



Review

Role of abiotic and biotic components in remediating environmental pollutants: A review

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Abstract

Increasing environmental pollutants due to various anthropogenic activities are of great concern nowadays since they affect the health of both terrestrial and aquatic ecosystems. Pollutants such as pesticides, heavy metals, fertilizers are non-biodegradable and persist in the environment for a longer duration affecting the health of living organisms. In this context, bioremediation technology is gaining considerable attention. Bioremediation technology involves various living organisms for the removal of toxic pollutants. This review discusses detoxification methods adopted by various microorganisms. An attempt has also been made to understand how these detox-mechanisms can be exploited to clean up the environment in a natural way. Finally, various environmental factors that regulate bioremediation processes and the methods to improve the rate of bioremediation have been mentioned. This comprehensive study may help to understand the fundamental aspects and future perspectives of microbial remediation of pollutants which could help in commercial success of waste management processes.

Keywords: Bioremediation; Biotechnology; Detoxification; Environmental pollution; Microbes

Introduction

Environmental pollution is of great concern worldwide due to increasing industrialization, urbanization, mining, usage of agro-chemicals and other anthropogenic activities [1]. Pollutants including both organic and inorganic compounds such as fertilizers, pesticides, heavy metals, herbicides, insecticides, greenhouse gases, hydrocarbons, oil, dyes, nuclear wastes, e-waste, etc. are reported to contaminate terrestrial and aquatic environments [2, 3]. These pollutants are known to enter the food chain of various living organisms causing adverse effects [4]. To control this prevailing condition, many research studies are being conducted to clean-up or reduce the increasing environmental pollutants [3]. Previously, the pollutants or wastes were disposed of in a traditional manner where they were dumped by digging

holes [5]. But this method of waste management has become very difficult and unsuccessful due to increasing demand for new places every time to dump the rapidly increasing environmental waste. Nowadays, microbial remediation is gaining attention since it is considered to be a successful cleaning or waste management technique, where the microorganisms are predominantly involved in detoxifying the environmental contaminants [6]. Thus, bioremediation is commonly defined as a biological mechanism that helps in recycling toxic wastes to non-toxic compound by any of the process such as degradation, eradication, immobilization, or detoxification [6]. Since bioremediation processes are environmental and budget friendly in relation to other physical and chemical methods, they have come into extensive usage in remediating technologies [3, 6]. Bioremediation technology generally employs various microorganisms which include bacteria, ciliates, algae and fungi [3]. The versatility of microbes with respect to their nutritional demands and also their metabolic activity confers them the ability to survive even in the harsh environmental conditions and contribute efficiently in bioremediation [2].

This review focuses on various microbial detoxification processes that help in altering the toxic pollutants to non-toxic materials by exhibiting a unique array of mechanisms. This review also talks about the abiotic factors which play predominant role in determining the biodegradation rate of pollutants and about various methods involved in regulating these abiotic factors for proper microbial activity. In addition, the limitations/challenges of bioremediation have been discussed. Thus, the current review will impart an updated information on the remediation strategies to remove environmental pollutants using microorganisms.

Detoxification mechanisms exhibited by various microorganisms

The various microorganisms used in bioremediation processes have been summarized in Table 1.

Following are the different mechanisms adopted by the microorganisms to detoxify the pollutants:

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Biosorption

Biosorption process involves binding of pollutants to the surface of organism which acts as absorbent (Fig. 1). The pollutants are reported to bind non-specifically to the functional groups of the proteins, and polysaccharides located on the surface of the cell [7].

Biosorption is facilitated in bacteria due to the presence of a peptidoglycan layer and extra polymer surface (EPS) (Fig. 1). Gram-positive bacteria have several layers of peptidoglycan, which predominantly consist of teichoic acid, whereas Gram-negative bacteria have a single layer of peptidoglycan [8] and it contains enzymes, glycoproteins, lipopolysaccharides and phospholipids. The functional groups of these compounds act as ligands and bind effectively with heavy metals and thus can absorb more metal ions than Gram-negative bacteria [9]. Extra polymer substances in the cell wall bind effectively to pollutants such as heavy metals thereby preventing their entry into the microbial intracellular environment, and protecting the cell from the metal toxicity [10]. It has been reported that *Staphylococcus hominis* strain AMB-2 can effectively bind to lead and cadmium, and is highly used in the biosorption process [11]. For improving the biosorption process by bacterial cells, the biological activities of EPS are being modified by acetylation, methylation, phosphorylation, carboxymethylation and sulphonylation [12]. Bacteria such as *Escherichia coli*, *Zooglea ramigera*, *Bacillus megaterium* and *Bacillus subtilis* are reported to be involved in successful biosorption of lindane by exhibiting hydrophobic interaction; Van der Waals and forces exhibited by the cell wall. *Bacillus pumilus* showed successful removal of tetrachlorodibenzo-p-dioxin, and some polychlorinated dibenzofurans by biosorption [13]. In eukaryotes, the mycelium of filamentous fungi *Phanerochaeta chrysosporium* has been observed to act as biosorbent for cadmium, lead and copper [14]. The process of biosorption depends on pH and availability of metal species. It has also been reported that fungi such as *Alternaria alternata* and *Penicillium aurantiogriseum* effectively remove cadmium and mercury, respectively, thereby acting as good biosorbents [15]. Different microalgal strains such as *Spirulina platensis*, *Chlorella vulgaris*, *Oscillatoria* sp. and *Sargassum* sp., could effectively remove metal ions and therefore act as good biosorbents [16]. In addition, non-living algae act as relatively better biosorbents of heavy metals in comparison to living algae [17].

(1) Bioaccumulation

Bioaccumulation process is addressed to be metabolically active which involves accumulation of pollutants by the living organisms. During the process of bioaccumulation, the pollutants enter the cytoplasm of the cell via the lipid bilayer with the aid of various transporters. The metal ion pollutants are sequestered by metal-binding proteins [18], and these sequestered heavy metals are finally removed

from the cell in the form of a non-toxic metal ion complex [19] (Fig. 1).

In the bacterial membrane, heavy metal bioaccumulation is achieved by endocytosis, ion channels, carrier-mediated transport and lipid permeation [20]. In most of the prokaryotes, heavy metal bioaccumulation has been facilitated by channel system, carrier-mediated and primary-active transporters. Glycerol facilitator channels of *Escherichia coli*, *Corynebacterium diphtheriae* facilitate in proper uptake of arsenic [21]. Proper uptake of heavy metals like nickel, cobalt and arsenic in *Helicobacter pylori*, *Staphylococcus aureus*, *Novospingobium armaticivorans* and *Rhodospseudomonas palustris* is facilitated by carrier-mediated transporters (symporters) belonging to the NiCoT family [22]. Similarly, primary active transporters such as MntA, CopA, which belong to P-type ATPase superfamily, have high efficiency in importing cadmium and copper ions as reported in *Lactobacillus plantarum* and *Enterobacter hirae* [23]. *Azospirillum lipoferum* reported to remove dicofol from soil by bioaccumulation process [13].

Sher and Rehman et al. (2019) [24] reported that fungi such as *Monodictys* and *Aspergillus niger* can accumulate chromium and lead. Dursun et al. (2003) [25] reported that *A. niger* could efficiently remove lead and copper by bioaccumulating 34.40 mg/g of Pb and 15.60 mg/g of Cu, respectively. Similarly, *Pichia stipitis* (yeast) was observed to bioaccumulate copper and chromium with uptake capacity of 15.85 mg/g and 9.10 mg/g, respectively [26]. It has been reported that bioaccumulation process act as major defence mechanism in metal-resistant protozoa such as ciliates that are present in highly polluted environments [27]. It has been reported that freshwater ciliates such as *Oxytricha trifallax*, *Paramecium caudatum* and *Euplotes mutabilis* show high efficiency in heavy metal accumulation [27]. *O. trifallax* has an ability to remove 91% of zinc, 90% of mercury, 94% of copper and cadmium and 88% of nickel from the medium after an incubation for 96 h. Similarly, *P. caudatum* can remove 82% of cadmium, 78% of mercury, 95% of zinc, 94% of copper and 76% of nickel from the medium after incubating for 96 h. Besides, *E. mutabilis* can minimize 60%, 82% and 95% of copper from the medium after 48 h, 72 h, and 96 h of incubation, respectively [28]. In addition, microalgae are also reported to remove heavy metals effectively by bioaccumulation [29]. Heavy metals such as copper and iron, metalloids and metallic nanoparticles are reported to get accumulated inside microalgae with the help of metal transport systems. Chelating proteins such as phytochelatin and metallothionein are also being reported in these protists, i.e., yeast, algae and ciliates which help in metal detoxification [30]. These microorganisms thus, use these chelating compounds to accumulate as well as sequester the metal ions in the vacuoles or intracellular spaces and release the non-toxic form of metal complexes into the environment [23].

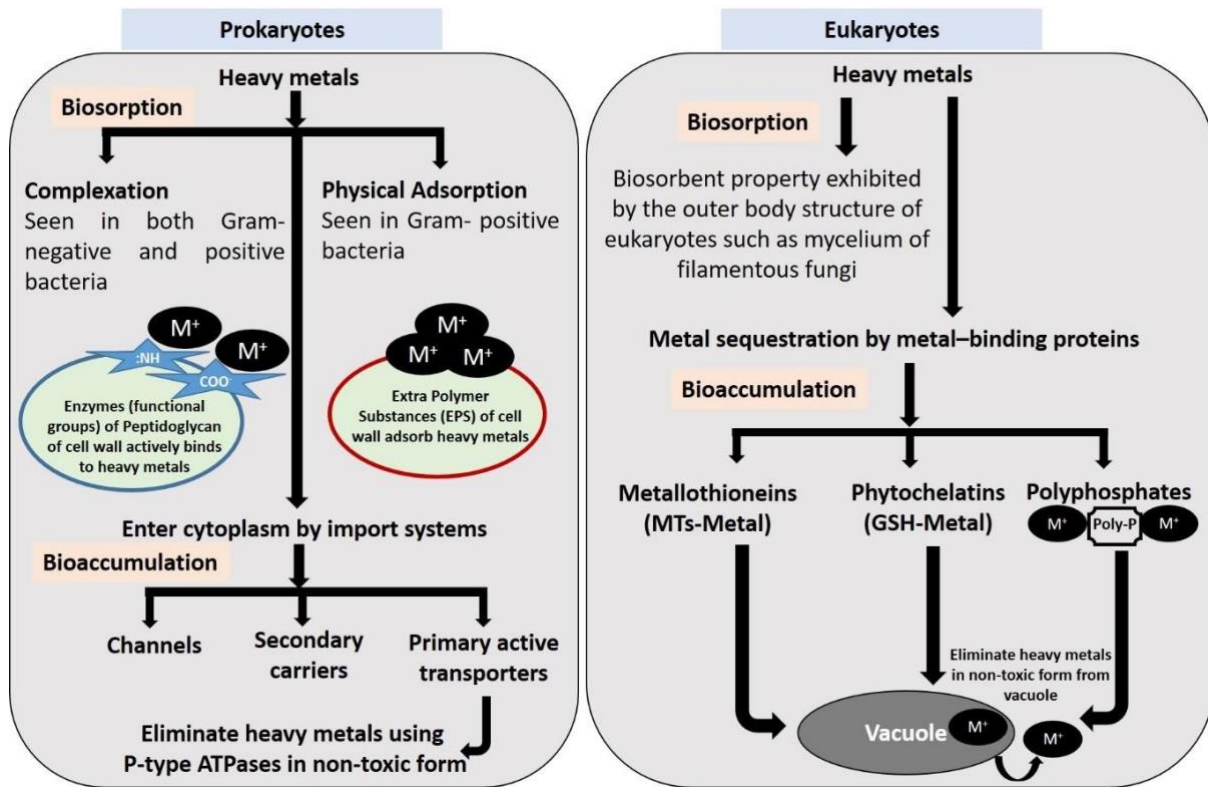


Figure 1: Representation of biosorption and bioaccumulation processes by microorganisms

(2) Bioleaching

Bioleaching is a process of disintegrating heavy metals by microbes. In this process, microbes can solubilize metal ions from ores and secondary wastes [31]. These are further purified using suitable techniques like membrane separation, ion exchange, adsorption and selective precipitation [32]. This method has been employed for extracting metals from ores [33]. Heavy metal is extracted by contact and non-contact mechanisms which involve several chemical enzymatic reactions [34]. The reactions include three major steps, i.e., (i) microbial oxidation-reduction reaction, (ii) formation of acids from organic and inorganic routes and (iii) heavy metal extraction from the matrix.

The property of bioleaching has been reported from bacteria and/or archaea inhabiting acidic environments (<pH 5.0) [35]. The sulfur- and iron- oxidizing bacteria, such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* have been reported to help in the bio-leaching of heavy metals like arsenic [30].

In eukaryotes, many acidophilic algae and fungi have been identified from acid mine drainage which is known to release various biological metabolites such as phosphatidylethanolamine that help in the formation of biofilms [36]. Biofilms help in inducing heavy metal resistance in acidophilic fungus such as *Acidomyces richmondensis* [37] and acidophilic algae such as *Delisea pulchra* [38]. Acidophilic fungi such as

Pullularia, *Penicillium*, *Spicaria*, *Rhodotorula glutinis* and *Rhodotorula rubra* have been reported to be present in the coal mine showing that they have bioleaching properties [39]. Also, high efficiency of bioleaching of heavy metals like gold, nickel and copper from electronic scrap by eukaryotic fungi such as *Aspergillus niger* has been reported [40].

(3) Biomineralization

Biomineralization involves mineral formation such as carbonates, oxides, silicates, sulphates and phosphates, employing different mechanisms exhibited by various living microorganisms [41]. The crucial factors that influence the mineral formation include the presence of highly variable and reactive interfaces such as EPS with different compositions and structures. The protein functional groups present on the microbial surface impart net negative charge [42] which act as ligands to bind and precipitate positively charged potential toxic metals to make them more stable and non-toxic compounds [43]. This mechanism is known as metal immobilization or complexation which is generally achieved by phosphate, carbonate or oxalate precipitation [44].

In recent reports, it has been indicated that biomineralization helps in remediating heavy metal pollutants [45]. *Bacillus* sp. can release free inorganic phosphate which helps in trapping toxic heavy metal ions by forming relatively non-toxic insoluble metal phosphate coat [43]. Similarly, it has been reported that calcium

carbonate (calcite) producing bacteria incorporates different divalent heavy metals into the calcite lattice thereby making these heavy metals less soluble and, thus, detoxifying the effect of heavy metals [46]. Also, many microorganisms are known to reduce polychlorinated biphenyls (PCBs) from industrial wastewaters by biomineralization processes which are facilitated by carbonate biomineralizing bacteria [47]. Bacteria, such as *Sporosarcina pasteurii* having ureolytic property, are also used to treat PCB in wastewater [48]. Microalgae such as diatoms have the ability to produce nano-patterned silica-based cell wall which is known as bio-silica which again traps the toxic heavy metals making them insoluble and non-toxic [49].

(4) Biotransformation

Biotransformation refers to a process of conversion of insoluble pollutants (xenobiotics) into soluble components and this can be achieved either by enzymatic or non-enzymatic methods. In non-enzymatic method, modification in the structure of a chemical compound has been reported that leads to the formation of a molecule with relatively more polarity and less toxicity [50]. This helps them acclimatize to changes in the environment. The various steps involved in this process are condensation, hydrolysis, isomerization, oxidation, reduction and methylation [51]. In enzymatic transformation, enzymes like oxidoreductases, oxygenases (mono and dioxygenases), laccases (multi-copper oxidases), peroxidases, lipases, cellulases and proteases present in fungi and bacteria, are known to degrade pollutants, i.e., heavy metals, pesticides, detergents, hydrocarbons, cyanide, azide, and hydroxides [5].

Bacteria such as *Acinetobacter* sp. and *Micrococcus* sp. oxidized toxic As (III) into non-toxic and less soluble As (V) [52]. In addition, microbes also transform pesticides into less toxic compounds by enzymatic biotransformation where it has been reported that *Arthrobacter* sp. can breakdown the herbicide Dalapon to pyruvate [53].

Both anaerobic and aerobic bacteria are involved in the biotransformation of a wide range of xenobiotics. *Mycobacterium vaccae* helps in catalyzing styrene, propyl-benzene-ethylbenzene, cyclohexane and benzene and acetone [54]. Bacteria namely *Pseudomonas*, *Bacillus*, *Cycloclasticus*, *Pseudo altermonas*, *Halomonas*, *Marinomonas* and *Dietzia* degrade PCB (polychlorinated biphenyls) and other polycyclic aromatic hydrocarbons (fluorine, phenanthrene, and pyrene) effectively [55]. Similarly, anaerobic bacteria such as *Methanosaeta concilii*, *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* are involved in degrading phthalate compound [56]. Similarly, lindane (hexachlorocyclohexane) which is a potent insecticide, is also being degraded by anaerobic and aerobic bacteria [57]. Hexachlorocyclohexane (HCH) is transformed into non-toxic compound by

anaerobic bacteria such as *Clostridium* sp., *Citrobacter freundii*, *Desulfo vibrio* sp. and *Dehalobacter* sp. and aerobic bacteria belonging to the family Sphingomonadaceae [58]. In addition, petroleum transformation is being reported where bacteria namely *Pseudomonas*, *Rhodococcus*, *Mycobacterium* and *Arthrobacter* are actively involved in degrading petroleum hydrocarbons [59]. Petroleum hydrocarbons are reported to be degraded by biotransformation process which is facilitated by fungi such as *Talaromyces*, *Neosartorya*, *Penicillium*, *Cephalosporium*, *Aspergillus* and *Amorphoteca* [53]. In addition, these fungi are used in remediation of oil spills. Recently, biotransformation process has been reported in ciliate, i.e., *Tetrahymena thermophila*, which is exploited in various pharmaceutical and food industries [60].

(5) Biostimulation

Biostimulation process involves addition of specific nutrients at the remediation site to induce the microbial activity in either liquid or gaseous form through injection. The added nutrients generally act as sources for phosphorus, nitrogen, carbon, oxygen or other electron acceptors or donors at the site [61]. The examples of nutrients that are used for this purpose include growth supplements, trace minerals and fertilizers or by supplying other environmental requirements namely temperature, oxygen and pH to enhance the microbial metabolic rate [62]. The pollutants present in small quantity can also stimulate the microbial activity by turning on the operons specific for the enzymes involved in bioremediation process. The major pollutants such as sulphate, petroleum hydrocarbons and polyester polyurethanes are successfully remediated through biostimulation process [63].

Sulfur-reducing bacteria such as *Ochrobactrum* sp. and *Desulfovibrio* sp. are predominantly being used in biostimulation and have been successfully used in treating hydrometallurgical leachates [64] (Fig. 2). Algae are predominantly used in biostimulation. Microalgae namely *Chlorophyta* sp., *Chlorella vulgaris*, *Cyanophyta* sp., *Scenedesmus quadricauda* and *Actadesmus dimorphus* are mostly used in agricultural sector. These algae help in the constant supply of oxygen and also in regulating plant hormones [65] (Fig. 2).

(6) Bioaugmentation

Bioaugmentation is similar to biostimulation where microorganisms showing specific metabolic activity are introduced to the polluted site to enhance the waste degradation, mainly in oil contaminated environment, which thereby increases the genetic diversity of microorganisms at the site [66]. Addition of microorganisms continuously to a bioreactor can enhance the process. Microbes present at the remediation site are collected, cultured, modified genetically, and then introduced back to the site. It is

predominantly used in treatment of wastewater. Bioaugmentation is predominantly used in those areas where groundwater and soil are polluted with chlorinated ethanes, namely tetrachloro- and trichloroethylene. Microorganisms used during this process have the ability to successfully degrade these pollutants to non-toxic compounds (chloride and ethylene) [67].

Bacteria namely *Novosphingobium* sp., *Hydrogenophaga intermedia* and *Bacteroidetes* sp. are used in bioaugmentation for wastewater treatment. They are known to degrade estradiol, petroleum hydrocarbons, dehydroabietic acid, phenol and atrazine [68] (Fig. 2). Bacteria such as *Acinetobacter* sp. and *Comamonas testosteroni* have high efficiency in degrading fluorinated and chlorinated compounds. Successful degradation of industrial wastes such as quinoline and pyridine, are facilitated by *Bacillus* sp., *Paracoccus* sp., *Shinellazoo gloeoids* and *Pseudomonas* sp. Bacteria such as *Acinebacter* sp. and *Sphingomonas*

sp. have the capacity to remove nicotine which is released from tobacco industry. In addition, bioaugmentation of *Paracoccus* sp. and *Pseudomonas* sp. facilitated removal of naphthalene, phenol, pyridine, quinoline and carbozole present in the cooking wastewater. Also, bioaugmentation of bacteria (*Comamonas* and *Pandoraea*) and fungi (*Aspergillus*), showed higher efficiency in removing lignin from the environment [69]. Diverse groups of eukaryotes are also known to successfully degrade hydrocarbons which include fungi (*Geomyces* sp.), algae and some protozoans (zooflagellates) [70]. These eukaryotes are known to be engaged in denitrification thereby helping in cleaning wastewater [71] (Fig. 2). The toxic compounds such as cyanides which are released from steel industries, are removed efficiently from the wastewater by applying bioaugmentation of cyanide-degrading yeast, i.e., *Cryptococcus humicolus* [69].

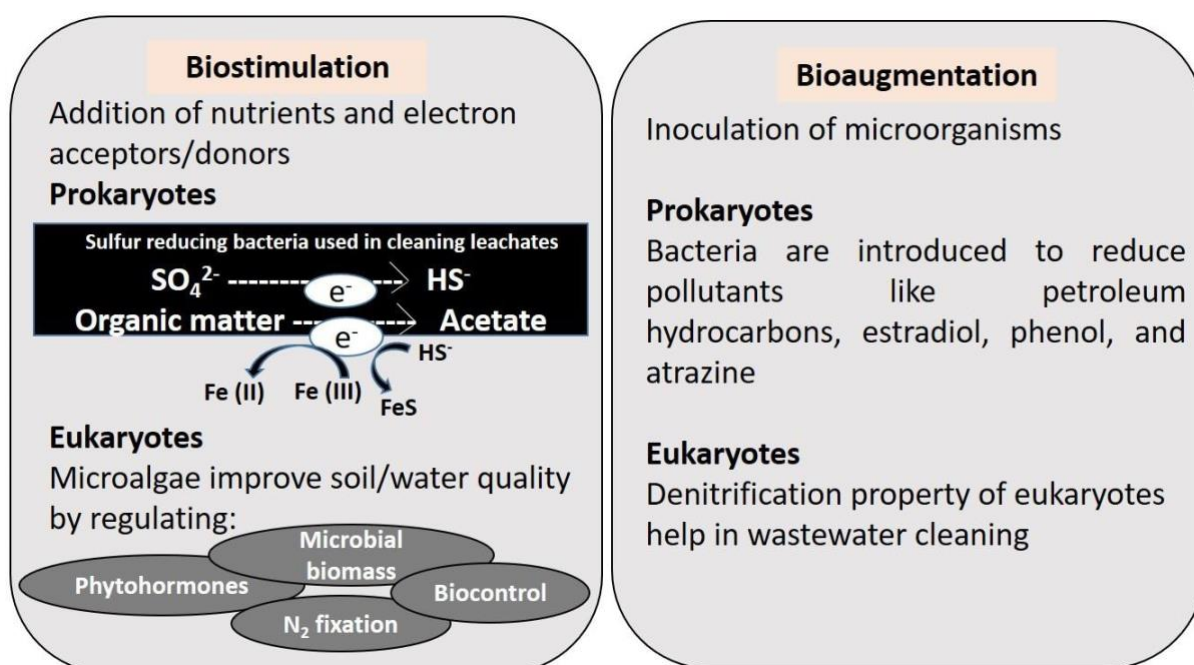


Figure 2: Representation of biostimulation and bioaugmentation processes by microorganisms

Table 1: Microorganisms used for bioremediation process.

Microorganism	Prokaryote/Eukaryote	Use in pollutant remediation	References
<i>Azospirillum lipoferum</i> s	Prokaryotes	Remove dicofol from soil by bioaccumulation process	[13]
<i>Staphylococcus hominis</i> strain AMB-2	Prokaryotes	Biosorption of Lead and Cadmium	[11]
<i>Escherichia coli</i> , <i>Corynebacterium diphtheriae</i>	Prokaryotes	Uptake of Arsenic	[21]
<i>Helicobacter pylori</i> , <i>Staphylococcus aureus</i> , <i>Novosphingobium armaticivorans</i> , <i>Rhodopseudomonas palustris</i>	Prokaryotes (bacteria)	Uptake of heavy metals namely Cobalt, Arsenic, and Nickel	[22]

Microorganism	Prokaryote/Eukaryote	Use in pollutant remediation	References
<i>Lactobacillus plantarum</i> , <i>Enterobacter hirae</i>	Prokaryotes	Importing Cadmium and Copper ions	[23]
<i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i> (iron and sulfur oxidizing bacteria)	Prokaryotes	Arsenic bioleaching	[30]
<i>Bacillus</i> sp.	Prokaryotes	Trap heavy metal ions by forming an insoluble metal phosphate coat	[43]
<i>Sporosarcina pasteurii</i>	Prokaryotes	Have ureolytic property, are used to treat PCB in wastewater	[48]
Calcium Carbonate (Calcite) producing bacteria	Prokaryotes	Detoxifying the heavy metal effect by forming calcite lattice	[46]
<i>Acinetobacter</i> sp., <i>Micrococcus</i> sp.	Prokaryotes	Oxidize toxic As (III) into harmless and less soluble As (III)	[52]
<i>Arthrobacter</i> sp.	Prokaryotes	Breakdown the herbicide Dalapon to pyruvate	[53]
<i>Mycobacterium vaccae</i>	Prokaryotes	Catalyse acetone, cyclohexane, styrene, benzene, ethylbenzene, and propylbenzene	[54]
<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Cycloclasticus</i> , <i>Pseudoaltermonas</i> , <i>Halomonas</i> , <i>Marinomonas</i> , <i>Dietzia</i>	Prokaryotes	Degrade PCB (polychlorinated biphenyls), and other polycyclic aromatic hydrocarbons (fluorine, phenanthrene, and pyrene)	[55]
<i>Methanospirillum hungatei</i> , <i>Methanosaeta concilii</i> , <i>Syntrophobacter fumaroxidans</i>	Prokaryotes	Degrading phthalate compound	[56]
<i>Clostridium</i> sp., <i>Citrobacter freundii</i> , <i>Desulfovibrio</i> sp., <i>Dehalobacter</i> sp. and aerobic bacteria belonging to family Sphingomonadaceae	Prokaryotes	Lindane (hexachlorocyclohexane) is transformed to non-toxic compound	[58]
<i>Rhodococcus</i> , <i>Pseudomonas</i> , <i>Arthrobacter</i> , <i>Mycobacterium</i>	Prokaryotes	Degrade petroleum hydrocarbons	[59]
<i>Ochrobactrum</i> sp., <i>Desulfovibrio</i> sp.	Prokaryotes	Used in biostimulation and treating hydrometallurgical leechates	[64]
<i>Novosphingobium</i> sp., <i>Bacteroidetes</i> sp., <i>Hydrogenophaga intermedia</i>	Prokaryotes	Used in bioaugmentation for wastewater treatment, degrade estradiol, petroleum hydrocarbons, dehydroabiatic acid, phenol, atrazine	[68]
<i>Acinetobacter</i> sp., <i>Comamonast estosteroni</i>	Prokaryotes	Degrade fluorinated and chlorinated compounds	[69]
<i>Bacillus</i> sp., <i>Paracoccus</i> sp., <i>Shinellazoo gloeoids</i> , <i>Pseudomonas</i> sp.	Prokaryotes	Degradation of industrial wastes such as quinoline and pyridine	[69]
<i>Acinebacter</i> sp., <i>Sphingomonas</i> sp.	Prokaryotes	Remove nicotine which is released from tobacco industry	[69]
<i>Paracoccus</i> sp., <i>Pseudomonas</i> sp.	Prokaryotes	Removal of naphthalene, phenol, pyridine, quinoline, and carbozole present in the coking wastewater.	[69]
<i>Comamonas</i> , <i>Pandoraea</i> , <i>Aspergillus</i>	Prokaryotes	Remove lignin from the environment	[69]
<i>Phanerochaeta chrysosporium</i>	Eukaryotes	Biosorbent for Cadmium, Lead and Copper.	[14]
<i>Alternaria alternate</i> , <i>Penicillium aurantiogriseum</i>	Eukaryotes	Biosorbents for Cadmium and Mercury	[15]

Microorganism	Prokaryote/Eukaryote	Use in pollutant remediation	References
<i>Spirulina platensis</i> , <i>Chlorella vulgaris</i> , <i>Oscillatoria</i> sp., <i>Sargassam</i> sp.	Eukaryotes	Biosorbents for metal ions	[16]
<i>Monodictys</i> , <i>Aspergillus niger</i>	Eukaryotes (fungi)	Accumulate Chromium and Lead	[24]
<i>Aspergillus niger</i>	Eukaryotes	Bioaccumulate Copper and Lead; Bioleaching of heavy metals namely nickel, copper and gold from electronic scrap	[25,40]
<i>Pichia stipitis</i> (Yeast)	Eukaryotes	Bioaccumulate Copper and Chromium	[26]
<i>Oxytricha trifallax</i> , <i>Paramecium caudatum</i>	Eukaryotes	Remove Zinc, Mercury, Copper and Cadmium, and Nickel	[27]
<i>Euplotes mutabilis</i>	Eukaryotes	Remove Copper	[28]
<i>Acidomyces richmondensis</i> , <i>Delisea pulchra</i>	Eukaryotes	Inducing heavy metal resistance	[37, 38]
<i>Pullularia</i> , <i>Penicillium</i> , <i>Spicaria</i> , <i>Rhodotorula glutinis</i> , <i>Rhodotorula rubra</i>	Eukaryotes	Bioleaching in coal mines	[39]
Diatoms	Eukaryotes	Detoxifying the heavy metal effect by forming Biosilica	[49]
<i>Aspergillus</i> , <i>Penicillium</i> , <i>Talaromyces</i> , <i>Amorphoteca</i> , <i>Neosartorya</i> , <i>Cephalosporium</i>	Eukaryotes	Degradation of petroleum hydrocarbons; Bioremediation of oil spills	[54]
<i>Tetrahymena thermophila</i>	Eukaryotes	Used in various food and pharmaceutical industries	[28]
<i>Chlorella vulgaris</i> , <i>Scenedesmus quadricauda</i> , <i>Chlorophyta</i> sp., <i>Cyanophyta</i> sp., <i>Actadesmus dimorphus</i>	Eukaryotes	Used in agricultural sector, help in constant supply of oxygen, and also in regulating plant hormones	[65]
Fungi (<i>Geomyces</i> sp.), algae and some protozoans (zooflagellates)	Eukaryotes	Degrade hydrocarbons and engaged in denitrification thereby helping in cleaning wastewater	[70, 71]
<i>Cryptococcus humicolus</i>	Eukaryotes	Remove toxic compounds such as cyanides which are released from steel industries	[69]

Role of abiotic factors in bioremediation

Environmental factors such as temperature, pH, moisture, solubility in water, soil structure, nutrients, oxygen content, site characteristics and redox potential, regulate the microbial interaction with targeted contaminants/pollutants [2]. Some of these important abiotic factors are discussed below:

Temperature

Temperature has been reported to regulate the growth of microorganisms involved in bioremediation. In general, high temperatures (50 °C-80 °C) are known to increase the microbial activity and therefore potential breakdown of organic pollutants. Microbial growth usually increases with increase in temperature. Elevated temperature also activates functional genes that are involved in degradation of environmental pollutants [72]. Raising temperature

decreases adsorption which increases the availability of organic pollutants to the microbes [73].

Moisture

Water is essential for all living organisms, and therefore facilitates microbial degradation of environmental pollutants. Low and high water content are known to disrupt the microbial activity. Low water content is known to limit the growth and activity of microbes due to low availability of nutrients, reduction in microbial movement and disruption in osmolarity. Whereas, the high water content disrupts the oxygen supply to the microorganisms and hence lower the aerobic degradation of pollutants including hydrocarbons [6]. The optimum moisture levels in water that helps in proper oxygen and other nutrient supply range from 50% to 70% of water holding capacity at which maximum aerobic microbial activity is facilitated [74].

Soil structure

Soil structure also plays an important role for soil microbes. The soil microorganisms which include bacteria, fungi, and viruses do play important roles in organic material degradation which acts as essential nutrient to the plant growth. The soil structure is determined mainly by the composition of minerals as mineral contents influence the porosity and moisture of the soil that determines the nutrient availability to the microorganisms. Proper functioning of the soil microbes requires 50% of soil porosity. The degradation activity of microbes has been reported to decrease with decrease in soil porosity/permeability, or under arid conditions [6]. In addition, soil aggregates also determine the growth and activity of the microbes. Growth of *Acidobacteria* was observed to be maximum in soil macroaggregates but minimum in soil microaggregates [75].

pH

The pH determines the acidity or alkalinity of the environmental ecosystem which influences the activity and growth of the microbes. pH determines the structure of microbial community. Microorganisms are known to exhibit wide range of pH tolerance. Soil or water ecosystems having neutral pH generally show greater microbial diversity whereas acidic environment reduces the microbial diversity [75]. The optimum pH that is known to enhance microbial growth and activity is 5.5–8.5 [74].

Nutrients

The major nutrients that determine the microbial activity are nitrogen, carbon, and phosphorous [6]. Nitrogen enrichment is necessary for increasing microbial communities. Carbon is another important nutrient which determines the structure and function of microbial communities. Decrease in carbon content lowers the catabolic activity of the microbes. Phosphorous is known to influence the community structure. Both nitrogen and phosphorous are known to act as nutrients for proper growth of microbes and carbon facilitates microbial catalytic activity [72].

Atmospheric carbon dioxide (CO₂)

CO₂ plays a major role in designing the environmental ecosystems especially of soil as it is known to interfere with the carbon cycle of the microorganisms. Microbial degradation rate was observed to decrease when exposed to high concentrations of CO₂. Also, it was observed that high concentration of CO₂ lowers the nitrogen availability to the microorganisms which actually acts as nutrient and therefore lowers the microbial growth and activity [76].

Oxygen

Oxygen positively influences the activity and growth of microbes and also enhances aerobic degradation of various toxic materials from the environment [75].

These abiotic factors are improved and optimized for proper activation of microorganisms by the following methods:

(1) Bio-Piling

Bio-piling involves soil excavation, shifting and heaping into piles. Air is forced into the bio-pile system either by positive or negative pressure [77]. In addition, the microbial activity is stimulated by microbial respiration, resulting in increased remediation of adsorbed petroleum contaminants [77]. It contains an aeration system, treatment bed, a leachate collection system, and an irrigation/nutrient system. Proper degradation of these contaminants requires regulated control of pH, heat, moisture, oxygen and nutrients. Run off is blocked by covering the soil with plastic which thereby prevents the evaporation and volatilization. This promotes solar heating which is used for commercial and industrial building purposes [67].

(2) Land Farming

Land farming is a simple method which requires very cheap and less equipment where the polluted soil is scarped and spread over a prepared bed and periodically tilled to degrade the contaminants. This process aims to activate microbes and facilitate degradation of contaminants (limited to the treatment of superficial 10–35 cm of soil) aerobically [6]. This method is used to treat spilled oil wastes [6]. It is very important for pesticide degradation, the scarped soil is placed between the clean and clay/concrete soil. This method thus helps in providing oxygen nutrition and moisture. It also maintains the pH near the neutral value thereby improving the soil quality.

(3) Composting

It's an ancient technology practiced even today at a very large scale. The basic principles required for the Integrated Solid Waste Management (ISWM) involves 4Rs principles, i.e., reductions, reuse, recycling and recovery methods [78]. Composting of piled up organic materials take place either aerobically or anaerobically where aerobic composting is considered to be the most efficient form of decomposition. At controlled conditions, composters could successfully breakdown large particles. Under optimal conditions, fungi, soil bacteria, protozoa and actinomycetes concentrate the organic matter and activate the composting process. The average temperature curve during aerobic composting showed three phases, which include (1) Mesophilic or moderate temperature phase, (2) thermophilic or high temperature phase and (3) cooling (maturation) phase [78].

(4) Biosparging

In this process, the oxygen concentration is elevated at the remediation site for successful pollutant degradation by microorganisms. This is facilitated by injecting the air

forcefully below the groundwater or into the soil subsurface, mainly at the saturated zone, to stimulate microbial activities resulting in aerobic degradation of pollutants [79]. This promotes movement of organic compounds from saturated to unsaturated zone and helps in biodegradation. Biosparging efficacy is determined by the permeability quality of the soil, which regulates pollutant bioavailability and degradability by microorganisms [79].

(5) Bioventing

This technique involves aerobic degradation of pollutants by injecting oxygen and introducing nutrients such as nitrogen and phosphorus to the contaminated site. Sufficient amount of oxygen is supplied via low air flow rate to sustain the microbial activity. Improved efficacy of bioventing process can be observed if the water table is situated deep below the surface and if the site has elevated temperature. It is predominantly used to remove contaminants namely gasoline, oil, petroleum, etc. [6].

Challenges or limitations of bioremediation

Despite having many advantages of bioremediation techniques, they have some limitations too which are as follows:

1. **Specificity:** Since biological processes are very specific, they are dependent on optimum mixture of abiotic as and biotic factors as mentioned above. This process is tedious since it requires more attention to maintain a suitable environment for the growth and activity of microorganisms [80].
2. **Time-consuming process:** Bioremediation process is found to be a time-consuming technique as compared to physicochemical method. Degradation of waste materials such as domestic and organic wastes takes minimum of six months to one year [80].
3. **Uncertainty of complete bioremediation and end product quality:** Since it is difficult to assess the performance of bioremediation, the complete remediation of waste and toxic materials cannot be determined. Also, it is difficult to ensure that the end product of bioremediation process is completely non-toxic to the environment [80].
4. **Energy sources:** Availability of reduced organic materials which serve as energy source to the microorganisms. Higher oxidation states of carbon in the organic material could serve as low energy source and thus negatively affect the degradation rate of microbes [80].
5. **Accessibility of pollutants:** The availability or accessibility of pollutants (mainly in soil) is determined by their toxicity level, adsorption and utilization properties. Many organic compounds such as pesticides and petroleum oil, hydrocarbons are not easily adsorbed by the microorganisms since they are present in more sorbet state in the environment. This reduces the bioavailability of these organic pollutants and thereby lowering their degradation [80].
6. **Microbial adaptability:** Some of the exogenous microorganisms are not able to adapt to the new environment and thus cannot act efficiently at the bioremediation site [80].
7. **Limited knowledge on metabolic pathways:** The microbial metabolic pathways shown during bioremediation process especially towards organic and heavy metal pollutants are still not completely known which limits the proper usage of microbes for bioremediation purposes [80].
8. **Enzyme activities:** The beneficial enzymes which are majorly involved in bioremediation process in microorganisms are still not completely unfolded [80].

Conclusion

Bioremediation process is a promising and upcoming technology used in the removal of contaminants. Since this technology uses various living microorganisms to improve the environmental conditions, this technology is relatively environment and budget-friendly. Although bioremediation technique has many advantages, it has some disadvantages too which needs to be addressed. The main disadvantages are that they are time-consuming and need optimum and strictly maintained environment suitable for microbial activity. Thus, the present review has explained various strategies exhibited by different microorganisms including both pro- and eukaryotes, i.e., bacteria, fungi, algae, ciliates and yeasts to detoxify various inorganic and organic environmental pollutants emphasising on improving various abiotic factors that regulate the microbial activity for degradation of pollutants. This bioremediation technology can be used at a large scale for various industrial applications and for environmental cleaning purposes.

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Conflicts of interest

The authors declare no conflicts of interest.

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