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

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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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V. Kumar et al. (eds.), *Rhizobiont in Bioremediation of Hazardous Waste*,
https://doi.org/10.1007/978-981-16-0602-1_11

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

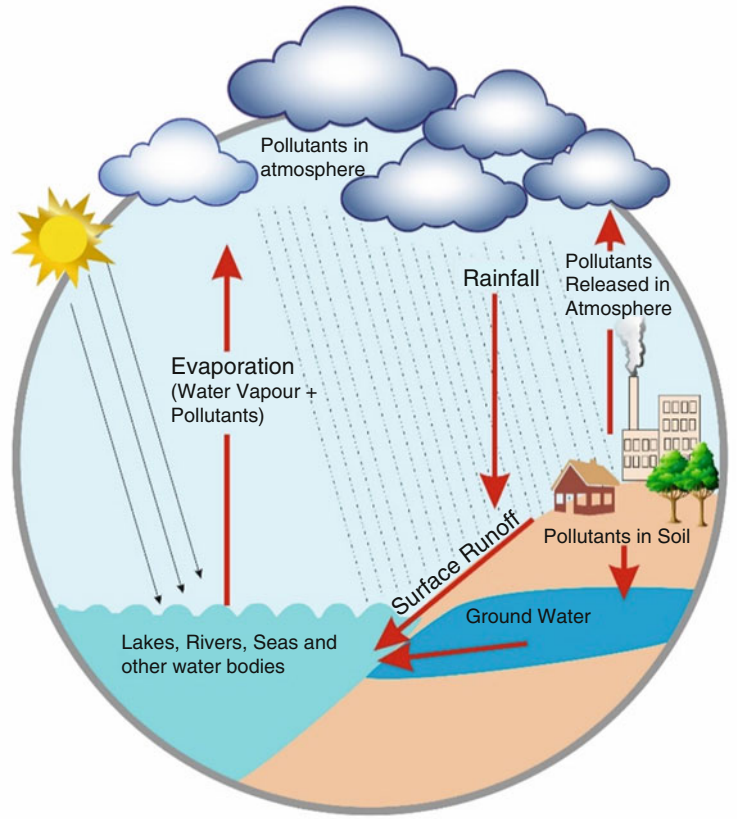

Fig. 11.1 Movement of toxic pollutants in the three spheres of the environment: lithosphere, hydrosphere and atmosphere

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).

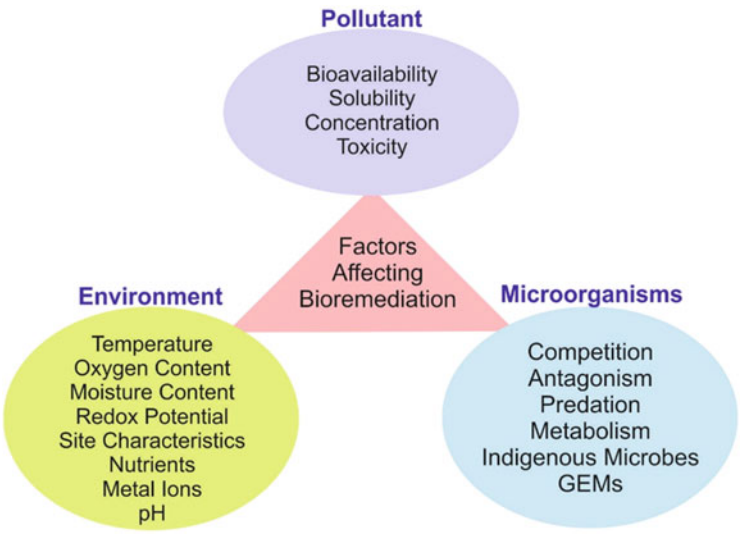

Fig. 11.2 Factors affecting microbial bioremediation

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

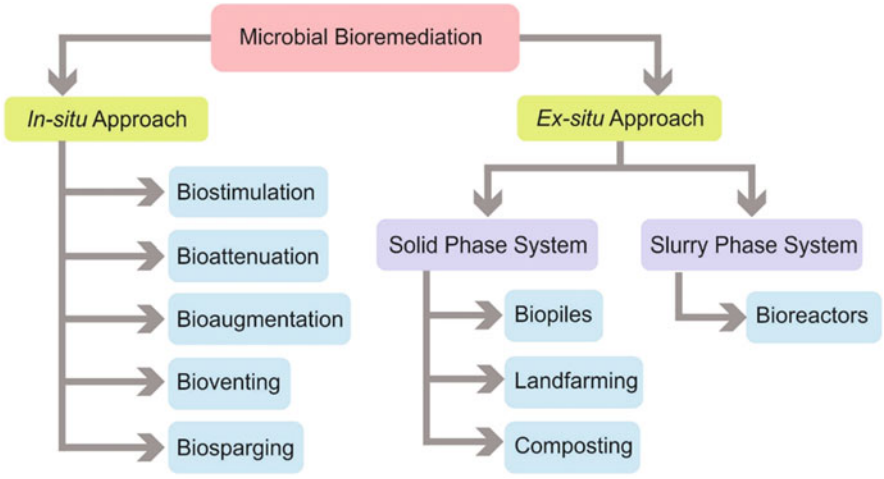

Fig. 11.3 Approaches in microbial bioremediation

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).

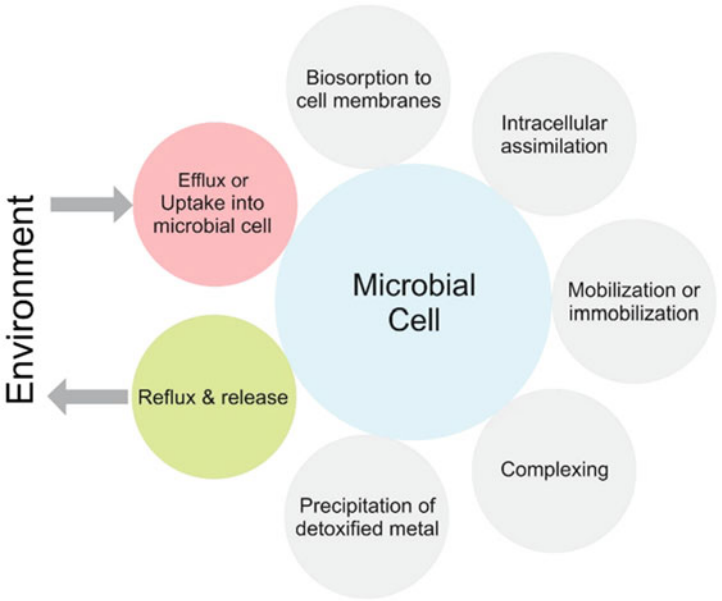

Fig. 11.4 Mechanisms possessed by microorganisms for dealing with metals and metal pollutants in the environment

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

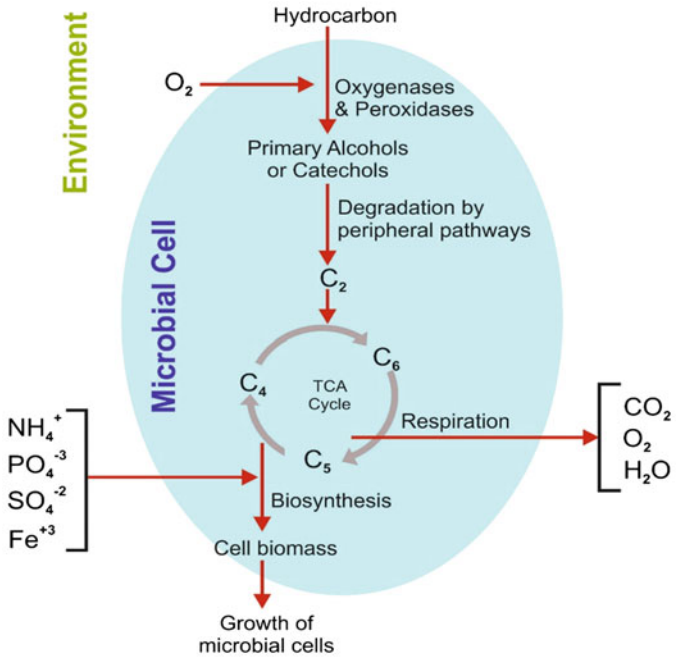

Fig. 11.5 Biodegradation of hydrocarbon compounds by microorganisms

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		
Aspergillus *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

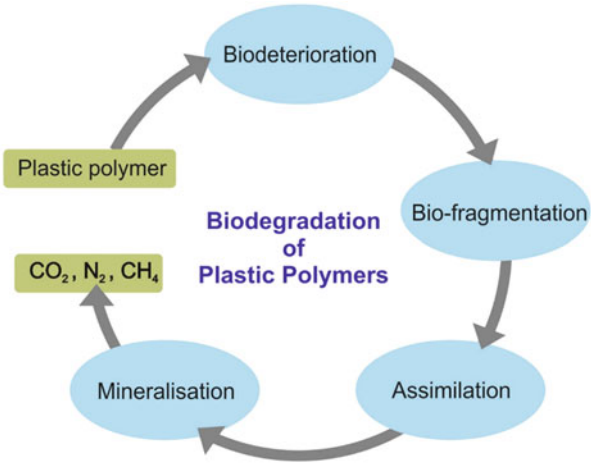

Fig. 11.6 The different steps in biodegradation of plastic polymers by microorganisms

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		
Asp*ergillus 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.

Acknowledgement The authors wish to thank Pankaj Lad for graphic designing of the diagrams and the valuable inputs in the chapter.

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