

Advances in microbial bioremediation and the factors influencing the process

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Abstract Irrational and rapid global human societal development has culminated to a condition of environmental deterioration. Accidental leakage and deliberate use of organic and inorganic chemicals have contaminated the environment up to the level of ecosystem. Advancements have been made in the field of research on bioremediation of the hazardous contaminants especially in last three decades. Microbial bioremediation has been the most understood biotechnological process of environmental restoration. Bacteria and fungi because of their inherent ability to adapt and grow in extreme environments have been employed for either removal or degradation of the chemical contaminants. Researchers all over the world are getting breakthroughs in finding new bacterial strains having plasmid linked degradation/reduction ability. Molecular biology and genetic engineering helped in crafting the microbes for the desired results on environment. Despite having favorable conditions, microbial remediation largely depends on

environmental factors and on the basic biological characters of microbes, especially bacteria being Gram-positive or Gram-negative. Metagenomic studies revealed the importance of microbial ecology as microbes work well in community, i.e., consortia. This review along with several other studies suggests the need of precision during microbial community identification, substrate specificity and the designing of microbes.

Keywords Environmental contamination · Climate change · Bioremediation · Plasmids · Metagenomics · Gram-positive bacteria · Gram-negative bacteria

Introduction

Environmental degradation signifies the damages of varying degrees to the soil, water and air characterized by substantial physicochemical alterations. Several remedies (natural and man-made) have been suggested in last three decades to mitigate the pollution from the environment. The literature is vast and is far beyond the capacity to be included here, however, e.g., Lovely (2003), Paul et al. (2005), Singh et al. (2006), Wei et al. (2007), Badri et al. (2009), Chojnacka (2010), Magan et al. (2010), Rajkumar et al. (2010), Glick (2010), Maphosa et al. (2012) and Zhang et al. (2012), delineating latest and upgraded techniques involving living organisms, specifically plants and microbes, for the removal and/or immobilization of contaminants, known as bioremediation. Phytoremediation (remediation through plants) is a novel approach (Shukla et al. 2009), however; it has certain technical shortcomings such as management of biomass (Wei et al. 2007) and the growth conditions as plants are restricted only to

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certain geographical regions. As far as the microbial species are concerned, only 5 % microbial diversity is known to date and 95 % is yet to explore. Successful microbial bioremediation is achieved when microbes interact within their niche, under the most favorable conditions (Riccardi et al. 2005). Biostimulation (enhancing the activity of native microbes), bioaugmentation (increasing the viable microbial counts), bioaccumulation (storage of toxic or nontoxic elements by the microbes) (Vasudevan et al. 2001; Tyagi et al. 2011), biosorption (removal of elements from the environment through adsorption) (Gupta et al. 2000) and the use of biofilms (Singh et al. 2006) are some of the examples of recent advancements in microbial bioremediation techniques. Bioremediation involves a number of species of microbes having different mechanisms depending upon the environmental factors and the nature of chemicals (Boopathy 2000) either to degrade or to remove the toxic contaminants from the environment. Elevated ambient atmospheric temperature (a climate change factor) may have impacts on the microbial activity (Margesin and Schinner 2001), especially on the upper layers of soils subjected to the contamination. In addition, such extreme environmental conditions may change microbial count and the diversity as well. In the present review, the current state of knowledge regarding microbial remediation along with the brief review of the factors influencing the processes has been reviewed.

Microbial bioremediation

Microbial bioremediation is the natural way to mitigate environmental pollution. The process includes the activities of aerobic and anaerobic microorganisms. Despite having different nutritional requirements, both life forms have basic need of organic carbon and a source of energy. There are several regions other than the typical ones, such as soil, water and sediments, which can be identified as the potential site of microbial actions. Such regions include the rhizospheric zone of plants, gravel-associated community, aquatic shorelines, subsurface layer of marshland and the weathered rocks. Higher microbial counts and diversity is directly associated with the higher content of organic matter in soil (Boopathy 2000), however; the presence of organic substances of high molecular weight such as polycyclic aromatic hydrocarbons (PAHs) may limit the microbial activity (Nam et al. 2001). On the contrary, Hesham et al. (2012) reported the degradation of chrysene and benzo(a)pyrene (PAHs of high molecular weight) by the yeast fungi (Table 2) up to 98.5 and 95.2 %, respectively, in soil.

In addition to this, several recent reports (Zhuang et al. 2012; Torres-Bojorges and Buitron 2012; Chiang et al. 2012; Yassine et al. 2013) suggest that the nature and occurrence of chemicals hardly disturb the bioremediation process. Generally, organisms rely upon associations with their neighbors for the sustenance of life (Badri et al. 2009). Microbes also interact with other organisms within their surroundings for organic carbon, energy and shelter, such as arbuscular mycorrhizal fungi (Harrison 2005) and plant-growth-promoting rhizobia (Bais et al. 2006), protecting the host organisms from several environmental toxic chemicals (Arriagada et al. 2007; Srivastava et al. 2010). Microbes have wider range of growing conditions and can withstand higher concentrations of chemical contaminants. For better understanding, microbial bioremediation can be classified on the basis of the nature of substrate undergoing either removal or degradation into two, viz., (1) microbial remediation of inorganic contaminants and (2) microbial remediation of organic contaminants (OCs).

Microbial remediation of inorganic contaminants

Major inorganic contaminants include toxic heavy metals and their compounds originated from various industrial processes, such as mining, metallurgy, power plants and chemical manufacturing processes (Goyal et al. 2003). In general, toxic elemental pollution is one of the major concerns of environmentalists and biologists because the disposal of toxic metals to the surface, subsurface soils and waters causes unacceptable health risks (Kaewsarn and Yu 2001). Microbiological removal of metal ions from the environment is a new biotechnique (Shukla et al. 2009) and the most cost-effective approach in mitigation of elemental pollution. Heavy metals and their removal are well-researched issues as evident from the existing research literatures (Lovely and Coates 1997; Lovely and Lloyd 2000; Garbisu and Alkorta 2003; Malik 2004; Shukla and Rai 2006; Kabata-Pendias and Mukherjee 2007; Kim et al. 2007; Srivastava et al. 2008; Badri et al. 2009; Rajkumar et al. 2013) and the references therein. It is imperative to know that microbes in any case cannot degrade the metal ions and are only able to transform metallic ions from higher to lower oxidation states to stabilize them (Garbisu and Alkorta 2003). Further microbes are able to metabolize, detoxify and accumulate metals mostly in cell wall like any other nutrient element. Table 1 presents few examples of toxic metal-remediating microbes. Microbes releasing chelating agents and acids altering physicochemical properties such as redox potential (Eh) of surroundings (Abou-Shanab et al. 2003) bring

Table 1 Microbes (bacteria and fungi) and the remediated elemental pollutants

Microbial species	Gram \pm	Occurrence	Remediated metal	References
<i>Pseudomonas putida</i>	Gram (–ve)	Soil and sediments	Cu	Wood and Wang (1985)
<i>Ralstonia eutropha</i>	Gram (–ve)	Soil and sediments	Ni resistant	Mergeay (1991)
<i>Acinetobacter</i> sp.	Gram (–ve)	Sediments	Cyanide salts	Finnegan et al. (1991)
<i>Helicobacter pylori</i>	Gram (–ve)	Soil and sediments	Ni	Hendricks and Mobley (1997)
<i>Pseudomonas stutzeri</i>	Gram (–ve)	Soil and sediments	Ni	Joerger et al. (2001)
<i>Staphylococcus carnosus</i>	Gram (+ve)	Soil and sediments	Cd and Ni	Samuelson et al. (2000)
<i>Pseudomonas</i> spp.	Gram (–ve)	Soil and sediments	U accumulator	Sar and D'Souza (2001)
<i>Aspergillus niger</i>	Fungi	Soil and sediments	Cr(VI)	Goyal et al. (2003)
<i>Saccharomyces cerevisiae</i>	Fungi	Sediments	Cr(VI)	Goyal et al. (2003)
<i>Gallionella ferruginea</i> ,	Gram (–ve)	Sediments	As (adsorption)	Katsoyiannis and Zouboulis (2004)
<i>Leptothrix ochracea</i>	Gram (–ve)			
<i>Lentinus sajor-caju</i>	Fungi	Sediments	U	Bayramoğlu et al. (2006)
<i>Salmonella</i> spp.	Gram (–ve)	Sediments	Zn(II)	Nweke et al. (2007)
<i>Desulfovibrio</i> spp., <i>Geobacter</i> spp.	Gram (–ve)	Soil	U(VI)	Cardenas et al. (2008)
	Gram (–ve)			
<i>Acidovorax</i> spp.	Gram (–ve)	Soil	U(VI)	Cardenas et al. (2008)
<i>Pseudomonas</i> spp.	Gram (+ve)	Wastewater	Cr(VI)	Srivastava et al. (2008)
<i>Aspergillus flavus</i>	Fungi	Soil	Cr(III)	Han and Gu (2010)
<i>Cenarchaeum</i> spp.	Gram (–ve)	Sediment and soil	NH ₄ ⁺ oxidizing	Hong et al. (2012)
<i>Trichoderma asperellum</i>	Fungi	Sediment	Cu (II)	Tan and Ting (2012)
<i>Geobacter bemidjensis</i>	Gram (–ve)	Sediment	Fe(III)	Luef et al. (2013)
<i>Desulfovibrio vulgaris</i> (ATCC 29579)	Gram (–ve)	Waste water	Cr(VI) reduction	Mabbett et al. (2002)

substantial changes by increasing the bioavailability of metal ions (Abou-Shanab et al. 2006). In addition, physicochemical reaction such as biosorption is the first step in the interaction between microbial cell and metal ions (Han and Gu 2010) followed by the physical adsorption, ion exchange and complexation (Fig. 1). Biochemical reactions during microbial transformation of metal ions involve the action of specific enzymes for oxidation, reduction, methylation, dealkylation and precipitation (Han and Gu 2010). Microbes have diversified conditions for their growth with ability to adapt to the changing environmental conditions, e.g., Naraian et al. (2012) reported that the multiple-drug-resistant strain of *Pseudomonas aeruginosa* T-3 isolated from tannery effluent had plasmid-encoded chromate ion resistance, exhibiting the adaptability feature of microbes. Plasmid-encoded copper and cadmium metal resistance has also been reported in *Pseudomonas putida* PhCN (El-Deeb 2009). Advancements in microbial remediation techniques and studies on plasmid-encoded biochemical information and genetic engineering helped in designing new strains of known bacterial species for targeted bioremediation, e.g., recombinant *Escherichia coli* expressing metallothionein gene (*Neurospora crassa*) for

Cd uptake, which was more rapid than the gene donor microbial species (Pazirandeh et al. 1995). *Staphylococcus xylosus* and *Staphylococcus carnosus* were designed by the introduction of polyhistidyl peptides encoding genes conferring Ni-binding capacity to these microbes (Samuelson et al. 2000).

Microbial remediation of organic contaminants (OCs)

Widespread and massive applications of organic compounds such as biocides, flame retardants, polymers, solvents, crude oil, explosives and chlorinated organic compounds have contaminated the environment, causing threats to most of the life forms on earth. Microbes have proven ability to degrade most of the OCs such as PCBs, PAHs and polybrominated biphenyl ethers (PBEs) in the environment (Hiraishi 2008; Fennell et al. 2011). Bio-remediation of OCs in environment is the most studied field of microbiology. Biodegradation of OCs refers to the catabolic activity of microbes transforming them into less or completely nontoxic residues (Margesin et al. 2000). The organic substrate is used by the microbes as the source of organic carbon and energy. Some of the important findings on bioremediation of hazardous



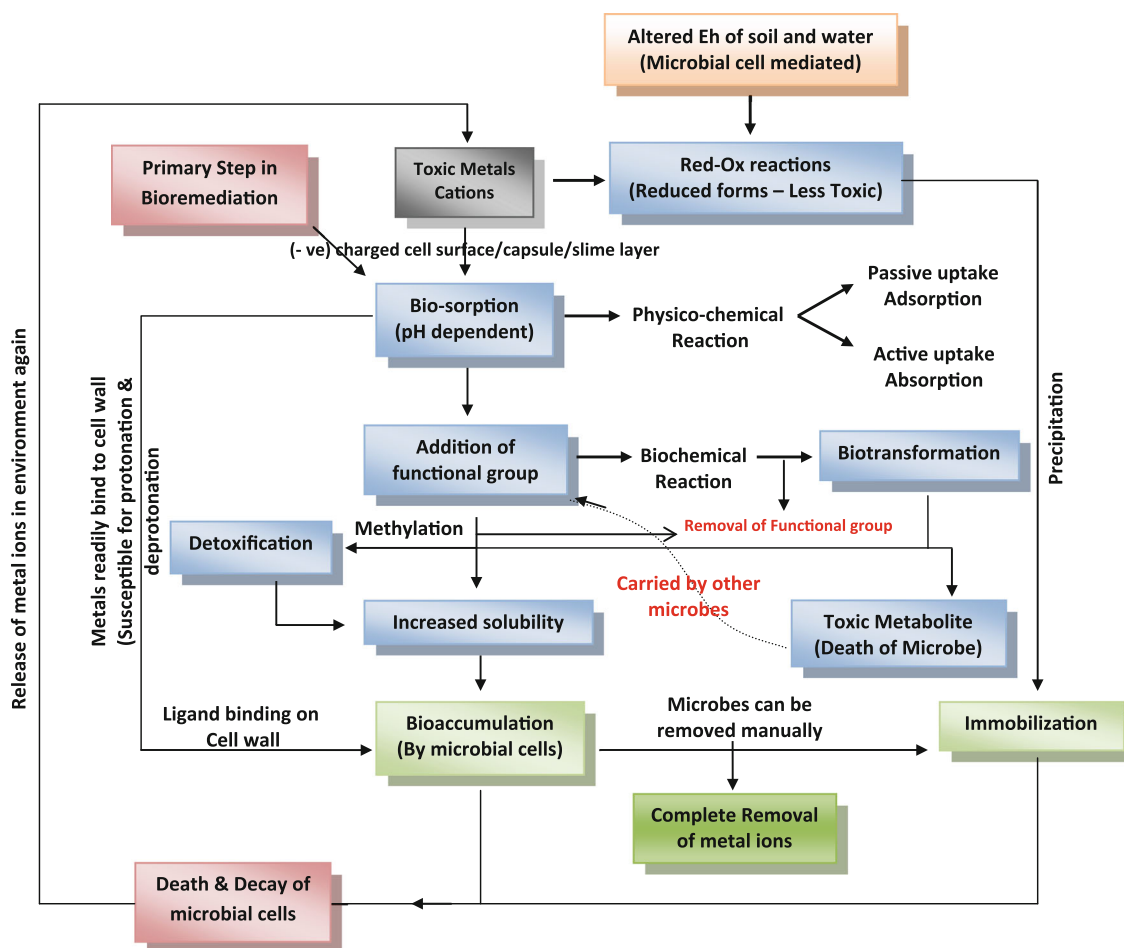


Fig. 1 Most preferable bioremediation mechanisms followed during the interaction between microbes and metal

organics and their mechanisms have been well reported by Watanabe et al. (2000); Widada et al. (2002); Li et al. (2004); Fragoeiro and Magan (2008); Magan et al. (2010); Fennell et al. (2011); Torres-Bojorges and Buitron (2012); Maphosa et al. (2012) and Weber and Leqqe (2013). Table 2 presents a brief index of some microbial species and their strains capable of degrading OCs. In general, single microbial species hardly degrades any organic substrate in isolation (Pieper and Reineke 2000) and works well in community. Interactions between microbes in the community ensure the exchange of genetic information between microbial species conferring resistance, tolerance and chemical-degrading ability. Most often the microbial species that are able to degrade OCs are misidentified owing to the lack of standard microbial identification techniques and laboratory practices in most of the parts of the world (Endy 2005), signifying the importance of researches on the microbial consortia via the tools of metagenomics and the standard genetic engineering protocols. In general,

microbes can degrade a variety of organic substances depending upon the plasmid-encoded catabolic processes (Table 3) and chromosomal genes (in case of bacteria) or the extracellular enzymatic activity (fungal degradation process). These processes are further subjected to the varying environmental conditions influencing the growth pattern of microbes (Watanabe 2001) (Fig. 2). In scientific perspective, genetic engineering is one of the elite biotechnological branches offering infinite combinations of genetic codes imparting the knowledge of designing of potentially degrading and substrate-specific microbes (Chaloupkova et al. 2003; Li et al. 2004; Ang et al. 2005; Wood 2008). For a successful bioengineered microbe, it is mandatory to identify the relevant species and their substrate-specific strains (Wood 2008). In addition, genetic modification with DNA reshuffling has provided enormous combinations enabling microbial cells to degrade even the most recalcitrant organic compounds. Apart from recombinant, microbes naturally degrade the recalcitrant OCs by triggering cometabolic



Table 2 Potentially hazardous organic chemicals and their degrading microbes (bacteria and fungi)

Microbial species	Gram \pm ve	Occurrence	Organic substrate	References
<i>Pseudomonas putida</i> (G7)	Gram (–ve)	Soil	Naphthalene (PAH)	Grimm and Harwood (1997)
<i>Pseudomonas putida</i> (GJ31)	Gram (–ve)	Soil	Chlorobenzenes	Mars et al. (1997)
<i>Klebsiella pneumonia</i> (RS-13)	Gram (+ve)	Water	N,N-dimethyl-p-phenylenediamine	Wong and Yuen (1998)
<i>Acetobacter liquifaciens</i> (S-1)	Gram (–ve)			
<i>Pseudomonas stutzeri</i>	Gram (–ve)	Soil	Naphthalene	Bosch et al. (2000)
<i>Burkholderia</i> spp.	Gram (–ve)	Soil	Polycyclic aromatic hydrocarbons	Grosser et al. (2000)
<i>Kluyveromyces marxianus</i> (IMB3)	Yeast (fungi)	Waste water	Remazol Black B	Meehan et al. (2000)
<i>Agrobacterium radiobacter</i> (S2)	Gram (–ve)	Waste water	Sulfonate benzene or Naphthalene	Contzen and Stolz (2000)
<i>Phanerochaete chrysosporium</i>	Fungi	Soil	4,4 dibromodiphenyl ether	Mikulasova et al. (2001)
<i>Vibrio cyclotrophicus</i> sp. nov. (ATCC 700982)	Gram (–ve)	Soil	Polyaromatic hydrocarbons	Hadlund and Staley (2001)
<i>Pseudomonas putida</i> (F1)	Gram (–ve)	Soil	Toluene and its derivatives	Leahy et al. (2003),
<i>Ralstonia picketti</i> (POK 01)	Gram (–ve)			Parales et al. (2000)
<i>Burkholderia cepacia</i> (G4)	Gram (–ve)			
<i>Gliocladium virens</i>	Fungi	Waste water	Lignocellulosic compounds	Murugesan, 2003
<i>Myceliophthora thermophila</i>	Fungi	Soil	Polycyclic aromatic hydrocarbons	Bulter et al. (2003)
<i>Acenotobacter</i> sp. (OK1)	Gram (–ve)	Water and soil	Hydrocarbons (Petroleum)	Koren et al. (2003)
<i>Dechloromonas</i> sp. (RCB)	Gram (–ve)	Water and soil	Benzene, Toluene, Xylene	Chakraborty et al. (2005)
<i>Rhodococcus</i> spp.	Gram (+ve)	Soil	Naphthalene	Alquati et al. (2005)
<i>Phanerochaete chrysosporium</i>	Fungi	Soil	Polyethylene, polypropylene	Zhou et al. (2007)
<i>Pleurotus sajorcaju</i> (MTCC 141)	Fungi	Waste water	Decoloration of effluent	Yadav and Yadav (2008)
<i>Sinorhizobium meliloti</i> (P221)	Gram (–ve)	Soil	Polycyclic aromatic hydrocarbons	Golubev et al. (2009)
<i>Stenotrophomonas maltophilia</i> (WZ2)	Gram (–ve)	Soil	Quinclorac (Herbicide)	Lü et al. (2009)
<i>Pseudomonas fluorescens</i>	Gram (–ve)	Waste water	Direct orange 102 dye	Pandey and Upadhyay (2010)
<i>Mycobacterium gilvum</i>	Gram (+ve)	Sediments	Pyrene, benzo(a)pyrene	Toyama et al. (2011)
<i>Saccharomyces cerevisiae</i>	Gram (–ve)	Soil	Chrysene, Benzo(a)pyrene, Methyl red (azo dye)	Hesham et al. (2012),Jadhav et al. (2007)

The strains mentioned in front of corresponding microbes have been adopted from the duly acknowledged literatures

processes utilizing other readily available sources of organic carbon and energy present in the vicinity. To find the optimum conditions for growth, microbes utilize the change of chemical gradients in the vicinity. Bacterial chemotaxis is one of the best examples whereby the bacterial cells move with the change of chemical gradients (Pandey and Jain 2002), e.g., *Pseudomonas putida* G7 is attracted by naphthalene (Grimm and Harwood 1997). Technological advancements in microbial bioremediation include the conversion of slow biodegradation process into a rapid by means of several modern methods including microbial fuel cells (MFCs) (Rabaey and

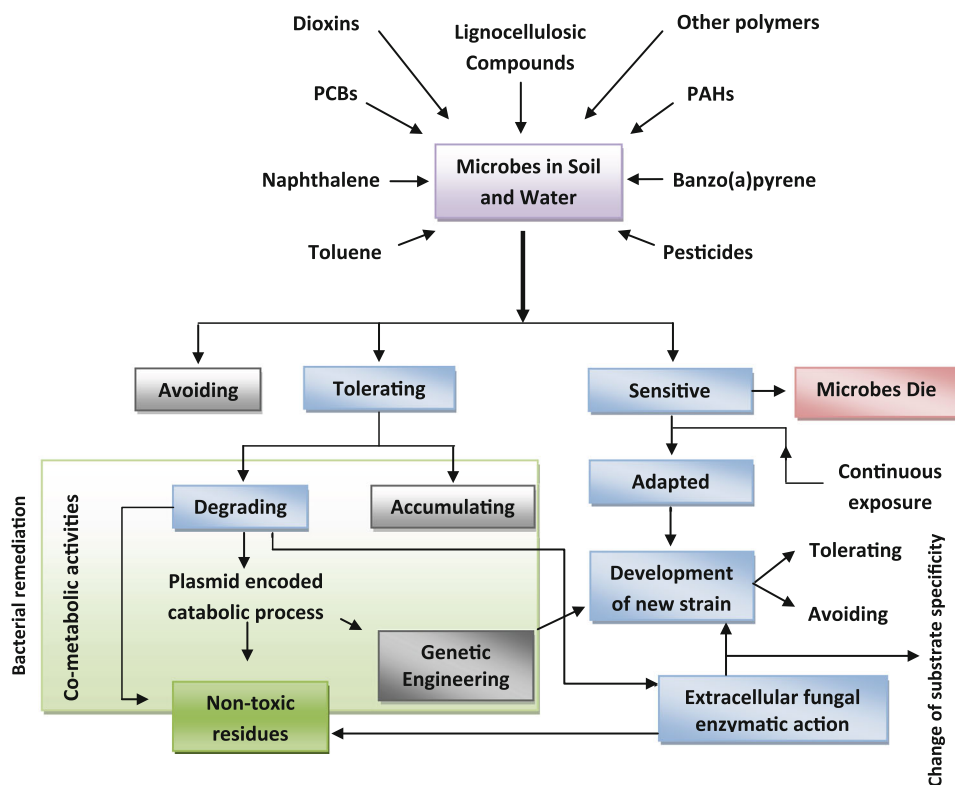
Verstraete 2005; Moris and Jin 2012), bioreactors (Robles-González et al. 2008), biofilms (Singh et al. 2006) and the use of microbial consortia for the degradation of recalcitrant organic compounds (Torres-Bojorges and Buitro 2012).

Fungi have, however, several advantages over bacterial degrading processes as the former can grow at higher concentrations of toxic organics (Fragoeiro 2005), e.g., *Phanerochaete chrysosporium* (a white rot fungi) metabolize lignocellulosic compounds by the activity of degrading enzymes such as *laccase* (LAC), lignin peroxidase (Lip) and manganese peroxidase (Schmidt et al.



Table 3 Some typical examples of plasmids encoded with the genes capable of catabolic degradations of organic compounds

Plasmid	Isolated from microbes	Organic substrate	References
<i>pMFB1</i>	<i>Pseudomonas pseudoalcaligenes</i> KF707	Biphenyls, toluene, benzene	Furukawa and Miyazaki (1986)
<i>bphA</i>	<i>Burkholderia</i> sp. LB400, <i>Rhodococcus globerulus</i> P6	PCBs, biphenyls, toluene, benzene	Barriault et al. (2002)
<i>IsoILR1</i>	<i>Burkholderia</i> sp. V106 A (Tom green)	<i>Cis</i> -1,1,dichloroethylene	Rui et al. (2004a)
<i>IsoLR1</i>	<i>Rhodococcus</i> sp. AD45	Epoxypropane, <i>cis</i> -DCE	Rui et al. (2004b)
<i>pNL1</i>	<i>Sphingomonas aromaticivorans</i> F199	Naphthalene, biphenyls, m-xylene, <i>p</i> -cresol	Romine et al. (1999)
<i>pADP-1</i>	<i>Pseudomonas</i> sp. ADP	Atrazine	Martínez et al. (2001)
<i>pWVO</i>	<i>Pseudomonas putida</i> PaW1	Toluene, Xylene	Greated et al. (2002)
<i>pCAR-1</i>	<i>Pseudomonas resinovorans</i> CA10	Carbazole	Maeda et al. (2003)
<i>pCAR-3</i>	<i>Sphingomonas</i> sp. KA1	Carbazole	Shintani et al. (2007)
<i>NAH-7</i>	<i>Pseudomonas putida</i> G7	PAHs, e.g., naphthalene	Yen and Serdar (1988)
<i>pDTG1</i>	<i>Pseudomonas putida</i> NCIB9816	PAHs, e.g., naphthalene	Yen and Serdar (1988)
<i>pND6-1</i>	<i>Pseudomonas</i> sp. ND6	Naphthalene	Li et al. (2004)
<i>pPhCN-1</i>	<i>Pseudomonas putida</i> PhCN	Phenol	El-Deeb (2009)
<i>pENH91</i>	<i>Alcaligenes eutropha</i> NH-9	3 CBA	Ogawan and Miyashita (1999)
<i>RP4::Tn4371</i>	<i>Enterobacter agglomerans</i> DK3	Biphenyl	De Rore et al. (1994)
<i>pTOM8</i>	<i>Alcaligenes xylosoxidans</i> 31A	Nickel resistance	Schmidt and Schlegel (1994)
<i>NikD</i>	<i>Helicobacter pylori</i>	Nickel resistance	Hendricks and Mobley (1997)

Fig. 2 Organic compounds degradation strategy followed by microbes

2005); similarly, catechol 2,3-dioxygenase (C-23O) produced by yeast fungi cleaves the benzene ring (Hesham et al. 2012). Table 2 presents few more examples of fungal degradation of OCs. The details of fungal role in bioremediation have been extensively reviewed by Gadd (2001). The study of factors influencing bioremediation by white rot fungi has been broadly described by Magan et al. (2010).

Role of Gram-positive and Gram-negative bacteria in bioremediation

It is interesting to understand the behavior of Gram-positive and Gram-negative bacteria during their interaction with toxic organic and inorganic pollutants in environment. Gram-positive bacteria are metabolically diverse and grow on the sites contaminated with aromatic hydrocarbons and toxic metals (Narancic et al. 2012). Tables 1 and 2 show certain examples of Gram-positive bacteria, e.g., *Staphylococcus* sp. and *Rhodococcus* sp., remediating environmental pollution. In addition to this, member of *Rhodococcus*, *Bacillus*, *Arthrobacter*, *Gordonia*, *Streptomyces* and *Nocardia* genus can degrade a variety of OCs, such as biphenyls, benzene (Narancic et al. 2012) and naphthalene (Al-quati et al. 2005). Studies show that few other Gram-positive bacteria such as *Corynebacterium* and *Mycobacterium* sp. withstand the anaerobic and extreme toxic conditions such as oil sludge (Lăzăroaie 2010). Typical examples of toxic metal tolerance in Gram-positive bacteria include *Bacillus megaterium* MB1 resistant to mercury because of TnMER11-resistant transposon (Huang et al. 1999). A comparative study of zinc toxicity on particular strains of Gram-positive and Gram-negative bacteria, viz., *Bacillus* sp., *Arthrobacter* sp. and *Salmonella* sp., by Nweke et al. (2007) showed that Gram-positive bacterial species are sensitive as compared with the Gram-negative species for the toxicity of zinc. In general, Gram-negative bacteria have better tolerance against metallic pollution (Minz et al. 1996; Nweke et al. 2007). Binding affinity with the metallic cations hardly differs between Gram-positive and Gram-negative bacteria, however; findings of Churchill et al. (1995) showed the different specificity in sorption of metal cations by Gram-positive bacteria (*Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*), whereby Co^{2+} was observed to be sorbed more than Ni^{2+} by both *M. luteus* and *E. coli* in a binary mixture of cations. Lăzăroaie (2010) also reported Gram-negative bacteria to be more tolerant to toxic mixture of saturated hydrocarbons, monoaromatic hydrocarbons and polyaromatic hydrocarbons as

compared with the Gram-positive bacterial cells. As evident from Tables 1 and 2, Gram-negative bacteria have wider range of toxicity tolerance of OCs and metal pollutants, however; few studies (Zahir et al. 2006; Segura et al. 2008) support that Gram-positive bacteria are even more tolerant for certain pollutants, such as hydrocarbons and PAHs. Although mechanistically Gram-negative bacteria are more tolerant because of the presence of an outer membrane made of lipopolysaccharide and proteins imparting it impermeability for toxic compounds, e.g., hydrocarbons that may be associated with the protection of microbes from being exposed to toxic elemental pollutants and OCs.

Factors affecting microbial remediation

Various biotic and abiotic factors affect the behavior and the growth of microbial cells, thereby affecting various biological processes occurring in a microbial community. Bioremediation process is subjected to the multiphasic heterogeneous surroundings (Boopathy 2000) influencing the rate of the reactions. Lack of information regarding the factors influencing the process often reduces the efficacy of process when implemented (Lovely 2003). Microbes have inherent ability of first rate to get adapted to the changing environment though they have certain limitations. In-depth understanding of microbial ecology is required for the improvement in the microbiological action and to predict the successful bioremediation process (Watanabe 2001). Figure 3 shows the environmental challenges that microbes face in their life. Three types of factors affecting the microbial processes have been shown in Fig. 3, viz., (1) physicochemical characteristics of environment or the abiotic factors, (2) biological factors or biotic factors and (3) climatic conditions whereby physicochemical and climatic conditions are among the major factors affecting the metabolic rates in microbes.

Physicochemical factors affecting bioremediation

Physicochemical factors include a set of parameters such as redox potential (Eh), pH, ionic strength, solubility, presence or absence of electron acceptors and donors, temperature and age of organometallic ions. Biosorption, a primary step in toxic metal removal by microbes, is a pH-dependent phenomenon whereby pH value influences the isoelectric point in a solution, affecting the net negative charge on the microbial cell surface. Additionally, this change brings changes in ionic state of ligands, e.g., a carboxyl residue, phosphoryl residues, S–H groups and amino acid groups



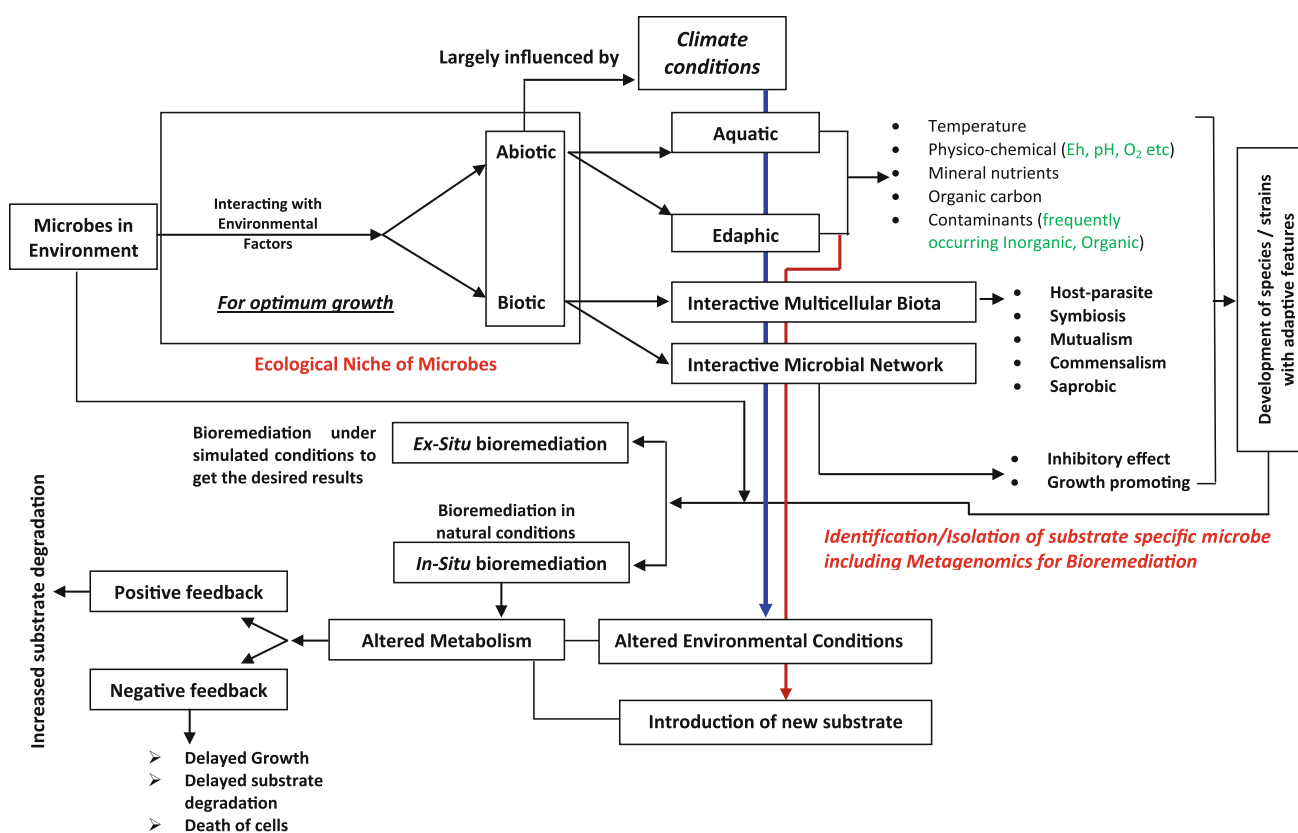


Fig. 3 Simple layout showing factors influencing the microbial ecology, thereby influencing microbial bioremediation processes

(Sag et al. 1995). Bioremediation techniques involve reduction of metal ions to insoluble form by the microorganisms from higher to lower oxidation state (pH dependent) as soluble metal ions can only undergo enzymatic reduction. Higher oxidation states of metals are generally soluble (Garbisu and Alkorta 2003). Solubility plays an important role in degradation of OCs as hydrophobic or sparingly soluble compounds persist in the environment for long time and are bio-unavailable (Pieper and Reineke 2000). pH values affect the solubility of metal ions, which increases with the decrease in pH of medium, thereby affecting adsorption by microbial cells (Blázquez et al. 2008). In addition, lower pH values are required for metal ions to get attached on the microbial cell surface (Rajendran et al. 2002; Han and Gu 2010), whereas alkaline medium favors precipitation of metal ions. Organic acid-producing microorganisms *Pseudomonas* sp. and *Burkholderia cepacia* produce gluconic acid and *Rhizobium* sp. and *Bacillus firmus* produce ketogluconic acid (Robles-Gonzalez et al. 2008), which lower the pH of the system, which increases the solubility of metal ions. Organometallic compounds increase mobilization of metal ions (Puzon et al. 2005). Bioremediation is

strongly inhibited by toxic metal ions, such as Zn^{2+} , which acts as a respiratory inhibitor in microbes (Beard et al. 1995). The presence of electron acceptors, e.g., oxygen in aerobic microbes and NO_3^{1-} , SO_4^{2-} and Fe(III) oxides in case of anaerobic microbes, also affects the biodegradation processes (Lovely 2003).

Biological factors affecting bioremediation processes

Biological factors are not as such very obvious, however; the importance of these factors is often realized while implementing bioremediation technique. There are several inherent characters of microbes that affect the substrate degradation, e.g., plasmid-encoded genes provide specificity for substrates and encode the specific enzymes (proteins), but it has been observed in nature that microbes, especially bacterial cells, have diversified specificity for different substrates (Mars et al. 1997). Bacterial chemotaxis is an advantageous behavior of bacteria for the degradation of recalcitrant organic compounds (Pandey and Jain 2002). As already mentioned earlier in this review, single microbial cell cannot achieve complete degradation of chemicals. Microbial communities required for bioremediation most often depend on complex multispecies

interactive networks as shown in Fig. 3. Organohalide respiratory bacteria thrive only in consortia, and their isolation and culture is very difficult. Maphosa et al. (2010) defined it as a function of metagenomics or community genomics whereby the metagenomics sequencing helps providing data of member species of consortia that support substrate degradation. Moreover, the optimum growth conditions of microbes are unpredictable (Ingham et al. 2007). Microorganisms are vital for the efficient functioning of ecosystems on earth, and factors affecting metabolism, composition and abundance of microbes and microbial communities may disturb the ecosystems (Nweke et al. 2007). Allelopathic response of terrestrial plants on the microbial community (Chakraborty et al. 2012) may negatively influence the degradation potential of soil microbes. Solubility of OCs and the absence of oxygen cause reduction in aerobic microbial activity. Certain microbes, however, utilize other sources of organic carbon, electron acceptor and energy as cometabolic substrate and degrade the recalcitrants, e.g., *Mycobacterium gilvum* reportedly degrade pyrene aerobically in the rhizospheric zone of *Phragmites australis* simultaneously with the degradation of benzo(a)pyrene (Toyama et al. 2011). Initiation of microbial degradation of xenobiotics depends on the toxic pressure exerted by the contaminants on the microbes that induce enzymatic modifications (Pandey and Jain 2002).

Climate change and bioremediation processes

Global climate change is characterized with the factors such as elevated CO₂ and atmospheric temperature. Soil microbial community plays an important role in cycling of carbon (C) as microbes exhibit increased ability to decompose soil organic matter at elevated CO₂ (Nie et al. 2013) and nutrients in ecosystems which are largely influenced by biotic and abiotic factors (Fig. 3). There is no direct evidence of any likely impacts of climate change on the bioremediation process, however; researches on soil microbes and climate change (Sowerby et al. 2005; Castro et al. 2010; Nie et al. 2013) suggest changes in the physicochemical properties of the microbial niche, which may alter microbial metabolic processes and thereby the bioremediation. Microbial extracellular enzyme production is linked to microbial activity as well as soil physicochemical properties (Sowerby et al. 2005), which are influenced by climatic conditions. One of the breakthrough findings belongs to Frey et al. (2013), whereby increased utilization efficiency of recalcitrant substrate in soils by the microbes at higher temperature as a positive

feedback to climate has been reported although the feedbacks from ecosystems to the climate have reportedly been regulated by microbial communities (Bardgett et al. 2008). Elevated ambient CO₂ level has been found associated with the increase in bacterial abundance (Castro et al. 2010) and decreased fungal abundance (Frey et al. 2008). Decrease in fungal biomass at warm and dry conditions decreases the fungal/bacterial biomass ratio in soil (Sowerby et al. 2005), indicating decreased magnitude of carbon cycling in an ecosystem which further disturbs the natural degradation process by naturally occurring microbes capable of degrading the hazardous chemicals cometabolically facilitated by the naturally available carbon. It has been predicted that if temperature of soil increases due to the global climate change, microbial enzymatic activity will rise substantially (Baldrian et al. 2013).

Future studies in bioremediation

Issues related to the metagenomics (community structure), identification of microbes and their implementation on the bioremediation under altered environmental conditions may be the main thrust of future studies. Since the urbanization is a global phenomenon, the urban waste treatment with naturally occurring and genetically modified microbes is not a cost-effective process as it needs a proper facility requiring extensive economic support. Although commercial use of waste treatment is now a realization, the incurred input of money is far greater than the output. Scientists are continuously making efforts to get the economical gains of microbial remediation of waste generated. These include the environmentally safe energy production from the waste through microbial activity, production of biofertilizers and recycling of material. Several other issues including mechanistic approach of degradation/removal, microbial response to changing environment, recalcitrant metabolites, proteomics and the chemistry of new xenobiotic compounds are some of the endless studies yet to investigate comprehensively. Adaptability features of microbes make it more difficult for the researchers to ascertain the specific pollution remediation technique; therefore, more precise techniques are required to identify microbes and the substrate specificity as well. Microbial resistance development for various organic chemicals through transformation of genetic information between microbes and the rate of this exchange may need a scientific surveillance study to ensure the complete safety of the living world from the extensively resistant strains.



Conclusion

Organic and inorganic contaminants deteriorated the environment on the earth because of their deliberate and accidental leakage or production and generation in the form of waste. Microbial bioremediation of these contaminations has attracted the researchers all over the world as microbes naturally offer plenty of opportunities for the cleaning-up of the polluted sites. Advancements in this field of science have widely been made. With the genetic engineering know-how, microbes are being designed precisely termed as “synthetic biology” by Endy (2005) whereby particular genes responsible for the degradation of contaminants are transferred to the microorganisms of common occurrence provided the correct identification and culturing of isolates. Thanks to metagenomics that provide information of the microbial community structure acting upon environmental pollutants. There are several biomolecular techniques available to identify microbes in consortia and to ascertain the degradation of xenobiotic compounds. We are now able to understand the degradation processes of most commonly used xenobiotics including polyaromatic hydrocarbons (PAHs), e.g., naphthalene (Alquati et al. 2005), benzene (Xiong et al. 2012), chrysene and benzo(a)pyrene (Hesham et al. 2012). For a successful bioremediation, knowledge of factors influencing the biological as well as physicochemical processes is must. Tools of molecular biology are being used to identify the target-specific microorganisms and their mechanisms of chemical degradation and still an open opportunity to understand the natural bioremediation process. Future prospects of researches in microbial bioremediation have still a lot of opportunities for the imminent scientists. Since climate is changing along with its associated impacts that can alter the microbial communities, it is predicted that climate change will alter the earth's ecosystem as a whole, however; reports indicate the increased microbial activity at elevated CO₂ and temperature. The climate change and microbial processes in nature will certainly be the interesting field of future research.

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