

## Research Article

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## Mode of Action, Mechanism and Role of Microbes in Bioremediation Service for Environmental Pollution Management

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### ABSTRACT

Environmental pollution is the global sensitive issues currently resulting ecological crisis, drastic climate change and biodiversity loss. Bioremediation is one of an ecofriendly and cost effective alternative strategy for removing different pollutant waste using microorganisms. Different types of ex-situ and in-situ bioremediation service these are biopiling, composting, Land farming, bioventing, biosparging, biostimulation, bioaugmentation are employed to treat heavy metal waste, Petroleum hydrocarbon, agro-industrial, dyestuff, agrochemicals, organic and volatile compound, lignocellulose biomass and nuclear waste. Several microorganisms (natural/exotic/ engineered) having specific metabolic capability and various enzyme production ability which fall under six main divisions include Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases (synthetases) are used during bioremediation process. Understanding the mechanism, mode of action and role of microorganisms in bioremediation process is essential to utilize microorganism potential and design waste management strategy.

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### Introduction

Environmental pollution currently became one of the global sensitive issues. Rapid population growth and human activities, urbanization, unsafe agricultural practices and rapid industrialization brought huge environmental pollution due the release of various types of wastes. Municipal and domestic solid waste and sewage, industrial effluent are source of pollutant containing of inorganic chemicals ( $\text{NH}_3$ ,  $\text{N}_2$ ,  $\text{P}$ ,  $\text{NO}_3$ ,  $\text{NO}_2$ ), Organic chemicals and volatile organic compound (Benzene, Toluene, Xylenes, Dichloromethane), Agro waste (Coffee pulp, bagasse, rice bran etc.), Heavy metals (Cd, Pb, Mercury, Cu, Pb, Chromium), Xenobiotic, Agrochemicals (Pesticide, fertilizer), Chlorinated compounds, Dyes stuff (Azodye, vat dye, triphenylmethane, Anthraquinone dye etc), Greenhouse gases, Hydrocarbons petroleum, Nuclear waste, Plastics, Polychlorinated, biphenyls, detergents, lubricants, nanoparticles, paints, disinfectants which contaminate agricultural soil, ground water, rivers and air in general terrestrial and aquatic ecosystem [26]. The report from the third world network showed that more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. Approximately  $6 \times 10^6$  chemical compounds have been synthesized with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use for different purpose [230]. In other study from industrial daily activities shows in each second about 310kg of toxic chemicals are entered into the air, land and water body with approximate amount 10 million tonnes per year globally [218]. At global scale, 140 billion metric tons of agriculture biomass waste is produced every year

like fronds, husk, shell, coffee (hull, husk, ground), (cob, stover, stalks, leaves), cotton (stalks), nuts (hulls), peanuts (shells), rice (hull/husk, straw, stalks), sugarcane (leavings, bagasse, molasses), vegetable wastes, etc [21]. In other study each year, human, livestock and crops by product generate approximately 38 billion metric tons of organic waste alone worldwide [56]. The estimated quantity of Municipal Solid Waste (MSW) generated also worldwide is 1.7 – 1.9 billion metric tons. Sources of waste generated 76% households, 18% institutions, commercial, factories, hotels, 6% is street sweeping. These municipal solid wastes composed of biodegradable and non-biodegradable waste consisting of high and low density polyethylene and organic lignocellulose waste. Often more than 50% of the solid waste produced is organic and biodegradable. Organic waste composed of the Lignocellulosic complex contains approximately 40 to 60% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin. Currently severe environmental pollution arises from non biodegradable plastic waste evokes our ecosystem with an estimated amount reached to 500 billion to 1 trillion plastic bags are utilized worldwide [183]. Annually 140 million tonnes of synthetic polymers are produced at a growing rate of 12% per year [90, 198]. The polyethylene bags or any other polyethylene based products are finally dumped into the landfills which pollute the environment at all [13, 93]. Other waste type is industry dye stuff, worldwide over 10,000 different dyes and pigments are used in dyeing and printing industries. The total world colorant production is estimated to be 8,00,000 tons per year and at least 10% of the used dyestuff enters the environment through waste [121, 162]. It is estimated that 2,80,000 tons of textile dyes are discharged in such industrial effluents every year worldwide [142]. Improper textile dye effluent disposal in aqueous ecosystems leads to the reduction

in sunlight penetration which in turn decreases the photosynthetic activity, dissolved oxygen concentration, water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems world-wide [242]. Environmental pollution has great devastating impact on the biodiversity at all. The contaminants causing ecological problems leading to imbalance in nature is one of great global concern [63,205]. Global warming, drastic climate change, microbial community lapse, soil mineral depletion, species extinction and biodiversity loss which leads to ecological crisis. Consequence results more than 1 billion people around the world cannot get healthy air to breathe, 3 million die annually due to air pollution [235]. Globally, over 1 million seabirds and thousands of sea mammals are killed by pollution every year. More than 3 million children under the age of 5 die annually from various environmental pollutions [45]. Biodiversity has also been significantly impacted, and more than 20% of bird species have become extinct in the last 200 years [240]. 39–50% of the land surface of our planet was changed and modified due to human activities such as agriculture and urbanization, resulted the atmospheric CO<sub>2</sub> concentration increased by 40% over the past 140 years [85]. Pollutant carcinogenic effect and their bioaccumulation and bio-magnification upset human and animal health like kidney damage, brain and nerve damage, testicular tissue destruction, respiratory problem, high blood pressure, gene defect, red blood cell destruction, liver problem, reproductive problem, depression and irritability, chromosome breakage, birth defect, psychopathological problem, stunt growth, unusual deformed birth and other complex problem on whole ecosystem biodiversity [147]. Several methods till now are implied and used for removing waste from environmental pollution using like chemical precipitation, photo catalysis, ion exchange, reverse osmosis, membrane processes, evaporation, purification, recycling, incineration, solvent extraction and adsorption [49]. Globally a huge budget is invested for the remediation of contaminated environment. The cost utilized for worldwide remediation of organic pollutants is projected to be USD 25-30 billion annually [143]. Remediation costs for sites contaminated with hazardous wastes in Europe are expected to exceed \$1.5 trillion in the near future [46]. In the United States, the Office of Technological Assessment (OTA) of the U.S. Congress estimates that the cost of cleaning up more than 300,000 highly contaminated sites will exceed \$500 billion [153]. There for cost effective and environmental friendly waste removal approach is timely important. Bioremediation is an optional and a modern concept for environmental pollution management highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding environment through the action of microorganisms and using their byproduct. Bioremediation can use biological agent i.e. Yeast, actinomycetes, fungi and bacteria to suppress or clear contamination [205]. Bioremediations not only a process of removing the pollutant from the environment but also it an eco-friendly and more effective process [195]. Generally it is less expensive method for the removal of hazardous contaminants. Bioremediation was first used commercially in 1972 to treat a Sun Oil gasoline pipeline spill in Ambler, Pennsylvania [176] and has been used almost as long as simple pump-and-treat technology. There are different bioremediation strategy these are bio mineralization, bio-sorption, bio-stimulation, rhizoremediation, mycoremediation, bioventing, bioreactor, composting, bio-augmentation, land farming, soil vapor extraction, soil washing and land filling [229]. The major mechanism of microorganism in mode of action for catalytic role using degrading enzymes or mineralizing various contaminants and converting non-toxic by-products during soil bioremediation processes [66,72,206]. Various enzymes are produced by microorganisms fall under six main divisions include

Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases (synthetases) during bioremediation process [124]. The purpose of bioremediation is to make environment free from pollution with help of environmental friendly microbes making the globe safe for the life of all inhabitants and proper functioning of biophysical networks of nature. This paper reviews the role and mechanism of microbes in bioremediation process for environmental pollution control and detoxification of hazardous toxic compounds.

## **Microbial Remediation Strategies**

### **Ex-Situ Bioremediation Strategies**

In cases where soils cannot be treated in the contaminated site for in situ due to regulatory reasons or the unavailability of sufficient land, risk to ground water or air pollution, soils must be excavated into another place and bio remediated. The ex-situ bioremediation can be carried out by bio piling, composting, bioreactor, land farming methods of ex-situ bio-remediation service.

### **Bio-Piling**

Bio-piles interchangeably called as bio-cells, bio-heaps, bio-mounds and compost piles. This type of Ex-situ bioremediation strategy is used to reduce concentrations of petroleum pollutants in excavated soils during the time of biodegradation. Biopiles involve soil excavation, shifting and heaping into piles. The soil is packed on a protective layer formed by a bottom inert liner. In this process, air is supplied to the bio-pile system during a system of piping and pumps that either forces air into the pile under positive pressure or draws air through the pile under negative pressure [40]. The microbial activity is enhanced through microbial respiration then the result in degradation of adsorbed petroleum pollutant became high [47,107]. The basic bio pile system includes a treatment bed, an aeration system, an irrigation/nutrient system and a leachate collection system. For proper degradation there should be control of moisture, heat, nutrients, oxygen, and pH. The irrigation system is buried under the soil and provides air and nutrient through vacuum. To prevent the run off the soil is covered with plastic and due to which evaporation and volatilization is also prevented and promote the solar heating. Bio-pile treatment takes 20 days to 3 month to complete the procedure [155].

### **Land Farming**

Land farming is a simple and less equipment requiring bioremediation approach in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants in general the practice is limited to the treatment of superficial 10–35 cm of soil [244]. Spilled oil and wood-preserving wastes have been bioremediated by land farming treatments [71,141]. Mostly for pesticide degradation, land farming is very important, the excavated soil is kept as sandwich layer in between clean and clay soil or concrete. It allows for natural degradation, it also provide oxygen, moisture and pH should also maintained by using lime.

### **Composting**

Composting is one of ex-situ bioremediation strategy and an ancient technology practiced today at every scale from the backyard compost pile to large commercial operations. Basic and acceptable principles in the integrated solid waste management (ISWM) under the 4Rs principles which are involved as reductions reuse, recycling and recovery methods [87]. Composting occurs through the activity of microorganisms naturally found in soils. Under natural conditions earthworms, nematodes and soil insects such as mites, sow bugs, springtails, ants, and beetles do most

of the initial mechanical breakdown of organic materials into smaller particles. Microorganism degrades the waste at elevated temperature that ranges from 55- 65°C. During the process of degradation microbes release heat and increase the temperature which leads to the more solubility of waste and higher metabolic activity in composts. Compost “happens” either aerobically (with oxygen) or anaerobically (without oxygen) when organic materials are mixed and piled together. Aerobic composting is the most efficient form of decomposition and produces finished compost in the shortest time. Under controlled conditions, composters break down large particles through grinding or chopping. Once optimal physical conditions are established, soil bacteria, fungi, actinomycetes and protozoa colonize the organic material and initiate the composting process. There is no fixed time to produce finished compost. Duration depends on feed stocks, composting method used and management. It can take as little as three months and as long as two years. During aerobic composting, the average temperature curve within the piles showed three classic phases. (1) Mesophilic (moderate temperature phase), (2) thermophilic (high temperature phase) and (3) cooling and maturation phase. Bulking and air access are very necessary, in addition to allowing some control over the C: N ratio, another key purpose of adding plant-based materials to a compost mixture can be to provide structure and channels for aeration [79,255,256].

### **In-Situ Bioremediation Strategies**

In situ bioremediation is the application of a biological treatment to clean up hazardous compounds present in the environment or contaminated site. The optimization and control of microbial transformations of organic contaminants requires the integration of many scientific and engineering disciplines. Bioremediation is carried in the contaminated area where enhanced microbial activities are achieved by creating a favorable environment to stimulate the natural or inoculated population of microorganisms and exploit their catabolic potential to grow and consume the contaminants as a food and energy source. Among the most important of the enzymes used by bacteria in degradation of organic compounds are oxygenases. Biodegradation or biotransformation rate is influenced by the type and concentration of specific contaminant present, Oxygen supply, moisture, temperature, pH, nutrient supply or bio-stimulation, bio-augmentation with strains containing desired catabolic properties and cometabolism [42]. There are different in-situ bioremediation techniques include bio slurping, bioventing bio-sparging, bio-augmentation, Microbial assisted phytoremediation.

### **Biosparging**

In biosparging air is injected below the ground water under pressure to increase the concentration of oxygen for microbial degradation of pollutant. Biosparging increase the aerobic degradation and volatilization [122]. This technique is very similar to bioventing in that air is injected into soil subsurface to stimulate microbial activities in order to promote pollutant removal from polluted sites. Unlike bioventing, air is injected at the saturated zone, which can cause upward movement of volatile organic compounds to the unsaturated zone to promote biodegradation. The effectiveness of biosparging depends on two major factors namely soil permeability, which determines pollutant bioavailability to microorganisms, and pollutant biodegradability [169].

### **Bioventing**

It is a technique to degrade any aerobically degradable compound. In bioventing the oxygen and nutrient like nitrogen and phosphorus is injected to the contaminated site. The distribution of these nutrient and oxygen in soil is dependent on soil texture. In bioventing enough oxygen is provided through low air flow rate for microbes.

Bioventing uses low air flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Bioventing is nothing but it is pumping of air into contaminated soil above the water table through well which sucked the air. Bioventing is more effective if the water table is deep from the surface and the area having high temperature. It is mainly used for the removal of gasoline, oil, petroleum etc. The rate removal of these substances is varied from one site to another site. This is just because of the difference in soil texture and different composition of hydrocarbons [179]. Effective bioremediation of petroleum contaminated soil using bioventing has been proved by many researchers [10,119].

### **Bio Augmentation**

It is one of the mechanisms of biodegradation. Microorganisms (natural/exotic/ engineered) having specific metabolic capability are introduced to the contaminated site for enhancing the degradation of waste. Therefore, bioaugmentation corresponds to an increase in the gene pool and thus the genetic diversity of the site. In principle, this genetic diversity could be increased by augmenting the microbial diversity [38,190]. This process can be enhanced by the continuous addition of microorganisms to a bioreactor for the above-ground treatment of ground waters. Commercial inoculants of enriched cultures consisting of one or more microbial species have been successfully used to colonize contaminated environments where the intrinsic microbial communities act on metals. In order to rapidly increasing the natural microorganism population growth and enhance degradation that preferentially feed on the contaminants site. Microbes are collected from the remediation site, separately cultured, genetically modified and returned to the site. Most commonly, it is used in municipal waste water treatment to restart activated sludge bioreactors. At sites where soil and ground water are contaminated with chlorinated ethanes, such as tetrachloro ethylene and trichloroethylene, bio-augmentation is used to ensure that the in situ microorganisms can completely degrade these contaminants to ethylene and chloride, which are nontoxic in nature [155]. Bioaugmentation is mainly undertaken in oil contaminated environments as an alternate strategy for bioremediation.

### **Bio Stimulation**

This kind of strategic is the addition of specific nutrients usually sources of carbon, nitrogen, and phosphorus, oxygen or other electron donors or acceptors at the site (soil/ground water) to stimulate the activity of indigenous microorganisms [179]. Amendments can be added in either liquid or gaseous form, via injection. Liquids can be injected into shallow or deep aquifers to stimulate the growth of microorganisms involved in the bioremediation. It is focus with in the stimulation of indigenous or naturally existing bacteria and fungus community. Firstly, by supplying fertilizers, growth supplements and traces minerals. By providing other environmental requirements like pH, temperature and oxygen to speed up their metabolism rate and pathway [95,101]. The Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. This type of strategic path is most of the time continued in the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the basic requirement for example, energy, cell biomass and enzymes to degrade the pollutant. All of them will need nitrogen, phosphorous and carbon [150]. The major contaminants that can be successfully remediated through biostimulation are petroleum hydrocarbons, sulphate and polyester polyurethanes [130,131].



## **The Beneficial Role of Microorganisms in Different Pollutant Waste Types**

### **Role of Microorganisms in Heavy Metal Bioremediation**

Heavy metals are environmental contaminants globally. They have polluted agricultural soils and caused detrimental effects on our ecosystem. Heavy metals at certain concentrations can have long-term toxic effects within ecosystems and have a clear negative influence on biologically mediated soil processes [120]. Toxic effects of heavy metals have been reported in plants, animals, humans, and microorganisms. There is more than 20 heavy metals, only few of them such as Cadmium (Cd), Copper (Cu), Argon (Ar), Silver (Ag), Chromium (Cr), Zinc (Zn), Lead (Pb), Uranium (Ur), Ra, Nickel (Ni) etc. is considered due to their toxicity potential [237]. Heavy metal stress poses severe threats to agricultural crops by inhibiting plant growth parameters and yield as documented by many researchers [41,66, 81,114, 125]. The non-biodegradable nature of metals enhances their availability and longevity in soils. The longer persistence of metals in soils causes carcinogenic and mutagenic effects and becomes part of our food chain [3, 5]. Heavy metal concentrations above the threshold limit also causes also disturbances in microbial activity and soil health [80]. Generally neurological disorders, Parkinson, Alzheimer, depression, schizophrenia, cancer, poor nutrition, lack of hormones balance, obesity, abortion, respiratory and cardiovascular disease, damage in organs (liver, kidneys and brain), anorexia, arthritis, hair loss, osteoporosis and death (in severe cases) are adverse effects of heavy metals in the human body are researched by many scholars [238]. For instance Cr (VI) by inhalation, may include irritating respiratory effects, possible circulatory effects, effects on stomach and blood, liver and kidney effects, and increased risk of death from lung cancer. Chromium in its hexavalent form is carcinogenic and the permissible limit is 0.05 ppm [144]. Hexavalent chromium is in general more toxic to organisms in the environment [182]. Inhalation and ingestion of Arsenic, Lead, Nickel Cadmium and Mercury cause irritation of respiratory system, Liver and Kidney damage, Loss of appetite, loss of hearing and muscle coordination, nausea and vomiting [8]. There are different report in microorganisms are important in heavy metal remediation highly efficient even at low metal concentrations. *Vibrio harveyi*, a normal inhabitant of the saline environment, is reported to possess the potential for bioaccumulation of cadmium up to 23.3 mg Cd<sup>2+</sup>/1g of dry cells. A reported a consortium of marine bacteria to efficiently remove mercury in a bioreactor in a disturbance-independent mechanism [23]. A new combination of genetic systems in bacteria for the potential degradation of phenol and heavy metals was also described. Bacteria also possess the properties of chelation of heavy metals, thus removing them from the contaminated environment by the secretion of exopolysaccharides which have been evident from the reports of a marine bacterium *Enterobacter cloacae* [92]. This bacterium has been reported to chelate up to 65% of cadmium, 20% copper, and 8% cobalt at 100mg/L of metal concentration. In line with that, certain purple nonsulfur bacterial isolates, e.g., *Rhodobium marinum* and *Rhodobacter sphaeroides*, have also been found to possess the potential of removing heavy metals like copper, zinc, cadmium, and lead from the contaminated environments either by biosorption or biotransformation. Thus, the bacteria have been designated for assessing pollution through their tolerance and biosorption of heavy metals. However, the genetic mechanisms of bioremediation towards toxic metals have been reduced for a smaller number of bacteria. For instance, *Bacillus spp.* and *Pseudomonas aeruginosa* have been used to alleviate Zn and Cu stress [101]. Moreover, symbiotic association between plants and *rhizobacteria* efficiently improve plant growth by increasing mineral nutrition and alleviating heavy metal toxicity

on target plants [222]. However, many mechanisms are adopted by plants to combat heavy metals. Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake [59].

### **Mechanism of Microbial Detoxification of Heavy Metal**

Microorganisms adopt different mechanisms to interact and survive in the presence of inorganic metals. Various mechanisms used by microbes to survive metal toxicity are biotransformation, extrusion, use of enzymes, production of exo-polysaccharide (EPS) and synthesis of metallothioneins [86,132] (Fig1 & Table 1). The mechanism involves several procedures, together with electrostatic interaction, ion exchange, precipitation, redox process, and surface complexation [209]. The major mechanical means to resist heavy metals by microorganism are metal oxidation, methylation, enzymatic decrease, metal-organic complexation, metal decrease, metal ligand degradation, metal flux pumps, demethylation, intracellular and extracellular metal sequestration, exclusion by permeability barrier, and production of metal chelators like metallothioneins and bio surfactants [106]. Detoxification mechanisms involved in fungi are different from eukaryotes [20]. Extracellular mechanisms involved are chelation, precipitation, and cell wall binding. Intercellular mechanisms include binding to sulfur compounds, organic acids, peptides, polyphosphates, and transport into intracellular compartments [20].

### **Bio sorption mechanisms**

Biosorption is the metal cations sorbed to negatively charge bacterial cell surface. The uptake of heavy metals by microbial cells through bio-sorption mechanisms can be classified into metabolism-independent bio-sorption, which mostly occurs on the cells exterior and metabolism-dependent bioaccumulation, which comprises sequestration, redox reaction, and species-transformation methods [67,234]. Bio-sorption can be carried out by dead biomass or living cells as passive uptake through surface complexation onto the cell wall and surface layers [52]. Bioaccumulation depends on a variety of chemical, physical, and biological mechanisms and these factors are intracellular and extracellular processes, where bio-sorption plays a limited and ill-defined role [52].

### **Intracellular Sequestration**

Intracellular sequestration is the complexation of metal ions by various compounds in the cell cytoplasm. The concentration of metals within microbial cells can result from interaction with surface ligands followed by slow transport into the cell. The ability of bacterial cells to accumulate metals intracellular has been exploited in practices, predominantly in the treatment of effluent treatment. Intracellular sequestration of heavy metal like Copper, Cadmium and Zinc ions with the help of cysteine rich low molecular weight proteins [76]. The rigid cell wall of fungi is made up of chitin, mineral ions, lipids, nitrogen-containing polysaccharide, polyphosphates, and proteins. They can decontaminate metal ions by energetic uptake, extracellular and intracellular precipitation, and valence conversion, with several fungi accumulating metals to their mycelium and spores.

### **Extracellular Sequestration**

Extracellular sequestration is the accumulation of metal ions by cellular components in the periplasm or complexation of metal ions as insoluble compounds. Cop0per-resistant *Pseudomonas syringae* strains produced copper-inducible proteins CopA, CopB (periplasmicproteins), and CopC (outer membrane protein) which bind copper ions and microbial colonies [24]. Another example of extracellular sequestration is metal precipitation as insoluble

complexes. Sulfate-reducing bacteria generate large amounts of hydrogen sulfide that causes precipitation of a number of metal cations [126,247]. *Klebsiella planticola* strain produced hydrogen sulfide from thiosulfate under anaerobic conditions and precipitated cadmium ions as insoluble sulfides [199].

### Methylation of Metals

Methylation increases metal toxicity as a result of increased lipophilicity and thus increased permeation across cell membranes. Microbial methylation plays a significant function in metal remediation. Methylated compounds are regularly explosive; for instance, Hg (II) can be bio methylated by some bacteria such as *Bacillus spp.*, *Escherichia spp.*, *Clostridium spp.*, and *Pseudomonas spp.* to gaseous methyl mercury. Bio methylation of selenium (Se) to volatile dimethyl selenide and arsenic (As) to gaseous arsines as well as lead (Pb) to dimethyl lead was witnessed in polluted top soil [177].

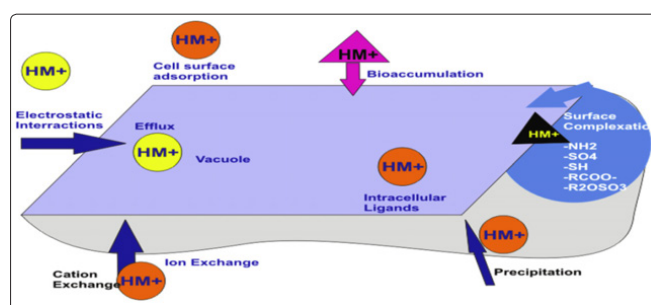
### Reduction of Heavy Metal Ions by Microbial Cell

Microbial cells can convert metal ion from one oxidation state to another, hence reducing their harmfulness [99]. Bacteria use metals and metalloids as electron donors or acceptors for energy generation. Metals in the oxidized form could serve as terminal acceptors of electrons during anaerobic respiration of bacteria. Reduction of metal ions through enzymatic activity could result in formation of less toxic form of mercury and chromium [19,232]. Direct enzymatic reduction (metal ion are reduced during the oxidation of organics). In direct enzymatic reduction (metal ion get reduced during Fe or S oxidation process).

### Active Transport of Metal Ions (Efflux)

Active transport or efflux is one of the mechanisms where microorganism heavy metal resistance systems. Bacteria exploit these systems to export metal ions from cells. Genetic determinants

of efflux systems can be localized on chromosomes [58,120,251] and on plasmids [62,158]. Some metal ions can enter the cell through the systems responsible for the uptake of essential elements for example, chromate is transported inside the cell via sulphate transportsystem [30], ions of cadmium, zinc, cobalt, nickel and manganese enter the cells of *Ralstonia metallidurans* (*Alcaligenes eutrophus*) using systems of magnesium transport [154] ATP hydrolysis [16] or electrochemical gradient [154] are used to export metal ions from the cell. Efflux systems contain proteins belonging to three families: RND (resistance, nodulation, cell division, CDF (cation diffusion facilitator) and P-typeATPases. P-type ATPases and CDF proteins of gram-negative bacteria transport specific substrates through the plasma membrane into the periplasm. It should be noted that PtypeATPases predominantly transfer metal ions with high affinity for sulphhydryl groups ( $\text{Cu}^{+}/\text{Ag}^{+}/\text{Zn}^{2+}/\text{Cd}^{2+}/\text{Pb}^{2+}$ ) while CDF-proteins specifically interact with ions of divalent metals ( $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Fe}^{2+}$ ). Next transport complexes formed by RND proteins transport cations from the periplasm across the plasma membrane [154].



**Figure 1:** Metal-Processing Features of Bacteria Required To Utilize In Bioremediation

**Table1: Some microorganisms involved in heavy metal detoxification**

Heavy metals	Sources	Heavy metal degrader	References
Cr	Tanneries, steel industries, flying ash from the burning of coal	<i>Pseudomonas mendoca</i> , <i>Cellulosimicrobium cellula ns</i> , <i>Oedogonium rivulare</i> , <i>Saccharomyces cerevisiae</i> , <i>Oscillatoria sp.</i> , <i>Arthrobacter sp.</i> , <i>Agrobacter sp.</i> , <i>Pseudomonas aeruginosa</i> S128, <i>Chlamydomonas sp. (algae)</i> , <i>Chlorella vulgaris (algae)</i> , <i>Zoogloea ramigera</i>	[105,98,33,100,175]
Pb	Herbicides, batteries, insecticides, aerial emissions from petrol	<i>Pseudomonas aeruginosa</i> , <i>Oedogonium rivulare</i> , <i>Saccharomyces cerevisiae</i>	[33,248,98]
Hg	Medical waste, coal burning, and Au-Ag mining	<i>Trichoderma Viride</i> , <i>And Humicola Insolens</i> , <i>Saccharomyces cerevisiae</i> , <i>Pseudomonas sp.</i> , <i>Escherichia sp.</i> , <i>Bacillus sp.</i> , <i>Clostridium sp.</i>	[248,133,170]
Ni	Battery manufacturing, steel alloys, kitchen appliances, surgical instruments, industrial effluents	<i>Pseudomonas aeruginosa</i> , <i>Oedogonium rivulare</i>	[224,33,226]
Cu	Pesticides and fertilizers usage	<i>Bacillus species</i> , <i>Pseudomonas aeruginosa</i>	[105,167]
Cd	Electroplating, plastic burning, phosphate fertilizer, paints and pigments	<i>Bacillus species</i> , <i>Pseudomonas aeruginosa</i> , <i>Micrococcus roseus</i>	[167,171,33]
As	Wood storage and pesticides	<i>Bacillus spp.</i>	[225]
Zn	Priming paints for metals, varnishes and pigments in aerospace paints.	<i>Escherichia coli</i> , <i>Aspergillus niger</i>	[69,227]

## Dye Stuff Pollution and Role of Microorganisms in Dyes Remediation

A dye is an organic compound containing both auxochrome and chromophore groups linked to benzene group. The history of dyeing can be divided into two great periods, the “preaniline”, extending to 1856 and the “post-aniline” period. Following these early syntheses, numerous azo dyes have been made and it is estimated that over 2,000 are in use. Of all the different types of dyes, azo dyes are the most useful and widely used colorants which accounts for 50% of all the industrial dyes produced worldwide. Azo dyes and several other groups of dyes are recalcitrant to conventional wastewater treatments and persist in the environment. Worldwide 100,000 types of textile dyes with an estimated of 7x10<sup>5</sup> metric tons are annually produced currently [15,184,210]. 10-25% of total textile dyes are lost during the dyeing process, and 2-20% of dyes are directly discharged as aqueous effluents in different environmental components. A large number of synthetic dyes with specific groups (azo, base, acid, triphenylmethane, anthracene etc) are widely used in textile, pharmaceutical, food, leather and cosmetic industries [32,156]. The residual dyes from different source such as textile industries, cosmetics, paper mills, pulp industries, dyeing and dye intermediates and bleaching industries, more than 80,000 tons of dyes and pigments are produced in these industries. The effluent which is untreated is one of the major sources of consumed metal dyes, phenol, aromatic amines [103,211,174]. Several aromatic amines are source of mutagens and carcinogens to human beings. These dyes can also affect the human health by causing nausea, ulceration of the skin, gastrointestinal tract, hemorrhagic and severe damage to reproductive system, kidney, liver, brain and central nervous system [212,242]. Many of the synthetic Azo dyes and their metabolites are toxic mutagenic and carcinogenic [147]. Azo dyes are reduced in mammals to carcinogenic aromatics, which are oxidized to N-hydroxy derivatives and finally give rise to electrophiles capable of forming covalent linkages with DNA

amines. Several numbers of microbes involved in bioremediation of different class of dye. *P. chrysosporium* has been reported to decolorize azo dyes, Congo red, Orange II and Tropaeolin [34]. *Flavodonflavus*, a basidiomycete isolated from the coastal marine environment, produces laccases, MnPs, and LiPs, and that efficiently degrades the dyes poly R, poly B, azure B. *Pleurotus eryngii* was reported to be involved in the decolorization of the industrial dyes Reactive Violet 5, Reactive Black 5, Reactive Orange 96, Reactive Red 198 and Reactive Blue 38 and 15 [74]. *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* decolorized Acid Red 97 and FF sky Blue dye more than 80% after 26 days. (Table2).

## Microbial Mode of Action in Degradation of the Complex Dye Structure

Both aerobic and anaerobic bacteria have different mechanisms of degradation. Enzymes play the key role in these biotransformation mechanisms. Oxidizing enzymes such as LiP, veratryl alcohol oxidase, laccase, and tyrosinase are well known to degrade textile dyes. However, reducing enzymes such as azo reductase, riboflavin reductases, DCIP reductase, and Green HE4B reductase also break the complex dye structures [200]. Peroxidases in particular, catalyze phenolic substrates result in radical formation by using hydrogen peroxide as the electron donor. Versatile peroxidases (VP) can oxidize not only Mn but also phenolic and nonphenolic aromatic compounds including dyes [74, 75]. Laccase is a copper-containing oxidase enzyme which performs oxidation of a wide range of organic pollutant substrates such as phenols, polyphenols, and anilines as well as highly recalcitrant environmental pollutants on the basis of a one-electron transfer mechanism [168, 185, 249]. Laccase couples the oxidation of substituted phenolic and nonphenolic chemical moieties with oxygen as an electron acceptor to form free radicals. These free radicals further undergo demethylation, depolymerization, repolymerization or quinoneformation [186].

**Table 2: Dye Degrader Microorganisms**

Classification of Dye	Dye degrader	Reference
<b>Based on chemical structure</b>		
Azodye dye	<i>P. rettgeri</i> , <i>Pseudomonas</i> sp, <i>Paenibacillus polymyxa</i> , <i>Micrococcus luteus</i> , <i>Micrococcus</i> sp. as <i>Bacillus vallismortis</i> , <i>Bacillus pumilus</i> , <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> and <i>Bacillus megaterium</i> , <i>Daphnia magna</i> , <i>Exiguobacterium indicum</i> , <i>Exiguobacterium aurantiacum</i> , <i>Bacillus cereus</i> and <i>Acinetobacter baumannii</i> .	[148,228,50,55,221]
<b>Metal complex azo dye</b>		
<b>Anthraquinone dye</b>	<i>Pseudomonas</i> sp, <i>Shewanella</i> sp., <i>Aeromonas</i> sp. <i>Rhodococcus</i> sp. <i>Klebsiella</i> sp.	[54,238,178,181,241]
<b>Phthalocyanine dye</b>	<i>Phanerochaete chrysosporium</i>	[12]
Triphenodioxazine	<i>Trametes hirsuta</i> , <i>Pleurotus pulmonarius</i> ECS-0190, <i>Bjerkander aadusta</i>	[73,104]
Formazon dye	<i>Bjerkander aadusta</i> , <i>Pleurotus ostratus</i>	[94]
<b>Based on usage/application</b>		
<b>Disperse dye</b>	<i>P. chrysosporium</i>	[202]
<b>Direct dye</b>	<i>P. chrysosporium</i>	[161]
<b>Sulphure dye</b>	<i>Acidithiobacillus thiooxidans</i>	[220]
<b>Acid dye</b>	<i>T. versicolor</i> , <i>Coriolopsis polyzona</i> , <i>Perenniporia ochroleuca</i> , <i>Perenniporia tephropora</i> , <i>Pycnoporus sanguineus</i> .	[243]



Vat dye	<i>Bacillus firmus</i> , <i>Bacillus macerans</i> , <i>Staphylococcus aureus</i> and <i>Klebsiella oxytoca</i>	[9]
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## Pesticide Pollution and Role of Microorganisms in Pesticide Bioremediation

Pesticide is substance intended for preventing, destroying, attracting, repelling or controlling any pest including unwanted species of plants or animals during production, storage, transport, distribution and processing of food, agricultural commodities or animal feeds or which may be administered to the animals for the control of ectoparasites. Pesticides are used in areas of agriculture, horticulture, fish farming, forestry, homes and gardens, food and commodity storage, animal husbandry, etc for pest control. Globally, the use of synthetic pesticides has increased rapidly in the last fifty years due to intensification of farming in order to obtain higher yields [236]. More than 55% of the land used for agricultural production in developing countries uses about 26% of the total pesticides produced in the world. Pesticides are necessary to protect crops and losses that may amount to about 45% of total food production worldwide [44]. About one third of the world's agricultural production is lost every year due to pests despite the pesticide consumption which is more than two million tons. Excess fertilizer, herbicides and pesticides when washed by rain into rivers causes serious danger to life. Excess phosphorus in fertilizer causing severe eutrophication. 98% of the pesticides imported were classified as acutely toxic for fish and crustaceans and 73% for amphibians. Organophosphorus poisoning is a global health problem with around 3 million poisonings and 200000 deaths annually [110,194]. Pesticides are problematic because they are designed to be persistent (for long-lasting action), and many are lipophilic often accumulating in animal's fatty tissue through food webs. The pesticides can be classified in many ways on the basis of use, toxicity, mode of entry, mode of action, chemistry and formulations. Classification based on the basis of use can be group as Acaricides, Algicide, Antifeedants, Avicides, Bactericides, Bird repellents, Chemosterilant, Fungicides, Herbicide softeners, Herbicides, Insect attractants, Insect repellents, Insecticides, Mammal repellents, Mating disrupters, Molluscicides, Nematicides, Plant activators, Plant growth regulators, Rodenticides, Synergists, Virucides and Miscellaneous. Pesticides can also be broadly classified according to their intended target pest and also by their chemical structure and properties in the form of fungicides, herbicides, insecticides, nematicides, rodenticides, fumigants, disinfectants, wood preservatives, and anti foliants. There are different classes of Pesticides. These are Organochlorine, Organophosphate, Carbamate, Pyrethroid, Thiocarbamates, Organotin, Di-nitrophenol [208]. Organochlorine pesticides group consists of, the polychlorinated derivatives of cyclohexane (Lindane), polychlorinated biphenyls (DDT, dicofol) and polychlorinated cyclodiene (Endosulfan). The effect of Organochlorine's in the environment is serious which can kill bees, pollination decline and the loss of bees that pollinate plants, and colony collapse disorder (CCD). A number of the Organochlorine pesticides have been banned from most uses worldwide, and globally they are controlled via the Stockholm convention on persistent organic pollutants. (POP's) Organochlorine pesticide include: aldrin, chlordane, DDT, dihedron, endrin, heptachlor, mirex and toxaphene. High level of DDT and its metabolites have been detected a human adipose tissues, blood plasma, liver, brain, placenta and even in breast milk. It is reported to be a potential endocrine disruptor in both avian and mammals. Resulting egg shell thinning, impaired male reproductive ability, interference with sex hormone, causes cancer and other nervous disease [25]. Organophosphorus (OP) pesticides are another class

of broad-spectrum insecticide used on a wide range of crops including vegetables, fruits, grains and ornamentals. They are designed to kill or repel pests but may be harmful and fatal to other organisms, including humans. Exhibits acute extreme toxicity to slight toxicity (Phorate 1.5 to 3.7 mg/Kg, temephos 8600mg/Kg). LD50 values may change with the purity of the compound. This Pesticide contributes significantly to cancer mortality [254]. Organophosphorus (OP) pesticides all act by inhibiting the nervous system enzyme acetylcholinesterase (AChE) and as such are termed anticholinesterase insecticides [88]. The adverse short-term effects of exposure to these chemicals have been studied mostly in the nervous system, which is the main target [60,61]. The majority of people are continually exposed to low OP concentrations, and long-term epidemiologic studies reveal linkage to higher risk of cancer development [22,245]. Organophosphates group was responsible for admission millions of people to hospitals with accidental poisoning, in addition to suicidal cases. Meanwhile, it was estimated that about 25 million agricultural workers suffering from poisoning every year in the third world countries [4]. Some commonly used organophosphates include monocrotophos, phosphamidon, methylparathion, fenitrothion, Phosphorothiates oxy demeton methyl, dimethoate, phorate, Phosalone, DDVP, Malathion, methyl parathion, chlorpyrifos, and diazinon. N-methyl carbamates are often grouped together with Organophosphorus because they act similarly. Which is active against a relatively narrower range of target organisms than the organophosphates, but they are highly toxic to such beneficial insects as honeybees. Common N-methyl carbamates include aldicarb and carbaryl [68, 204]. The other types of insecticide are synthetic pyrethroid which used primarily in structural pest control and agriculture, function much like organochlorines. However they are fairly short-lived in the environment and are less acutely toxic to humans. The toxicity of these chemicals ranges from 80 to 4000mg/Kg body weight and toxicity varies with the ratio of isomers and test animals/species. Toxicity to the insects can be increased by synergists (e.g. Piperonyl butoxide, sesamex) (9:1) Typical pyrethroids include permethrin, cypermethrin, cyfluthrin, lambda-cyhalothrin). Cypermethrin is a synthetic pyrethroid insecticide that has high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light. It is used to control many pests including lepidopterous pests of cotton, fruit and vegetable crops and is available as an emulsifiable concentrate or wettable powder [109]. Emamectin benzoate is a salt with benzoic acid is widely used in controlling lepidopterous pests and sea lice. Thiamethoxam is also considered as moderately hazardous to human as it is harmful if swallowed. It is nontoxic to fish and algae, mildly toxic to birds and highly toxic to midges and bees [53]. The current study involves use of three Endosulfan, Chlorpyrifos and Cypermethrin insecticide widely used in pest control of crops including cereals, fruit, oil seeds, potato, tea and vegetables. For example the Pesticide Registration Council of Ethiopia has registered a total of 171 pesticides consisting of 86 insecticides, 45 herbicides, 22 fungicides and 18 miscellaneous groups [217]. Of these, 159 are currently in use. The largest proportion of pesticide use in Ethiopia has been for the control of bollworms and other pests such as the cotton aphid and the cotton whitefly in cotton. Horticultural crops such as vegetables and fruits have also become heavy users of pesticides in recent years [151]. Various studies suggested that a wide range of microorganisms are capable of degrading pesticides. Most notable among the

pesticide degrading bacteria are *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Alcaligenes*, *Arthrobacter* [96]. Several fungi such as *Agrocybesemiobicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velupites*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, *Stereum hirsutum*, and *Avatha discolor* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated and organophosphorus compounds [11]. Microbes which were commonly reported in pesticides bioremediation include *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Pandora* sp., *Phanerochaete chrysosporium*, *Mycobacterium* sp., *Agrocybe semiobicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velupites*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, *Stereum hirsutum*, and *Avatha discolor* [29]. These microorganisms have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, and dicarboximide, chlorinated and organophosphorus compounds. Several classes of pesticides such as lindane, atrazine, diuron, terbutylazine, metalaxyl, DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, etc. have been degraded to different extent by white-rot fungi [172] (Table 3).

**Table 3: Pesticide Degrader Microorganisms**

Pesticide	Microorganism	Reference
<b>Organochlorine class</b>		
DDT	<i>Alcaligenes eutrophus</i> , <i>Aerobacter aerogenes</i> , <i>Sphingobacterium</i> sp., <i>Penicillium miczynskii</i> , <i>Aspergillus sydowii</i> , <i>Trichoderma</i> sp., <i>Penicillium raistrickii</i> , <i>Aspergillus sydowii</i> and <i>Bionectria</i> sp., <i>Aerobacter aerogenes</i> , <i>Trichoderma viridae</i> , <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp., <i>Arthrobacter</i> sp., <i>Bacillus</i> sp., <i>Pseudomonas</i> sp., <i>Sphingobacterium</i> sp., <i>P. Chrysosporium</i> , <i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> , <i>Enterobacter cloacae</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas putida</i> , <i>Bacillus species</i> , <i>Hydrogenomonas</i> , <i>Saccharomyces cerevisiae</i> , <i>Phanerochaete chrysosporium</i> , <i>Trichoderma viridae</i> , <i>Serratia marcescens</i> DT-1P	[14,48, 115,157,159,164, 165,246,]
Endosulfan	<i>Aspergillus terreus</i> , <i>Bacillus</i> sp., <i>Cheatosartorea stromatoides</i> , <i>Cladosporium oxysporum</i> , <i>Fusarium ventricosum</i> , <i>Klebsiella oxytoca</i> KE-8, <i>Klebsiella pneumoniae</i> KE-1, <i>Mycobacterium</i> sp., <i>ESD</i> , <i>Pandora</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas spinosa</i> , <i>Pseudomonas cepacia</i>	[82,193,214,189,116,136].
Aldrin, lindane	<i>Bacillus</i> sp., <i>Exiguobacterium aurantiacum</i> , <i>Pandora</i> sp., <i>Pseudomonas pseudoalcaligenes</i>	[160,91]
<b>Organophosphorus class</b>		
Cadusafos, dichlorvos, ethoprophos, fenamiphos, malathion, methyl-parathion, parathion, phospho-midon, quinalphos, Chlorpyrifos	<i>Acinetobacter radioresistens</i> , <i>Arthrobacter</i> sp., <i>Aulosira fertilissima</i> , ARM 68, <i>Flavobacterium</i> sp., <i>Fusarium oxysporum</i> , <i>Nostoc muscorum</i> , ARM221, <i>Pseudomonas putida</i> (ep1), <i>Sphingomonas paucimobilis</i> , <i>Enterobacter</i> sp., <i>Klebsiella</i> sp., <i>Alcaligenes faecalis</i>	[84,108,113,213,215, 216,253]
Carbofuran	<i>Novosphingobium</i> sp.	[252]
Carbamate Aldicarb	<i>Rotylenchulus reniformis</i>	[139]
Dieldrin	<i>Pseudomonas</i> sp.	[127]
1,4- Dichlorobenzene	<i>Pseudomonas</i> sp., <i>Sphingomonas paucimobilis</i>	[201]
Lindane	<i>Pleurotus ostreatus</i> , <i>Streptomyces</i> sp., <i>Ganoderma australe</i>	[17,180]
DDD	<i>Trichoderma</i> sp.	[159]
Heptachlor epoxide	<i>Phanerochaete chrysosporium</i> , <i>Phlebia</i> sp.	[7, 250]
Heptachlor O	<i>Bjerkandera</i> sp., <i>Trichoderma viridae</i> , <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp., <i>Bacillus</i> sp.	[165]
Aldrin O	<i>Pseudomonas</i> sp. 105	[165]
Chlorpyrifos	<i>Enterobacter</i> sp., <i>Enterobacter aerogenes</i> , <i>Escherichia coli</i> , and <i>Klebsiella pneumonia</i>	[188,117]



Malathion	<i>Bacillus Thuringiensis, Pseudomonas putida</i>	[57, 128,129]
Heptachlor and lindane	<i>P. chrysosporium., P. eryngi, Pleurotus florida and Pleurotus sajor-caju.</i>	[7]
<b>Synthetic-pyrethroid pesticides</b>		
Allethrin, beta-cyfluthrin, cyper-methrin, flumethrin,permethrin	<i>Acidomonas sp., Aspergillus niger,Pseudomonas sp., Pseudomonas,stutzeri, Serratia sp.,</i>	[60,118,163,196]
Fungicides		
Pentachlorophenol	<i>Pseudomonas sp.</i>	[239]
Herbicides		
2,4-D	<i>Pseudomonas sp.</i>	[146]
Alachlor	<i>Streptomyces capoamus, Streptomyces galbus, Streptomycesbikiniensis, Streptomyces taxa</i>	[187]

### Microorganisms in Petroleum Hydrocarbons and Chlorinated Compound Bioremediation

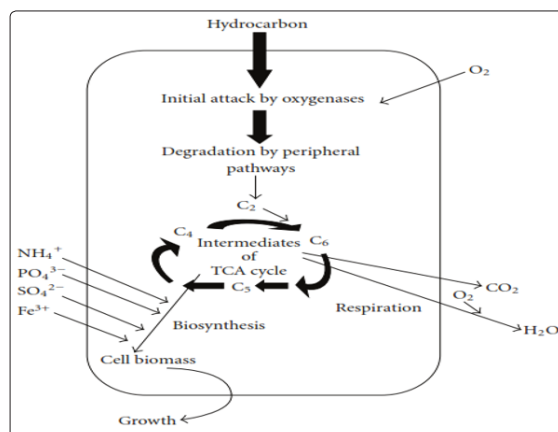
Petroleum hydrocarbons comprise a diverse group of compounds, including alkanes, alkenes, and heterocyclic and aromatic constituents. Another important group of pollutants are the poly nuclear aromatic hydro carbons (PAHs), which are commonly found near coal conversion facilities and petroleum plants [135] short-chain alkanes tend to be volatile and are readily stripped from groundwater whereas mono aromatic hydro carbons such as benzene, toluene, ethyl benzene, and xylenes (which are collectively known as BTEX) tend to be relatively soluble and are transported over longer distances by groundwater. PAHs are the principal constituents of creosote, which is a complex mixture of about 200 compounds also containing phenolic and heterocyclic. Chlorinated aliphatic and aromatic compounds make up an important group of organic pollutants that are both ubiquitous and relatively persistent in aquifers. Common volatile organic compounds (VOCs) in the chlorinated solvents group include tetra chloro ethylene, trichloroethylene, dichloroethylene, and vinyl chloride or chloroethylene. All of these VOCs are potential carcinogens. Groundwater contamination by 1,1,1-trichloroethane (TCA) and chlorinated methanes, such as carbon tetrachloride (CCl<sub>4</sub>) and chloroform (CHCl<sub>3</sub>), is also common. Chlorinated solvents generally have higher specific gravity than water and tend to sink to the bottom of the aquifer if present in a separate organic phase. Crude oil is the most important organic pollutant in the environment, as 1.7-8.8 X 10<sup>6</sup> tonnes of petroleum hydrocarbons are being released to the marine and estuarine environments annually [137]. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [78]. Soil contamination with hydrocarbons causes' extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [1]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies [123, 231]. Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the

aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides [35]. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [18,173]. Several bacteria are even known to feed exclusively on hydrocarbons [223]. *Acinetobacter* sp. was found to be capable of utilizing n-alkanes of chain length C10–C8 as a sole source of carbon [223]. Bacterial genera, namely, *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia*, and *Mycobacterium* isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation [27]. The degradation of poly aromatic hydrocarbons by *Sphingomonas* was reported by Daugulis and McCracken [36]. Some novel marine bacterial species like *Cycloclasticus spirillensus*, *Lutibacterium anuloderans*, and *Neptunomonas naphthovorans* have also been utilized in enhanced biodegradation of PAHs in a marine environment [31]. Similarly, *Achromobacter denitrificans*, *Bacillus cereus*, *Corynebacterium renale*, *Cyclophobicus sp.*, *Moraxella sp.*, *Mycobacterium sp.*, *Burkholderia cepacia*, *Pseudomonas fluorescens*, *Pseudomonas paucimobilis*, *P. putida*, *Brevundimonas vesicularis*, *Comamonas testosteroni*, *Rhodococcus sp.*, *Streptomyces sp.*, and *Vibrio sp.* have been isolated from marine resources and are capable of degrading naphthalene by the process of mineralization. (Table 4) However, bacteria belonging to the genus *Cycloclasticus* play the major role in biodegradation of hydrocarbons. Bacterial isolates like *Sphingomonas paucimobilis* EPA505 have been found to utilize fluoranthene as their sole carbon source. Some of the important genera of bacteria that are capable of degrading petroleum oil include *Acinetobacter*, *Marinococcus*, *Methylobacterium*, *Micrococcus*, *Nocardia*, *Planococcus*, and *Rhodococcus*. In terms of commercial applications, a consortium has been developed by [39] by using Arctic bacteria like *Agreia*, *Marinobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, and *Shewanella* for significant degradation of crude oil and its components.

### Mechanism of Petroleum Hydrocarbon Degradation

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. It is main principle of aerobic degradation of hydrocarbons [51]. The initial intracellular attack of organic pollutant is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. The tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various bio syntheses and growth is synthesized by gluconeogenesis. The degradation

of petroleum hydrocarbons can be mediated by specific enzyme system. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants [77]. The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied. In microorganisms such P450 multiplicity can only be found in few species [257]. Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons (Fig 2). Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [18,173].



**Figure 2:** Main Principle of Aerobic Degradation of Hydrocarbons by Microorganisms

**Table 4: Hydrocarbon Degrading Microorganisms**

Hydrocarbon Substrates	Enzymes	Microorganisms	References
C1–C8 alkanes alkenes and cycloalkanes	Soluble Methane Monooxygenases	<i>Methylococcus</i> , <i>Methylosinus</i> , <i>Methylocystis</i> , <i>Methylomonas</i> , <i>Methylocella</i>	[134]
C1–C5 (halogenated) alkanes and cycloalkanes	Particulate Methane Monooxygenases	<i>Methylobacter</i> , <i>Methylococcus</i> , <i>Methylocystis</i>	[134]
C5–C16 alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	AlkB related Alkane Hydroxylases	<i>Pseudomonas</i> , <i>Burkholderia</i> , <i>Rhodococcus</i> , <i>Mycobacterium</i>	[97]
C10–C16 alkanes, fatty acids	Eukaryotic P450	<i>Candida maltose</i> , <i>Candida tropicalis</i> , <i>Yarrowialipolytica</i>	[83]
C5–C16 alkanes, cycloalkanes	Bacterial P450 oxygenase system	<i>Cinetobacter</i> , <i>Caulobacter</i> , <i>Mycobacterium</i>	[233]
C10–C30 alkanes	Dioxygenases	<i>Acinetobacter sp</i>	[138]

### The Role of Microbes in Domestic and Agricultural Lignocellulos Wastes Remediation

In nature, lignocellulose is derived from wood, grass, agricultural residues, forestry wastes, and solid municipal wastes. Lignocellulosic complex contains three types of polymers approximately 40 to 60% cellulose, 20 to 40% hemicellulose, and 10 to 25% lignin. Contaminated soil is removed from the ground and mixed with natural materials such as cow manure, activated sludge, and corn cobs. These additives provide the biomass necessary for microbial growth and worm growth. Each year, human, livestock, and crops by product produce approximately 38 billion metric tons of organic waste worldwide [56]. Hydrolytic enzyme producing microorganisms play an important role in the recycling of agricultural wastes. Especially cellulase, lacase, lignin peroxidase, manganese peroxidase, hemicellulase enzyme are vitally important in degradation of cellulose, hemicellulose and lignin containing agricultural waste. Composting is one of the bioremediation method where the compost generated by bioconversion of agro residues offers several benefits such as enhanced soil fertility and soil health which can lead to increased agricultural productivity, improved soil biodiversity, reducing ecological risks and a healthier environment. These virtues make composting an ideal option for processing of the enormous quantities of agro wastes that are generated in the

world. Composting is the biological degradation and stabilization of organic substrate under conditions that allow development of thermophilic temperature as a result of biologically produced heat [64]. During composting, mesophilic population builds up initially by the utilization of simple nutrients, which raises the temperature of the piles. Thermophilic microbes proliferate in the second phase. The final product is stable, free of pathogens and plant seeds and can be beneficially applied to land. Composting is essentially a microbiological phenomenon that depends highly on temperature fluctuation within the windrows. The temperature within a composting mass determines the rate at which many of the biological processes take place and plays a selective role on the evolution and the succession of the microbiological communities [152]. In biological terms the operating temperature ranges are as follows: > 55°C to maximize sanitation, 45–55°C to maximize the biodegradation rate, and 35–40°C to maximize microbial diversity [207]. During aerobic composting, the average temperature curve within the piles showed three classic phases. Generally, composting proceeds through three phase: 1) mesophilic (moderate temperature phase), 2) thermophilic (high temperature phase) and 3) cooling and maturation phase. Mesophilic phase, Psychrophilic and mesophilic microorganisms in waste piles tended to increase during the first 25 days of the composting cycle. During this phase, the temperature increased to reach 40 to 50°C as a consequence

of biodegradation of organic compounds [2, 152]. Thermophilic phase: This important step occurred between days 30 to 110 of the composting process, during which the temperature exceed the tolerance limit of mesophilic microorganisms and promoted development of thermogenic microorganisms. According to [207] temperatures above 55°C are important to maximize sanitization. Meanwhile, temperature between 45 and 55°C are to improve the degradation rate and between 35 and 40°C to increase microbial diversity. Control of the temperature and its maintenance below 65°C inside the windrow during composting is assured by ventilation and watering. As mentioned by [70 & 145] temperatures above 60°C affect seriously the decomposition rate of the waste organic matter as a result of microbiological activity lowering. In Cooling phase, microbial activity slows down and as the process nears completion, the material approaches ambient air temperature. Finished compost takes on many of the characteristics of humus, the organic fraction of soil. The material will have been reduced in volume by 20 to 60%, the moisture content by 40% and the weight by up to 50%. One of the key challenges in composting is to retain as much nitrogen as possible. Composting may contribute to the greenhouse effect because carbon dioxide (CO<sub>2</sub>), methane (NH<sub>4</sub>) and nitrous oxide (NO<sub>2</sub>) will be emitted to the atmosphere during composting. During the curing phase the need for turning is greatly reduced. However, there is still a need for low levels of oxygen for microbial activity. Therefore, it is necessary to construct curing piles and/or windrows to a size that will allow for passive airflow through the windrow. A recommended size for a curing pile is 1.5 meters tall and 3-4 meters wide. During the curing phase, the windrows should be kept in dry areas, away from excess moisture. Exposure to excess moisture during this phase may cause the curing piles to become anaerobic. The temperature began to decrease after the twelfth week. This decrease resulted in a beginning of depletion of organic matters; during this phase the C/N ratio in the different windrows

tended to stabilize by the end of the 4th month of composting, average temperatures inside the different windrows marked a real fall with values of approximately 30°C. The temperature remained low in spite of the watering and the turning of windrows. Composting has been used as a means of disposal of organic wastes like paddy straw, sugarcane trash and other agricultural wastes. Natural succession of microflora takes place during composting. Several fungi like *Trichoderma harzianum*, *Pleurotus ostreatus*, *Polyporus ostriformis* and *Phanerochaete chrysosporium* are known to play important role in composting of lignocellulosic materials [203]. Though fungi, bacteria and actinomycetes play unique and important roles during composting, mixed cultures of microorganisms enhance the rate of lignocellulose degradation due to their synergistic activity through utilization of intermediate degradation products [112]. The consortium of four hypercellulolytic fungal cultures namely *Aspergillus nidulans*, *Trichoderma viride*, *Phanerochaete chrysosporium* and *Aspergillus awamori* were used for composting of paddy straw in perforated pits. Thermophilic fungal consortium of *A. nidulans*, *Scytalidium thermophilum* and *Humicola sp.* was found highly effective in degradation of soybean trash and paddy straw mixture during summer months [111]. Similarly a consortium of thermophilic microorganisms *Scytalidium thermophilum*, *Humicola insolens* and *Sporotrichum thermophilum* are also being used. The contaminants may be degraded using different mechanisms like mineralization by microbial activity, transformation to non-toxic products, volatilization, and formation of humus and inert by products, such as carbon dioxide, water and salts. The critical parameters and efficiency of compost in bioremediation is essentially determined by process parameters like pH, temperature, C: N ratio, moisture content and recalcitrance of the material to be composted. Bulking agents like peat moss, pine wood shavings, bran flakes accelerate composting by favoring the growth of aerobic microorganisms [6, 149, 166] (Table.5)

**Table 5: Composting Microorganisms**

	Composting phase	Microorganisms	Reference
1	Thermophilic phase(day 45)	<i>Curtobacterium citreum</i> , <i>Stenotrophomonas rhizophila</i> , <i>Stenotrophomonas maltophilia</i> , <i>Microbacterium foliorum</i> , <i>Xanthomonas oryzae</i> , <i>Pseudoxanthomonas taiwanensis</i> , <i>Bacillus ginsengihumi</i> , <i>Serratia marcescens</i> , <i>Serratia odorifera</i> , <i>Rhabditidae spp.</i> , <i>Panagolamidae sp.</i> , <i>Diplogasteridae Sp.</i> , <i>Cephalobidae sp.</i> , <i>Mononchoides sp.</i> , <i>Ditylenchus filimus</i> .	[79,191]
2	Mesophilic phase(day 139)	<i>Xenophilus azovorans</i> , <i>Bacillus licheniformis</i> , <i>Pseudomonas mendocina</i> , <i>Rhodococcus rhodochrous</i> , <i>Bacillus sp.</i> , <i>Paenibacillus sp.</i> , <i>Actinomycetes</i> , <i>Aspergillus fumigatus</i> , <i>Feecal coliforms</i> , <i>Pseudomonas Sp.</i> , <i>Streptococcus sp.</i> , <i>Proteus Sp.</i> , <i>Serratia Sp.</i>	[79, 192, 219]
3	Psychophilic phase	<i>Aspergillus fumigatus</i> , <i>Emericella Sp.</i> , <i>Aspergillus ochraceus</i> , <i>Aspergillus terreus</i> , <i>Penicillium oxalicum</i> , <i>Thermoactinomyces sp.</i> , <i>Cladosporium sp.</i> , <i>Mycotypha sp.</i> , <i>Scopulariopsis sp.</i> , <i>Coprinus sp.</i> , <i>Cephalosporium sp.</i> , <i>Trichothecium sp.</i>	[37, 79, 219]

## Conclusion

Environmental pollution due to different pollutant source from industry, agriculture and urbanization activities leads to ecological crisis, biodiversity loss and drastic climate change. There are different waste removal strategies utilized to tackle these global problems till now. According to cost effectiveness

and ecofriendly approach waste treatment and detoxification method seek optional and alternative approach. Bioremediation is an incremental technology using the potential of biological agent employed for eradication of environmental pollutant and detoxification of hazardous toxic contaminant. It has evolved over 20 years in use of commercial life and application in remediation



service. Through exsitu and in situ bioremediation method like biopiling, composting, land farming, bioventing, biosparging, biostimulation, bioaugmentation, are employed to treat heavy metal waste, hydrocarbon petroleum, chlorinated compound and plastic waste, organic and volatile compound pollutant, organic and inorganic municipal solid waste, lignocellulose organic agricultural based waste, industry effluent waste, nuclear waste and different dyestuff and agrochemical waste like pesticide and fertilizer. Microorganisms produce versatile class of enzymes belonged in six class Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases (synthetases) are used degradation and mineralization during bioremediation process. Understanding the mechanism, mode of action and role of microorganism in bioremediation process is essential to utilize microorganism potential and design waste management strategy.

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