-friendly, appreciably biodegradable, and multidimensional compounds, behave as additives or having surface-additive properties. They are generated by fungi, bacteria, and yeast. They are more active at very low dose

and quite stable at severe environmental state like temperature (high or low), pH, and salinity. Moreover, these surfactants also increase the efficacy and/or amount of associated genes and enzymes in microbes, facilitating bioremediation of pollutants. Thus, it reduces also the toxicity generated from pollutants toward microorganisms [104].

Bacillus sp., *Pseudomonas* sp., *Candida tropicalis,* and *Citrobacter freundii* like microbes were isolated in laboratory as probable sources of biosurfactants to generate compounds like rhamnolipids, sophorolipids, and surfactin during bioremediation [105, 106]. Biosurfactants from *Pseudomonas* sp. CQ2 obtained from China (Chongqing oilfield) using ammonium nitrate and soybean oil as nitrogen and carbon sources with optimal bioleaching conditions could efficiently eliminate heavy metal Cd, Cu, and Pb from contaminated with removal efficiencies ranges from 56.9 to 78.7%. The effectiveness of biosurfactants was better than known chemical surfactants such as SDS or Tween-80 for their low-toxicity and low critical micelles concentration (CMC) [107, 108].

Bacillus nealsonii S2MT (naturally potential biosurfactant producer) was isolated from the sediment of Yanqi Lake, Beijing, China, has successful heavy engine oil-polluted soil (10–40 mg/L concentrations of contamination) remediation potential. Surfactin (powerful biosurfactants) generated from this strain has reduced surface tension, strong stability, better emulsifying abilities, therapeutic applications, and effective over wide range of environmental conditions [109].

10.8 Eco-Friendly Nanomaterials: Mitigation of Pollutants by Microbial Nanotechnology

Nanomaterials (NMs) have special physical and specific chemical properties, and scientists in different fields of environmental science, especially dealing with bioremediation, have given much attention in application of nanoparticles (nanoscale particles). When nature is exposed to elevated concentrations of pollutants (such as heavy metals and salts), that are hazardous to most microorganisms, desirable level of bioremediation may not always be achieved. Nanotechnology, a diverse field, has wide environmental benefits, including pollution prevention, remediation and treatment, pollutant exact sensing, and detection [110]. NMs employed in bioremediation have a lower toxicity to native microorganisms and boost microbial biodegradation activity (Fig. 10.6).

"Remediate" stands for the resolution of the crisis, and "bioremediation" means the utilization of various biological microorganisms (such as plants, bacteria, fungi, yeast, or their enzymes) to mineralize the pollutant, on-site or off-site remediation, and change it into non-harmful forms [111]. However, these bio-based remedial technology are extremely convenient, high throughput, and cost-effective and cause less environmental impact, and their inflexible procedures produce highly

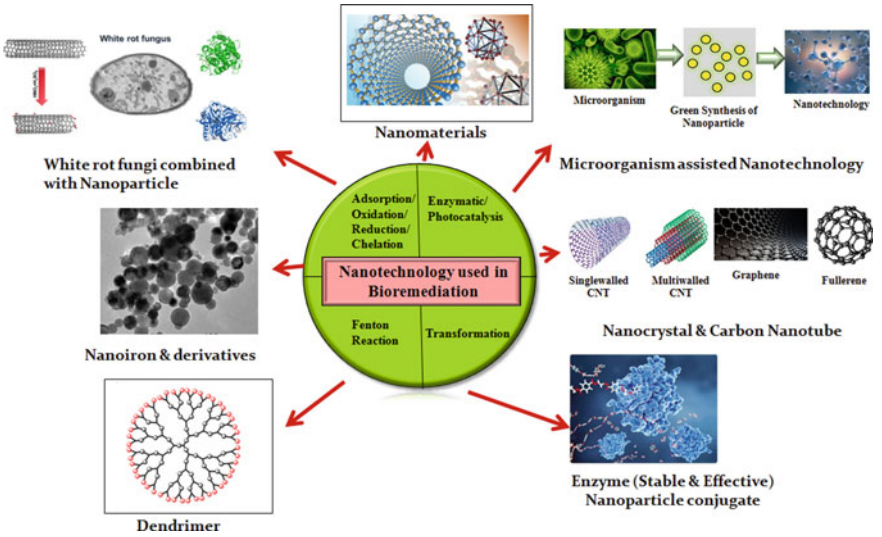

Fig. 10.6 Nanotechnology used in bioremediation

toxic by-products that cause environmental unenviable changes as well as deterioration of the microbes used in the method [112]. Bioremediation of pollutants using bio-nanomaterials is one environmentally acceptable and low cost-effective way for overcoming this barrier. In terms of environmental science, NMs have a variety of eco-friendly applications, such as substances that provide a better environment from contaminated sources in both large-scale and some portable applications.

10.8.1 Bioremediation with Nanomaterial

For in situ applications, NMs exhibit a number of desirable features or characteristics. NMs can be employed in bioremediation in waste water treatment, radioactive waste remediation, heavy metal remediation, hydrocarbon remediation, and solid waste remediation. Nanoparticles may be capable of penetrating relatively small gaps in the subsurface and remain suspended in groundwater because of their small size and exclusive surface coating property, permitting them to penetrate further and achieve greater distribution than bigger, macro-sized particles [113]. The prevalence of near sub-soil regions with constituents dissimilar from bulk regions can also motivate the chemical reactivity of materials, stimulating the involvement of interfacial free energy to the conversion of free energy of dissolution–precipitation reactions [114]. Nano-remediation methods mostly involve the application of reactive NMs (like metal oxides, carbon containing nanotubes, nanoscale size zeolites, carbon fibers, and bimetallic nanoparticles) for the detoxification and trans-degradation of contaminants.

10.8.2 Nano-Iron and Its Derivatives in Bioremediation

Iron nanoparticles behave as green nanoparticles in bioremediation owing to its redox potential property while combining with water. It is less toxic in nature and has magnetic susceptibility [115]. The elimination of As (III), a highly mobile, toxic, and prevalent arsenic species in anoxic ground water, was studied using nanoscale zero-valent iron (NZVI) [116]. Arsenic (V) has also been eradicated from ground level of water employing a colloidal reactive barrier material made of nanoscale zero-valent iron. With dissolved water and oxygen, iron also conducts "redox" reactions. Heavy metals like chromium and arsenic, pesticides (DDT, Lindane), chlorinated solvents (DCE, TCE, and PCE), and organic compounds such as nitrates have all been removed with nZVI [117]. The ability of powdered zero-valent iron to dechlorinate DDT and its linked compounds at optimum temperature has been examined [118]. Specifically, DDT, DDD [1,1-dichloro-2,2-bi's(p-chlorophenyl) ethane], and DDE [2,2-bi's(p-chlorophenyl)-1, 1-dichloroethylene] conversion by powdered zero-valent iron in buffered anaerobic aqueous solution was shown at 20 °C, in the presence and absence of nonionic surfactant like Triton X-114. *Noaea mucronata* of Chenopodiaceae family is the finest lead accumulator. This plant also accumulates for copper, zinc, and nickel as a native plant accumulator. CD accumulator plant is *Marrubium vulgare*, but the finest Fe accumulator plant is *Reseda lutea.* Nanoparticles prepared from *N. mucronata* has the best bioaccumulation ability in waste water [119]. The "ferragels" of supported zero-valent iron nanoparticles quickly dispel and immobilize Pb (II) and Cr (VI) from aqueous solution, reducing lead to Pb (0) and chromium to Cr (III) while oxidizing iron to goethite (–FeOOH). Nickel–iron nanoparticles with a large surface area have been utilized to dehalogenate trichloroethylene (TCE). At room temperature, the power of powdered zero-valent iron to dechlorinate DDT and other correlated compounds (such as DDD [1,1-dichloro-2,2-bi's(p-chlorophenyl) ethane] and DDE [2,2-bi's(p-chlorophenyl)-1, 1-dichloroethylene]) has been shown [118]. At nearly 20 °C, the outcome of powdered zero-valent iron on buffered aqueous anaerobic solution was shown in the presence and absence of nonionic surfactant Triton X-114. The role of iron nanoparticles in removal of various contaminants was shown by using Zero-valent powder iron, Iron sulfide nanoparticle, Iron nanoparticle, Fe nanocomposite, Colloidal zero-valent powder iron, and CMC4-stabilized ZVI nanoparticle in removal of Azo dye orange (II), Nitrate, arsenic (V), Herbicide: molinate, Lindane, PAHs, lead, zinc, Nickel (II), Chromium (VI), Copper (II), DDT, Cadmium, Cobalt (II), Perchlorate, etc [116–118, 120–123].

10.8.3 Carbon Nanotubes and Nanocrystals in Bioremediation

Nanomaterials based on carbon such as carbon nanotube(s) (CNT(s)) and nanocrystals allow innovative solutions to address a wide array of environmental issues [116].

Table 10.2 Removal of pollutants using carbon nanotubes (CNTs)

Types of nanoparticles	Contaminant removed	References
Carbon-based nanotubes	Lead (II), Arsenic, Atrazine, Nickel	[124]
	Organic compounds present in pesticides, dyes and several pharmaceuticals, medicines, or drugs	[111]
	Trihalomethanes	[125]
	Trichloroethane	[122]
	Methylene blue adsorption	[126]
CNTs $KMnO_4$ oxidized	Cadmium (II)	[127]
MWCNTs (multi-walled carbon tubes)	Organochlorines, Benzene, Toluene	[110]

Adsorbent particles interact with their carbon atoms on the adjoining walls of carbon nanotubes, which feature cylindrical pores. The size and structure of pores play a part in the interaction between molecules and solid surfaces. The elimination of ethyl benzene from aqueous solution has been examined using NMs such as multi-walled carbon nanotubes (MWCNTs), single-walled carbon nanotubes (SWCNTs), and hybrid carbon nanotubes (HCNTs) [124]. Organic chemicals found in colored dyes, insecticides, pharmacy medicines, and treatment medications eluted in activated sludge and wastewater were transformed by multi-walled or single-walled carbon nanotubes [122]. Relevance of carbon nanotubes was discussed in Table 10.2.

10.8.4 Enzyme Nanoparticles in Bioremediation

In bioremediation, enzymes act as biocatalysts. Enzymes' utility as cost-efficient alternatives to synthetic catalysts is restricted due to their short catalytic lifetimes and lack of stability. Binding enzymes to magnetic iron nanoparticles is a good strategy to improve their steadiness, endurance, and reusability. After attaching enzymes to magnetic iron-based nanoparticles, a magnetic field can be used to quickly separate them from reactants or end products. To make core shell magnetic nanoparticles, two separate catabolic enzymes, trypsin and peroxides, were also used as MNPs [128–131]. MNP enzyme conjugates have been discovered to be more viable, competent, and cost-effective [127]. We know that less stability of enzymes is incurred due to its oxidation property. Enzyme-conjugated nanoparticles shield the enzyme active site and thus prevent its oxidation. NMs also minimize the cell interaction of enzymes through steric hindrances and thus decrease the surface energy.

10.8.5 Dendrimers in Remediation

Dendrimers are highly branching, monodispersive macromolecules that have just lately been identified as polymer members [112]. Due to their increased reactivity, surface area, and lower toxicity, dendrimers containing NPs composites can be employed to improve catalytic activity. Polyamidoamine (PAMAM) dendrimers are a new group of nanoparticles that can be approved as water-soluble chelators [128]. Simple filtration unit for the elimination of organic pollutants based on TiO_2 (Titanium dioxide) porous ceramic filters with an alkylated poly(ethyleneimine) hyperbranched polymer, poly dendrimer (propylene imine), or cyclodextrin-impregnated pore, forming hybrid inorganic /organic filter modules with great mechanical strength and also surface area [131].

10.8.6 Microorganism-Mediated Nanotechnology

The concurrent use of microbes and biofabrication of nanomaterials makes nanotechnology more eco-friendly [132]. Because chemically generated nanoparticles may have drawbacks, green nanoparticle synthesis using fungal, plant extracts, and bacteria released enzymes could be a feasible alternative. They behave as reductive agents for the metal complex salt and produces metallic nanoparticles. *Aspergillus tubingensis* (STSP 25)-biofabricated iron oxide nanoparticles were produced from the *Avicennia officinalis* (rhizosphere) in the Sundarbans forest of India [133, 134]. With a regeneration ability of nearly five cycles, the synthesized nanoparticles were capable to eliminate higher than 90% of constituent heavy metals [copper (II), lead (II), nickel (II), and zinc (II)] from wastewater [134]. *Escherichia* sp. SINT7, a copper-resistant bacteria, was used to create copper nanoparticles. The generation of nanoparticles with the help of microbes has provided a low-cost and environment sustainable technique [134, 135].

10.8.7 Bioremediation of White Rot Fungi Combined with Nanoparticles

White rot fungi (known as WRF) are one of the most significant microbes in natural ecosystem [136]. They are recognized for having a potent enzyme system that can mineralize lignin and carbohydrates like hemicelluloses and cellulose in wood, which is important for forest biogeochemical cycles [137]. Due to the effective creation of metallic nanoparticles in bioremediation in the agricultural field, WRF has also been recognized as a possible biological reserve for biosynthesis [138]. For example, the *Trametes versicolor* was challenged with hazardous cadmium ions and in situ reduced, stable CdS nanoparticles were produced, highlighting the prospective of

WRF not only in bioremediation but also in large-scale production of metallic nanoparticles [139].

10.9 Microbial Community Proteomics: Microbial Interaction with Environment

The metaproteomic procedures offer an expensive avenue to investigate the bioremediation functions of the major dominant bacteria with other minor communities in contaminated soil, air, and water (in situ method) and better suited than traditional "artificial" laboratory setups [140]. The metaproteomic science has been extensively applied to portray microbial metabolic functions needed for bioremediation of pollutants. The metaproteome of cadmium-contaminated soil using the gel-based analysis provides information about some related proteins [141]. The biostimulation process of the dominant members of Geobacter community and their metabolic reactions to energy yield was demonstrated by metaproteomic study on the uranium-contaminated aquifer [142]. The members of the family of Betaproteobacteria (indigenous aquifer microbiota) and the Firmicutes dominate the contaminated aquifer during biostimulation with emulsified vegetable oil [143]. Metaproteomic analysis of the autochthonous bacteria was done during biodegradation of organic pollutants like chlorobenzene [144]. *Bacillus* sp. along with *Synechococcus*, *Sphingomonadales*, *Clostridium* sp., *Ralstonia solanacearum,* etc., are resistant to hydrocarbon contamination as revealed by metaproteomic survey of hydrocarbon amended soil [145]. Metaproteomic approach was employed to validate the inhibitory level of cadmium in a continuous flow of wastewater treatment in bioreactor and the biological response of an unsequenced bacterial population [146]. Metaproteomics has also happen to an imperative research field of stimulated sludge wastewater treatment using enzymes, sludge extracellular proteins in sludge digestion, and transport proteins. Both gel-based technology and non-gel-based proteomic approach were employed to identify the key players of metabolic pathways.

Metal-enriched extremely acidic (pH < 3) waters or Acid Mine Drainage (AMD) is a hazardous ecological crisis in the mining industry. The final release of water must be done after removal of associated metals and also to raise the pH. The in situ bioremediation of an AMD site was done by quantitative metaproteomic analysis (2033 proteins were identified) of the natural microbial community (Leptospirillum group II) and their related biofilm production with low complexity [147]. The Fe-oxidizing Leptospirillum (both group II with group III) chemoautotrophic bacterial communities used in AMD biofilms were employed in community genomic and metaproteomic approaches [148] to identify methyl-accepting chemotaxis proteins and methyl-independent response signal transduction.

10.10 Discussion

The application of nano-bioremediation, using better technology engineered NMs, can deliver low charge, high efficiency, minimization of chemical sludge, effective, and time cutback in situ removal processes for large-scale removal of pollutants directly and also aid in microbial degradation of waste and toxic materials. Due to the powerful potential of NMs in catalyzing biodegradation of waste and toxic materials, it is worthwhile that relevance of nanoparticles will elevate in future in sustainable development. Moreover, various health hazards or risks are also associated with NMs and have widespread ecological implications. This may reduce the relevance of NMs for environmental bioremediation. Thus, to fulfill this nanotechnology more advantageous than hazardous, regular monitoring and close intervention need to be applied sooner.

The impact of genetic structural variation on CRISPR targeting is not very specific to CRISPR as other genome editing technologies employing TALENs and ZFNs can also be used. Therapeutic genome editing is not the only tool to study impact of human genetic variation and also in treatment regime of several genetic diseases. Different variation in drug-target genes can alter drug binding property, and various genetic variants can greatly influence the rate of drug metabolism. For safer personalized, non-toxic, more effective therapeutic treatment for patients, it is necessary to optimize gRNAs by minimizing off-target potential to minimize adverse outcomes. Thus, CRISPR targeting TALENs and ZFNs should be practiced in both laboratory and clinical translation medicine setup to validate and identify the exact effects of genetic variation.

The metaproteomic science has been broadly useful to study microbial populations from different environmental habitats to provide new insights into diverse metabolic pathways, microbial diversity, metabolic potential, signal transduction cascade, ecological attributes, and microbe–environment network of interactions. Furthermore, because of the diversity and complexity of varied environmental setups, this technology still faces great drawbacks in the research of environmental microbial communities: to preserve the sample, to sort the low-abundant to high-abundant proteins, to work with low microbial populations, to critically separate the rare species, to employ the best sample collection method, and difficulty in protein extraction and proper taxonomic assignments of proteins. In environmental microbiology, the study of system biology along with biomarker discovery is needed for the precise quantitative informatics of a pre-determined group of proteins in various environmental samples. Metaproteomics in conjugation with other omics provide widespread knowledge and insight into real microbe population, their metabolic pathways, and functioning of genes and proteins.

Microbes possess many unique properties such biosurfactant production, secondary metabolites synthesis, network of biofilm formation, and other in consortium to resist the stressful environmental conditions. These properties of pollutant-resistant bacteria may be employed judicially in bioremediation. Research on multi-species biofilm producing communities has been noted for their pollutant tolerance

and bio-mineralization characteristics. Thus, microbial village population showed quite promising result in bioremediation and every knots of these applied systematic need to be evaluated for better applicability.

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References

1. Li, Y., & Li, B. (2011). Study on fungi-bacteria consortium bioremediation of petroleum contaminated mangrove sediments amended with mixed biosurfactants. *Advanced Materials Research, 183–185*, 1163–1167.
2. Nikolopoulou, M., & Kalogerakis, N. (2018). Biostimulation strategies for enhanced bioremediation of marine oil spills including chronic pollution. *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation*, pp. 1–10.
3. Hall, E., Bernhardt, E., Bier, R., et al. (2018). Understanding how microbiomes influence the systems they inhabit: Moving from a correlative to a causal research framework. *bioRxiv*, p. 065128.
4. Montiel-Rozas, M. M., Domínguez, M. T., Madejón, E., et al. (2018). Long-term effects of organic amendments on bacterial and fungal communities in a degraded Mediterranean soil. *Geoderma, 332*, 20–28.
5. von Netzer, F., Granitsiotis, M. S., Szalay, A. R., et al. (2018). Next-generation sequencing of functional marker genes for anaerobic degraders of petroleum hydrocarbons in contaminated environments. *Anaerobic Utilization of Hydrocarbons, Oils, and Lipids*, pp. 1–20.
6. Philp, J. C., & Atlas, R. M. (2005). Bioremediation of contaminated soils and aquifers. In R. M. Atlas & J. C. Philp (Eds.), *Bioremediation: Applied microbial solutions for real-world environmental cleanup* (pp. 139–236). American Society for Microbiology (ASM) Press.
7. Maila, M. P., & Colete, T. E. (2004). Bioremediation of petroleum hydrocarbons through land farming: Are simplicity and cost-effectiveness the only advantages? *Reviews in Environmental Science and Bio/Technology, 3*, 349–360. https://doi.org/10.1007/s111157-004-6653-z
8. United States Environmental Protection Agency (USEPA). (2006). In situ and ex situ biodegradation technologies for remediation of contaminated sites. EPA/625/R-06/015.
9. Khan, F. I., Husain, T., & Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management, 71*, 95–122. https://doi.org/10.1016/j.jenvman.2004.02.003
10. Whelan, M. J., Coulon, F., Hince, G., et al. (2015). Fate and transport of petroleum hydrocarbons in engineered biopiles in polar regions. *Chemosphere, 131*, 232–240. https://doi.org/10.1016/j.chemosphere.2014.10.088
11. Delille, D., Duval, A., & Pelletier, E. (2008). Highly efficient pilot biopiles for on-site fertilization treatment of diesel oil-contaminated sub-Antarctic soil. *Cold Regions Science and Technology, 54*, 7–18. https://doi.org/10.1016/j.coldregions.2007.09.003
12. Azubuike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2016). Bioremediation techniques-classification based on site of application: Principles, advantages, limitations and prospects. *World Journal of Microbiology & Biotechnology, 32*(11), 180. https://doi.org/10.1007/s11274-016-2137

13. El Fantroussi, S., & Agathos, S. N. (2005). Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Current Opinion in Microbiology, 8*, 268–275.
14. Magalhães, S. M. C., Jorge, R. M. F., & Castro, P. M. L. (2009). Investigations into the application of a combination of bioventing and biotrickling filter technologies for soil decontamination processes—A transition regime between bioventing and soil vapour extraction. *Journal of Hazardous Materials, 170*, 711–715. https://doi.org/10.1016/j.jhazmat.2009.05.008
15. Burgess, J. E., Parsons, S. A., & Stuetz, R. M. (2001). Developments in odour control and waste gas treatment biotechnology: A review. *Biotechnology Advances, 19*, 35–63. https://doi.org/10.1016/S0734-9750(00)00058-6
16. Kim, S., Krajmalnik-Brown, R., Kim, J.-O., et al. (2014). Remediation of petroleum hydrocarbon contaminated sites by DNA diagnosis-based bioslurping technology. *Science of the Total Environment, 497*, 250–259. https://doi.org/10.1016/j.scitotenv.2014.08.002
17. Kao, C. M., Chen, C. Y., Chen, S. C., et al. (2008). Application of in situ biosparging to remediate a petroleumhydrocarbon spill site: Field and microbial evaluation. *Chemosphere, 70*, 1492–1499. https://doi.org/10.1016/j.chemosphere.2007.08.029
18. Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. C. R. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation, 22*, 231–241. https://doi.org/10.1007/s10532-010-9394-4
19. Shah, A. A., Kato, S., Shintani, N., et al. (2014). Microbial degradation of aliphatic and aliphatic-aromatic co-polyesters. *Applied Microbiology and Biotechnology, 98*(8), 3437–3447.
20. Mohanty, A., Misra, M., & Hinrichsen, G. (2000). Biofibres, biodegradablepolymers and biocomposites: An overview. *Macromolecular Materials and Engineering, 276*, 1–24.
21. Tokiwa, Y., Calabia, B. P., Ugwu, C. U., et al. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences, 10*(9), 3722–3742.
22. Sharma, M., & Dhingra, H. K. (2016). Poly-β-hydroxybutyrate: A biodegradablepolyester, biosynthesis and biodegradation. *British Microbiology Research Journal, 14*(3), 1–11.
23. Jin, H. J., Lee, B. Y., Kim, M. N., et al. (2000). Thermal and mechanicalproperties of mandelic acid-copolymerized poly (butylene succinate)and poly (ethylene adipate). *Journal of Polymer Science: Polymer Physics, 38*(11), 1504–1511.
24. Lam, C. X., Hutmacher, D. W., Schantz, J. T., et al. (2009). Evaluation of polycaprolactone scaffold degradation for 6 months in vitro and in vivo. *Journal of Biomedical Materials Research. Part A, 90*(3), 906–919.
25. Saminathan, P., Sripriya, A., Nalini, K., et al. (2014). Biodegradation of plastics by *Pseudomonas putida* isolated from garden soil samples. *Journal of Advanced Zoology, 1*(3), 34–38.
26. Muhamad, W. N. A. W., Othman, R., Shaharuddin, R. I., et al. (2015). Microorganism as plastic biodegradation agent towards sustainable environment. *Advances in Environmental Biology, 9*, 8–14.
27. Comăniță, E.-D., Hlihor, R. M., Ghinea, C., et al. (2016). Occurrence of plastic waste in the environment: Ecological and health risks. *Environmental Engineering and Management Journal, 15*, 675–685.
28. Vijaya, C., & Reddy, R. M. (2008). Impact of soil composting using municipal solid waste on biodegradation of plastics. *Indian Journal of Biotechnology, 7*, 235–239.
29. Rujnić-Sokele, M., & Pilipović, A. (2017). Challenges and opportunities of biodegradable plastics: A mini review. *Waste Management Journal,* 35(2), 132–140, 2564–2569.
30. Priyanka, N., & Archana, T. (2012). Biodegradability of polythene and plastic by the help of microorganism: A way for brighter future. *Journal of Environmental & Analytical Toxicology, 1*, 12–15.
31. Ahmed, T., Shahid, M., Azeem, F., et al. (2018). Biodegradation of plastics: Current scenario and future prospects for environmental safety. *Environmental Science and Pollution Research, 25*, 7287–7298. https://doi.org/10.1007/s11356-018-1234-9
32. Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic. *FEMS Microbiology Ecology, 82*(2), 254–267.

33. Eich, A., Mildenberger, T., Laforsch, C., & Weber, M. (2015). Biofilm and diatom succession on polyethylene (PE) and biodegradable plastic bags in two marine habitats: Early signs of degradation in the pelagic and benthic zone? *PloS One, 10*, e0137201. https://doi.org/10.1371/journal.pone.0137201
34. De Tender, C. A., Devriese, L. I., Haegeman, A., et al. (2015). Bacterial community profiling of plastic litter in the Belgian part of the North Sea. *Environmental Science & Technology, 49*(16), 9629–9638.
35. Urbanek, A. K., Rymowicz, W., & Mirończuk, A. M. (2018). Degradation of plastics and plastic-degrading bacteria in cold marine habitats. *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-018-9195-y
36. Maes, T., Van der Meulen, M., Devriese, L. I., et al. (2017). Microplastics baseline surveys at the water surface and in sediments of the North-East Atlantic. *Frontiers in Marine Science, 4*(135).
37. Bryant, J. A., Clemente, T. M., Viviani, D. A., et al. (2016). Diversity and activity of communities inhabiting plastic debris in the North Pacific Gyre. *mSystems, 1*(3), e00024–e00016.
38. Pauli, N. C., Petermann, J. S., Lott, C., et al. (2017). Macrofouling communities and the degradation of plastic bags in the sea: an in situ experiment. *Royal Society Open Science, 4*(10), 170549.
39. Monteiro, C., Castro, P. L., & Xavier Malcata, F. (2011). Biosorption of zinc ions from aqueous solution by the microalga *Scenedesmus obliquus*. *Environmental Chemistry Letters, 9*(2), 169e176.
40. Monteiro, C. M., Castro, P. M. L., & Malcata, F. X. (2012) Metal uptake by microalgae: Underlying mechanisms and practical applications. *Biotechnology Progress, 28*(2), 299e311.
41. Wilde, K. L., Stauber, J. L., Markich, S. J., et al. (2006, August). The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.). *Archives of Environmental Contamination and Toxicology, 51*(2), 174–85. https://doi.org/10.1007/s00244-004-0256-0. PMID: 16583260.
42. Talebi, A. F., Tabatabaei, M., Mohtashami, S. K., et al. (2013). Comparative salt stress study on intracellular ion concentration in marine and salt-adapted freshwater strains of microalgae. *Notulae Scientia Biologicae, 5*(3), 309e315.
43. Perales-Vela, H. V., Peña-Castro, J. M., & Cañizares-Villanueva, R. O. (2006, June). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere, 64*(1), 1–10. https://doi.org/10.1016/j.chemosphere.2005.11.024. PMID: 16405948.
44. Lamaia, C., Kruatrachuea, M., & Pokethitiyooka, P. (2005). *Toxicity and accumulation of lead and cadmium and lipid microbiology* (pp. 2583–2594). Springer.
45. Godlewska-żyłkiewicz, B. (2001). Analytical applications of living organisms for preconcentration of trace metals and their speciation. *Critical Reviews in Analytical Chemistry, 31*(3), 175e189.
46. Volesky, B. (2007). Biosorption and me. *Water Research, 41*(2007), 4017–4029.
47. Raza, Z. A., Abid, S., & Banat, I. M. (2018). Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. *International Biodeterioration and Biodegradation*, *126*(September 2017), 45–56.
48. Kasirajan, S., & Ngouajio, M. (2012). Polyethylene and biodegradable mulches for agricultural applications: A review. *Agronomy for Sustainable Development, 32*, 501–529.
49. Xu, J., & Guo, B. H. (2010). Microbial succinic acid, its polymer poly(butylene succinate), and Applications *14*, 347–388.
50. Baker, M. I., Walsh, S. P., Schwartz, Z., et al. (2012). A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *100B*(5), 1451–1457.
51. Hajiali, F., Tajbakhsh, S., & Shojaei, A. (2018). Fabrication and properties of polycaprolactone composites containing calcium phosphate-based ceramics and bioactive glasses in bone tissue engineering: A review. *Polymer Reviews, 58*(1), 164–207.
52. Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances, 26*(3), 246–265.

53. Denef, V. J., VerBerkmoes, N. C., Shah, M. B., et al. (2009). Proteomics-inferred genome typing (PIGT) demonstrates inter-population recombination as a strategy for environmental adaptation. *Environmental Microbiology, 11*, 313–325.
54. Thrash, J. C., Temperton, B., Swan, B. K., et al. (2014). Single-cell enabled comparative genomics of a deep ocean SAR11 bathytype. *ISME Journal, 8*, 1440–1451.
55. Segata, N., Boernigen, D., & Tickle, T. L. (2013). Computational meta'omics for microbial community studies. *Molecular System Biology, 9,* 666.
56. Kobir, A., Shi, L., Boskovic, A., et al. (2011). Protein phosphorylation in bacterial signal transduction. *Biochimica et Biophysica Acta, 1810*, 989–994.
57. Vanacek, P., Sebestova, E., Babkova, P., et al. (2018). Exploration of enzyme diversity by integrating bioinformatics with expression analysis and biochemical characterization. *ACS Catalysis, 8*(3), 2402–2412.
58. Malla, M. A., Dubey, A., Yadav, S., et al. (2018). Understanding and designing the strategies for the microbe mediated remediation of environmental contaminants using omics approaches. *Frontiers in Microbiology.*
59. Nolte, T. M., Pinto-Gil, K., Hendriks, A. J., et al. (2018). Quantitative structure–activity relationships for primary aerobic biodegradation of organic chemicals in pristine surface waters: Starting points for predicting biodegradation under acclimatization. *Environmental Science: Processes & Impacts, 20*(1), 157–170.
60. Arora, T., Broglia, E., Thomas, G. N., et al. (2014, February). (2014) Associations between specific technologies and adolescent sleep quantity, sleep quality, and parasomnias. *Sleep Medicine, 15*(2), 240–247. https://doi.org/10.1016/j.sleep.2013.08.799 PMID: 24394730.
61. Jaiswal, S., Singh, D. K., & Shukla, P. (2019). Gene editing and system biology tools for pesticide bioremediation: A review. *Frontiers in Microbiology, 10,* 87. https://doi.org/10.3389/fmicb.2019.00087.
62. Ravikrishnan, A., Nasre, M., & Raman, K. (2018). Enumerating all possible biosynthetic pathways in metabolic networks. *Scientific Reports, 8*(1), 9932.
63. Gonzalez-Garcia, R. A., Aispuro-Castro, R., Salgado-Manjarrez, E., et al. (2017). Metabolic pathway and flux analysis of H_2 production by an anaerobic mixed culture. *International Journal of Hydrogen Energy, 42*(7), 4069–4082.
64. Sulpice, R., & McKeown, P. C. (2015). Moving toward a comprehensive map of central plant metabolism. *Annual Review of Plant Biology, 66*, 187–210.
65. Marzorati, M., Balloi, A., De Ferra, F., et al. (2010). Identification of molecular markers to follow up the bioremediation of sites contaminated with chlorinated compounds. *Methods in Molecular Biology, 668*, 219–134.
66. Das, S., Dash, H. R., Mangwani, N., et al. (2014). Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. *Journal of Microbiol Methods, 103*, 80–100.
67. Kong, W., Meldgin, D. R., Collins, J. J., et al. (2018). Designing microbial consortia with defined social interactions. *Nature Chemical Biology*, p. 1.
68. Borja, A. (2018). Testing the efficiency of a bacterial community-based index (microgAMBI) to assess distinct impact sources in six locations around the world. *Ecological Indicators, 85*, 594–602.
69. De Sousa, C. S., Hassan, S. S., Pinto, A. C., et al. (2018). Microbial omics: Applications in biotechnology. In *Omics technologies and bio-engineering* (pp. 3–20).
70. Khan, F., Sajid, M., & Cameotra, S. S. (2013). In silico approach for the bioremediation of toxic pollutants. *Journal of Petroleum & Environmental Biotechnology, 4*, 2.
71. Paul, D., Pandey, G., Pandey, J., et al. (2005). Accessing microbial diversity for bioremediation and environmental restoration. *Trends in Biotechnology, 23*, 135–142.
72. Pieper, D. H., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Microbiology, 11*, 262–270.
73. Butt, H., Jamil, M., Wang, J. Y., et al. (2018). Engineering plant architecture via CRISPR/Cas9-mediated alteration of strigolactone biosynthesis.

74. Bier, E., Harrison, M. M., O'Connor-Giles, K. M., et al. (2018). Advances in engineering the fly genome with the CRISPR-Cas system. *Genetics, 208*(1), 1–18.
75. Yadav, R., Kumar, V., Baweja, M., et al. (2018). Gene editing and genetic engineering approaches for advanced probiotics: A Review. *Critical Reviews in Food Science and Nutrition, 58*(10), 1735–1746.
76. Waryah, C. B., Moses, C., Arooj, M., et al. (2018). Zinc fingers, TALEs, and CRISPR systems: A comparison of tools for epigenome editing. In *Epigenome editing* (pp. 19–63). Humana Press.
77. Basu, S., Rabara, R. C., Negi, S., et al. (2018). Engineering PGPMOs through gene editing and system biology: A solution for phytoremediation? *Trends in Biotechnology.*
78. Dangi, A. K., Sharma, B., Hill, R. T., et al. (2018). Bioremediation through microbes: System biology and metabolic engineering approach. *Critical Reviews in Biotechnology, 39*(1), 79–98.
79. Stein, H. P., Navajas-Pérez, R., & Aranda, E. (2018). Potential for CRISPR genetic engineering to increase xenobiotic degradation capacities in model fungi. In *Approaches in bioremediation* (pp. 61–78).
80. Dai, Z., Zhang, S., Yang, Q., et al. (2018). Genetic tool development and systemic regulation in biosynthetic technology. *Biotechnology for Biofuels, 11*(1), 152.
81. Komor, A. C., Badran, A. H., & Liu, D. R. (2017). CRISPR-based technologies for the manipulation of eukaryotic genomes. *Cell, 168*, 20–36.
82. Mali, P., Yang, L., Esvelt, K. M., et al. (2013). RNA-guided human genome engineering via Cas9. *Science, 339*, 823–826.
83. Cong, L., Ran, F. A., Cox, D., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*, 819–823.
84. Cox, D., Platt, R. J., & Zhang, F. (2015). Therapeutic genome editing: Prospects and challenges. *Nature Medicine, 21*, 121–131.
85. Karvelis, T., Gasiunas, G., & Siksnys, V. (2017). Harnessing the natural diversity and in vitro evolution of Cas9 to expand the genome editing toolbox. *Current Opinion in Microbiology, 37*, 88–94.
86. Niti, C., Sunita, S., Kamlesh, K., et al. (2013). Bioremediation: An emerging technology for remediation of pesticides. *Research Journal of Chemistry and Environment, 17*, 88–105.
87. Bhatt, P. (2018). Insilico tools to study the bioremediation in microorganisms. In *Handbook of research on microbial tools for environmental waste management* (pp. 389–395).
88. Covino, S., Stella, T., & Cajthaml, T. (2016). Mycoremediation of organic pollutants: Principles, opportunities, and pitfalls. In *Fungal applications in sustainable environmental biotechnology* (pp. 185–231).
89. Dvořák, P., Nikel, P. I., Damborský, J., et al. (2017). Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnology Advances, 35*(7), 845–866.
90. Mengeling, B. J., & Turco, S. J. (1998). Microbial glycoconjugates. *Current Opinion in Structural Biology, 8*(5), 572–577. https://doi.org/10.1016/s0959-440x(98)80146-2
91. Varjani, S. J., & Upasani, V. N. (2016). Carbon spectrum utilization by an indigenous strain of Pseudomonas aeruginosa NCIM 5514: Production, characterization and surface active properties of biosurfactant. *Bioresource Technology, 221*, 510–516.
92. Moya, R. I., Tsaousi, K., Rudden, M., et al. (2015). Rhamnolipid and surfactin production from olive oil mill waste as sole carbon source. *Bioresource Technology, 198*, 231–236.
93. Gaur, N., Flora, G., Yadav, M., et al. (2014). A review with recent advancements on bioremediation-based abolition of heavy metals. *Environmental Sciences: Processes and Impacts, 16*, 180–193.
94. Wawrzynczyk, J., Szewczyka, E., Norrlow, O., et al. (2007). Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge characterization of the extracted polysaccharides/glycoconjugates by a panel of lectins. *Journal of Biotechnology, 130*, 274–281.
95. Li, J. Q., Liu, W. Z., Cai, W. W., et al. (2019). Applying rhamnolipid to enhance hydrolysis and acidogenesis of waste activated sludge: Retarded methanogenic community evolution and methane production. *RSC Advances, 9*, 2034e2041.

96. Ndlovu, T., Khan, S., & Khan, W. (2016). (2016) Distribution and diversity of biosurfactant-producing bacteria in a wastewater treatment plant. *Environmental Science and Pollution Research, 23*, 9993–10004.
97. Fang, Y., Hozalski, R. M., Clapp, L. W., et al. (2002). Passive dissolution of hydrogen gas into groundwater using hollow-fiber membranes. *Water Research, 36*, 3533–3542.
98. Vogt, C., & Richnow, H. H. (2013). Geobiotechnology II. Bioremediation via *in situ* microbial degradation of organic pollutants (pp. 123–146). Springer.
99. Banat, I. M., Franzetti, A., Gandolfi, I., et al. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology, 87*, 427–444.
100. Singh, P., Saini, H. S., & Raj, M. (2016). Rhamnolipid mediated enhanced degradation of chlorpyrifos by bacterial consortium in soil-water system. *Ecotoxicology and Environmental Safety, 134*, 156–162.
101. Karanth, N. G. K., Deo, P. G., & Veenanadig, N. K. (1999). Microbial productions of biosurfactants and their importance. *Current Science, 77*, 116–126.
102. Abdul, S. J., & Das, N. (2013). Enhanced biodegradation of lindane using oil-in-water bio-microemulsion stabilized by biosurfactant produced by a new yeast strain, *Pseudozyma* VITJzN01. *Journal of Microbiology and Biotechnology, 23*, 1598–1609.
103. Gaur, V. K., Bajaj, A., Regar, R. K., et al. (2018). Rhamnolipid from a *Lysinibacillus sphaericus* strain IITR51 and its potential application for dissolution of hydrophobic pesticides. *Bioresource Technology, 272*, 19–25.
104. Liu, L., Li, S., Garreau, H., et al. (2000). Selective enzymatic degradationsof poly (L-lactide) and poly (ε-caprolactone) blend films. *Biomacromolecules, 1*(3), 350–359.
105. Liu, Z.-F., Zeng, G.-M., Wang, J., Zhong, H., Ding, Y., & Yuan, X.-Z. (2010). Effects of monorhamnolipid and Tween 80 on the degradation of phenol by Candida tropicalis. *Process Biochemistry, 45*(5), 805–809.
106. Mishra, S., Lin, Z., Pang, S., et al. (2021). Biosurfactant is a powerful tool for the bioremediation of heavy metals from contaminated soils. *Journal of Hazardous Materials, 15*(418), 126253. https://doi.org/10.1016/j.jhazmat.2021.126253 Epub 2021 Jun 2.
107. Liang, Y. S., Yuan, X. Z., Zeng, G. M., Hu, C. L., Zhong, H., Huang, D. L., Tang, L., & Zhao, J. J. (2010). Biodelignification of rice straw by Phanerochaete chrysosporium in the presence of dirhamnolipid. *Biodegradation, 21*(4), 615–624.
108. Sun, W., Zhu, B., Yang, F., et al. (2020, February). Optimization of biosurfactant production from Pseudomonas sp. CQ2 and its application for remediation of heavy metal contaminated soil Chemosphere. *265*, 129090. https://doi.org/10.1016/j.chemosphere.129090. Epub 2020 Nov 30.
109. Phulpoto, I. A., Yu, Z., Hu, B., et al. (2020). Production and characterization of surfactin-like biosurfactant produced by novel strain Bacillus nealsonii S2MT and it's potential for oil contaminated soil remediation. *Microbial Cell Factories, 19*(1), 145. https://doi.org/10.1186/s12934-020-01402-4
110. Hua, S., Gong, J. L., Zeng, G. M., et al. (2017). Remediation of organochlorine pesticides contaminated lake sediment using activated carbon and carbon nanotubes. *Chemosphere, 77*, 65–76.
111. Xu, J., & Bhattacharyya, S. D. (2005). Membrane based bimetallic nanoparticles for environmental remediation: Synthesis and reactive properties. *Environmental Progress, 24*, 358–366.
112. Ponder, S. M., Darab, J. G., & Mallouk, T. E. (2000). Remediation of Cr(VI) and Pb(II) aqueous solutions using supported, nanoscale zero-valent iron. *Environmental Science & Technology, 34*(12).
113. Rajan, S. (2011). Nanotechnology in groundwater remediation. *International Journal of Environmental Science and Technology, 2*, 182–187.
114. Kanatzidis, M. G., & Poeppelmeier, K. R. (2007). Report from the third workshop on future directions of solid-state chemistry: The status of solid-state chemistry and its impact in the physical sciences. *Progress in Solid State Chemistry, 36*, 1–133.

115. Bolade, O. P., Williams, A. B., & Benson, N. U. (2020). Green synthesis of iron-based nanomaterials for environmental remediation: A review. *Environmental Nanotechnology, Monitoring & Management, 13*, 100279. https://doi.org/10.1016/j.enmm.2019.100279
116. Choe, S., Chang, Y. Y., Hwang, K. Y., et al. (2000). Kinetics of reductive denitrification by nanoscale zero-valent iron. *Chemosphere, 41*, 1307–1311.
117. Li, X. Q., & Zhang, W. X. (2007). Iron nanoparticles: The core shell structure and unique properties for Ni (II) sequestration. *Langmuir, 22*, 4638–4642.
118. Feitz, A. J., Joo, S. H., Guana, J., et al. (2005). Oxidative transformation of contaminants using colloidal zerovalent iron. *Colloids and Surfaces A, 265*, 88–94.
119. Mohsenzadeh, F., & Chehregani Rad, A. (2012). Bioremediation of heavy metal pollution by nano-particles of noaea mucronata. *International Journal of Bioscience, Biochemistry and Bioinformatics, 2*, 85–89.
120. Xiong, Z., Zhao, D., & Pan, G. (2007). Rapid and complete destruction of perchlorate in water and ionexchange brine using stabilized zero-valent iron nanoparticles. *Water Research, 41*, 3497–3505.
121. Feng, J., Hu, X., Yue, P. L., et al. (2003). Degradation of azo-dye orange II by a photo assisted Fenton reaction using a novel composite of iron oxide and silicate nanoparticles as a catalyst. *Industrial and Engineering Chemistry Research, 42*, 2058–2066.
122. Kshitij, C. J., Zhuonan, L., Hema, V., et al. (2016). Carbon nanotube based groundwater remediation: The case of trichloroethylene. *Molecules, 21*, 953–967.
123. Paknikar, K. M., Nagpal, V., Pethkar, A. V., et al. (2005). Degradation of lindane from aqueous solutions using iron sulfide nanoparticles stabilized by biopolymers. *Technology of Advanced Materials, 6*, 370–374.
124. Li, Y. H., Wang, S., Wei, J., et al. (2002). Lead adsorption on carbon nanotubes. *Chemical Physics Letters, 357*, 263–266.
125. Lu, C., Chung, Y. L., & Chang, K. F. (2005). Adsorption of trihalomethanes from water with carbon nanotubes. *Water Research, 39*, 1183–1189.
126. Ahsan, M. A., Jabbari, V., Imam, M. A., et al. (2020). Nanoscale nickel metal organic framework decorated over graphene oxide and carbon nanotubes for water remediation. *Science of the Total Environment, 69*, 134214. https://doi.org/10.1016/j.scitotenv.2019.134214
127. Li, Y. H., Wang, S., Luan, Z., et al. (2003). Adsorption of cadmium (II) from aqueous solution by surface oxidized carbon nanotubes. *Carbon, 41*, 1057–1062.
128. Schrick, B., Hydutsky, B. W., Blough, J. L., et al. (2004). Delivery vehicles for zerovalent metal nanoparticles in soil and groundwater. *Chemistry of Materials, 16*(11), 2187–2193.
129. Qiang, Y., Sharma, A., Paszczynski, A., et al. (2007). Conjugates of magnetic nanoparticle-enzyme for bioremediation. In *Proceedings of the 2007 NSTI Nanotechnology Conference and Trade Show* (vol. 4, pp. 656–659).
130. Ruffini-Castiglione, M., & Cremonini, R. (2009). Nanoparticles and higher plants. *Caryologia, 62*, 161–165.
131. Wang, C. B., & Zhang, W. X. (1997). Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs. *Environmental Science & Technology, 31*(7), 2154–2156.
132. Mahanty, S., Chatterjee, S., Ghosh, S., et al. (2020). Synergistic approach towards the sustainable management of heavy metals in wastewater using mycosynthesized iron oxide nanoparticles: Biofabrication, adsorptive dynamics and chemometric modeling study. *Journal of Water Process Engineering, 37*, 101426. https://doi.org/10.1016/j.jwpe.2020.101426
133. Govarthanan, M., Jeon, C. H., Jeon, Y. H., et al. (2020). Non-toxic nano approach for wastewater treatment using Chlorella vulgaris exopolysaccharides immobilized in iron-magnetic nanoparticles. *International Journal of Biological Macromolecules, 162*, 1241–1249. https://doi.org/10.1016/j.ijbiomac.2020.06.227
134. Noman, M., Shahid, M., Ahmed, T., et al. (2020). Use of biogenic copper nanoparticles synthesized from a native Escherichia sp. as photocatalysts for azo dye degradation and treatment of textile effluents. *Environmental Pollution, 257*, 113514. https://doi.org/10.1016/j.envpol.2019.113514

135. Cheng, S., Li, N., & Jiang, L. (2019). Biodegradation of metal complex Naphthol Green B and formation of iron–sulfur nanoparticles by marine bacterium Pseudoalteromonassp CF10-13. *Bioresource Technology, 273*, 49–55. https://doi.org/10.1016/j.biortech.2018.10.082
136. Chen, G., Guan, S., Zeng, G., et al. (2013). Cadmium removal and 2,4-dichlorophenol degradation by immobilized *Phanerochaete chrysosporium* loaded with nitrogendoped TiO_2 nanoparticles. *Applied Microbiology and Biotechnology, 97*, 3149–3157.
137. Hou, J., Dong, G., Ye, Y., et al. (2014). Laccase immobilization on titania nanoparticles and titania-functionalized membranes. *Journal of Membrane Science, 452*, 229–240.
138. Xu, P., Zeng, G., Huang, D., et al. (2013). Synthesis of iron oxide nanoparticles and their application in *Phanerochaete chrysosporium* immobilization for Pb (II) removal. *Colloids and Surfaces A: Physicochemical and Engineering, 419*, 147–155.
139. Sanghi, R., & Verma, P. (2009). A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. *Chemical Engineering Journal, 155*, 886–891.
140. Wilmes, P., Heintz-Buschart, A., & Bond, P. L. (2015). A decade of metaproteomics: Where we stand and what the future holds. *Proteomics, 15*, 3409–3417.
141. Singleton, I., Merrington, G., Colvan, S., et al. (2003). The potential of soil protein-based methods to indicate metal contamination. *Applied Soil Ecology, 23*, 25–32.
142. Wilkins, M. J., Verberkmoes, N. C., Williams, K. H., et al. (2009). Proteogenomic monitoring of geobacter physiology during stimulated uranium bioremediation. *Applied and Environment Microbiology, 75*, 6591–6599.
143. Chourey, K., Nissen, S., Vishnivetskaya, T., et al. (2013). Environmental proteomics reveals early microbial community responses to biostimulation at a uraniumand nitrate-contaminated site. *Proteomics, 13*, 2921–2930.
144. Benndorf, D., Balcke, G. U., Harms, H., et al. (2007). Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. *ISME Journal, 1*, 224–234.
145. Bastida, F., Jehmlich, N., & Lima, K. (2016). The ecological and physiological responses of the microbial community from a semiarid soil to hydrocarbon contamination and its bioremediation using compost amendment. *Journal of Proteomics, 135*, 162–169.
146. Carla, M. R., Lacerda, L. H. C., & Kenneth, F. R. (2007). Metaproteomic analysis of a bacterial community response to cadmium exposure. *Journal of Proteome Research, 6*, 1145–1152.
147. Ram, R. J., VerBerkmoes, N. C., & Thelen, M. P. (2015). Community proteomics of a natural microbial biofilm. *Science, 308*, 1915–1920.
148. Goltsman, D. S., Denef, V. J., Singer, S. W., et al. (2009). Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "leptospirillum rubarum" (group II) and "leptospirillum ferrodiazotrophum" (group III) bacteria in acid mine drainage biofilms. *Applied and Environment Microbiology, 75*, 4599–4615.