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Rhizobiont in Bioremediation of Hazardous Waste

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Current Approaches in Bioremediation of Toxic Contaminants by Application of Microbial Cells; Biosurfactants and Bioemulsifiers of Microbial Origin

Amrita Kharangate-Lad and Nezlyn Cressida D'Souza

Abstract

The increase in global human population has resulted in swift and extensive urbanization and industrialization. These anthropogenic activities along with natural phenomena result in the release of toxic compounds in the environment. These toxic compounds are recalcitrant in nature and accumulate in the environment, contaminating the soil and aquatic ecosystems. They pose a risk to human health and ecosystem through the contamination of drinking water, ingestion through the food chain and reduction in water and food quality. Microorganisms such as bacteria, fungi, yeast and algae possess various mechanisms that metabolize and detoxify these toxic pollutants. In this chapter, we emphasize the use of these microorganisms for bioremediation of toxic pollutants like heavy metals such as Cd, Hg, Pb, Zn, Cu and others; polyaromatic hydrocarbons and petroleum-based hydrocarbons; plastic polymers and recalcitrant dyes and agro-based compounds. Apart from naturally occurring microorganisms, genetically engineered microorganisms have been designed to degrade these recalcitrant toxic compounds. Bioremediation using both these natural and genetically engineered microbes is an economic and eco-friendly alternative to conventional physicochemical technologies.

Keywords

Biosurfactants · Bioemulsifiers · Hydrocarbons · Microbes · Toxic pollutants

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11.1 Introduction

Environmental pollution from toxic metals, organic pollutants and other hazardous materials has affected the natural ecosystem and human health. Anthropogenic activities like industrialization, mismanagement of toxic waste and natural activities like hurricanes, storms and volcanic eruptions are responsible for the discharge of toxic pollutants into the environment. Due to the expense and inefficiency of chemical methods, bioremediation using nanoparticles, microorganisms or their components is an eco-friendly and economical alternative for reclaiming the environments that are contaminated with toxic pollutants.

Bioremediation is detoxification of toxic xenobiotic compounds using living organisms including plants (phytoremediation) and microorganisms such as algae, bacteria and fungi (microbial remediation). The toxic compounds usually include pesticides, plastics, polyaromatic hydrocarbons (PAHs), heavy metals and toxic metal contaminants discharged in soil and aquatic environments due to anthropogenic activities (Das and Dash 2014). Due to the interaction between air, water and land, the toxic pollutants move in the environment and are transported beyond geographical boundaries by air and water currents (Fig. 11.1).

11.2 Microbial Cells in Bioremediation of Toxic Pollutants

Microorganisms are extensively studied for their role in bioremediation of toxic pollutants. The indigenous bacteria from contaminated sites are stimulated by providing optimum conditions of growth such as pH and nutrients such as phosphorous and sulphur by addition of compost and biochar (Ojuederie and Babalola 2017). This stimulates the microorganisms and makes the environment more favourable for bioremediation enabling the microbes to metabolize the toxic pollutants more efficiently (Das and Dash 2014). The efficacy of biodegradation of the toxic pollutants during bioremediation therefore depends on the nutrient availability, oxygen, temperature and pH of the surrounding environment. These factors influence the chemistry of the pollutant such as viscosity and volatility thereby affecting the bioavailability of the toxic pollutant to the microorganisms.

11.3 Factors Affecting Bioremediation

The factors that govern the efficiency of the microbial bioremediation are of two types: abiotic and biotic factors. The abiotic factors include environmental influences such as soil type, oxygen content, temperature, pH, presence of electron acceptors, nutrients and metal ions.

The metabolic abilities of the microorganisms and the physicochemical properties of the pollutant are the major properties that determine the fate of the target pollutant. Environmental factors like soil structure and site characteristics, pH, temperature, moisture, redox potential, oxygen content and availability of nutrients affect the

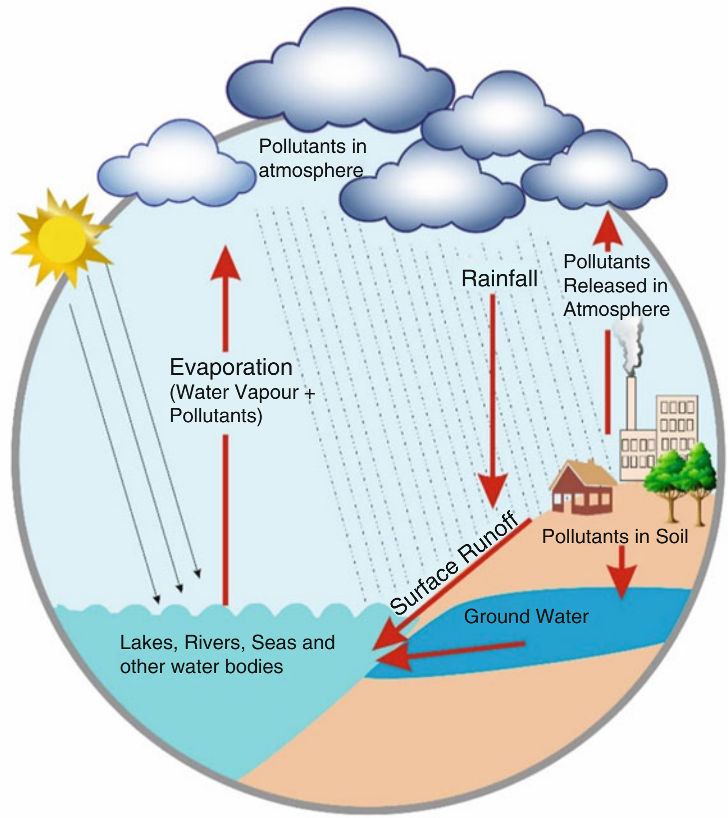

Fig. 11.1 Movement of toxic pollutants in the three spheres of the environment: lithosphere, hydrosphere and atmosphere

growth and interaction of the microorganisms with the pollutant (Fig. 11.2). Whereas the physicochemical properties such as the structure and toxicity of the pollutant govern the bioavailability of the compound to the microorganisms.

11.3.1 Availability of Nutrients

Essential nutrients, mainly nitrogen and phosphorus play a crucial role in microbial growth, reproduction and degradation of the toxic pollutant. Supplementing microorganisms with these essential nutrients has been reported to significantly impact the metabolic activity and increase the degradative capacity of microorganisms in the cold environments since biodegradation in cold environment is limited due to lack of nutrient availability. Similar improvement in degradation of hydrocarbons was reported on addition of nutrients (Abatenh et al. 2017).

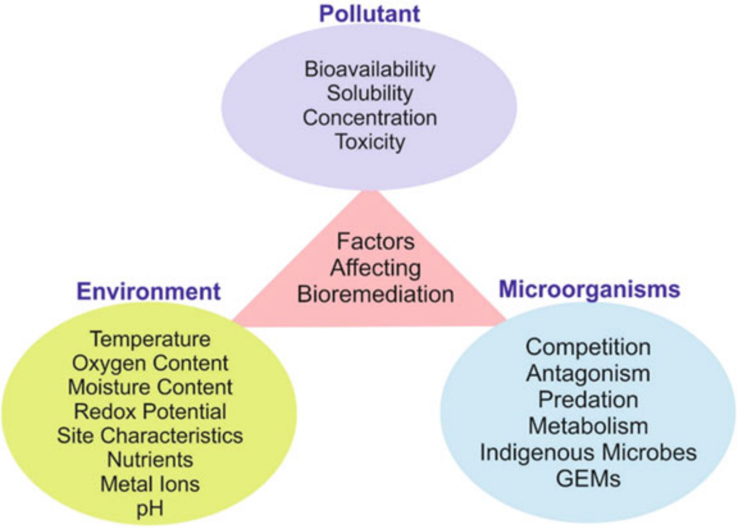

Fig. 11.2 Factors affecting microbial bioremediation

11.3.2 Temperature

Temperature is the most vital factor that determines the survival of the microorganism as well as the bioavailability of the pollutant. In colder regions of the Arctic, it becomes difficult to employ microorganisms for a cleanup as the sub-zero temperature freezes the microbial transport channels and the cytoplasm rendering the microbe metabolically inactive (Abatenh et al. 2017). All enzymes have an optimum temperature below and above which the rate of conversion for the pollutant will not be as effective as at optimum temperature. The metabolic activity of a microorganism increases with the increase in temperature. At a specific temperature the metabolic activity reaches a maximum which is known as the optimum temperature. The metabolic activity of microorganisms is slow at temperatures below and above the optimum temperature. Thus, temperature either increases or decreases the rate of bioremediation as it directly influences physiological activities of the microbes.

11.3.3 Oxygen Content

Oxygen requirement of microorganisms differs depending upon the nature of the microorganisms and widely affects their ability to degrade complex compounds. Biological degradation of various complex compounds has been carried out by both aerobic and anaerobic microorganisms (Abatenh et al. 2017). However, presence of oxygen is significant for the degradation of hydrocarbons by the enzymes oxygenases.

11.3.4 Moisture Content

Availability of water is an important factor as most microorganisms have ion transfer mechanisms at the cell surface level. The uptake of these ions depends upon their solubility in water. Moisture around the cells renders the pollutant more accessible for biosorption by microbial cells (Abatenh et al. 2017). Moisture influences the osmotic pressure, pH and the kind and amount of solubility of nutrients and pollutant; therefore, it directly influences the rate of degradation of the pollutant.

11.3.5 pH of Soil

The pH of the surrounding environment affects the growth and survival of the microorganism as it has no means of adjusting its inherent pH to that of surroundings. The pH also affects the structure and characteristics of the pollutant and thus its bioavailability to the microorganisms. A pH of 6.5–8.5 is optimal for biodegradation in most terrestrial and aquatic ecosystems (Abatenh et al. 2017).

11.3.6 Site Characterization

A detailed study of the site of contamination is needed to decide the best bioremedial strategies when employing microorganisms. It is necessary to study the extent of contamination in the vertical and horizontal zones of the site in addition to the abiotic parameters of the site (Abatenh et al. 2017). This helps determine the techniques to be used for sampling and analysis.

11.3.7 Metal Ions

Metals ions form an integral part of the biochemical components of the microorganisms. They are necessary in small amounts either in biosynthesis of new cell components or for carrying out metabolic activities. Limitation of these essential metal ions is known to have adverse effects on the rate of biodegradation of a compound. Microorganisms therefore have evolved strategies such as production of siderophores and metallothionein proteins to acquire these metal ions from the environment (Davis et al. 2003).

The microorganisms degrading the pollutant often face competition (from other microorganisms for carbon and energy sources), antagonistic interactions (from bacteriocins) and predation (from bacteriophages and protozoa). These negative interactions result in a decrease in production of enzymes by the microorganisms as well as it reduces the population of effective microbes responsible for degradation of the pollutants. These affect the degradative capacity of the microorganisms towards the toxic pollutant (Abatenh et al. 2017). Furthermore, the microorganism needs to maintain its ability to degrade the pollutant without undergoing changes at a gene level (mutations) that may cause it to lose its affinity to the target pollutant.

11.4 Types of Microbial Bioremediation

Bioremediation techniques can be carried out by either in-situ or ex-situ approach. The in-situ treatment involves treating of the contaminated area without excavation of the contaminated site. In-situ treatment uses processes like biostimulation, bioattenuation, bioaugmentation, bioventing and biosparging. In-situ treatments are usually more desirable as it involves less cost and prevents disturbance of the environment. However, it faces limitations due to its inability to penetrate desired depth. Therefore, to make it more desirable diffusion of oxygen is allowed by means of external pipes and pump systems.

Ex-situ involves excavation of the soil from contaminated area. It mainly involves two major processes: the solid phase and slurry phase systems. The solid phase systems involve approaches such as biopiles, landfarming and composting (Fig. 11.3). The slurry phase system uses the bioreactor technique (Abatenh et al. 2017; Kumar et al. 2018a).

11.4.1 Biostimulation

Biostimulation involves the injection of nutrients at the site of contamination in order to stimulate the indigenous and naturally occurring microbial population. This involves the use of minerals, fertilizers, compost and growth supplements and providing environmental conditions such as pH, temperature and oxygen for

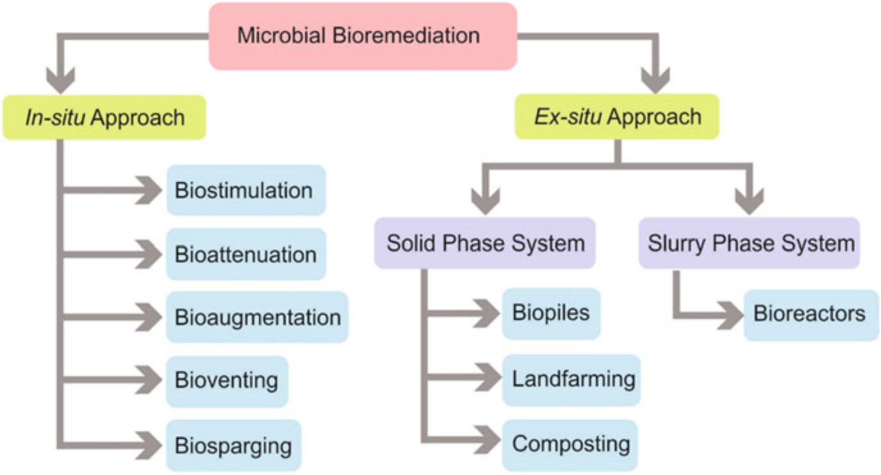

Fig. 11.3 Approaches in microbial bioremediation

optimum functioning of metabolic processes. The presence of small amounts of the pollutant can also trigger enzyme operons required in bioremediation (Abatenh et al. 2017). These nutrients provide the basic elements such as carbon, nitrogen and phosphorous that are needed for cell biomass and energy to produce enzymes that degrade the pollutant (Kumar et al. 2018a).

11.4.2 Bioattenuation

Bioattenuation or natural attenuation is the eradication of pollutant from the surrounding environment. Biologically it involves aerobic and anaerobic biodegradation; plant, animal or microbial uptake. Physical processes (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption) result in clean up of the pollutant, and chemical mechanisms (complexation and ion exchange) result in abiotic transformation. Bioattenuation relies on nature to clean up the environmental pollutant. Microorganisms metabolize the pollutant as a source of carbon and energy converting them into water and harmless gases. Soil particles interact with the pollutant binding to it strongly and keeping them from entering the groundwater. The movement of pollutant through soil and into groundwater results in dilution of the pollutant. Volatile pollutants can evaporate from soil on exposure to sunlight and air (Abatenh et al. 2017). If bioattenuation has not completely cleared the pollutant, then bioremediation using biostimulation or bioaugmentation can be considered (Kumar et al. 2018a).

11.4.3 Bioaugmentation

Microorganisms that have a capacity to degrade the target pollutant are added to augment the biodegradative capacity of the natural and indigenous microbial populations at the contaminated site. GEMS or genetically engineered microorganisms are microbes that are collected from site of bioremediation and genetically modified to increase the efficiency of degradation. This technique has been specifically proven successful for chlorinated ethenes, such as tetrachloroethylene and trichloroethylene and ensures complete removal of these contaminants from the contaminated sites or their conversion to non-toxic forms (Abatenh et al. 2017; Kumar et al. 2018a).

11.4.4 Bioventing

Vents or wells in the soil are engineered to carry oxygen and nutrients to the soil to stimulate the growth of either the natural microorganisms or the introduced microorganisms. It can only be used for compounds that undergo aerobic degradation such as fuel residuals, volatile compounds and petroleum hydrocarbons (Abatenh et al. 2017; Sutar and Kumar 2012).

11.4.5 Biosparging

Biosparging refers to injecting air under pressure to increase the level of oxygen in groundwater for stimulating the indigenous population of microbes to degrade the contaminants. Biosparging enhances the interaction in the saturation zone and therefore increases the contact between soil and groundwater (Abatenh et al. 2017).

11.4.6 Biopiles

Soils contaminated with the pollutants are piled to form mounds and air is supplied to the biopile system by means of pumps. This enhances the microbial activity through microbial respiration resulting in efficient degradation of pollutants. This is a commonly used technique for aerobic degradation of petroleum pollutants (Abatenh et al. 2017; Sutar and Kumar 2012).

11.4.7 Landfarming

In landfarming, the contaminated soil is excavated, spread over an area and periodically tilled until pollutants are degraded. Tilling stimulates indigenous microorganisms and facilitates aerobic degradation of contaminants. This technique has limitations as it is effective upto10–35 cm of soil (Sutar and Kumar 2012). Efficient cleaning ability and low maintenance and monitoring costs make it a feasible option for bioremediation.

11.4.8 Composting

In this technique contaminated soil is mixed with known proportions of organic compost, manure or agricultural waste. These organic materials allow and support microbial population that degrades the contaminants (Kumar et al. 2018a; Sutar and Kumar 2012). The elevated temperature generated during composting is characteristic of this process.

11.4.9 Bioreactor

It involves the use of slurry reactors or aqueous reactors. The contaminated soil, sediment or sludge or contaminated water is introduced in the reactor (Kumar et al. 2018a; Sutar and Kumar 2012). A slurry bioreactor mixes the contaminants with water and gas to facilitate biodegradation by the indigenous microorganisms. The disadvantages include excavation and pre-treatment of the contaminated soil or water before being introduced into the bioreactor which is economically expensive.

11.5 Mechanisms of Interaction Between Microbial Cells and the Metal Pollutant

Heavy metals such as mercury, cadmium, nickel, cobalt, chromium, arsenic, lead have found their way into the environment due to natural and anthropogenic activities. For potential application of microbial cells in bioremediation, the microbial cells should not be inhibited by the toxic pollutant and should possess either one or more of the metal pollutant processing mechanisms (Kumar et al. 2016b). These mechanisms include uptake of the metal by means of metallothionein or metal sequestering proteins or by acquisition and interaction with the toxic pollutants by means of extracellular polymers (extracellular polymeric substances (EPS), biofilms, capsules, slime or sheath), biosorption into the cell membrane, intracellular assimilation, mobilization and immobilization, bioaccumulation, complexing and precipitation of the metal, efflux, reflux and release of the detoxified or transformed metal pollutant (Fig. 11.4). On uptake, the metal pollutant may be processed in mechanisms either dependent on the metabolic pathway of the organism or independently by using the metal pollutant processing mechanisms (Das and Dash 2014).

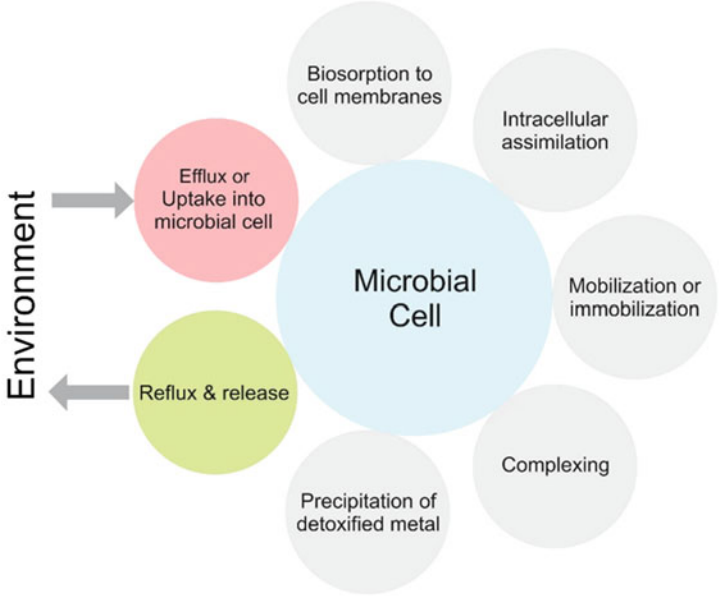

Fig. 11.4 Mechanisms possessed by microorganisms for dealing with metals and metal pollutants in the environment

11.6 Bioremediation of Toxic and Heavy Metals by Microorganisms

Bioremediation of heavy metals has been reported using bacteria, fungi and algae (Table 11.1). Microbial cells are negatively charged owing to the presence of negatively charged groups like hydroxyl groups, phosphate groups, carbonyl groups, sulphate groups and uronic acid of carboxyl groups on the surface of the bacterial cell wall. These bind to the heavy metal ions resulting in biosorption. *Enterobacter cloacae* has been reported to chelate cadmium, copper and cobalt. *Rhodobium marinum* NW16, *Rhodobacter sphaeroides* KMS24, purple non-sulphur bacteria have exhibited potential to remove zinc, copper, cadmium and lead from contaminated environment by bioaccumulation and precipitation (Panwichian et al. 2011). Research studies show that *Desulfovibrio desulfuricans,* a *s*ulphate-reducing bacterium can convert sulphate to hydrogen sulphate. This hydrogen sulphate reacts with heavy metals such as Zn and Cd and transforms them into insoluble forms of these metal sulphides (Chibuike and Obiora 2014).

Reports on viable and dead cells of *Mucor rouxii* demonstrated their ability to absorb cadmium, lead, zinc and nickel. It also established that the viable cells were more effective at low pH and optimum biosorption was achieved by the dead biomass and live cells above a pH of 4.0 (Yan and Viraraghavan 2003). Yeast *Saccharomyces cerevisiae* is used in bioremediation of contaminated wastewaters and is reported to remove toxic metals by biosorption. Detoxifying mechanisms like mobilization, immobilization and transformation by using metal-binding peptides called phytochelatins have been studied and reported in yeasts like *Schizosaccharomyces pombe* and *Candida* sp. (Bahafid et al. 2017; Wifak et al. 2017). Some strains of yeast such as *Hansenula polymorpha, Saccharomyces cerevisiae, Pichia guilliermondii, Rhodotorula pilimanae, Rhodotorula mucilage* and *Yarrowia lipolytica* have exhibited conversion of Chromium (VI) to Chromium (III) (Chatterjee et al. 2012; Ksheminska et al. 2008).

The large biomass of algae allows them a greater biosorption capacity in comparison to both bacteria and fungi (Mustapha and Halimoon 2015). Burdin (1985) reported ability of the algae to bioaccumulate heavy metals such as aluminium, cadmium, chromium, cobalt, copper, gold, lead, manganese, nickel, silver, tin and zinc. Many marine algae such as *Durvillaea potatorum, Ecklonia radiata* and *Laminaria japonica* have been reported to exhibit a higher biosorption capacity for heavy metals in comparison with zeolites or activated carbon sorbents (Kumar et al. 2013). Brown marine algae was studied to be effective in bioremediation of Cd, Ni and Pb due to presence of carboxyl, sulphonate, amino and sulphydryl groups on its surface (Davis et al. 2003). *Euglena gracilis*, a single celled alga has been reported for the bioaccumulation of Zn and *Chlorella vulgaris* and *Scenedesmus acutus* have been studied for bioaccumulation of Zn, Cr and Cd (Travieso et al. 1999). Biosorption of cadmium ions by *Spirulina platensis* has been demonstrated by using its dry biomass (Al-Homaidan et al. 2015). Marine algae have also shown to react differently to cadmium: *Tetraselmis suecica* exhibited affinity for cadmium, *Gracilaria fisheri* accumulated cadmium (II) and copper (II) while *Dunaliella salina*

Table 11.1 Microorganisms used in bioremediation of toxic metals at contaminated sites

Toxic Metal degrading microorganisms		
Microorganism	Pollutant	Reference
Bacteria		
Acidithiobacillus ferrooxidans	Cu	Rehan and Alsohim 2019
Alcaligenes sp.	Pb	Acosta-Rodríguez et al. 2018
Bacillus cereus strain XMCr-6 *Bacillus cereus*	Cr (VI)	Kanmani et al. 2012; Dong et al. 2013; Coelho et al. 2015
Bacillus megaterium	Ni	Acosta-Rodríguez et al. 2018; Igiri et al. 2018
Bacillus pumilus	Cd, Pb	Fulke et al. 2020
Bacillus subtilis	Cr (VI)	Balamurugan et al. 2014
Bacillus subtilis, Bacillus cereus	Cobalt, cadmium, zinc	Abdelatey et al. 2011
Bordetella sp.	Cadmium	Abou-Shanab et al. 2003
Desulfovibrio desulfuricans	Cr (VI), Cu, Ni	Igiri et al. 2018
Enterobacter cloacae B2-DHA	Cr (VI)	Rahman et al. 2015
Enterobacter cloacae	Cu, Cd, Co	Iyer et al. 2005
Frankia	Cu	Rehan and Alsohim 2019
Kocuria flava	Cu	Coelho et al. 2015
Pseudomonas aeruginosa	Organic and inorganic Hg	De et al. 2008; Das and Dash 2014
Pseudomonas putida	Cr (VI)	Balamurugan et al. 2014
Pseudomonas sp..	Phenols and aromatic compounds	Selvaratnam et al. 1997
Pseudomonas sp.	Co, Cd, Zn	Abou-Shanab et al. 2003
Pseudomonas veronii	Cd, Zn, Cu	Vullo et al. 2008; Coelho et al. 2015
Rhodobium marinum NW16, *Rhodobacter sphaeroides* KMS24	Cd, Cu, Pb, Zn	Panwichian et al. 2011
Sporosarcina ginsengisoli	As (III)	Achal et al. 2012; Coelho et al. 2015
Staphylococcus aureus	Chromate	Aguilar-Barajas et al. 2008
Vibrio harveyi	Cd, Pb	Mire et al. 2004; Abd-Elnaby et al. 2011
Fungi		
Aspergillus fumigatus	Pb	Kumar Ramasamy et al. 2011
Aspergillus niger	Zn, Hg, Co, Pb, Cd, Cu, Ni	Acosta-Rodríguez et al. 2018
Aspergillus versicolor	Ni, Cu	Coelho et al. 2015; Tastan et al. 2010
Aspergillus versicolor	Ni, Cu	Tastan et al. 2010; Coelho et al. 2015
Coprinopsis atramentaria	Cd, Pb	Igiri et al. 2018
Gloeophyllum sepiarium	Cr (VI)	Achal et al. 2011
Mucor rouxii	Pb, Cd, Ni, Zn	Yan and Viraraghavan 2003

(continued)

Table 11.1 (continued)

Toxic Metal degrading microorganisms		
Microorganism	Pollutant	Reference
Penicillium chrysogenum	Cr (VI)	De et al. 2008
Penicillium sp.	Pb	Igiri et al. 2018
Pleurotus ostreatus HAAS	Pb, Cd, Cr	Acosta-Rodríguez et al. 2018
Rhizopus oryzae (MPRO)	Cr (VI)	De et al. 2008; Sukumar 2010
Rhizopus stolonifer	Pb, Cd, Cu, Zn	Acosta-Rodríguez et al. 2018
Yeast		
Candida sp.	Ni, Zn, Pb, Cd, Cu, Co, Hg, Ag, As	De et al. 2008; Acosta-Rodríguez et al. 2017; Igiri et al. 2018
Saccharomyces cerevisiae	Pb, Cd	Farhan and Khadom 2015; Bahafid et al. 2017
Schizosaccharomyces pombe	Cu	Bahafid et al. 2017
Algae		
Chlorella vulgaris	Zn, Cd, Cu, Pb, Tributylin (TBT)	Travieso et al. 1999; De et al. 2008
Euglena gracilis	Zn	Travieso et al. 1999
Hydrodictyon, Oedogonium and *Rhizoclonium* sp.	As	Coelho et al. 2015; Srivastava and Dwivedi 2015
Rhodotorula mucilaginosa	Hg, Cu, Pb	Acosta-Rodríguez et al. 2018
Scenedesmus acutus	Cd, Zn, Cr	Travieso et al. 1999
Spirogyra sp. and *Cladophora* sp.	Pb (II), Cu (II)	Lee and Chang 2011; Coelho et al. 2015
Spirogyra sp. and *Spirulina* sp.	Cr Cu, Fe, Mn, Zn	Mane and Bhosle 2012; Coelho et al. 2015
Spirulina platensis	Cd	Al-Homaidan et al. 2015
Bacterial consortium		
Acinetobacter sp. and *Arthrobacter* sp.	Cr	De et al. 2008
Viridibacillus arenosi B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47	Lead, cadmium, copper	Kang et al. 2016

exhibited tolerance to cadmium. *Chlamydomonas* produces phytochelatins which sequester many metals and they have potential application in bioremediation of heavy metals (Kumar et al. 2013).

Pseudomonas sp. have been reported to degrade a wide range of toxic compounds including compounds of cobalt, zinc, cadmium; organic and inorganic mercury; phenols and other aromatic compounds and tributyltin in the aquatic environments. At low concentration of heavy metals, *Vibrio harveyi* which is a common bacterium of the saline environment exhibited bioaccumulation of cadmium (Abd-Elnaby et al. 2011) and precipitation of divalent lead into lead phosphate (Mire et al. 2004; Rehan and Alsohim 2019). Bacteria such as *Citrobacter freundii* avoid toxicity of metals by converting divalent lead to lead phosphate. Other bacteria such as *Acidithiobacillus* *ferrooxidans* and *Frankia* detoxify copper by precipitating the metal by forming metal phosphate complexes (Rehan and Alsohim 2019).

The ability of bacteria to produce EPS is an important feature in metal sequestration and therefore in bioremediation. Exopolysaccharides produced by bacteria protect it against environmental stresses such as salinity, heavy metal toxicity, desiccation, presence of antibiotics, etc. Bacterial EPS such as alginate from *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, sphingans from *Sphingomonas paucimobilis*, hyaluronan from *Pseudomonas aeruginosa*, *Pasteurella multocida* and attenuated strains of *Streptococci*, xanthan from *Xanthomonas campestris*, galactopol from *Pseudomonas oleovorans* and fucopol from *Enterobacter* A47 are some of the heteropolysaccharides that have potential applications in metal sequestration and reduction of metal from contaminated sites (Gupta and Diwan 2016). The bacteria, *Rhodobium marinum* NW16, *Rhodobacter sphaeroides* KMS24 were found to be more efficient in the removal of heavy metals from contaminated shrimp ponds when incubated for production of EPS (Panwichian et al. 2011).

Bioremediation of heavy metals is more efficient when a consortium of bacterial strains is used in comparison with a single strain. Kang et al. (2016) reported that a bacterial consortium containing *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47 were more effective in bioremediation of soil contaminated with Pb, Cd and Cu due to the synergistic effect of bacterial consortium. The bacterial consortium showed greater resistance to the heavy metals in comparison to using a single strain. Bioremediation studies using consortium of marine bacteria exhibited efficient removal of mercury in the bioreactor (De et al. 2008). Therefore, a consortium of microbial isolates is metabolically more effective in biosorption of metals and therefore more potent in field applications (Table 11.1).

11.7 Microbial Mechanism of Degradation of Hydrocarbon Pollutants

Hydrocarbon pollutants are mainly of two types: polycyclic aromatic hydrocarbons and petroleum-based hydrocarbons (crude oil-based hydrocarbon). Polycyclic aromatic hydrocarbons are unsaturated hydrocarbons that contain two or more aromatic rings. These are generated by incomplete combustion of organic material such as wood, petroleum, coal, natural gas. Crude oil-based hydrocarbons or petroleum hydrocarbons usually include the *n*-alkanes and cyclohexanes which are saturated hydrocarbons (Kumar et al. 2018b). These are found to contaminate the soil and water due to spillages from oil tankers, shipping activities, storm water and industrial discharge. The degradation pathway employed by microorganisms for the degradation of these hydrocarbons and most of the organic pollutants involves the oxidation of the pollutant by cell oxygenases and peroxidases (Das and Dash 2014). The resulting catechol in case of PAHs and primary alcohols in case of crude oil-based hydrocarbons undergo degradation by the peripheral pathways of the cell forming intermediates that enter the central intermediary pathway like the Tricarboxylic acid

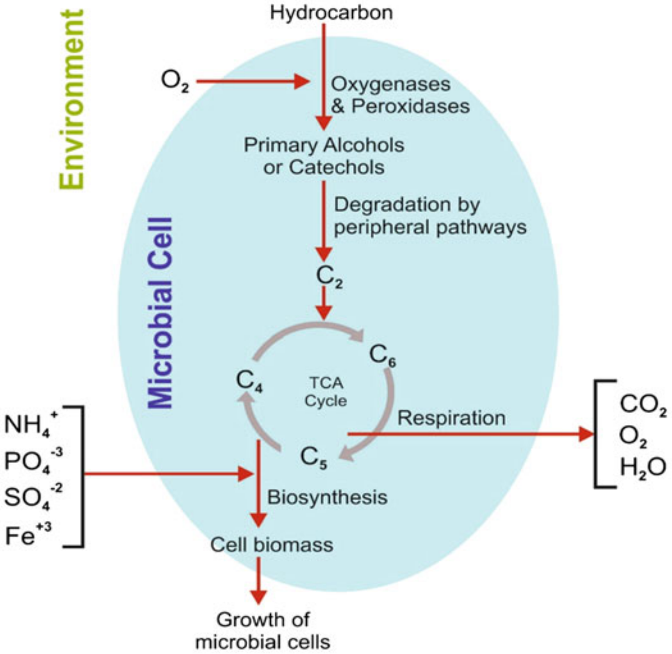

Fig. 11.5 Biodegradation of hydrocarbon compounds by microorganisms

pathway (TCA) (Fig. 11.5). The central precursor molecules of the TCA cycle (acetyl-CoA, succinate, pyruvate) then enter the biosynthesis pathway for sugar synthesis by gluconeogenesis and by formation of cell biomass.

In the biodegradation of hydrocarbons, the genera *Pseudomonas* is found to be the most prominent member that is capable of degrading wide number of polyaromatic hydrocarbons and petroleum hydrocarbons. *Pseudomonas stutzeri* was found to be a very dominant organism in the petroleum pipelines which had an ability to utilize aromatic hydrocarbons such as toluene, phenol, xylene and naphthalene.

11.7.1 Bioremediation of Polyaromatic Hydrocarbons by Microorganisms

Polyaromatic hydrocarbons are of environmental concern owing to their persistence in nature and their toxigenic, mutagenic and carcinogenic properties. PAHs include recalcitrant compounds such as naphthalene, benzopyrene, phenanthrene, anthracene, etc. Many bacteria, fungi and algae have been studied for their ability to metabolize and degrade these PAHs (Bhatia et al. 2018). Table 11.2 shows the list of bacterial, fungal and algal microorganisms that have the potential to degrade polyaromatic hydrocarbons. The bioremediation potential of the microorganisms

Table 11.2 Microorganisms used in bioremediation of polyaromatic hydrocarbons (PAHs)

Polyaromatic hydrocarbon degrading microorganisms		
Microorganism	Polyaromatic hydrocarbon (PAH)	Reference
Bacteria		
Achromobacter xylosoxidans DN002	Mono and poly aromatic hydrocarbons	Xu et al. 2018
Cycloclasticus sp.	Naphthalene, Phenanthrene, pyrene	Ghosal et al. 2016; Bhatia et al. 2018
Lutibacterium anuloederans	Phenanthrene	Chung and King 2001; Das and Dash 2014
Mycobacterium sp.	Naphthalene, Phenanthrene	Ghosal et al. 2016; Bhatia et al. 2018
Neptunomonas naphthovorans	Naphthalene	Hedlund et al. 1999; Das and Dash 2014
Pseudomonas sp.	Naphthalene, Phenanthrene	Ghosal et al. 2016; Bhatia et al. 2018
Sphingomonas paucimobilis EPA505	Phenanthrene	Das and Dash 2014; Ghosal et al. 2016
Fungi		
Aspergillu*s terreus*	Pyrene, Benzopyrene, Phenanthrene	Capotorti et al. 2004; Capotorti et al. 2005; Cerniglia and Sutherland 2010
Cunninghamella elegans	Naphthalene, anthracene, phenanthrene	Cerniglia and Sutherland 2010
Fusarium sp.	Benzopyrene	Cerniglia and Sutherland 2010
Ganoderma lucidum	Phenanthrene, pyrene	Agrawal et al. 2018
Irpex lacteus	Pyrene	Cajthaml et al. 2008
Phanerochaete chrysosporium	Fluorene	Cerniglia and Sutherland 2010
Phanerochaete sordida	Creosote	Cerniglia and Sutherland 2010
Pleurotus ostreatus	Creosote, pyrene, anthracene, fluorene, and dibenzothiophene	Bezalel et al. 1996; Bogan et al. 1999; Cerniglia and Sutherland 2010
Scopulariopsis brevicaulis	Phenanthrene, benzopyrene	Mao and Guan 2016
Trametes versicolor	Anthracene, benzopyrene	Cerniglia and Sutherland 2010
Algae		
Chlamydomonas sp.	Lindane, naphthalene, phenol	Ardal 2014
Chlorella sp.	Lindane, chlordimeform	Ardal 2014
Dunaliella sp.	Naphthalene	Ardal 2014
Elkatothrix viridis	Anthracene	El-Sheekh et al. 2012
Lyngbya lagerlerimi	Phenol	El-Sheekh et al. 2012
Nitzschia sp. and *Skeletonema costatum*	Phenanthrene and fluoranthene	Hong et al. 2008
Nostoc linckia	Naphthalene	El-Sheekh et al. 2012

(continued)

Table 11.2 (continued)

Polyaromatic hydrocarbon degrading microorganisms		
Microorganism	Polyaromatic hydrocarbon (PAH)	Reference
Scenedesmus obliquus	Phenanthrene, naphthalene, Sulphonic acid	Safonova et al. 2005
Selenastrum capricornutum	Benzo[a]pyrene	Ardal 2014
Volvox aureus	2-methythie 3-phenyl quinazlin-4- 3H	El-Sheekh et al. 2012
Microbial consortium		
Burkholderia cepacia GS3C, *Pandoraea pnomenusa* GP3B *Pseudomonas* GP3A *Sphingomonas* GY2B,	Phenanthrene and Methylphenanthrenes	Gupta et al. 2015
Bacillus pumilus *Staphylococcus warneri*	Phenanthrene, Pyrene and Benzo[a]anthracene	Moscoso et al. 2012
Serratia marcescens L-11, *Streptomyces rochei* PAH-13 *Phanerochaete chrysosporium* VV-18	Fluorene, anthracene, phenanthrene and pyrene	Sharma et al. 2016
Rhodococcus sp. ASDC1 *Bacillus* sp. ASDC2 *Burkholderia* sp. ASDC3	Chrysene	Vaidya et al. 2018
Aeromonas hydrophila *Bacillus megaterium* *Raoultella ornithinolytica,* *Serratia marcescens*	Acenaphthene, fluorene	Alegbeleye et al. 2017
Aphanocapsa sp., *Chlorella minutissimma,* *Citrobacter* sp. SB9, *Pseudomonas aeruginosa* SA3, *Bacillus subtilis* SA7	PAH in crude oil effluents	Godsgift and Fagade 2016

may be DNA based or plasmid based. *Cycloclasticus* sp. are the most common and widely studied bacteria that have the potential to degrade multiple PAH compounds (Wang et al. 2018). Marine bacteria such as *Cycloclasticus spirillensus, Lutibacterium anuloederans and Neptunomonas naphthovorans* have been studied for their ability to degrade PAHs in the marine environment (Das and Dash 2014). Bacteria such as *Mycobacterium* sp., *Moraxella* sp., *Burkholderia cepacia, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas paucimobilis, Bacillus cereus, Rhodococcus* sp., *Streptomyces* sp., *Achromobacter denitrificans, Brevundimonas vesicularis, Comamonas testosteroni, Vibrio* sp., *Sphingomonas, Brevibacterium, Arthrobacter, Nocardioides* have exhibited their ability to degrade naphthalene and phenanthrene (Ghosal et al. 2016). *Sphingomonas paucimobilis* EPA505 has also shown an ability to grow on fluoranthene utilizing it as the sole carbon source (Das and Dash 2014). Research studies have shown other bacteria such as *Sphingobacterium, Alteromonas, Streptomyces* and fungi *Irpex lacteus, Aspergillus fumigatus* can be used either individually or as a consortium with other PAH degrading microorganisms for bioremediation of PAH-contaminated environments (Bhatia et al. 2018). The degradation of the polyaromatic hydrocarbon, like in case of other substrates also depends upon the pH of the environment. The degradation of *Burkholderia cocovenenans* increases from 40% to 80% when the pH is changed from 5.5 to neutral (Mahjoubi et al. 2017).

The fungi *Pleurotus ostreatus, Phanerochaete chrysosporium, Phanerochaete laevis* HHB-1625*, Rigidoporus lignosus, Aspergillus terreus, Cunninghamella elegans, Fusarium* sp.*, Trametes versicolor, Phanerochaete sordida* have been studied for their ability to degrade various polyaromatic compounds (Bogan et al. 1996; Cerniglia 1982; Cerniglia and Sutherland 2010). Though degradation of PAHs by bacteria and fungi has been widely studied, much less is known about the degradation of these compounds by algae. *Scenedesmus obliquus*, a green alga has been reported to degrade phenanthrene by biotransformation (Safonova et al. 2005). *Nostoc linckia, Elkatothrix viridis* and *Volvox aureus* degraded naphthalene, anthracene and 2-methythie 3-phenyl quinazlin-4-3H, respectively. *Nitzschia* sp. and *Skeletonema costatum* biodegrade phenanthrene and fluoranthene by bioaccumulation of these compounds inside the cells (Hong et al. 2008). The algae *Prototheca zopfi* has also been reported to degrade polyaromatic hydrocarbons extensively.

Degradation of PAH has been found to be more effective on application of consortium of microorganisms to PAH-contaminated soils. Microbial communities from the rhizosphere have been reported to degrade PAHs in contaminated soils by synergistic action between the microorganisms (Bisht et al. 2015). Investigations using a consortium of *Staphylococcus warneri* and *Bacillus pumilus* in the degradation of Phenanthrene, Pyrene and Benzo[a]anthracene were found to yield results with the removal of about 80–90% of the aromatic compound in 3 days in a bioreactor (Moscoso et al. 2012). Bacterial consortium *Serratia marcescens* L-11, *Streptomyces rochei* PAH-13 and *Phanerochaete chrysosporium* VV-18 were found to be 85–100% effective against soil contaminated with fluorene, anthracene, phenanthrene and pyrene within a period of 30 days when the soil was amended with compost (Sharma et al. 2016). A bacterial algal consortium with *Chlorella minutissimma* and *Aphanocapsa* sp. as the algal counterparts and *Citrobacter* sp. SB9, *Pseudomonas aeruginosa* SA3, *Bacillus subtilis* SA7 as the bacterial inoculants was studied for its efficiency in degradation of PAHs from crude oil effluents. A combination of *Chlorella minutissimma* and the bacterial inoculants was found to be the most effective in comparison with all the algal and bacterial inoculants used together or when *Aphanocapsa* sp. was used along with the bacterial inoculants. Therefore, the success and efficiency of the consortium depends on the synergistic action between the inoculants (Godsgift and Fagade 2016).

11.7.2 Bioremediation of Crude Oil-Based Hydrocarbons by Microorganisms

Crude oil-based hydrocarbons pose a major threat to humans as well as to the terrestrial and marine ecosystems. Bioremediation approaches for the removal of these crude oil-based hydrocarbons have received much attention largely due to their efficacy in detoxifying the contaminants effectively. The interaction and biodegradation of hydrocarbon substrates depend essentially on the adhesion mechanisms of the bacterial cell that include the outer membrane proteins and lipids, fimbriae, pili and extracellular polymers and capsules. It has been reported that in *Acinetobacter* sp. RAG-1 (Table 11.3), the utilization of Alkane is dependent upon the presence of fimbriae. However, it is not just the bacteria with hydrophobic cell surface that degrade the pollutants. Bacteria with hydrophilic cell surface have also been demonstrated to metabolize hydrocarbon pollutants owing to modifications in their outer membranes. These hydrophilic hydrocarbon degrading bacteria possess more potential in degrading the hydrocarbon as it involves direct assimilation and action on the hydrocarbon substrate in comparison with the hydrophobic bacteria. This is due to the high cell surface hydrophobicity which promotes cell aggregation and biofilm formation (Prakash et al. 2014).

Bioremediation of hydrocarbon involves two approaches: Bioaugmentation and biostimulation. Bioaugmentation involves introduction of highly efficient hydrocarbon degrading bacteria to degrade the hydrocarbon (Mahjoubi et al. 2017), whereas biostimulation is the stimulation of the indigenous bacteria by modifying the environmental conditions. Though biodegradation of the hydrocarbons has been studied in bacteria yeast and fungi, bacteria are the major class of microorganisms involved in biodegradation of hydrocarbons. Hydrocarbon biodegradation by various marine strains that has been studied includes bacteria belonging to the genera *Acinetobacter, Achromobacter, Alcanivorax, Alkanindiges, Alteromonas, Arthrobacter, Burkholderia, Dietzia, Enterobacter, Kocuria, Micrococcus, Marinococcus, Methylobacterium, Marinobacter, Mycobacterium, Pseudomonas, Pandoraea, Nocardia, Planococcus, Rhodococcus, Staphylococcus, Streptococcus, Streptobacillus, Sphingomonas* and *Vibrio* (Tremblay et al. 2017). A wide number of pseudomanads are capable of degrading a wide variety of petroleum-based hydrocarbons (Varjani and Upasani 2012; Wu et al. 2018; Muriel-Millán et al. 2019).

Bioremediation of oil spills by novel bacterial isolates, capable of degrading crude oil has been reported which can utilize these hydrocarbons as a source of carbon and energy. *Vibrio* and *Acinetobacter* sp. reported by Kharangate-Lad and Bhosle (2014) were capable of growing on crude oil and produced EPS which were capable of bioemulsifying hydrocarbons. Surface sediment bacteria, *Halomonas* sp. MS1 isolated from the Kish Island in the Persian Gulf showed a significant ability to utilize crude oil as the sole source of carbon and energy and making it a potentially important bacteria in bioremediation of crude oil contaminated sites (Sadeghi et al. 2016). Interestingly bacteria such as A*lkanindiges* sp. which are rare in non-polluted sediments show a dominance in these sediments when polluted with diesel. Similarly, bacteria belonging to the obligate hydrocarbonoclastic (OHCB) group such as *Alcanivorax, Marinobacter, Thallassolituus, Cycloclasticus, Oleispira* were undetectable or few in number before pollution. However, they were found to be abundant and dominating the site after pollution with petroleum oil. These rare-to-dominant phenomenon of hydrocarbon degrading bacteria play a crucial role in the biotransformation and bioremediation of the crude oil hydrocarbons. Though bacteria utilizing wide range of crude oil components like *Dietzia* sp. and *Achromobacter xylosoxidans* DN002 have been reported, no bacteria can degrade the entire spectrum of petroleum hydrocarbons (Xu et al. 2018). Therefore, efficient removal of crude oil requires combined action of multiple bacteria degrading various hydrocarbons.

Commercial consortiums have been developed for bioremediation of hydrocarbons with bacteria such as *Agreia, Marinobacter, Pseudoalteromonas, Pseudomonas, Psychrobacter* and *Shewanella*. This consortium has been reported to efficiently degrade crude oil and its components. Bacterial consortium developed using *Ochrobactrum* sp., *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* could effectively degrade 3% of crude oil by 83%. Significantly, when exogenous *Bacillus subtilis* was applied with indigenous bacterial consortium, it effectively accelerated the degradation of crude oil (Xu et al. 2018). A bioaugmentation field study, on the treatment of diesel oil-contaminated soil demonstrated that with exogenous consortium containing *Aeromonas hydrophila, Alcaligenes xylosoxidans, Gordonia* sp., *Pseudomonas fluorescens, Pseudomonas putida, Rhodococcus equi, Stenotrophomonas maltophilia* and *Xanthomonas* sp. a high biodegradation efficiency of 89% was observed in 365 days. Other bacterial consortiums that effectively degrade hydrocarbon pollutants have been mentioned in Table 11.3. Bacterial-fungal consortiums have found to be very efficient in the degradation of both PAH and petroleum-based hydrocarbons (Tang et al. 2012).

Fungi such as *Amorphoteca, Graphium, Neosartorya, Talaromyces* and yeast like *Candida, Yarrowia* and *Pichia* have been isolated from petroleum contaminated soils and that exhibit hydrocarbon degradation. Other genera such as *Aspergillus, Cephalosporium, Penicillium, Rhizopus, Paecilomyces, Pleurotus, Alternaria, Mucor, Talaromyces, Gliocladium, Fusarium* and *Cladosporium* have also been reported to have potential to degrade crude oil hydrocarbons. The yeast species isolated from contaminated water, *Candida lipolytica, Geotrichum* sp., *Torulopsis, Rhodotorula mucilaginosa* and *Trichosporon mucoides* were also seen to degrade petroleum compounds (Das and Chandran 2011).

Algae such as *Prototheca zopfi* has been studied for its ability to utilize crude oil and mixed hydrocarbon substrates. It also was reported to extensively degrade *n*-alkanes and isoalkanes (Das and Chandran 2011).

11.8 Bioremediation of Plastic Polymers by Microorganisms

With growth in industrialization and population, synthetic plastic pollution poses a major problem to the environment. Among the global plastic usage, 80% are petrochemical plastics that include polyethylene (PE), polypropylene (PP),

Table 11.3 Microorganisms used in bioremediation of crude oil-based hydrocarbons

Crude oil and crude oil component degrading bacteria		
Microorganism	Pollutant	Reference
Bacteria		
Achromobacter xylosoxidans DN002	Mono and polyaromatic hydrocarbons	Xu et al. 2018
Alcanivorax sp.	*n*-alkanes	Xu et al. 2018
Brevibacillus laterosporus	Diesel	Amina and Chibani 2016
Dietzia sp.	*n*-alkanes (C6–C40)	Xu et al. 2018
Halomonas sp. MS1	Crude oil	Sadeghi et al. 2016
Pseudomonas aeruginosa	*n*-alkanes	Wu et al. 2018; Muriel-Millán et al. 2019
Fungi		
Aspergillus sp.	Petroleum hydrocarbon	Al-Nasrawi 2012; Al-Hawash et al. 2018b
Beauveria bassiana		Al-Nasrawi 2012
Cochliobolus lunatus	Crude oil	Al-Nasrawi 2012
Cunninghamella echinulate *Cunninghamella elegans*	Crude oil	Rudd et al. 1996
Fusarium solani	Crude oil	Al-Nasrawi 2012
Meyerozyma guilliermondii	Gasoline	Sangale et al. 2019
Mortierella sp.		Sangale et al. 2019
Penicillium sp. *Penicillium documbens*	Crude oil	Al-Nasrawi 2012; Govarthanan et al. 2017; Al-Hawash et al. 2018a
Scolecobasidium obovatum	Crude oil	Mahmoud and Bagy 2018
Yeast		
Candida lipolytica *Candida glabrata* *Candida krusei*	*n*-alkanes	Das and Chandran 2011; Burghal et al. 2016
Geotrichum sp.	Crude oil	Das and Chandran 2011
Rhodotorula mucilaginosa	Crude oil	Das and Chandran 2011
Trichosporon mucoides	Crude oil	Das and Chandran 2011
Saccharomyces cerevisiae	Crude oil	Burghal et al. 2016
Polysporus sp. S133	Crude oil	Burghal et al. 2016
Algae		
Prototheca zopfi	*n*-alkanes and isoalkanes	Das and Chandran 2011
Fucus vesiculosus	Petroleum waste	Aditi et al. 2015
Consortium		
Burkholderia cepacia GS3C, *Pandoraea pnomenusa* GP3B *Pseudomonas* GP3A *Sphingomonas* GY2B	Alkanes, alkylcycloalkanes, alkylbenzenes	Tang et al. 2012

(continued)

Table 11.3 (continued)

Crude oil and crude oil component degrading bacteria		
Microorganism	Pollutant	Reference
Ochrobactrum sp., *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*	Crude oil	Xu et al. 2018
Brachybacterium sp., *Cytophaga* sp., *Sphingomonas* sp., *Pseudomonas* sp.	Oil spills	Angelim et al. 2013
Micrococcus sp., *Bacillus* sp., *Corynebacterium* sp., *Flavobacterium* sp., *Pseudomonas* sp.	*n*-alkane	Rahman et al. 2003
Alteromonas putrefaciens, *Klebsiella pneumoniae, Pseudomonas fragi* *Moraxella saccharolytica,*	Diesel hydrocarbon	Sharma and Rehman 2009
Acinetobacter faecalis, *Staphylococcus* sp. *Neisseria elongate*	Crude petroleum oil	Mukred et al. 2008
Brachybacterium sp., *Cytophaga* sp., *Pseudomonas* sp. *Sphingomonas* sp.,	Oil spills	Angelim et al. 2013
Aeromonas hydrophila, *Alcaligenes xylosoxidans,* *Gordonia* sp. *Pseudomonas fluorescens,* *Pseudomonas putida,* *Rhodococcus equi,* *Stenotrophomonas maltophilia,* *Xanthomonas* sp.	Diesel	Xu et al. 2018

polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) which pollute the soil and water environment.

Biodegradation of plastic polymers by microorganisms proceeds via four important steps, biodeterioration, biofragmentation, assimilation and mineralization (Fig. 11.6). Biodeterioration involves initial colonization by microorganisms by adhesion thereby affecting the physical, chemical and mechanical properties of the plastic. Abiotic factors play a synergistic role in initializing the degradation. Microorganisms colonize and produce biofilm or EPS that invade the polymeric pores resulting in grooves and cracks. Therefore, weakening the polymeric structure of the plastic and physically deteriorating the polymer. The release of corrosive compounds during metabolism such as sulphuric acid (*Thiobacillus* sp.), nitrous acid (*Nitrosomonas* sp.) or nitric acid (*Nitrobacter* sp.) by chemolithotrophic bacteria and production of organic acids such as citric, fumaric, oxalic, gluconic, glutaric, oxaloacetic and glyoxalic acids affects the microplastic matrix resulting in chemical deterioration of the polymer. In biofragmentation the polymeric plastic is cleaved

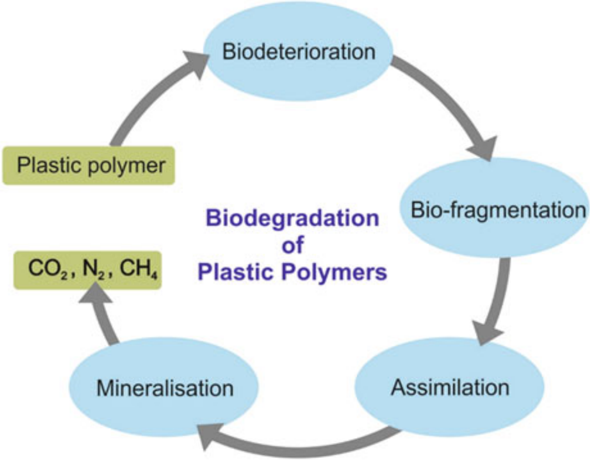

Fig. 11.6 The different steps in biodegradation of plastic polymers by microorganisms

into oligomers, dimers or monomers by the action exo-enzymes or free radicals produced by the microorganisms. Bacteria that degrade plastics usually contain the enzyme oxygenases that catalyses the addition of an oxygen molecule to the polymeric chain converting it to a less recalcitrant molecule such as an alcohol or peroxyl group. Assimilation involves the absorption of molecules across the cell cytoplasm for metabolic process to form cell biomass or cell structures. Mineralization is the complete degradation of the absorbed molecules into oxidized metabolites such as carbon dioxide, nitrogen, methane and water vapour (Dussud et al. 2018).

In studies involving biodegradation of plastic polymers, *Pseudomonas* and *Clostridium* are the most dominant bacteria that can metabolize plastics like polyethene, PVC, PHB (Table 11.4) (Ghosh et al. 2013). Studies on *Rhodococcus* sp. demonstrate the ability to degrade plastic by 8% of its dry weight in 30 days (Urbanek et al. 2018). Other bacteria known to degrade plastic polymers are *Acidovorax* sp., *Alcaligenes* sp., *Brevibacillus borstelensis, Comamonas acidovorans, Diplococcus* sp., *Moraxella* sp., *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Micrococcus* sp., *Thermomonospora fusca, Schlegelella thermodepolymerans* and *Amycolatopsis* sp. (Ghosh et al. 2013; Kathiresan 2003). Bacteria degrading plastic polymers such as *Alcanivorax, Shewanella, Moritella, Psychrobacter*, *Pseudomonas* and *Tenacibaculum* that exhibited biodegradation ability against polyester PCL have been reported from the deep-sea sediments. The highest biodegradation capacity for PCL was observed in *Pseudomonas* and *Rhodococcus* (Urbanek et al. 2018).

Bacteria such as *Phormidium, Pseudophormidium, Bacteroides, Lewinella, Proteobacteria, Arcobacter and Colwellia* sp. isolated from the surface of PET bottles and microplastic polymers have been identified for their ability to degrade these polymers. Analysis of enzymatic profiles of most plastic degrading microorganisms suggests that the presence of the enzyme lipase plays a crucial role in the ability of these microorganisms to degrade plastic polymers as it catalyses

Table 11.4 Microorganisms used in bioremediation of plastic polymers

Plastic polymer degrading microorganisms		
Microorganism	Pollutant	Reference
Bacteria		
Alcanivorax	Monofilament fibres of PCL, PHB/V, PBS	Sekiguchi et al. 2011
Arcobacter sp., Colwellia sp.	LDPE	Urbanek et al. 2018
Bacillus brevis	Polycaprolactone	Urbanek et al. 2018
Ideonella sakaiensis	PET	Urbanek et al. 2018
Moritella sp.	PCL	Sekiguchi et al. 2011
Ochrobactrum sp.	PVC	Ghosh et al. 2013
Phormidium, Lewinella	PET	Urbanek et al. 2018
Proteobacteria, Bacteroides	Microplastics	Urbanek et al. 2018
Pseudomonas sp.	PCL, commercially available bag based on potato and corn starch monofilament fibres of PCL, PHB/V, PBS	Sekiguchi et al. 2011
Psychrobacter sp.	PCL	Sekiguchi et al. 2011
Rhodococcus sp.	PCL, commercially available bag based on potato and corn starch	Sekiguchi et al. 2011
Rivularia	PP, PE	Urbanek et al. 2018
Shewanella sp.	PCL	Sekiguchi et al. 2011
Stanieria, Pseudophormidium	PET	Urbanek et al. 2018
Streptomyces sp.	PHB, poly(3-hydoxybutyarate-co-3-hydroxyvalerate), and starch or polyester	Ghosh et al. 2013
Tenacibaculum sp.	Monofilament fibres of PCL, PHB/V, PBS	Urbanek et al. 2018
Zalerion maritimum	PE	Urbanek et al. 2018
Fungi		
Aspergillus versicolor, Aspergillus sp. *Aspergillus sydowii*	LDPE PVC	Urbanek et al. 2018; Sangale et al. 2019
Clonostachys rosea, Trichoderma sp.	PCL, commercially available bag based on potato and corn starch	Urbanek et al. 2018
Ochrobactrum anthropi strain L1-W	di-2-ethylhexyl phthalate	Nshimiyimana et al. 2020
Pleurotus ostreatus	PE	Rodrigues da luz et al. 2019
Myceliophthora sp.	Polyethylene	Ibrahim 2013
Penicillium chrysogenum	Polythene	Sangale et al. 2019
Trichoderma viride	LDPE	Munir et al. 2018

(continued)

Table 11.4 (continued)

Plastic polymer degrading microorganisms		
Microorganism	Pollutant	Reference
Pestalotiopsis microspora	Polyurethane	Russell et al. 2011
Yeast		
Candida rugosa	Polyurethane	Russell et al. 2011
Pseudozyma sp.	Poly-butylene succinate or poly-butylene succinate-co-adipate	Kitamoto et al. 2011
Algae		
Anabaena spiroides	Polyethylene	Kumar et al. 2017
Scenedesmus dimorphus	Polyethylene	Kumar et al. 2017
Navicula pupula	Polyethylene	Kumar et al. 2017
Consortium		
Vibrio alginolyticus, Vibrio parahaemolyticus	PVA-LLDPE	Urbanek et al. 2018

the hydrolysis of ester bonds. Microbial lipases can efficiently hydrolyse polyesters of PCL. Therefore, lipase producing strains *Agreia, Cryobacterium*, *Polaromonas, Micrococcus, Subtercola, Leifsonia* and *Flavobacterium* from the marine environment have potential to degrade plastic polymers. Other microbial enzymes like cutinases, ureases, depolymerases (PHA-depolymerases, PHB-depolymerases PLA depolymerases, PCL depolymerases), esterases, proteinases (proteinase K against PLA) and dehydratases produced by microorganisms also aid in degradation of plastic polymers. Recent studies on the bacterium *Ideonella sakaiensis* have shown the presence of a novel enzyme PETase (Urbanek et al. 2018).

Fungi, *Clonostachys rosea* and *Trichoderma* sp. have been reported to degrade plastic polymers. *Aspergillus versicolor* and other *Aspergillus* sp. actively degrade LDPE plastic polymers. Saprophytic fungi capable of degrading polyurethane, *Agaricus bisporus*, *Marasmius oreades*, *Cladosporium cladosporioides, Xepiculopsis graminea* and *Penicillium griseofulvum* were isolated from floating plastic litter from the shorelines of Lake Zurich, Switzerland (Brunner et al. 2018). *Alternaria* sp., *Aspergillus niger, Geomyces pannorum, Nectria* sp., *Phoma* sp., *Paraphoma* sp., *Penicillium* sp., *Plectosphaerella* sp. and *Neonectria* sp. are fungi that utilized polyurethane as the sole source of carbon. Yeast *Candida rugosa* has been reported to have polyurethane degrading enzymes and *Pseudozyma* sp. exhibits ability to degrade poly-butylene succinate or poly-butylene succinate-co-adipate films (Kitamoto et al. 2011).

The microalgae *Anabaena spiroides* (blue-green alga), *Scenedesmus dimorphus* (Green microalga) and *Navicula pupula* (Diatom) are being considered as the novel solutions for degradation of polyethylene (Kumar et al. 2017). Microalgae promote efficient biodegradation of plastic polymers by using its enzymes and toxins (Bhuyar et al. 2018).

Microbial consortium using microorganisms with enzymatic profiles necessary for degradation of LDPE and polyethylene has been obtained from culture collection and tested for their potential application in degradation of these plastic polymers (Skariyachan et al. 2016). A consortium of marine bacteria *Vibrio alginolyticus* and *Vibrio parahaemolyticus* when incubated with polyethylene for 15 days showed disintegration of the polymer in the form of grooves and cracks. Studies on bacterial consortium are focused on biodegradation of plastic polymers using indigenous microbial population and biostimulation, by incorporating microbial strains that produce specific plastic degrading enzymes. Research on bacterial consortium also suggests that tailored consortia can thrive in the plastic mixtures and participate in their biodegradation (Syranidou et al. 2019).

11.9 Bioremediation of Recalcitrant Agro-Chemicals by Microorganisms

The rise in the global population has increased the demand for food supplies and therefore involves incessant use of fertilizers, pesticides, fungicides, insecticides and herbicides in farming. Pesticides and herbicides are chemicals that are used to control insects and unwanted weeds, respectively. It is necessary to use these in moderate amounts, only as required in order to control the pests and weeds. However unrestricted use of these pesticides has led to their accumulation in the soil as well as in the water bodies resulting in problems due to biomagnification. Landfilling and pyrolysis of these xenobiotic compounds lead to formation of toxic intermediates. Therefore, bioremediation of these compounds using microorganisms is a promising technique for the removal of these compounds from the soil and marine environment.

The bacteria involved in degradation of pesticides include *Alteromonas undina*, *Alteromonas haloplanktis, Bacillus diminuta, Flavobacterium* sp., *Arthrobacter* sp., *Azotobacter* sp., *Burkholderia* sp., *Pseudomonas* sp., *Raoultella* sp., and *Bacillus sphaericus.* These have been reported to degrade herbicidal and fungicidal compounds (Table 11.5) (Uqab et al. 2016). The biodegradation of these xenobiotic compounds involves complete oxidation of the compound to carbon dioxide and water with the release of energy for the microbes. Bacterial strains of *Pseudomonas fluorescens* and *Bacillus polymyxa* from the Kyrgyzstan pesticide dumping sites exhibited high rate of degradation of aldrin. These dumping sites also showed an abundance of bacteria belonging to the genera *Pseudomonas, Bacillus* and *Micrococcus*. Reports on biodegradation of endosulfan highlight the degradative abilities of *Klebsiella* sp*., Acinetobacter* sp*., Alcaligenes* sp*., Flavobacterium* sp. and *Bacillus* sp. in degradation of this compound. The microbial action on endolsulfan results in the production of intermediates (endosulfan diol, endosulfan ether, and endosulfan lactone) of lesser toxicity than the original compound. Bioremediation of pesticide and related compounds by using microorganisms is preferred due to the production of less toxic intermediates. However, in soils where the innate microbial population is unable to degrade these compounds, addition of external microflora

Table 11.5 Microorganisms used in bioremediation of recalcitrant agro-chemicals

Recalcitrant agro-chemical degrading microorganisms		
Microorganism	Pollutant	Reference
Bacteria		
Acidomonas sp.	Allethrin	Paingankar et al. 2005
Aminobacter sp. MSH1	2,6-dichlorobenzamide (BAM)	Ellegaard-Jensen et al. 2017
Bacillus amyloliquefaciens IN937a *Bacillus pumilus* SE34	Propamocarb and Propamocarb hydrochloride	Ardal 2014
Bacillus polymyxa	Aldrin	Uqab et al. 2016
Bacillus thuringiensis	Melathion	Javaid et al. 2016
Micrococcus	Aldrin	Uqab et al. 2016
Mycobacterium chlorophenolicum	PCB	
Ochrobactrum anthropi NC-1	Phenmedipham	Pujar et al. 2019
Ochrobactrum anthropi Strain SH14	Azoxystrobin	Feng et al. 2020
Pseudomonas sp.	Organophosphates, neonicotinoids, endosulfan, atrazine	Uqab et al. 2016; Doolotkeldieva et al. 2018
Rhizobium meliloti	Chlorinated phosphates	Javaid et al. 2016
Sphingobium japonicum	Hexachlorocyclohexane	Javaid et al. 2016
Stenotrophomonas maltophilia	Endosulfan, DDT	Javaid et al. 2016
Shewanella sp.	Methyl parathion	Javaid et al. 2016
Fungi		
Aspergillus sp.	Endosulfan, organophosphates	Frazar 2000
Fusarium proliferatum CF2	Allethrin	Bhatt et al. 2020
Mortierella sp. LEJ701	Diuron	
Mortierella sp. LEJ701, *Aminobacter* sp. MSH1	2,6-dichlorobenzamide (BAM)	Ellegaard-Jensen et al. 2017
Phanerochaete	Aldrin, DDT, etc.	Uqab et al. 2016
Pleurotus ostreatus	Aldrin, DDT, etc.	Uqab et al. 2016
Pleurotus sp.	Endosulfan, chlorothalonil paraquat	Camacho-Morales and Sánchez 2016
Variovorax sp. SRS16 *Arthrobacter globiformis* D47 *Mortierella* sp. LEJ702	Diuron	Ellegaard-Jensen et al. 2017
Algae		
Chlorella sp..	Mirex, chlordimeform	Ardal 2014
Chlamydomonas sp.	Toxaphene, methoxychlor	Ardal 2014
Chlorococcum sp.	Mirex	Ardal 2014
Cylindrotheca sp.	DDT	Ardal 2014
Dunaliella sp.	Mirex	Ardal 2014
Euglena gracilis	DDT, parathion	Ardal 2014
Scenedesmus obliquus	DDT, parathion	Ardal 2014

(continued)

Table 11.5 (continued)

Recalcitrant agro-chemical degrading microorganisms		
Microorganism	Pollutant	Reference
Selenastrum capricornutum	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene Naphthalene, 2,6-dinitrotoluene, phenanthrene, di-*n*-butylphthalate, Pyrene	Ardal 2014
Consortiums		
Bacillus sp. *and Chryseobacterium joostei*	Lindane, methyl parathion, and carbofuran	Javaid et al. 2016
Pseudomonas putida (NII 1117), Klebsiella sp., *(NII 1118), Pseudomonas stutzeri (NII 1119), Pseudomonas aeruginosa (NII 1120)*	Chlorpyrifos	Sasikala et al. 2012

capable of degrading these compounds has been recommended. The biodegradation depends on enzymatic abilities of the microbes as well as the factors such as pH, temperature, nutrients, oxygen, etc. *Pseudomonas* sp. have been reported to degrade organophosphate compounds and neonicotinoids (Doolotkeldieva et al. 2018; Uqab et al. 2016). Immobilization of bacteria on alginate and other matrix have been used to achieve degradation of various pesticides under different flow rates and environmental conditions (Javaid et al. 2016).

In case of fungi, they make minor changes in the structure of these compounds during degradation, making them more accessible for degradation by other microbes. The fungal species *Flammulina velutipes, Stereum hirsutum, Coriolus versicolor, Dichomitus squalens, Hypholoma fasciculare, Auricularia auricula, Pleurotus ostreatus, Avatha discolor* and *Agrocybe semiorbicularis* have exhibited the ability to degrade pesticides such as chlorinated organophosphorus compounds triazine, dicarboximide and phenylurea (Uqab et al. 2016). Reports on white rot fungi especially *Phanerochaete* such as *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Pleurotus ostreatus*, *Phellinus weirii* and *Polyporus versicolor* have shown the ability to degrade aldrin, chlordane, mirex, gamma-hexachlorocyclohexane (g-HCH), heptachlor atrazine, terbuthylazine, lindane, metalaxyl, dieldrin, diuron, dichlorodiphenyltrichloroethane (DDT), etc. *Aspergillus* sp., *Fusarium oxysporum*, *Penicillium chrysogenum* and *Trichoderma* sp. have shown effective biodegradation of organophosphate pesticides (Frazar 2000; Uqab et al. 2016). *Aspergillus* sp. have also been reported to actively degrade endosulfan (Bhalerao and Puranik 2007). Oliveira et al. (2015) reported the fungal species *Penicillium citrinum*, *Aspergillus fumigatus, Aspergillus terreus* and *Trichoderma harzianum* that could tolerate and degrade chlorfenvinphos.

A fungal-bacterial consortium of *Mortierella* sp. LEJ701 and *Aminobacter* sp. MSH1 was used for the degradation of 2,6-dichlorobenzamide (BAM) and it was observed that the mineralization of the compound proceeded faster than when these strains were used individually. Degradation of agro-chemicals has been found to be most effective on using the bacterial and fungal consortiums than using the microorganisms individually. Biosorption by *Aspergillus niger* and *Mycobacterium chlorophenolicum* has been studied for the removal of polychlorinated phenols (PCP) from aqueous solutions and was found to be pH dependent.

Algal cells such as *Chlamydomonas* sp., *Chlorella* sp., *Chlorococcum* sp., *Cylindrotheca* sp., *Dunaliella* sp., *Euglena gracilis, Scenedesmus obliquus, Selenastrum capricornutum* have been reported to degrade agro-chemicals (Table 11.5) (Ardal 2014).

The algae either metabolize these toxic pollutants using them as energy source or utilize cytochrome P450, a specialized family of monooxygenase enzymes to oxidize herbicides and pesticides. Biotransformation of these agro-chemicals has been reported in *Chlorella* sp. using the cytochrome P450. The presence of P450 has also been demonstrated in the presence of herbicide Metflurazon in the algae *Chlorella fusca* and *Chlorella sorokiniana.* Research on green algae degrading phenol, lindane, DDT, chlordimeform has also been reported (Priyadarshani et al. 2011).

Bacterial consortiums have been preferred for bioremediation of soils contaminated with mixed pesticides. *Bacillus* sp. and *Chryseobacterium joostei* have been used together to treat soils contaminated with lindane, methyl parathion and carbofuran. Abraham and others (Abraham et al. 2014) reported the use of a ten strain bacterial consortium containing *Alcaligenes* sp. JAS1, *Ochrobactrum* sp. JAS2, *Sphingobacterium* sp. JAS3 isolated from chlorpyrifos contaminated soil; *Enterobacter ludwigii* JAS17, *Pseudomonas moraviensis* JAS18 and *Serratia marcescens* JAS16 isolated from monocrotophos containing soil and *Klebsiella pneumoniae* JAS8, *Enterobacter cloacae* JAS7, halophilic bacterial strain JAS4, *Enterobacter asburiae* JAS5 isolated from endosulfan contaminated soil in the biodegradation of organophosphorus and organochlorine pesticides. Similar studies using a consortium isolated from chlorpyrifos contaminated soil containing the bacteria *Pseudomonas putida* (NII 1117), *Klebsiella* sp., (NII 1118), *Pseudomonas stutzeri* (NII 1119), *Pseudomonas aeruginosa* (NII 1120) in biodegradation of chlorpyrifos have been reported (Sasikala et al. 2012). The bacterial consortium using *Acinetobacter* sp., *Bacillus* sp., *Citrobacter freundii, Flavobacterium* sp., Pseudomonads (*Pseudomonas putida, Pseudomonas aeruginosa* and *other Pseudomonas sp*). *Stenotrophomonas* sp., *Proteus* sp., *Proteus vulgaris* and *Klebsiella* sp. was seen to be effective in degradation of methyl parathion and p-nitrophenol (Pino et al. 2011).

11.10 Microorganisms Used in Bioremediation of Dye Compounds

Rapid urbanization and industrialization have led to an increase in the use of fast dyes in industries such as textiles, plastic, food, etc. About 50% of the dye used is released in the industrial effluent. Azo dyes are a potential hazard to the environment due to their bio-recalcitrant, toxic, carcinogenic and mutagenic effects on living organisms. Commonly applied techniques for the removal of the dye involve physical, chemical and decolourization processes which pose a cost issue. Green technologies using microorganisms such as bacterial and fungal biomass provide a low-cost solution.

Various bacteria capable of degrading dyes have been reported. These include lactic acid bacteria, *Pseudomonas, Staphylococcus arlettae, Micrococcus luteus, Listeria denitrificans* and *Nocardia atlantica, Bacillus megaterium*. Basidiomycetous fungi such as *Trametes pubescens* and *Pleurotus ostreatus* and other fungal species such as *Aspergillus tamarii, Aspergillus ochraceus, Aspergillus niger, Fusarium oxysporum, Penicillium purpurogenum* and *Trichoderma lignorum* have also been identified for their role in biodegradation of dyes (Table 11.6) (Patel and Gupte 2016; Rani et al. 2014).

Significant findings have also been reported using consortium of bacteria containing two aerobic strains of bacteria and *Pseudomonas putida* (MTCC1194) in degradation of a mixture of azo dyes from textile effluents (Senan and Abraham 2004). Bacterial consortium of *Neisseria* sp., *Vibrio* sp., *Bacillus* sp., *Bacillus* sp. and *Aeromonas* sp. reportedly showed a degradation of the dyes that ranged from 65% to 90% with decolourization of the dye. This was significantly more than when the monocultures were used proving that bacterial consortium is more efficient in treating dye effluents (Karim et al. 2018).

11.11 Bioremediation of Toxic Pollutants Using Genetically Modified Microorganisms

Xenobiotic compounds cannot be easily degraded by the naturally occurring autochthonous population of microorganisms. These toxic pollutants persist in nature owing to their hydrophobic nature which makes it difficult for the microorganisms to take it up as they lack the uptake transport pathways for such compounds. Recombinant DNA technology involves introducing the desired gene by gene manipulation and plasmid DNA resulting in the development of strains of genetically modified microbes that are efficient in bioremediation. Many genetically modified strains have been used in bioremediation of various complex and toxic anthropogenic compounds especially from the genera *Pseudomonas* and *E. coli* (Table 11.7). This is due to the simple nature of *E. coli* and easy ability to manipulate its genome. While *Pseudomonas* sp. have a natural ability to degrade complex compounds, the genome can be further modified to yield more efficient strains. Marine bacteria have been efficiently transformed by inserting gene coding for

Table 11.6 Microorganisms used in bioremediation of dye compounds

Dye compound degrading microorganisms		
Microorganism	Compound	Reference
Bacteria		
Acinetobacter baumannii	Azo dyes effluents	Kumar et al. 2016a
Bacillus firmus	Vat dyes, textile effluents	Adebajo et al. 2016
Bacillus macerans	Vat dyes, textile effluents	Adebajo et al. 2016
Bacillus pumilus HKG212	Textile dye (Remazol black B), Sulphonated di-azo dye reactive red HE8B, RNB dye	Das et al. 2015
Bacillus sp. ETL-2012	Textile dye (Remazol black B), Sulphonated di-azo dye reactive red HE8B, RNB dye	Shah 2013
Bacillus subtilis strain NAP1, NAP2, NAP4	Oil-based based paints	Phulpoto et al. 2016
Bacillus cereus	Azo dyes effluents	Kumar et al. 2016a
Exiguobacterium indicum	Azo dyes effluents	Kumar et al. 2016a
Exiguobacterium aurantiacum	Azo dyes effluents	Kumar et al. 2016a
Pseudomonas aeruginosa	Textile dye (Remazol black B), Sulphonated di-azo dye reactive red HE8B, RNB dye	Das et al. 2015
Klebsiella oxytoca	Vat dyes, textile effluents	Adebajo et al. 2016
Listeria denitrificans	Textile azo dyes	Hassan et al. 2013
Micrococcus luteus	Textile azo dyes	Hassan et al. 2013
Nocardia atlantica	Textile azo dyes	Hassan et al. 2013
Staphylococcus aureus	Vat dyes, textile effluents	Adebajo et al. 2016
Fungi		
Myrothecium roridum IM 6482	Industrial dyes	Jasinska et al. 2015
Pycnoporus sanguineous	Industrial dyes	Yan et al. 2014
Phanerochaete chrysosporium	Industrial dyes	Yan et al. 2014
Penicillium ochrochloron	Industrial dyes	Shedbalkar and Jadhav 2011
Trametes trogii	Industrial dyes	Yan et al. 2014

Table 11.7 Genetically modified microorganisms used in bioremediation processes

Genetically modified bacteria used for bioremediation		
Microorganism	Pollutant	Reference
Bacteria		
Corynebacterium glutamicum	As	De et al. 2008
Deinococcus geothermalis	Mercury (II), Fe (III)-nitrilotriacetic acid, Uranium (VI), and Chromium (VI).	Brim et al. 2003
Deinococcus radiodurans	Toluene, ionic Mercury	Brim et al. 2003
Deinococcus radiodurans DR1-bf +	Uranium and heavy metals	Manobala et al. 2019
E. coli	*cis*-1,2-dichloroethylene	Kumar et al. 2013
E. coli JM109	Cd	Deng et al. 2007
Escherichia coli ArsR -ELP153AR	As	De et al. 2008
Escherichia coli	Polychlorinated benzene (PCB), benzene and toluene	Kumamaru et al. 1998
Escherichia coli FM5/pKY287	Trichloroethylene (TCE) and toluene	Winter et al. 1989
Nocardia sp.	Crude oil	Balba et al. 1998
Pseudoalteromonas haloplanktis TAC125	Aromatic compounds	Papa et al. 2009
Pseudomonas aeruginosa PA142 *P. aeruginosa* JB	2-chlorobenzoate	Kumar et al. 2013
Pseudomonas putida DLL-1	Methyl parathion	Kumar et al. 2013
Sphingomonas sp. CDS-1	Methyl parathion	Kumar et al. 2013
Sphingomonas sp. *CDS-1*	Organophosphate and carbamate-degrading	Kumar et al. 2013
Sulphate-reducing bacteria (SRB)	Chromate	Das and Dash 2014
Synechococcus sp.	Heavy metals	Das and Dash 2014
Thalassospira lucentensis	Hydrocarbons	Das and Dash 2014
Fungi		
Fusarium solani	DDT	Kumar et al. 2013
Gliocladium virens	Paraoxon and diisopropylfluorophosphate	Kumar et al. 2013
Pichia pastoris	Azo dyes, anthraquinone dyes	Kumar et al. 2013
Trichoderma atroviride	Dichlorvos pesticide	Kumar et al. 2013
Yeast		
Saccharomyces cerevisiae CP2 HP3	Cd, Zn	De et al. 2008

(continued)

Table 11.7 (continued)

Genetically modified bacteria used for bioremediation		
Microorganism	Pollutant	Reference
Algae		
Chlamydomonas reinhardtii	Cd	De et al. 2008; Kumar et al. 2013; Igiri et al. 2018
Chlorella sorokiniana ANA9	Heavy metals	Kumar et al. 2013
Laminaria japonica	Pb	Kumar et al. 2013
Nitella pseudoflabellata	Chromium (VI)	Kumar et al. 2013
Phaeodactylum tricornutum	PET	Moog et al. 2019

metallothionein. This has been successfully used in bioremediation of metal contaminated environments. Genetically modified marine Antarctic bacterium, *Pseudoalteromonas haloplanktis* TAC125 has reportedly shown promise in bioremediation of aromatic compounds (Table 11.7). A genetically modified strain of *Pseudomonas putida* ENV2030 was obtained by mutation and was reported to degrade an organophosphorus compound paraoxon by utilizing it as a sole source of carbon and nitrogen. The strain from *Acinetobacter* sp. YAA was mutated by several rounds of mutagenesis to increase the activity of aniline dioxygenase against aniline, 2,4-dimethylaniline and 2-isopropylaniline. *Burkholderia cepacia* strain V350F and V350M are mutants that produce the enzyme 2,4-dinitrotoluene dioxygenase that is reported to have significant activity against m-nitrophenol, o-nitrophenol, o-methoxyphenol and o-cresol. The bacteria *Agrobacterium radiobacter* AD1 was reported to efficiently degrade TCA at contaminated sites. Genomic shuffling has increased the degradation potential of *Sphingobium chlorophenolicum* ATCC 39723 for the pesticide pentachlorophenol. The most significant application for bioremediation involves protein engineering for large subunit of the hybrid enzyme of biphenyl dioxygenase from *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia cepacia* LB400 that results in enhanced degradation of polychlorobiphenyls (Kumamaru et al. 1998; Kumar et al. 2013). Toxic mercury-degrading gene from terrestrial bacteria has been used to transform marine bacteria for their applications in field for bioremediation of mercury contaminated environments (Das and Dash 2014). *Deinococcus radiodurans* is genetically modified bacteria and the most radiation resistant bacteria that has been designed to digest toluene and ionic mercury from nuclear wastes (Brim et al. 2003). The plasmid from *Deinococcus radiodurans* has been reportedly used to transform *Deinococcus geothermalis,* another radiation resistant bacterium that can reduce Mercury (II), Fe (III)-nitrilotriacetic acid, Uranium (VI) and Chromium (VI). A recombinant strain of *Deinococcus radiodurans* DR1-bf + has gained importance as a potential bacterium for the bioremediation of uranium and heavy metals due to its ability to form biofilms (Manobala et al. 2019). Genes for metallothioneins and phytochelatins from fungi and plants have been cloned in *Escherichia coli* which demonstrates an enhanced binding of heavy metals. Genetically engineered bacteria such as *E. coli* JM109*, Mesorhizobium huakuii, Pseudomonas putida* and *Caulobacter crescentus* that bioaccumulate Cd^{+2} by presence of phytochelatins and metal-binding proteins have been reported. Other bacteria that have been engineered and are being studied for bioremediation have been mentioned in Table 11.7. Modifications in the active sites of enzymes of microorganisms such as *Pseudomonas putida*, *Bacillus megaterium*, *Burkholderia cepacia* strain LB400, *Comamonas testosteroni* B-365 and *Rhodococcus globerulus* P6 by genetic manipulation has resulted in increased efficiency of these enzymes in degrading the target pollutant. *Halobacteriaceae* family protein MBSP1that had biosurfactant activity when used to transform *E. coli Rosetta* ™ (DE3) demonstrated significant increase in hydrocarbon degradation (Araújo et al. 2020).

Saccharomyces cerevisiae has been genetically modified to express P450 cytochrome complexes to express genes to degrade dioxins. Another yeast, *Hansenula polymorpha* has been genetically modified for bioremediation of chromate. The fungi *Fusarium solani* has been genetically modified to improve production of dehalogenase enzyme that is crucial in degradation of DDT. Other fungi that have been genetically modified for the degradation of target pollutants are included in Table 11.7.

A brown algae *Laminaria japonica* from the marine ecosystem was chemically modified for the removal of lead from the wastewaters. The green algae, *Chlorella sorokiniana* ANA9 was used in removal of heavy metals from soil. It was reported to play a crucial role in preventing the diffusion of toxic Cd^{+2} in the soil. The toxicity of chromium (VI) in contaminated waters can be reduced by using *Nitella pseudoflabellata.* To enhance ability of *Chlamydomonas* to bind metals, a foreign metallothionein gene was expressed in *Chlamydomonas reinhardtii.* This enhanced the ability of the strain to absorb Cd by two-fold in comparison to the wild strain in damp soils and aquatic ecosystems (Kumar et al. 2013). Marine microalgae, *Phaeodactylum tricornutum*, has been modified to carry polyethylene terephthalate, PETase gene from *Ideonella sakaiensis* is an eco-friendly method for recycling strategies (Moog et al. 2019).

11.12 Bioremediation of Toxic Pollutants Using Microbial Biosurfactants and Bioemulsifiers

Bioemulsifiers and biosurfactants are surface active compounds which are amphiphilic in nature and promote emulsification of two immiscible phases. These biomolecules are produced by microorganisms such as yeast, bacteria and fungi. They find potential applications in environmental bioremediation, industrial processes and food processing industries.

Although the terms biosurfactants and bioemulsifiers have been used interchangeably with each other, they differ based on their physicochemical properties of interaction and the physiological role they play in bringing the miscibility of the two phases. Bioemulsifiers and biosurfactants both by virtue of being amphiphilic biomolecules possess hydrophilic and hydrophobic structural moieties which allow them to dissolve in polar and non-polar solvents.

Biosurfactants can be either glycolipids which includes rhamnolipids, sophorolipids or trehalose lipids, wherein the sugars are linked to β-hydroxy fatty acids. Lipopeptides biosurfactants such as iturin and fungicin contain cycloheptapeptides with amino acids linked to fatty acids of different chain lengths. Other biosurfactants include polymeric and particulate surfactants. The surface activity of biosurfactants is due to their ability to lower the surface and interfacial tension between the two phases (liquid- air), (liquid- liquid), (liquid- solid). This is carried out by adsorption onto the different surfaces stimulating more interaction and mixing of the immiscible phases. Biosurfactants possess critical micelle concentration (CMC) that form stable emulsions. CMC is the minimum concentration of biosurfactant that is needed to reduce the surface tension to a minimum level and forms micelles. Biosurfactants have low toxicity, high biodegradability and exhibit diversity. Thus, they find potential applications as wetting, foaming and solubilizing agents in industrial processes (Uzoigwe et al. 2015).

A marine strain of *Pseudomonas aeruginosa* isolated from oil-contaminated sea water was found to produce biosurfactant that was effective in degrading hydrocarbons such as 2-methylnaphthalene, tetradecane, hexadecane, octadecane, heptadecane and nonadecane. Hydrocarbonoclastic bacteria that are ubiquitous in the marine environment have the ability to degrade aliphatic and aromatic fractions of crude oil. A mixture of biosurfactants produced by these bacteria stimulate the degradation of these hydrocarbons. Biosurfactant producing strains *Acinetobacter haemolyticus* and *Pseudomonas* ML2 showed a significant reduction in hydrocarbons up to 75%. A lipopolypeptide from *Bacillus subtilis* was found to be stable at varying temperatures, pH and salt concentrations and therefore exhibited potential in applications for bioremediation of hydrocarbons in the marine environment. Biosurfactants such as surfactin, lichenysin and rhamnolipids are found to be effective in hydrocarbon degradation. The biosurfactants produced by *Acinetobacter venetianus* ATCC 31012 exhibited removal of 89% of the crude oil by emulsification (Uzoigwe et al. 2015).

The biosurfactants from *Candida sphaerica* exhibited a bioremediation efficiency of 95% for iron, 90% for zinc and 79% for lead. The surfactant interacted with the heavy metal ion resulting in their detachment from the soil. *Candida* sp. could bioaccumulate nickel and copper by producing biosurfactants (Luna et al. 2016). Heavy metal removal was found to be productive when biosurfactants like surfactin, rhamnolipid, sophorolipids were used for bioremediation of copper and zinc. *Rhodotorula mucilaginosa* was studied for the removal of metal with an efficiency up to 95% due to its ability to form biofilms. The production of biofilm plays a crucial role in bioremediation as biofilms are a direct result of EPS formation which contains molecules that possess surfactant or emulsifying properties (Grujić et al. 2017; El-Masry et al. 2004). The biosurfactant EPS isolated from *Vibrio* sp. emulsified the hydrocarbon hexadecane and xylene by reducing the surface tension between the two immiscible phases (Kharangate-Lad and Bhosle 2014).

In microbial cells, apart from special components produced by cells, many EPS produced by yeast, bacteria and fungi are bioemulsifiers in nature. *Halobacillus trueperi* has been reported to produce an EPS that possesses bioemulsifying properties with the hydrocarbon hexadecane (Kharangate-Lad and Bhosle 2015). This EPS bioemulsifier on characterization was found to be glycopeptide in nature. Bioemulsifiers efficiently emulsify two immiscible liquids and form stable emulsions at low concentration. Significantly, it is important to understand that though all biosurfactants bioemulsify all bioemulsifiers do not reduce surface tension. Therefore, it can be suggested that though all biosurfactants are bioemulsifiers, all bioemulsifiers are not biosurfactants.

Research has shown that efficient stabilization property of bioemulsifiers is a function of their chemical composition. It has been reported that in *Acinetobacter* sp. RAG-1 (Table 11.8), the utilization of alkane is dependent upon the presence of fimbriae. Microorganisms producing biosurfactants and bioemulsifiers that have potential applications in the field of bioremediation have been listed in Table 11.8. An alanine-containing bioemulsifier has been reported in *A. radioresistens* KA53. Alasan is a complex of alanine in association with polysaccharides and proteins. It is a secreted by the cell and remains cell bound and has the ability to emulsify a wide range of hydrocarbons such as long chains alkanes and aromatics, solubilization of polyaromatic hydrocarbons (PAHs) and paraffins and crude oils (Uzoigwe et al. 2015). *Acinetobacter* sp. also exhibited a bioemulsifier that was composed of 53% protein, 42% polysaccharide and only 2% lipid. Owing to the significant ability of this bioemulsifier to emulsify hydrocarbons and solvents, it showed potential for bioremediation studies. *Stenotrophomonas maltophilia* UCP 1601 showed production of bioemulsifier that had excellent dispersion capacity and formed stable oil in water emulsions. The EPS produced by *Halomonas eurihalina*, which was rich in uronic acid and was composed of smaller fractions of carbohydrates and protein components had significant ability to bioemulsify and detoxify hydrocarbons. Similar bioemulsifiers produced by *Klebsiella* sp. were seen to exhibit bioremediation potential.

Relatively a smaller number of filamentous fungi have been identified for the production of biosurfactants. These fungi include *Aspergillus niger, Cunninghamella echinulate, Fusarium* sp., *Penicillium chrysogenum* SNP5*, Rhizopus arrhizus* and *Trichoderma* sp. (Silva et al. 2018). Filamentous fungi are less extensively used in bioremediation due to their slow growth. However, they are excellent producers of biosurfactants and bioemulsifiers and promote dispersion of hydrophobic compounds that aids in bioavailability and biodegradation of these compounds (Table 11.8).

Mannoproteins are glycoproteins that are produced by the yeast in their cell walls. Mannoproteins of *Kluyveromyces marxianus, Saccharomyces cerevisiae* exhibit significant emulsifying properties. These mannoproteins could form stable emulsions with hydrocarbons, solvents and waste oil suggesting potential application in bioremediation. Other yeasts which have been reported for the production of biosurfactants are *Rhodotorula glutinis*, *Candida* sp., *Yarrowia lipolytica*,

Table 11.8 Biosurfactant and bioemulsifier producing microorganisms used in bioremediation processes

Microorganisms producing biosurfactants and bioemulsifiers used in bioremediation			
Microorganism	Biosurfactant/ bioemulsifiers	Pollutant	Reference
Bacteria			
Acinetobacter calcoaceticus BD4 13	Emulsan (polysaccharide-protein bioemulsifier)	Oil in water emulsion stabilization	Uzoigwe et al. 2015
Acinetobacter radioresistens KA53	Alanine (alanine-based glycoprotein bioemulsifier)	Oil in water emulsion stabilization	Uzoigwe et al. 2015
Acinetobacter sp. ATCC 31012 (RAG-1)	Emulsan (Glycolipopeptide bioemulsifier)	Insoluble toxic pollutants, heavy metals, hydrocarbon emulsification	Ron and Rosenberg 2001
Arthrobacter sp.	Trehalose, corynemycolates	*n*-alkane	Uzoigwe et al. 2015
Bacillus licheniformis	Lichenysin	Oil recovery	Uzoigwe et al. 2015
Bacillus subtilis K1	Lipoprotein Subtilisin	Oil recovery	Pathak and Keharia 2014
Halomonas eurihalina	Uronic acid rich glycopeptides	Emulsification and detoxification of hydrocarbons	Martínez-Checa et al. 2002
Klebsiella sp.	Uronic acid rich glycopeptides	Emulsification and detoxification of hydrocarbons	Uzoigwe et al. 2015
Nocardia farcinica BN26	Trehalose	Toxic pollutants	Uzoigwe et al. 2015
Pseudomonas aeruginosa DS10-129	Rhamnolipid	Toxic pollutants	Uzoigwe et al. 2015
Rhodococcus erythropolis	Trehalose	Dissolution of hydrocarbons (*n*-alkanes)	Uzoigwe et al. 2015
Rhodococcus sp.	Mycolates, corynemycolates	Oil recovery	Uzoigwe et al. 2015
Rhodococcus wratislaviensis BN38	Trehalose	Toxic pollutants	Tuleva et al. 2008
Fungi			
Aspergillus niger	Glycolipid	Hydrocarbon degradation	Silva et al. 2018
Aspergillus ustus	Glycolipoprotein	Hydrocarbon degradation	Silva et al. 2018
Cunninghamella echinulate	Carbohydrate-protein-lipid complex	Hydrocarbon degradation	Silva et al. 2018
Fusarium sp.	Trehalose	Hydrocarbon degradation	Silva et al. 2018

(continued)

Table 11.8 (continued)

Microorganisms producing biosurfactants and bioemulsifiers used in bioremediation			
Microorganism	Biosurfactant/ bioemulsifiers	Pollutant	Reference
Penicillium chrysogenum SNP5	Lipopeptide	Hydrocarbon degradation	Silva et al. 2018
Ustilago maydis	Glycolipid	Hydrocarbon degradation	Bhardwaj et al. 2013
Yeast			
Candida lipolytica	Lipopolysaccharide	*n*-alkane dissolution	Uzoigwe et al. 2015
Kluyveromyces marxianus	Mannoproteins	Hydrocarbon emulsification	Uzoigwe et al. 2015
Saccharomyces cerevisiae	Mannoproteins	Hydrocarbon emulsification	Uzoigwe et al. 2015
Torulopsis sp	Sophorolipids	Hydrocarbon emulsification	Uzoigwe et al. 2015
Algae			
Cyanobacteria	Trehalose dicorynomycolate, lipid based bioemulsifier	Hydrocarbon biodegradation	Alizadeh-Sani et al. 2018
Diatoms	Lipid based bioemulsifier	Hydrocarbon biodegradation	Alizadeh-Sani et al. 2018
Phormidium sp.	Lipid-protein-carbohydrate bioemulsifiers	Hydrocarbon biodegradation	Alizadeh-Sani et al. 2018

Pseudozyma rugosa, Trichosporon asahii, *Wickerhamomyces anomalus* and *Kurtzmanomyces* sp. (Bhardwaj et al. 2013; Silva et al. 2018).

A variety cyanobacteria belonging to *Oscillatoriales* produce bioemulsifiers. *Phormidium* sp. ATCC 39161 have been successfully used to yield hydrocarbon and oil emulsions in water. The bioemulsifier showed fractions of lipid, proteins and carbohydrates and showed significant stability of oil in water emulsions (Alizadeh-Sani et al. 2018). Marine algae and diatoms are increasingly being explored for their potential to produce bioemulsifiers. These bioemulsifiers are EPS based lipid bioemulsifiers that have potential application in bioremediation and industries.

11.13 Conclusion

Microbial bioremediation, although a cost effective and eco-friendly technique for biodegradation of recalcitrant toxic compounds, faces issues due to the biotic and abiotic factors affecting biodegradation. In the natural environment, constant fluctuations in oxygen, nutrient, pH and temperature occur that hinders and reduces the efficiency of microbial bioremediation. Despite these limitations the advantages outweigh the disadvantages and bioremediation using microbes and their components have been successfully implemented in cleanup of many toxic contaminants. Naturally occurring microbes and genetically designed microbes are important tools for successful cleanup of contaminated sites using green technology. However, considering the lacunae in the efficiency of microbial cell mediated bioremediation, integrated approaches involving microorganisms, nanoparticles and physical methods are now being explored.

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Remediation of Toxic Environmental Pollutants Using Nanoparticles and Integrated Nano-Bio Systems

19

Amrita Kharangate-Lad and Nezlyn Cressida D'Souza

Abstract

Rapid industrialization and urbanization have led to the release of many toxic contaminants in the environment. Environmental pollution is a major concern as it affects health and well-being of all organisms. The toxicity and health hazards associated with the environmental pollutants requires clean-up from the soil and water. Remediation using nanotechnology is an effective alternative to conventional physicochemical methods as it is eco-friendly and economical. In this chapter we emphasize the use of nanoparticles in the remediation of toxic environmental pollutants such as heavy metals, hydrocarbons, antibiotics, hormones, dyes, and recalcitrant agro-based and organohalide compounds. Nanoparticles are synthesized biogenically which involves its synthesis using plants and microorganisms. Nanobioremediation is a technology that integrates microbial bioremediation and nanoremediation. This chapter provides a brief outlook on applications of nanobioremediation through integrated nano-bio systems in removal of organic or inorganic toxic pollutants and discusses the implications of using these integrated systems on a larger scale. The benefit of this collaboration is the development of more efficient, cost-effective, environmentally friendly solutions in remediation of toxic environmental pollutants.

Keywords

Nanoparticles · Microbes · Synthesis · Toxic pollutants · Remediation

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19.1 Introduction

Environmental pollution is undeniably a major issue that the society faces. The environment gets polluted with toxic compounds released due to industrialization and unplanned urbanization. Toxic pollutants like heavy metals, particulate matter, industrial effluents, sewage, pesticides, fertilizers, oil spills, and other organic compounds are a few examples of environmental pollutants. The use of conventional physical and chemical technologies in the treatment of contaminated soils and water is expensive as they have high energy requirements and usually leave behind hazardous waste residues. Therefore, cost-effective bioremediation technologies that use microorganisms to detoxify and transform pollutants have been developed for the treatment of polluted soils and water (Abatenh et al. 2017; Kumar et al. 2018; Tomei and Daugulis 2013). Another technology that is being explored currently is the use of nanoparticles in cleaning up toxic pollutants from contaminated sites. Nanoparticles have highly desirable properties that favour their application in remediation of recalcitrant pollutants from soil and water.

19.2 Properties of Nanoparticles

Nanoparticles possess unique physicochemical properties and therefore, nanotechnology has gained a lot of attention in the last decade. Nanoparticles are nanoscopic particles having dimensions within the range of 1–100 nm. Due to their nanoscale size these particles exhibit exceptional physicochemical properties such as high energy, large specific surface area, and unique visible properties (Fig. 19.1). Nanoparticles by virtue of their small size have properties that differ from the bulk metal. They exhibit physical and chemical properties that are a virtue of their nanosize. The nano-size of the particles imparts them with unique chemical properties such as large surface per unit area and high reactivity. Nanoparticles exhibit plasmon resonance as owing to their small size they have the ability to confine their electrons and produce quantum effects. The media used in the synthesis of nanoparticles and the number of bioactive compounds present in the media directly influence the chemical and morphological properties of nanoparticles (Yadav et al. 2017).

Based on the chemistry, the nanoparticles are broadly classified into organic and inorganic. Organic nanoparticles include carbon nanoparticles (like carbon nanotubes (CNT)), while inorganic nanoparticles include metals (such as nanoscale zerovalent iron (nZVI), palladium, cadmium), noble metals (gold and silver), semiconductors (copper, zinc oxide, and titanium oxide), and magnetic nanoparticles (maghemite, magnetite) (Guerra et al. 2018).

Nanoparticles have different ways of interacting with each other, some tend to remain as individual moieties, while others tend to group together, depending upon the attractive or repulsive forces acting between them. Agglomeration of nanoparticles is often linked with oxidation-induced-instability, which leads to decrease in the surface area and reactivity of the particles. Coating of nanoparticle with suitable stabilizers increases their adsorbing capacity while decreasing their

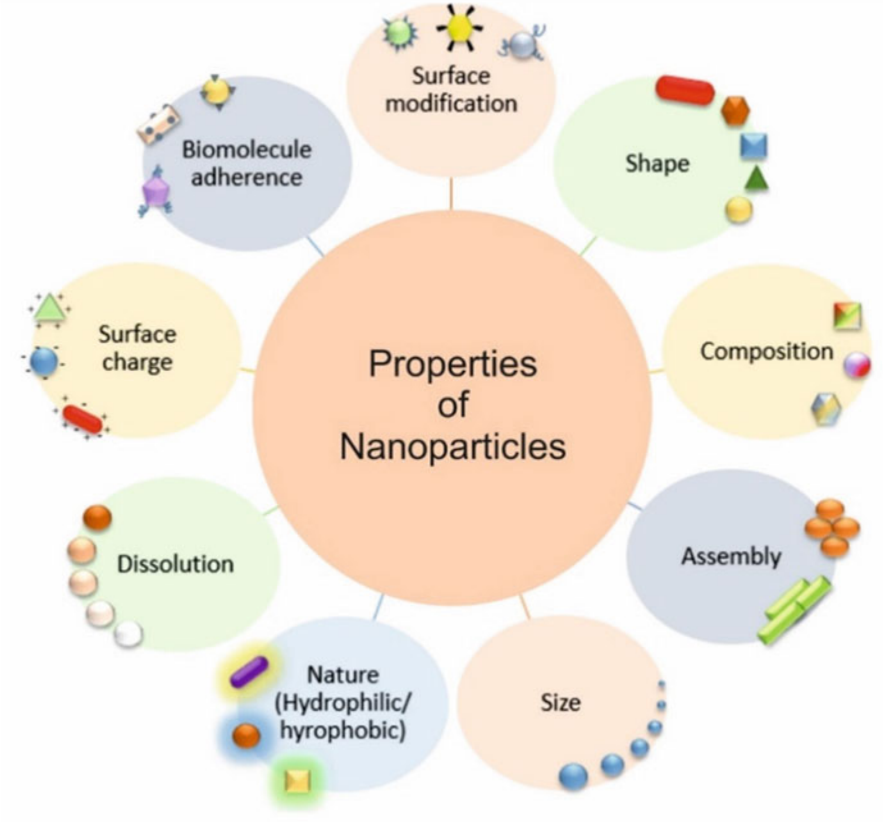

Fig. 19.1 Properties of nanoparticles

tendency to agglomerate. Immobilization with organic polymers such as lactate, gaur gum, gellan gum, etc. enhances their interaction with the toxic compounds by improving nanoparticle mobility, stability, and reactivity. Nanoparticles can be designed to exhibit specific physical properties and chemical composition in order to target specific pollutants (Sakulchaicharoen et al. 2010).

19.3 Nanoparticles and Nanomaterials

Morphologically, nanoparticles can be spheres, sheets, cylinders, and tubes (Pal et al. 2019). However, they differ from nanomaterials owing to their dimensions. Nanomaterials have been defined as “a manufactured or natural material that possesses unbound, aggregated, or agglomerated particles where external dimensions are between 1 and 100 nm size range”, according to the EU Commission (Jeevanandam et al. 2018). Nanomaterials therefore have a length of 1–1000 nm in at least one dimension. Based on the shape, size, structure, and composition, nanomaterials can be classified into nanoclusters, nanofibres, nanotubes, nanoshells, and nanocomposites.

Nanomaterials have successfully demonstrated their ability to degrade environmental pollutants from soil and ground/surface water. Metal-based nanomaterials composed of silver (Ag) nanoparticles have been used for disinfection of water and soil from *Escherichia coli* and *Pseudomonas aeruginosa*. Titanium dioxide (TiO_2) nanoparticles have been proven efficient against *Escherichia coli,* MS-2 phage and hepatitis B virus. Other titanium-based nanoparticles have been reportedly used for remediation of aromatic hydrocarbons (phenanthrene), chlorinated compounds (2-chlorophenol), organic pollutants (endotoxins), microorganisms (*Escherichia coli* and *Staphylococcus aureus*), dyes (Rhodamine B), and biological nitrogen (nitric oxide) (Guerra et al. 2018). Binary mixed oxides, iron nanoparticles, and bimetallic nanoparticles have been used for the treatment of water containing methylene blue, heavy metals, and chlorinated and brominated compounds, respectively (Jeevanandam et al. 2018). Due to their minute size and surface properties, they can enter very small spaces in the soil and attain access to contaminants well below the subsurface level. Since movement of these nanoparticles is governed by Brownian motion, they can remain suspended in groundwater facilitating wider transport of the particles and more efficient interaction with the contaminant (Tratnyek and Johnson 2006).

19.4 Synthesis of Nanoparticles

The modification of surface properties of the nanoparticle improves its interaction and compatibility with the pollutant of interest. Nanoparticles, in general, are synthesized using two approaches: top-down and bottom-up. The top-down approach centres on gradual degradation of bulk materials to nanosized particles, while the bottom-up approach involves assembly of atoms and molecules into a nanoparticle (Narayanan and Sakthivel 2010). The former approach involves usage of expensive and energy-intensive physical methods, while the latter can be further sub-classified into chemical and biological methods. The employment of high-priced toxic solvents and extreme physical conditions (such as high pressure and temperature) in chemical synthesis of nanoparticles renders the chemical process as environmentally unfriendly (Narayanan and Sakthivel 2010). Biological methods, which utilizes plants and microbes (algae, bacteria, yeasts, actinomycetes, and fungi) serve as an eco-friendly and less expensive alternative to top-down synthesis of nanoparticles (Fig. 19.2) (Sastry et al. 2003).

19.4.1 Biogenic Production of Nanoparticles Using Plants

The biosynthesis of nanoparticles generally entails oxidation–reduction reactions. Plants and microorganisms have enzymes and produce antioxidizing and reducing

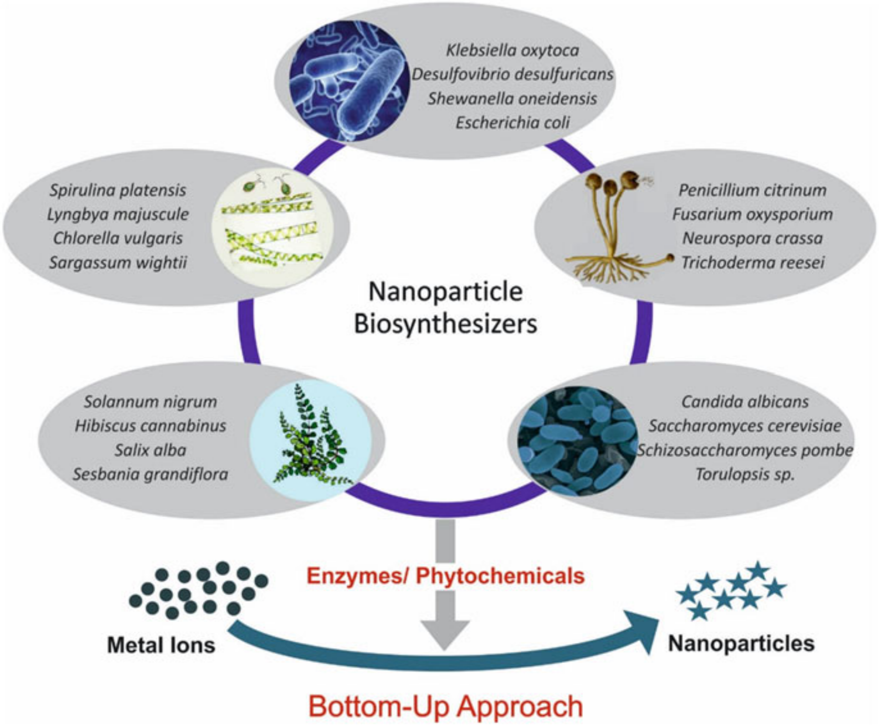

Fig. 19.2 Biogenic synthesis of nanoparticles using bottom-up approach

phytochemicals, which facilitate reduction and detoxification of accumulated metal ions. In the synthesis of metal/metal oxide nanoparticles, plant extracts (from roots, leaves, flowers, stems) are reported to be effective as they contain phytochemicals such as amides, aldehydes, ascorbic acids, carboxylic acids, flavones, ketones, phenols, and terpenoids. These compounds have been reported to have ability to reduce metal salts into metal nanoparticles. Plants such as Alfalfa, oats, Tulsi, lemon, Aloe vera have been reported to be used in production of silver and gold nanoparticles (Singh et al. 2016). Through optimization of process parameters, nanoparticles of desired sizes and morphologies can be biosynthesized (Iravani 2014). The application of whole plants or their phytochemical extracts in synthesis of nanoparticles has been demonstrated through numerous studies (Hussain et al. 2016). Biogenic production using plants is a preferred method as it is easier to scale-up (Megharaj et al. 2011).

19.4.2 Biogenic Production of Nanoparticles Using Microorganisms

Microbes possess highly versatile and effective catalytic mechanisms that enable degradation of various toxic compounds (Watanabe 2001). Additionally, microbes are ubiquitous, much easier to culture and manipulate, have high growth rates, and require low cost investment. Silver nanoparticles have been biogenically produced by using bacteria such as *Arthrobacter gangotriensis, Aeromonas* sp. SH10, *Bacillus amyloliquefaciens, Bacillus cecembensis, Bacillus indicus, Bacillus cereus, Corynebacterium* sp. SH09, *Escherichia coli, Enterobacter cloacae, Geobacter* sp., *Lactobacillus casei, Phaeocystis antarctica, Pseudomonas proteolytica,* and *Shewanella oneidensis.* Gold nanoparticles have been reported to be extensively produced using *Bacillus subtilis* 168, *Bacillus megaterium* D01, *Desulfovibrio desulfuricans*, *Escherichia coli* DH5a, *Plectonema boryanum* UTEX 485, *Rhodopseudomonas capsulate,* and *Shewanella alga*. Fungi have been used to synthesize zinc, gold, silver, and titanium metal/metal oxide nanoparticles. Biosynthesis of silver and gold nanoparticles by *Saccharomyces cerevisiae* has been reported (Singh et al. 2016).

Microorganisms synthesize inorganic nanoparticles by assimilating metal ions from the surrounding environment and converting them to their elemental form using enzymes. Heterotrophic sulphate reducing bacteria have been reported to reduce gold-(I)-thiosulphate complex (Au $\left(S_2O_3\right)_2^{3-}$) to elemental gold (Au^0) (Lengke and Southam 2006). Similarly, *Stenotrophomonas maltophilia* SELTE02 mediated transformation of selenite (SeO_3^{2-}) to elemental selenium (Se^0) (Gregorio et al. 2005). Microbial biosynthesis can be achieved through intracellular and extracellular routes. The intracellular method consists of transporting metal ions into the cell, followed by enzymatic reactions, whereas the extracellular method involves adsorption of the metal ions on the cell surface, followed by enzyme-moderated reduction (Zhang et al. 2011). Microorganisms are known to synthesize various types of nanoparticles including metallic nanoparticles (gold, silver, cobalt, chromium, and other metals), magnetic, non-magnetic nanoparticles, and alloy nanoparticles (Li et al. 2011).

The biogenic method of nanoparticle production is an enzyme-mediated "green" synthesis of nanoparticles (Fig. 19.2). It allows elimination of costly, inefficient methods that involve use of expensive, hazardous solvents (such as sodium borohydride, ammoniacal silver nitrate) and produce toxic by-products. Furthermore, the particles generated by biogenic processes have greater surface area and higher catalytic reactivity (Mohanpuria et al. 2008). Table 19.1 lists microorganisms involved in the biogenic synthesis of various nanoparticles.

19.5 Nanoparticles: Mechanism of Action

Nanoparticles exhibit surface and chemical properties that are distinct from the bulk metal due to their nano-size. The nano-size increases the mechanical, magnetic, electrical, and optical properties of the nanoparticles. Nanoparticles catalyse processes such as adsorption and chemical reactions such as photocatalysis, precipitation, reduction, oxidation, and hydrogen peroxide dependent oxidation (Fenton reactions). The adsorption capacity of nanoparticles is being used to develop sorbents like carbon nanotubes and zeolites that have demonstrated efficient removal

Table 19.1 Biogenic production of nanoparticles by microorganisms

Biogenic production of nanoparticles by microorganisms		
Nanoparticle	Microorganism	References
Iron (Fe)	*Pleurotus* sp.	Mazumdar and Haloi 2011
	Bacillus subtilis	Sundaram et al. 2012
	Escherichia coli	Arcon et al. 2012
	Klebsiella oxytoca	Anghel et al. 2012
	Chaetomium globosum	Elcey et al. 2014
Zinc (Zn)	*Desulfobacteriaceae*	Labrenz et al. 2000
	Lactobacillus sp.	Selvarajan and Mohanasrinivasan 2013
	Streptomyces sp.	
	Aspergillus terreus	Raliya and Tarafdar 2013
	Sphingobacterium thalpophilum	Raliya and Tarafdar 2014; Rajabairavi et al. 2017
Palladium (Pd)	*Bacillus sphaericus*	Creamer et al. 2007
	Desulfovibrio desulfuricans	Gurunathan et al. 2015
	Bacillus benzeovorans	Omajali et al. 2015
	Shewanella sp.	Zhang and Hu 2017
	Geobacter sulfurreducens	Yates et al. 2013
Copper (Cu)	*Penicillium aurantiogriseum*	Honary et al. 2012
	Penicillium citrinum	Cuevas et al. 2015
	Penicillium waksmanii	Tiwari et al. 2016
	Stereum hirsutum	Parveen et al. 2016
	Bacillus cereus	Parveen et al. 2016
	Pseudomonas sp., *Serratia* sp.	Parveen et al. 2016
Silver (Ag)	*Bacillus licheniformis*	Kalimuthu et al. 2008
	Trichoderma reesei	Nanda and Saravanan 2009
	Cladosporium cladosporioides	Balaji et al. 2009
	Candida sp.	Kumar et al. 2011
	Streptomyces naganishii	Alani et al. 2012
Gold (Au)	*Rhodococcus* sp., *Streptomyces* sp.	Ahmad et al. 2003
	Aspergillus fumigatus, A. flavus	Gupta and Bector 2013
	Rhodopseudomonas capsulata	He et al. 2007
	Stenotrophomonas maltophilia	Nangia et al. 2009
	Penicillium brevicompactum	Mishra et al. 2011
Titanium (Ti)	*Lactobacillus* sp.	Prasad et al. 2007
	Bacillus subtilis	Vishnu Kirthi et al. 2011
	Aspergillus flavus	Rajakumar et al. 2012
	Aspergillus terreus	Raliya and Tarafdar 2014
	Bacillus amyloliquefaciens	Khan and Fulekar 2016
Cadmium (Cd)	*Escherichia coli*	Sweeney et al. 2004
	Rhodopseudomonas palustris	Bai et al. 2009
	Fusarium sp.	Reyes et al. 2009
	Bacillus amyloliquefaciens	Singh et al. 2011
	Bacillus licheniformis	Shivashankarappa and Sanjay 2015

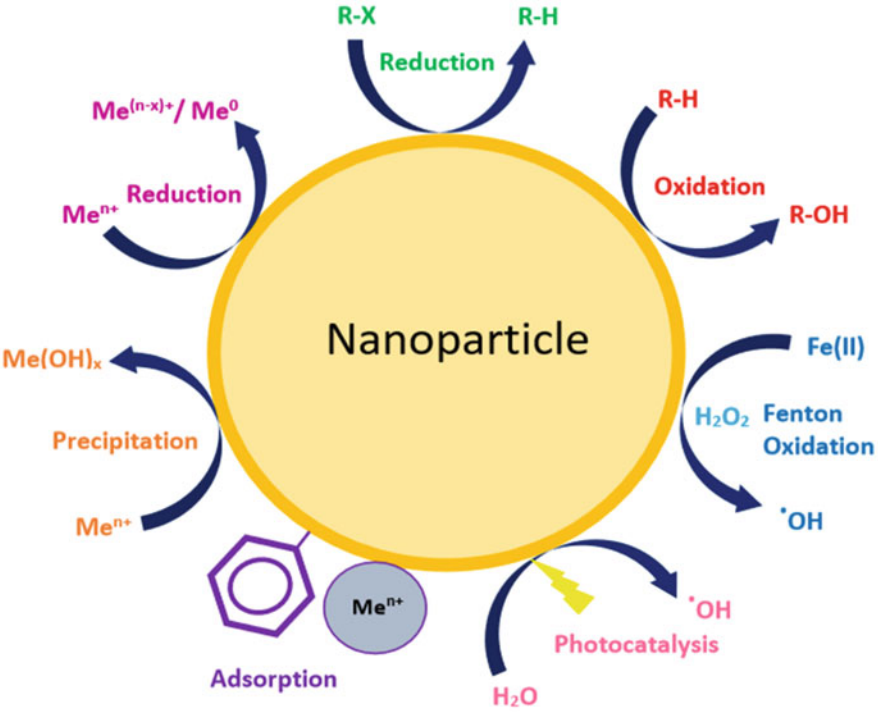

Fig. 19.3 Mechanisms catalysed by nanoparticles in degrading toxic pollutants

of anions and metals from drinking water (Yaqoob et al. 2020). Nanoparticles mediate the transformation of toxic metals into their respective hydroxides by precipitation. Nanoparticles play a crucial role in nanobioremediation by catalysing reactions and generating hydrogen ions that aid the microorganisms to further detoxify and transform the compound. The high reactivity and larger surface area for interaction offer the nanoparticles the characteristic property of having high oxidation potential. Metal oxide nanoparticles have been shown to catalyse oxidation reactions that can transform toxic pollutants such as hydrocarbons. Nanoparticles can absorb photons and act as nanophotocatalysts. The larger surface area of nanoparticles increases the oxidation of organic pollutants at the surface of the nanoparticles resulting in their degradation. TiO_2 is the most commonly used nano-photocatalyst in treatment of wastewater, due to its low cost, chemical stability, easy availability, and zero toxicity. Composite nanomaterials like copper oxide doped (CuO-doped) zinc oxides (ZnO) have been used to catalyse the Fenton oxidation. Fenton oxidation is a H_2O_2-assisted photo-decomposition of toxic pollutant in the presence of solar radiation (Banik and Basumallick 2017). Figure 19.3 summarizes the mechanisms catalysed by nanoparticles in degradation of toxic pollutants.

19.6 Remediation of Toxic Metals Using Nanoparticles

Heavy metals constitute a group of metals and metalloids that have atomic density higher than 4000 kg/m^3 (Edelstein and Ben-Hur 2018). Although plants need various heavy metals such as molybdenum, zinc, iron, etc. for basic metabolic activities, at higher concentrations, these metals are harmful to the plant (Vardhan et al. 2019). Heavy metals are toxic to humans even at low concentrations. Due to urbanization, population explosion, and rapid industrialization, soil and water resources end up as heavy metal sinks. Bioremediation measures thus play an important role in restoring balance within these contaminated environments, failure of which would lead to bioaccumulation and biomagnification of these toxic metals at the cellular level.

Nanomaterials such as bimetallic nanoparticles, metal oxides, nanoscale zeolites, and carbon nanotubes have been considered for metal remediation. Iron oxide nanoparticles have been extensively studied in metal detoxification owing to their ability to be separated easily from the reaction media and due to their low toxicity. Immobilization of accumulated heavy metals in soil plays a crucial role in their transformation and detoxification. Nanoparticles such as nano-alginate, bentonite-nZVI, zerovalent iron nanoparticles, nanocarbon, and dendrimers have been used to immobilize the heavy metals in soil (Helal et al. 2016).

Nano-hydroxyapatite, a phosphate-based nanomaterial has demonstrated significant results in clean-up of Pb contaminated soils. It has also been reported to immobilize other heavy metals such as Cr, Zn, Cd, and Cu. Studies on both graphene oxide nanoparticles and nZVI nanoparticles have been reported in relation to increasing the bioavailability of arsenic and other metals in polluted soils (Baragaño et al. 2020). Similar studies using graphene as the adsorbent showed removal of arsenic by 80% from wastewater. The advantages of this nanomaterial are its ability to be recycled and reused. A nanocomposite with reduced graphene oxide and Fe_3O_4 has been studied for the removal of Pb^{2+} ions from water by co-precipitation and has been found to be efficient. Other graphene-based nanomaterials have been successfully used for the removal of heavy metals such as Cu (II), Fe (II), Mn (II), and Pb (II). Activated carbon nanomaterial has been successfully applied in the removal of heavy metals such as Zn^{+2} and Al^{+3} ions and has demonstrated an efficiency up to 92%. In wastewater treatment, it showed an absorption capacity of up to 50% for Cu^{+2} ions. Other activated carbon nanomaterials have been successfully employed in remediation of heavy metals such as Pb^{+2}, Cd^{+2}, Cu^{+2}, Ni^{+2}, and Zn^{+2} (Baby et al. 2019). The role of various environmental factors affecting the remediation of heavy metals by iron-based nanoparticles has been studied. This study also focused on the toxicity of these nanoparticles on the living organisms (Latif et al. 2020). Hydrous iron oxide-based nanomaterials have been found to remove heavy metals like Pb (II), Cu (II), Cd (II), and Ni (II). However, they have been found to be most effective in removal of arsenic (V) from drinking water. Nanomaterials based on manganese oxides have been used for the treatment of metals such as Tl (I), U, Pb (II), Zn (II), Hg (II), Cd (II), and Cu (II) and have now been considered promising in removal of heavy metals from polluted water. Polyvinylpyrrolidone-coated magnetic nanoparticles (PVP-Fe_3O_4 nanoparticles) have shown significant efficiency (100%) in the removal of Cd, Cr, Ni, and Pb. Hexavalent chromium (VI) is a prevalent inorganic pollutant that is known to be toxic, mutagenic, and carcinogenic (Valko et al. 2005). Remediation of Cr (VI) involves its reduction to trivalent Cr (III), a less toxic, non-carcinogenic form that can be precipitated into hydroxides. This reaction serves as a key step for the removal of Cr (VI) from aqueous solutions (Mabbett et al. 2004). Iron nanoparticles such as ferromagnetic carbon-coated nanoparticles have been used to remove 95% of Cr (VI) from aqueous solutions. Table 19.2 shows the use of nanoparticles in heavy metals remediation.

19.7 Remediation of Hydrocarbons Using Nanoparticles

Hydrocarbon pollution refers to the contamination of the environment by organic polycyclic aromatic hydrocarbons (PAH) or crude oil (petroleum hydrocarbons) compounds. Oil pollution has severe environmental and ecological consequences. Crude oil and its derivatives are considered as some of the most pervasive environmental pollutants. The leakage of crude oils into aquatic systems results in wide-ranging contamination. Oil sludge is a thick complex emulsion made up of various petroleum hydrocarbons, heavy metals, sediments, and water. It is composed of alkanes, aromatics, resin, and asphaltenes and contains volatile and semi-volatile organic compounds, which are reported to be genotoxic (Srivastava et al. 2019). Unintentional and deliberate spillage of oil into the environment leads to transfer of toxic organic materials into the food chain.

Due to their unique physicochemical properties, nanoparticles have gained tremendous interest in oil biodegradation. Nanoparticles can decrease the hydrophobicity of these hydrocarbon compounds and thus increase their bioavailability for bioremediation. Graphene-doped TiO_2 nanoparticles have demonstrated adsorption and photodegradation of phenanthrene, fluoranthene, and benzopyrene to phthalic and benzoic acid; oxygenated and hydroxylated PAHs; acyclic hydrocarbons and alcohols (Bai et al. 2017). Similarly, iron hexacyanoferrate (FeHCF) nanoparticles have been employed in degradation of anthracene, chrysene, and fluorine. It was also established that aromatic hydrocarbon adsorption by the FeHCF followed a molecular weight dependent trend: anthracene > phenanthrene > fluorene > chrysene > benzopyrene (Shanker et al. 2017). Bimetallic magnetic Pt/Pd nanoparticles supported on magnetic silica reduced anthracene to non-toxic partially hydrogenated species. Furthermore, compared to monometallic nanoparticles, the bimetallic particles displayed enhanced activity post recycling (Zanato et al. 2017). Nanoparticles are efficient in degrading organic compounds such as benzophenone and phenol. Benzophenone-3 is an ultraviolet absorber, and thus they can be degraded using catalytic ozonation. Manganese oxide and cobalt oxide nanoparticles immobilized on ceramic membrane displayed remarkable

Table 19.2 Nanoparticles used in remediation of heavy metals

Nanoparticles used in remediation of heavy metals		
Pollutant	Nanoparticle	References
Copper (Cu)	Amino-functionalized magnetic nanoparticles	Hao et al. 2010
	Polydopamine nanoparticles	Farnad et al. 2012
	Maghemite nanoparticles	Rajput et al. 2017
	nZVI	Boente et al. 2018
	Graphene oxide–manganese oxide nanoparticles composite	Wan et al. 2018
Chromium (VI) (Cr)	Chitosan-nZVI (nanoscale zerovalent iron) nanoparticles	Geng et al. 2009
	Magnetite–maghemite nanoparticles	Chowdhury and Yanful 2010
	Cerium oxide nanoparticles	Contreras et al. 2015
	CTAB modified magnetic nanoparticles	Elfeky et al. 2017
	Magnetic alkaline– Lignin−dopamine nanoparticles	Dai et al. 2019
Arsenic (As)	Magnetite–maghemite nanoparticles	Chowdhury and Yanful 2010
	Iron oxide nanoparticles	Shipley et al. 2011
	Cupric oxide nanoparticles	Reddy et al. 2013
	γ-Aluminium oxide nanoparticles	Ghosh et al. 2019
	Magnetic iron-titanium binary oxide composite	Deng et al. 2019
Cadmium (Cd)	Cerium oxide nanoparticles	Contreras et al. 2015
	Iron phosphate (vivianite) nanoparticles	Qiao et al. 2017
	EDTA functionalized iron oxide nanoparticles	Huang and Keller 2015
	Silica-coated iron oxide nanoparticles	Ghafoor and Ata 2017
	Graphene oxide–manganese oxide nanoparticles composite	Wan et al. 2018
Lead (Pb)	Iron phosphate (vivianite) nanoparticles	Liu and Zhao 2007
	Cerium oxide nanoparticles	Contreras et al. 2015
	EDTA functionalized iron oxide nanoparticles	Huang and Keller 2015
	L-cysteine stabilized nZVI nanoparticles	Bagbi et al. 2017
	Maghemite nanoparticles	Rajput et al. 2017
Mercury (Hg)	Carboxymethyl cellulose stabilized iron sulphide nanoparticles	Gong et al. 2012
	Citrate-coated gold nanoparticles	Ojea-Jiménez et al. 2012
	Silica-coated magnetite nanoparticles	Mohmood et al. 2016
	Inorganic magnetic sulphide nanoparticles	Patel et al. 2018

catalytic ozonation of benzophenone-3 (Guo et al. 2016). Reduced graphene oxide silver nanoparticles (rGO-Ag) were demonstrated to successfully breakdown phenol (Bhunia and Jana 2014). ZnO and magnetite-zinc oxide (Fe_3O_4-ZnO) hybrid nanoparticles have been studied in the degradation of phenols and the hybrid nanoparticles were found to be more efficient (Feng et al. 2014). UV-irradiated semiconductor catalysts such as titanium dioxide have been used in photodegradation of organic contaminants (Banik and Basumallick 2017). Calcium peroxide nanoparticles have been reported to exhibit remarkable degradation of benzene and toluene (Mosmeri et al. 2017; Qian et al. 2013). Removal of benzene from aqueous solutions has also been carried out using magnetite nanoparticles. The nano-magnetic particles have been reported to decompose 98.7% and 94.5% of the benzene in batch and continuous conditions, respectively. Furthermore, these particles can easily desorb benzene and be reused for its removal (Amin et al. 2013). Magnetite nanoparticles catalysed decomposition of p-nitrophenol to aromatic intermediates such as benzoquinone and hydroquinone, which were further oxidized by hydrogen peroxide. The nanoparticles exhibited the ability to be magnetically separated from the sludge and reused (Sun and Lemley 2011). Magnesium doped magnetite nanoparticles have been reported to effectively decompose hexachlorobenzene, a precursor of carcinogenic and mutagenic compounds (Su et al. 2014).

The remediation of petroleum-based hydrocarbons using nanoparticle-stabilized surfactants has been explored (Ali et al. 2020). Iron nanoparticles produced biogenically from *Vaccinium floribundum* showed a significant removal of up to 86% and 88% in the total petroleum hydrocarbons from polluted soil and water, respectively (Murgueitio et al. 2018). Raw and distillate forms of petroleum oil were treated with nanocarbon scavengers which efficiently sequestered these hydrocarbons by 80% and 91%, respectively (Daza et al. 2017). Polyvinylpyrrolidone (PVP) coated magnetite nanoparticles (NP) have demonstrated remediation of lower chain alkanes by 70% and higher chain alkanes by 65% after incubation of 1 h. However, the efficiency of degradation of these alkanes increased significantly on incubation of the nanoparticles with oil degrading bacteria (Alabresm et al. 2018). Table 19.3 enlists integrated systems studied for hydrocarbon degradation. Other studies have shown significant results in degradation of hydrocarbons using nanobioremediation, which has been discussed further in this chapter.

19.8 Remediation of Hormones, Antibiotics, and Medicinal Drugs Using Nanoparticles

Antibiotics and hormones are emerging pollutants. Modern healthcare and their extensive use in farming results in their release in effluent and wash waters leading to contamination of groundwater and soil. Their recalcitrant nature makes them persist in the soil and water and lead to serious effects on the environment and human health.

Table 19.3 Nanoparticles used in remediation of hydrocarbon pollutants

Nanoparticles used in remediation of hydrocarbons		
Pollutant	Nanoparticle	References
Polycyclic aromatic hydrocarbons (PAHs)		
Pyrene	Haematite nanoparticles	Jorfi et al. 2016
Phenanthrene, fluoranthene, benzopyrene	Titanium dioxide nanoparticles	Bai et al. 2017
Anthracene, phenanthrene, chrysene, fluorene, and benzopyrene	Iron hexacyanoferrate nanoparticles	Shanker et al. 2017
Anthracene	Bimetallic magnetic Pt/Pd nanoparticles	Zanato et al. 2017
Benzophenone-3	Manganese oxide and cobalt oxide nanoparticles coated on ceramic membrane	Guo et al. 2016
Phenol	Magnetite and zinc oxide hybrid nanoparticles Reduced graphene oxide silver nanoparticles (rGO-Ag)	Feng et al. 2014; Bhunia and Jana 2014
Petroleum-based hydrocarbons		
Total petroleum hydrocarbons	Iron nanoparticles	Murgueitio et al. 2018
Raw and distillate forms of petroleum oil	Nanocarbon nanoparticles	Daza et al. 2017
Alkanes	Polyvinylpyrrolidone (PVP) coated magnetite nanoparticles	Alabresm et al. 2018

19.8.1 Remediation of Antibiotics

Nanoparticles have been reported to clean up even low concentrations of these compounds. Many nanoparticles like zerovalent iron nanoparticles have exhibited the ability to remove antibiotics such as sulfacetamide from the aqueous solution by 61% (Table 19.4). Bimetallic $CuFe20_4$ nanoparticles have been efficiently used for the removal of amoxicillin from aqueous solutions. Photocatalytic degradation of ciprofloxacin has also been reported using graphene oxide/nanocellulose composite. Similar degradation studies have also been reported on ciprofloxacin using zerovalent iron nanoparticles. Tetracycline degradation was successfully carried out by nanoscale zero-valent iron (nZVI) and Fe/Ni bimetallic nanoparticles in aqueous solution. Although Fe/Ni nanoparticles performed better that nZVI, the removal efficiency of both the particles was reported to decrease with increase in pH, thus asserting that the degradation process is pH-dependent (Dong et al. 2018b). UiO-66, a zirconium based nanoparticle doped with cobalt was reported to adsorb and degrade tetracycline via photocatalysis. Factors affecting the adsorption process included dosage of the nanoparticles, coexisting ions, pH, and initial concentration of the antibiotic (Cao et al. 2018). The Oxone process has been utilized to degrade organic compounds. In this process, Oxone [peroxymonosulfate (PMS)] is

Table 19.4 Nanoparticles used in remediation of antibiotics, medicinal drugs, and hormones

Nanoparticles used in remediation of antibiotics, medicinal drugs, and hormones		
Pollutant	Nanoparticle	References
Antibiotics		
Amoxicillin	Copper-iron bimetallic nanoparticles	Malakootian et al. 2019
Ceftriaxone	Fe_3O_4 nanoparticles	Malakootian et al. 2019
Sulfacetamide	Zerovalent iron nanoparticles	Malakootian et al. 2019
Tetracycline	Fe/Ni bimetallic nanoparticles Cobalt-doped UiO-66 nanoparticles Martite nanoparticles Cu/Fe bimetallic particle ZnS nanoparticles Titanium oxide nanocomposite	Dong et al. 2018b; Cao et al. 2018; Soltani et al. 2018; Malakootian et al. 2019
Ciprofloxacin	Zinc oxide nanoparticles Fe-doped zinc oxide nanoparticles Titanium oxide nanoparticles Cupric oxide nanoparticles Graphene oxide/ nanocellulose composite Zerovalent iron nanoparticles	Gharaghani and Malakootian 2017; Das et al. 2018; Malakootian et al. 2020
Levofloxacin	Zn oxide nanoparticles, Graphene oxide sheets	El-Maraghy et al. 2020
Hormones		
Estrone	Polystyrene nanoparticles Titanium dioxide nanoparticles	Akanyeti et al. 2017; Czech and Rubinowska 2013
17-ß-Estradiol	Manganese oxide nanoparticles Magnetic biochar nanoparticles	Han et al. 2014; Dong et al. 2018a
Medicinal drugs		
Paracetamol	Activated carbon nanoparticles	Baby et al. 2019
Carbamazepine	Haematite nanoparticles Magnetite nanoparticles	Rajendran and Sen 2018; Liu et al. 2018
Ibuprofen	Nanoscale zerovalent iron (nZVI)	Machado et al. 2013

combined with a source of transition metals to generation sulphate ions, which catalyse the redox process. Martite (α-Fe_2O_3) nanoparticles have been found to activate the Oxone process to degrade the recalcitrant antibiotic (Soltani et al. 2018).

The degradation of ciprofloxacin, another commonly used antibiotic, was proved both spectrophotometrically and microbiologically by the loss of antibiotic activity, using Fe-doped-ZnO nanoparticles (Das et al. 2018). Immobilized nanoparticles allow easy placement and removal of aqueous contaminants. Moreover, it resolves complications related to separation of catalysts from the solution, as well as allows reusability of nanoparticles. ZnO immobilized on a glass plate exhibited nano-photocatalysis of ciprofloxacin with high removal efficiency (Gharaghani and Malakootian 2017). Similarly, TiO_2 nanoparticles immobilized on a glass plate were shown to adsorb and photodegrade ciprofloxacin and also resulted in decreased sludge production (Malakootian et al. 2019). Other antibiotics such as ampicillin, amoxicillin, and penicillin were reduced by bentonite-supported Fe/Ni nanoparticles (Weng et al. 2018).

19.8.2 Remediation of Medicinal Drugs

Medicinal drugs such as paracetamol, carbamazepine, and ibuprofen not just pollute water bodies, but pose a significant threat to aquatic communities. As in case of other organic compounds, a variety of nanoparticles have been researched for their applicability in degrading these recalcitrant compounds. Ibuprofen reduction can be carried out by nZVI nanoparticles. While effective on lab scale, simulation of the nanoremediation with ibuprofen contaminated soil concluded that the degradation process is slower in aqueous systems, but without any significant impact on contaminant degradation efficiency (Machado et al. 2013). Haematite nanoparticles were demonstrated to be effective adsorbents of carbamazepine and thus offer a potential remediation method for the adsorptive elimination of drugs from contaminated aquatic systems (Table 19.4) (Rajendran and Sen 2018). Magnetite nanoparticles were also confirmed to effectively remove carbamazepine (Liu et al. 2018). Nanoparticles have also been proved effective in reducing chlorhexidine, a commonly used antiseptic component. Photocatalytic degradation of chlorhexidine was successfully concluded using titanium dioxide nanoparticles as photocatalyst (Das et al. 2014). Activated carbon nanoparticles have been reportedly used for the bioremediation of methylene and paracetamol from hospital wastewaters (Baby et al. 2019).

19.8.3 Remediation of Hormones

Naturally synthesized and synthetic hormones are one of the most potent trace contaminants released into soil and water bodies. Apart from excretion and disposal of hormones, application of animal faeces as manure to fields allows transmission of endogenous hormones into soil and ultimately in water bodies. These hormones and endocrine disruptors are known to affect the reproductive health of various animals by disrupting sexual function and promoting abnormal sexual development. As with other organic compounds, nZVI, under oxidative conditions, was found to adsorb and degrade 17β-estradiol (Table 19.4) (a popular endocrine disrupting chemical) and 17α-ethinylestradiol (a synthetic birth control drug) into less bioactive estrone (Jarosova et al. 2015). Biochar, a pyrogenic black carbon product of biomass pyrolysis can be used to derive nanosized biochar particles due to their nanoporous structure, good stability, multiple functional groups, and easy availability. Magnetic biochar nanoparticles (Mag-BCNPs) are a composite of nanosized biochar nanoparticles and magnetic iron nanoparticles (Fe_3O_4). This composite was demonstrated to effectively reduce 17β-estradiol. Post remediation, the Mag-BCNPs can be recovered using a magnet, and the biochar can be desorbed by purging with ozone, thus allowing easy regeneration of the nanoparticles (Dong et al. 2018a). Carboxymethyl cellulose (CMC) stabilized manganese oxide nanoparticles have also been studied for their 17β-estradiol degrading activity (Han et al. 2014). Estrones can be removed from wastewater using TiO_2-assisted photocatalysis (Czech and Rubinowska 2013). A hybrid polystyrene nanoparticle-ultrafiltration system with renewable nanoparticles acts as a lucrative solution to filtering estrone from contaminated water. However, due to the higher permeability of the filtration system compared to that of nanofiltration/reverse osmosis systems, estrone removal capacity of 40% was achieved. However, replacing the filtration system with a finer one can increase success of the designed system (Akanyeti et al. 2017). Table 19.4 shows the use of nanoparticles in remediation of antibiotics, medicinal drugs, and hormones.

19.9 Remediation of Dyes and Organic Solvents Using Nanoparticles

With the onset of industrialization, the synthesis and utilization of various organic compounds have increased exponentially. These organic compounds include dyes which are released into the environment through anthropogenic activities. These pollutants are known to have neurotoxic, mutagenic, and carcinogenic effects on biological systems (Das and Chandran 2011). Various studies using nanoparticles have been carried out on degradation of the dyes. Nanomaterials such as reduced graphene oxide, (rGO)-TiO_2 has been successfully applied for the bioremediation of Alizarin Red S. Other studies have also reported the use of graphene oxide nanomaterials in association with TiO_2 or ZnO in photodegradation of methylene blue. Bimetallic nanoparticles of iron (Fe) and nickel (Ni) showed significant results in cleaning up of the azo dye Orange G from wastewater (Foster et al. 2019). Similarly, remarkable efficiency was exhibited when absorbent made of ZnO nanoparticle was used to remove azo dyes, amaranth, and methyl orange from aqueous solution (Zafar et al. 2019). Tara and others (Tara et al. 2020) have summarized the nanoparticles used in the removal of Azo dyes and the efficiency of removal of these

Table 19.5 Nanoparticles used in remediation of dyes and organic solvents

Nanoparticles used in remediation of dyes, and organic solvents		
Pollutant	Nanoparticle	References
Dyes		
Dye containing effluents	Nickel nanoparticles	Kale and Kane 2017
Azo dyes	ZVI nanoparticles Cerium loaded copper oxide Iron nanoparticle	Tara et al. 2020
Methylene blue, Congo red	Copper oxide nanoparticles Copper oxide nanoneedles Graphene oxide sheets	Tara et al. 2020
Cationic and anionic dyes	Nickel doped zinc oxide nanoparticle	Tara et al. 2020
Coomassie brilliant blue, Amido black 10B	Copper oxide nanoneedles Graphene oxide sheets	Tara et al. 2020
Malachite green, methyl violet	Magnetic NPs coated with poly (dimethylaminoethyl acrylamide) and methyl acrylate on modified with Fe_3O_4 nanoparticles	Tara et al. 2020
Methylene blue	Copper oxide nanoparticles activated with montmorillonite clay	Tara et al. 2020
Methylene blue, methyl orange, rhodamine B, organic dyes	Silver nanocomposite, Silver nanoparticles	Tara et al. 2020; Bhakya et al. 2015
Amaranth and methyl orange	ZnO nanoparticles	Zafar et al. 2019
Orange G	Fe and nickel nanoparticles	Foster et al. 2019
Organic solvents		
TCE	Zerovalent iron nanoparticles	Ibrahem et al. 2012
p-Nitrophenol	Magnetite nanoparticles	Sun and Lemley 2011
Hexachlorobenzene	Magnetic micro/nano Fe_xO_y-CeO_2 composite Mg-doped iron oxide nanoparticles	Jia et al. 2010; Su et al. 2014
Benzene	Magnetite nanoparticles Calcium peroxide nanoparticles	Amin et al. 2013; Mosmeri et al. 2017
Toluene	Calcium peroxide nanoparticles Tungsten doped titanium dioxide nanoparticles	Qian et al. 2013; Poorkarimi et al. 2017

dyes by the respective nanoparticles. Table 19.5 summarizes different nanoparticles that are involved in remediation of dyes and organic solvents.

Nanoscale iron nanoparticles have shown significant efficiency in removal of chlorinated organic solvents contaminating groundwater (Zhang and Elliott 2006). Zerovalent iron nanoparticles have been used for the remediation of trichloroethylene (TCE). It was considered an efficient reducing catalyst and resulted in fast degradation of TCE from contaminated soils (Ibrahem et al. 2012).

19.10 Remediation of Agro-Based Compounds Using Nanoparticles

Agro-based compounds such as herbicides, fungicides, pesticides, weedicides are extensively used in agriculture. These compounds leach and contaminate the surrounding soil and water bodies. Due to the complex chemical structure of these compounds they are recalcitrant in nature and tend to persist in the environment for a long time. Nanoparticles have been found to be efficient in removal of pesticides from contaminated sites by adsorption and photodegradation of these compounds (Table 19.6). Nanomaterials such as zinc oxide, titanium dioxide, and silver have been reported to photocatalyse the degradation of pesticides. Metal nanocomposites and polymer matrix nanocomposites have been successfully used in the removal of organic and inorganic pollutants. Iron nanoparticles have been successfully used in remediation of carbamate pesticides such as methomyl. Fe_3O_4 nanoparticles have

Table 19.6 Nanoparticles used in remediation of agro-based compounds

Nanoparticles used in remediation of agro-based compounds		
Pollutant	Nanoparticle	References
Aldrin	Fe_3O_4 nanoparticles	Sahithya and Das 2015
Alachlor	Nanoscale zerovalent iron (nZVI)	Bezbaruah et al. 2009
Eldrin	Fe_3O_4 nanoparticles	Sahithya and Das 2015
Lindane	Fe_3O_4 nanoparticles, silver nanoparticles	Sahithya and Das 2015
	Nanoscale zerovalent iron (nZVI)	Elliott et al. 2009
Organophosphate pesticides	TiO_2, ZnO, Au-TiO_2, CuO nanoparticles	Sahithya and Das 2015
Atrazine	Nanoscale zerovalent iron (nZVI)	Bezbaruah et al. 2009
Chlorpyrifos	Silver and gold nanoparticles	Bootharaju and Pradeep 2012
DDT	Mixed metal oxides nanoparticles	Manav et al. 2018
Profenofos (organophosphorous pesticide)	Fe/Ni bimetallic nanoparticles	Mansouriieh and Khosravi 2015
Malathion	Iron oxide nanoparticles and iron oxide/ gold nanoparticles Magnetite nanoparticles and gold coated magnetic nanoparticles	Fouad et al. 2015
Acetamiprid	Fe/Ni bimetallic nanoparticles	Marcelo et al. 2016

been applied for their efficiency in clean-up of organochlorine pesticides such as aldrin, endrin, and lindane. Silver nanoparticles have also been efficient in remediation of pesticide lindane. Organophosphate pesticides such as monocrotophos, malathion, chlorpyrifos, dichlorvos have been photo-catalytically degraded using nanoparticles such as titanium dioxide, zinc oxide, gold-titanium dioxide, and copper oxide, respectively.

Gold-titanium dioxide (Au-TiO_2) nanoparticles have been reported to photocatalyse the degradation of chloridazon (Sahithya and Das 2015). Rapid anoxic degradation of pesticide alachlor (2-Chloro-2′,6′-diethyl-*N*-(methoxymethyl)-acetanilide) has been reported using nZVI. The reaction resulted in dechlorinated alachlor, which was significantly more biodegradable in comparison to its parent compound (Bezbaruah et al. 2009) (Table 19.6). The nZVI nanoparticles have been documented to degrade lindane (γ-hexachlorocyclohexane), a commonly used organochlorine pesticide, into benzene, diphenyl, and di/tetra/penta forms of the compound (Elliott et al. 2009). Silver and gold nanoparticles have exhibited remarkable ability to decompose chlorpyrifos, an organophosphorothioate pesticide, to 3,5,6-trichloro-2-pyridinol and diethyl thiophosphate at room temperature. These nanoparticles have also demonstrated an ability to be regenerated and reused (Bootharaju and Pradeep 2012). Catalytic degradation of Profenofos (an organophosphorous pesticide) and acetamiprid (a neonicotinoid insecticide) using Fe/Ni bimetallic nanoparticles has been reported (Marcelo et al. 2016; Mansouriieh and Khosravi 2015). $MgCeO_3$ and $MgFe_2O_4$ are mixed metal oxides that have been studied for degradation of DDT (dichlorodiphenyltrichloroethane), a well-known harmful organochlorine insecticide. Both mixed oxides degraded DDT to less toxic compounds DDD (dichlorodiphenyldichloroethane), DDE (dichlorodiphenyldichloroethylene), and simpler compounds within the first reaction hour (Manav et al. 2018). Zerovalent metal nanoparticles, semiconductor nanoparticles, and some bimetallic nanoparticles have been documented to treat environmental pollutants such as azo dyes, pesticides (chlorpyrifos and organochlorines), nitroaromatics, hormones, and antibiotics (Foster et al. 2019).

19.11 Remediation of Organohalide Compounds Using Nanoparticles

Organohalide compounds such as polybrominated diphenyl ethers (PBDEs), dioxins, polychlorinated biphenyls (PCBs), and chlorinated aliphatic hydrocarbons (CAH) are omnipresent, highly toxic fat-soluble compounds. Being insoluble in water, their low bioavailability hinders biodegradation by aquatic microbes. PCBs are toxic organohalide compounds classified as persistent organic pollutants. Various nanoparticles have been studied for their remediation. Pd/Fe bimetallic nanoparticles and nZVI have been reported to catalyse hydro-dechlorination of hexachlorobiphenyl (Chen et al. 2014). Carbon-modified titanium dioxide (CM-*n*-TiO_2) nanoparticles were reportedly used in photocatalytic degradation of PCB mixtures Aroclor 1254 and Aroclor 1260 (Shaban et al. 2016). Surfactants enhance

Table 19.7 Nanoparticles used in remediation of organohalide compounds

Polychlorinated biphenyls (PCBs)		
Pollutant	Nanoparticle	References
2-chlorobiphenyl	Palladium–aluminium (Pd/Al) bimetallic nanoparticles	Yang et al. 2013
Penta, hexa, hepta, and octobiphenyls	Nanoscale zerovalent iron (nZVI)	Gomes et al. 2014
2,2′,4,4′,5,5′-hexachlorobiphenyl	Nanoscale zerovalent iron (nZVI)	Chen et al. 2014
Aroclor 1254 and Aroclor 1260	Carbon-modified titanium dioxide (CM-*n*-TiO_2) nanoparticles	Shaban et al. 2016

solubilization of hydrophobic organic compounds, hence their integration with nanoparticles may lead to increase in bioremediation efficiency. In a combined electro-remediation set-up consisting of electrodialysis, non-ionic detergents (Saponin and Tween-80), and nZVI nanoparticles, degradation of up to 76% PCBs was reportedly achieved within 5 days. However, in a set-up with just surfactant and nanoparticles, the remediation efficiency was low, thus supporting the assumption that the surfactant will not always enhance nanoparticle activity (Gomes et al. 2014). Similar observations were made pertaining to remediation of 4-chlorophenol and 2-chlorobiphenyl using palladium-aluminium (Pd/Al) bimetallic nanoparticles (Table 19.7). While the nanoparticles by themselves were capable of degrading the PCBs, addition of ionic and non-ionic surfactants led to decrease in hydrodechlorination of the organic compounds (Yang et al. 2013). Polychlorinated aliphatic compounds such as trichloroethylene can be remediated using nanoparticles. Polymethyl methacrylate coated nZVI have also been reported capable in reducing trichloroethylene (Wang and Chiu 2009). Bimetallic Ni/Fe nanoparticles immobilized in hydrophilic nylon-66 membrane have been reported to completely dechlorinate trichloroethylene within 25 min. The immobilization of nanoparticles improved the reactivity and longevity of the nanoparticles (Parshetti and Doong 2009).

19.12 Nanobioremediation Using Integrated Nano-Bio Systems

Microbial bioremediation or nanoremediation, by itself offers multiple advantages over conventional treatment methods, but also has multiple noteworthy limitations. Microbial degradation of toxic compounds is a time-consuming process that can take over a year. Its effectiveness varies with conditions such as pH, nutrients, temperature, and other environmental conditions (Abatenh et al. 2017; Kumar et al. 2018). Integration of nanotechnology with microbial bioremediation can overcome these limitations and provide a better alternative to pollutant remediation. The reaction of the toxic pollutant with nanoparticles transforms the pollutant into compounds more easily metabolized by microorganisms. Therefore, nanobioremediation is a promising alternative in remediation technologies involving detoxification of toxic pollutants.

Nanobioremediation can be described as a combination of nanotechnology and bioremediation systems, wherein the former enhances the effectiveness of the latter. Therefore, nanobioremediation offers the benefits of both nanotechnology and bioremediation. The application of nanobioremediation can be done using two approaches. The sequential approach involves subjecting the contaminant to nanoparticles followed by exposure to the bioagent. The nanoparticles break down the contaminants to simpler compounds, allowing the bioagent to metabolize it further. In the concurrent or combined approach, the nanoparticle and biological agent is injected into the system simultaneously (Tratnyek and Johnson 2006; Karn et al. 2009). While both these methods seem to be practical alternatives to conventional technologies, more research and development is needed for their execution on a larger scale.

The most significant advantage of using nanoparticles in remediation is their application in both in situ and ex situ conditions. In ex situ remediation, the contaminated soil is excavated, while the contaminated groundwater is extracted and treated with nanoparticles on-site or off-site. In in situ treatment methods, nanoparticles are directly injected at the site of contamination where it degrades the contaminant. Compared to conventional methods, nanobioremediation is best suited for in situ treatment of contaminated soil and groundwater as the exceptional properties and colloidal nature of nanoparticles allow deep penetration up to the water table, a feat that is not possible to achieve with ex situ methods. Furthermore, nanoparticles enhance the reaction rates of microorganisms, thus allowing better degradation of pollutants (Karn et al. 2009). The ability of nanoparticles to degrade pollutants in association with biological systems presents opportunity for development of more efficient nano-bio integrated technologies. Apart from treatment and remediation of contaminated sites, nanoparticles can be applied to pollution sensing and pollution prevention.

19.13 Nanobioremediation of Toxic Heavy Metals

Integrated systems have great scope in metal bioremediation. Nanoparticles in combination with microorganisms have been used successfully to remediate heavy metals. Nanoscale zerovalent iron (nZVI) is smaller and more reactive than microscale zerovalent iron (ZVI) and therefore offers elevated reactive surface area, higher reactivity, and better injectability into aquifers, thus making them valuable in bioremediation of polluted sites (Li et al. 2006). However, due to their tendency to agglomerate and form larger particles, the reactivity of the nanoparticles decreases. Thus, direct application of free nZVI in remediation of contaminated soil and water could lead to overall decrease in efficiency of nanoparticles and leaching of iron into aquatic bodies. The immobilization of nZVI onto biodegradable, porous materials (beads) helps to overcome agglomeration. The toxic pollutant diffuses into beads and comes in contact with the entrapped nanoparticles. Similar studies on

Table 19.8 Nano-Bio integrated systems for remediation of Chromium (VI)

Integrated systems for remediation of toxic Chromium (VI)			
Metal pollutant	Nanoparticle	Bioagent	References
Bacteria			
Chromium (VI)	CNTs	*Shewanella oneidensis* MR-1	Yan et al. 2013
	CNTs	*Pseudomonas aeruginosa*	Pang et al. 2011
	Bio-Pd	*Clostridium pasteurianum* BC1	Chidambaram et al. 2010
Consortium			
Chromium (VI)	nZVI	Whey microbiota	Nemecek et al. 2016
Chromium (VI)	nZVI	Consortia of *Bacillus subtilis*, *Escherichia coli,* and *Acinetobacter junii*	Ravikumar et al. 2016

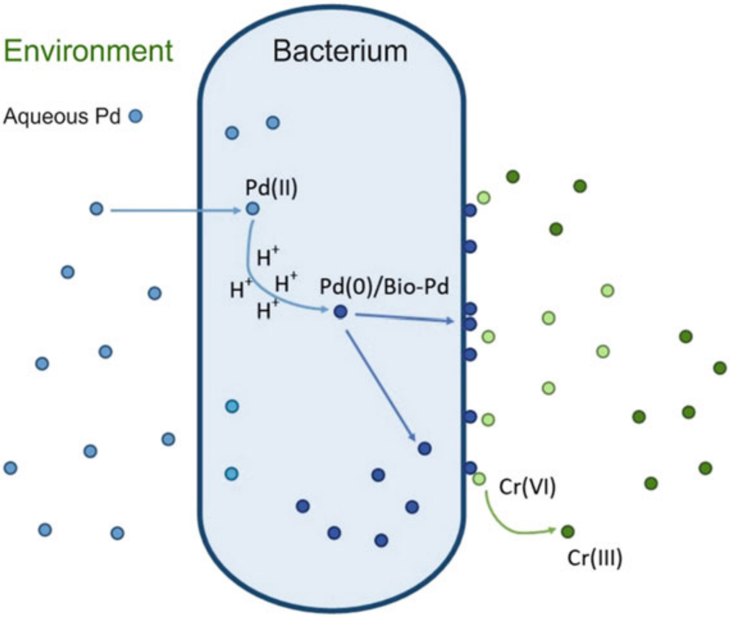

Fig. 19.4 Integrated Bio-Pd system in chromium remediation. The anaerobic hydrogen-producing bacterium accumulates aqueous palladium and reduces it to Bio-Pd, which further reduces toxic chromium (VI) to its less toxic form, Cr (III)

immobilization of nZVI on calcium alginate (nZVI−C − A beads), in presence of microbial biofilm has been shown to be effective in reducing Cr (VI) to Cr (III) (Ravikumar et al. 2016). Table 19.8 depicts various studies carried out using integrated nano-bio systems for remediation of chromium (VI).

The Bio-Pd (bio-reduced-Pd) integrated system is an innovative method for in situ remediation of toxic metals (Fig. 19.4). Bio-Pd generation is a novel system wherein bacterium, in the presence of hydrogen, reduces and precipitates accumulated palladium, Pd (II) into Pd nanoparticles (Pd (0)). The two-step remediation system involves synthesis of Pd nanoparticles (bio-Pd) followed by reaction with the contaminant. Although this two-step process is suitable for ex-situ remediation, hydrogen supply acts as a technical and commercial challenge. *Clostridium pasteurianum* BC1, an anaerobic hydrogen producer, allows coupling of in situ production of H_2 with in situ synthesis of nanoparticles, which further catalyses the degradation of contaminants. The bio-Pd synthesized by *Clostridium pasteurianum* BC1 successfully demonstrated the reduction of Cr (VI) to Cr (III) (Chidambaram et al. 2010).

Nanomaterials have been used to augment the metal-reduction efficiency of microorganisms. Compared to suspended cells, immobilized microorganisms allow continuous and stable bioreduction of Cr (VI). However, the alginate used for immobilization can lower the reduction efficiency due to its effect on electron and mass transport. Impregnation of organic nanomaterial such as carbon nanotubes (CNTs) has proved to enhance the mechanical strength and bioreduction capacity. The immobilization of *Shewanella oneidensis* MR-1 in alginate/CNT beads was shown to yield a higher reduction efficiency compared to the controls, possibly due to accelerated electron transfer (Yan et al. 2013). CNT-modified polyvinyl alcohol and sodium alginate matrix using immobilized *Pseudomonas aeruginosa* also enhanced bioreduction efficiency of the microorganism (Pang et al. 2011). Additionally, these modified beads can be reused effectively multiple times. This technique of CNTs impregnated immobilization can be applied to various other biological reduction processes and can be tested on various other pollutants.

19.14 Nanobioremediation of Polycyclic Aromatic Hydrocarbons (PAHs)

Hydrocarbons and organic pollutants are persistent in nature. The chances of degrading these compounds increase on application of nano-bio integrated systems. Hydrophobic polycyclic aromatic compounds (PAH) such as carbazole and indenopyrene are toxic and carcinogenic environmental pollutants. *Sphingomonas* sp. XLDN2–5 cells magnetically immobilized in Fe_3O_4 nanoparticle containing gellan gum matrix were proved to be effective in degrading carbazole. The addition of Fe_3O_4 nanoparticles to immobilized bacterial cells significantly enhanced its biodegradation activity (Wang et al. 2007). Yeast strains of *Candida tropicalis* NN4 have been reported to enhance indenopyrene biodegradation activity of zerovalent iron nanoparticles by producing glycolipid biosurfactant (Ojha et al. 2019). *Rhodococcus erythropolis* IGTS8 combined with magnetite (Fe_3O_4) nanoparticles have been found to effectively desulphurize dibenzothiophene (DBT), a recalcitrant organo-sulphur compound released on combustion of fossil fuels, to 2-hydroxybiphenyl (2-HBP). The nanoparticles have been reported to enhance cell growth rate, in comparison with the controls containing either the bacteria or the nanoparticles (Karimi et al. 2017). Immobilized *Pseudomonas*

Table 19.9 Integrated nano-bio systems for remediation of polycyclic aromatic hydrocarbons

Integrated nano-bio systems for remediation of polycyclic aromatic hydrocarbons			
Nanoparticle	Bioagent	Pollutant	References
Polycyclic aromatic hydrocarbons			
Fe^0 nanoparticles (ZVI)	*Candida tropicalis* NN4	Indenopyrene (InP)	Ojha et al. 2019
Pd/nFe bimetallic nanoparticles	Laccase enzyme from *Trametes versicolor*	Triclosan	Bokare et al. 2010
Magnetite (Fe_3O_4) nanoparticles	*Pseudomonas delafieldii*	Dibenzothiophene	Shan et al. 2005
Fe_3O_4 nanoparticles + gellan gum gel beads	*Sphingomonas* sp.	Carbazole	Wang et al. 2007
Magnetite nanoparticles	*Rhodococcus erythropolis* IGTS8	Dibenzothiophene (DBT)	Karimi et al. 2017
Nitro-aromatic compound			
Fe^0 nanoparticles (ZVI)	Methanogenic anaerobes	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	Oh et al. 2001

delafieldii coated with magnetite nanoparticles are also effective in degrading DBT (Shan et al. 2005). Successful degradation of a pollutant depends on its chemical structure. Some pollutants can be degraded completely, while others are broken down to less toxic moieties.

Triclosan, a halogenated aromatic hydrocarbon compound, is a commonly used antibacterial and antifungal component of soaps, toothpaste, and deodorants. Although triclosan is not considered as chemical pollutant of high concern currently, its degraded by-products have been detected in soil, wastewater, drinking water, and even human breast milk. Triclosan has been reported to bioaccumulate in various aquatic species, raising concern of it being an emerging toxic contaminant (Dhillon et al. 2015). Complete biotransformation of triclosan to non-toxic oligomers has been reported via sequential nanobioremediation. Pd/nFe bimetallic nanoparticles, under anaerobic conditions, dechlorinated triclosan to 2-phenoxyphenol, which, in the absence of nanoparticles, was oxidized into non-toxic products by laccase enzyme isolated from *Trametes versicolor* (Bokare et al. 2010). Table 19.9 lists some of the integrated nano-bio systems used for bioremediation of polycyclic aromatic hydrocarbons.

19.15 Nanobioremediation of Petroleum-Based Hydrocarbons

Although the studies conducted on nanobioremediation of oil sludge are few, researchers have demonstrated successful degradation of aliphatic and aromatic hydrocarbons of oil sludge using an integrated system of nanoparticles and microorganisms. The nanoparticles decrease the hydrophobicity of the hydrocarbons allowing them to be accessible to the microorganisms for degradation. The bacteria,

Table 19.10 Integrated systems for remediation of petroleum-based hydrocarbon pollutants

Integrated systems for remediation of petroleum-based hydrocarbon pollutants			
Nanoparticle	Bioagent	Pollutant	References
Fe_2O_3 and Fe_3O_4 nanoparticles	*Thalassospira* sp.	Oil sludge	Rizi et al. 2017a
Fe_2O_3 and Fe_3O_4 nanoparticles	*Chromohalobacter* sp.	Oil sludge	Rizi et al. 2017b
Consortium			
ZnO nanoparticles	*Bacillus, Pseudomonas, Achromobacter,* and *Microbacterium*	Crude oil	Ismail et al. 2013
NiO nanoparticles	*Halomonas xianhensis* A, *Halomonas zincidurans* B6, *Pseudomonas stutzeri* ATCC 17588, and *Halomonas salifodinae* BC7	Crude oil	El-Sheshtawy et al. 2017

Thalassospira sp. and *Chromohalobacter* sp. isolated from an oil site in the Persian Gulf has been studied to successfully degrade oil sludge into various non-toxic compounds. The addition of Fe_2O_3 and Fe_3O_4 nanoparticles, at low concentrations, further enhanced degradation of the compounds, without negatively impacting bacterial growth rate (Rizi et al. 2017a, b).

The interaction between nanoparticles and bacteria may not always be synergistic; nanoparticles can have toxic effects on the microbe and thus reduce its bioremediation potential. Crude oil degradation by the isolated bacteria: *Bacillus, Pseudomonas, Achromobacter,* and *Microbacterium sp.* was deemed successful; but addition of ZnO nanoparticles to *Pseudomonas* decreased its growth rate and lowered bioremediation potential (Ismail et al. 2013). This reduction can be attributed to inhibitory effect of the nanoparticles on the bacterium.

Low bioavailability of crude oil and their poor aqueous solubility obstructs microbial degradation. Nevertheless, evolved mechanisms allow many aquatic microbes to utilize these hydrophobic compounds as a source of carbon. Crude oil degrading bacteria produce biosurfactants and bioemulsifiers that permit bacterial adhesion to hydrocarbons and increase aqueous solubility of crude oil components, thus enhancing its biodegradation. Microcosms containing consortia of *Halomonas xianhensis, Halomonas zincidurans, Pseudomonas stutzeri,* and *Halomonas salifodinae*, along with nickel oxide (NiO) nanoparticles and biosurfactants were found to degrade different membered ring polyaromatics, and 90% of the crude oil sample, compared to microcosms with either biosurfactants or nanoparticles (El-Sheshtawy et al. 2017). Table 19.10 lists some of the integrated nano-bio systems used for remediation of petroleum-based hydrocarbons.

Nanoparticle assisted microbial degradation of hydrocarbon plays a significant role in hydrocarbon decontamination. However, many aspects of nanobioremediation need to be studied such as the innate properties of the nanoparticles, their impact on the microorganisms, the reaction conditions and whether sequential or combined treatment systems need to be applied. Also, the effect of biosurfactants and bioemulsifiers on the biodegradation of hydrocarbon contaminated sites needs to be evaluated prior to large-scale application of the integrated system.

19.16 Bioremediation of Organic Solvents and Antibiotics

The organic solvent, trichloroethylene (TCE), is an industrial solvent and is a common soil contaminant which is toxic at low levels. Due to its mobility in groundwater and high density, remediation of TCE can be challenging. Combination of nanoscale zerovalent iron (nZVI) along with bacteria *Dehalococcoides* sp. (Table 19.11) has demonstrated successful dechlorination and degradation of TCE to non-toxic compounds in comparison to controls that either contained the microorganism or the nanometal. It was also reported that the combination enhanced the rate of degradation as the nZVI stimulated bacterial growth by providing the bacteria with hydrogen which was utilized to dehalogenate the chlorinated compound (Xiu et al. 2010).

Antibiotics find their way into soil and aquatic systems due to their incessant use in farming and poultry. Due to their continual input into the environment, antibiotics have been classified as pseudo-persistent organic pollutants (Ma and Zhai 2014). These compounds resist biodegradation due to their antimicrobial properties. While nanoparticles and a few microorganisms are known to individually degrade these compounds to certain extent, integrated remediation can offer a cost-effective and more efficient alternative. A combined nano-bio system using MgO nanoparticles has been reported to accelerate the rate of degradation of the antibiotic Cefdinir, by enhancing the cell membrane permeability of yeast *Candida* sp. (Table 19.11) (Adikesavan and Nilanjana 2016). Table 19.11 also enlists some of the other contaminants that have been studied for their remediation using an integrated system of nanoparticles and microbes. Thus, nanoparticles are known to assist microbial activities, but their effect on microbial reaction rates is yet to be studied.

19.17 Bioremediation of Organohalide Compounds

Organohalide compounds are the most persistent toxic compounds that contaminate the soil and water ecosystems. Under aerobic conditions, nZVI has been reported to enhance the degradation of polybrominated diphenyl ethers (PDBE) such as tetrabromo-diphenyl-ether (BDE-47) by *Pseudomonas stutzeri* with increase in incubation time (Huang et al. 2016). PDBEs such as di-, tri-, and deca-BDEs can be degraded into bromophenols by subjecting it to nZVI followed by biological treatment using *Sphingomonas* sp. PH-07. The combination of *Sphingomonas* sp. PH-07 and nZVI is also effective in degrading tetrachlorodibenzo-p dioxin (TCDD), the most toxic dioxin compound (Kim et al. 2012). Nanoparticles integrated with biosurfactant producing bacteria can potentially remediate PCBs. The sequential treatment of PCBs with Pd/Fe nanoparticles followed by bioremediation with

Table 19.11 Nano-bio integrated systems for remediation of recalcitrant organic pollutants

Nano-bio integrated systems for remediation of recalcitrant organic pollutants			
Nanoparticle	Bioagent	Pollutant	References
Organic solvents			
Fe^0 nanoparticles (ZVI)	*Methanosarcina thermophila*	Carbon tetrachloride and chloroform	Novak et al. 1998
nZVI	*Dehalococcoides* sp.	Trichloroethylene (TCE)	Xiu et al. 2010
Antibiotics			
MgO nanoparticles	*Candida* sp. SMN04	Cefdinir (antibiotic)	Adikesavan and Nilanjana 2016
Organohalide compounds			
nZVI	*Sphingomonas* sp. PH-07	Polybrominated diphenyl ethers (PBDEs)	Kim et al. 2012
Pd/nFe bimetallic nanoparticles	*Sphingomonas wittichii* RW1	2,3,7,8-Tetrachlorodibenzo-p dioxin	Bokare et al. 2012
Carboxymethyl cellulose (CMC)-Pd/nFe bimetallic nanoparticles	*Sphingomonas* sp. NM05	γ-Hexachlorohexane (insecticide)	Singh et al. 2013
Magnetite nanoparticles	*Enterobacter aerogenes*	Organophosphate (pesticide)	Daumann et al. 2014
Pd/nFe bimetallic nanoparticles	*Burkholderia xenovorans*	Polychlorinated biphenyls (PCBs)	Le et al. 2015
Pd^0 nanoparticles	*Shewanella oneidensis* MR-1	Polychlorinated biphenyls (PCBs)	Windt et al. 2005
nZVI	*Dehalobacter* sp., *Sedimentibacter* sp., *Dehalogenimonas* sp.	Chlorinated aliphatic hydrocarbons (CAH)	Koenig et al. 2016
nZVI	*Pseudomonas stutzeri*	Polybrominated diphenyl ethers (PBDEs)	Huang et al. 2016
nZVI	*Bacillus* sp.	Polychlorinated biphenyls (PCBs)	Zhang et al. 2016

biosurfactant producing *Burkholderia xenovorans* resulted in formation of less toxic and harmless compounds (Le et al. 2015). The Bio-Pd-*Shewanella oneidensis* MR-1 system serves as a self-sustaining model for remediation, wherein the bacteria adsorb palladium nanoparticles, reduce it to zerovalent state, and precipitate it. These bio-reduced Pd (bio-Pd) nanoparticles were able to dechlorinate a wide range of chlorinated PCB congeners, without any selectivity (Windt et al. 2005). Culture medium dominated by organochlorine respiring bacteria *Dehalobacter* sp., *Sedimentibacter* sp., and *Dehalogenimonas* sp. when combined with nZVI completely reduce CAH such as dichloroethane and trichloroethane to ethane (Koenig et al. 2016). Carboxymethyl cellulose (CMC) stabilized bimetallic nanoparticles (CMC-Pd/nFe0) integrated with *Sphingomonas* sp. have been demonstrated to degrade γ-hexachlorohexane (γ-HCH), a commonly used insecticide. The stabilized nanoparticles demonstrated to have a biostimulatory effect on the bacterial cells (Singh et al. 2013). Likewise, zerovalent iron (ZVI) has been reported to enhance *Methanosarcina thermophila* mediated dechlorination of compounds such as chloroform and carbon tetrachloride, by serving as an electron donor (Novak et al. 1998). Table 19.11 lists nano-bio systems that are used for remediation of recalcitrant organic compounds.

19.18 Fate of Nanoparticles

While it is implicit that nanoparticles have various applications in the field of bioremediation, the extensive use of these nanoparticles and their subsequent release into the environment is a major concern, especially in cases of in situ remediation. Therefore, the zerovalent iron nanoparticles are currently the only nanoparticles on field application (Kharangate 2015). These particles, once released into the environment, may find channels to enter the food chain, deposit in various organisms, and manifest toxic effects. Nanoparticles have been studied to have toxicity affects that can be attributed to its size. Even nanoparticles of inert metals such as gold have shown to have toxic effects on biological systems (Jeevanandam et al. 2018). Due to the shortage of investigations of the toxicological effects of these nanoparticles, a sub-speciality of nanotechnology, known as nanotoxicology has been developed (Colvin 2003; Niazi and Gu 2009).

Compared to plants, invertebrates, and vertebrates, microorganisms exhibit widespread and diverse reactions to nanoparticles (Oberdorster et al. 2009). The toxic effects depend on the nature of the nanoparticle and its interaction with the microbial species. In case of rhizosphere resident bacteria *Pseudomonas chlororaphis* O6, CuO nanoparticles were demonstrated to neutralize the charge on the EPS colloids and release ions that disrupt the homeostatic control within the cell. Similar mechanisms of disrupting homeostatic mechanisms of the cell have been observed with ZnO nanoparticles (Dimpka et al. 2011).

Application of nanoparticles in remediation processes results in the discharge of these nanoparticles in the soil and aquatic ecosystems wherein they encounter and interact with various microorganisms, which are sensitive to these particles. Due to their nano-size, large surface area, and high reactivity, nanoparticles can destroy the microbial membrane and generate reactive oxygen species (ROS) that are known to disrupt cellular processes (Cecchin et al. 2016). Studies conducted on nanoparticles released in natural aqueous environments confirm that the nanoparticles react and conjugate with biological molecules such as DNA, RNA, proteins as well and lysophospholipids which facilitate their solubilization in water (Ke and Qiao 2007; Niazi and Gu 2009). Since microbes can assimilate nanoparticles, it can lead to accumulation in the food chain and ultimately build-up to toxic levels in humans.

Recovery of released nanoparticles from natural systems seems impossible, extensive research is needed to understand their potential toxic effects on various biological systems, prior to their large-scale application in nanobioremediation of contaminated sites.

Furthermore, soil microorganisms play an important role in regulating biogeochemical cycles and also possess natural mechanisms to degrade contaminants. Drastic reduction in populations of these beneficial organisms can lead to ecological imbalance. Despite their adaptability, nanoparticles used for in situ remediation can have a bactericidal effect on the inherent microbiota. Thus, determining the interactions between native microorganisms, the contaminant and the nanoparticle to be used prior to application is imperative for sustainable remediation of polluted sites.

19.19 Conclusion

Integrated systems have a lot of potential in remediating various organic and inorganic contaminants from polluted environments. Nanobioremediation has the ability to reduce the overall cost for environmental clean-up on large-scale application. While ex situ nanobioremediation is still at an infant stage, in situ application of nanoremediation and nanobioremediation to soil and ground/surface water can reduce contaminant to near-zero levels. Microbe-assisted intracellular/extracellular synthesis of biogenic nanoparticles acts as a novel, environment friendly, cost-effective strategy to supply tailored nanoparticles for remediation projects. Additionally, the possibility of intentionally tuning nanoparticles opens up new avenues in nanoremediation. Although this approach is a favourable alternative to conventional methods, nano-toxicity associated environmental risks are still unclear and need further research. Moreover, the effect of parameters like pH, temperature, ionic strength, presence of competing or inhibitory substances, etc. on remediation efficiency of the nanobioremediation method needs to be evaluated. But once these gaps are filled, integrated nano-bio systems can be applied on a larger scale.

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