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Potential of Microorganisms in Clean-up the Environment

A.A. Haleem Khan[1], A. Sadguna[2], V. Divya[3], Sadia Begum[4], Naseem*[5], Ayesha.G.Siddiqui[6]

[1]Department of Botany, Narendra Degree & PG College, Armoor, Nizamabad, A.P
[2]Department of Microbiology, SSR College, Nizamabad - 503001, A.P
[3]Department of Biotechnology, Girraj Govt College, Nizamabad - 503002, A.P
[4]Department of Zoology, Women's College, Nizamabad - 503002, A.P
[5]Department of Pharmaceutical Chemistry, Telangana University, Nizamabad
[6]Department of Microbiology, Dayanand Science College, Latur-413512, MH.
*Corresponding Author: Prof. Naseem, Mobile:9440037022, Fax:08461-222212

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Abstract

Microorganisms are ubiquitous in nature and make up the biodiversity on earth. They perform several important processes in cycling of nutrients, degradation of various compounds globally. Knowledge of microbial activities helps mankind to find various strategies to utilize agricultural natural resources in long term sustainable manner. In sustainable agriculture bacteria and fungi are being utilized as biofertilizers. Plant growth promoting rhizobacteria (PGPR) play important role in soil fertility. Use of PGPR as biofertilizers should be increased to stop deterioration of soil fertility due to extensive application of chemical fertilizers. Change in living standards contributes increased dependence on chemicals in day today life, These chemicals are used from our soaps, detergents, disinfectants, insecticides, like DDT, pollute receiving water bodies through sewage. Various microorganisms can be used for bioremediation of such waste water containing recalcitrant and xenobiotic compounds. The microbial activities that help us to design an excellent new strategy of life leading to sustainable future are presented.

Keywords: *Biofertilizers, Biopesticides, Microbial Biostimulants, PGPR, Siderophores*

1. Introduction

The review is presented as

1. **Microorganisms in Sustainable agriculture**
2. **Biofertilizers or microbial biostimulants or inoculants**
3. **Biopesticides**
4. **Siderophores**
5. **Microorganisms in Renewable energy sources**
6. **Biofuels**
7. **Bioelectricity**
8. **Microorganisms in Pollution control**
9. **Biodegradation of pollutants**
10. **Bioremediation**
11. **Bioaugmentation**

Sustainable development (SD) is employed to fulfill the human needs with preserving the environment, consequently the necessities could be met for generations together (ELF: Environment, Local people, Future). The Brundtland Commission previously known as the World Commission on Environment and Development (WCED), in the UN General Assembly recognized that environmental problems were global in nature and determined as common interest of all the nations. The term 'sustainable development' was first used by WCED (Brundtland Commission) in 1987. There are many dimensions of sustainable development viz., environmental, social, economic and political [1-2]. The most important concern today is environmental sustainability upon which other three are dependent.

Microorganisms are ubiquitous in nature and make up the biodiversity on earth. They perform several important processes in cycling of nutrients and degradation of various compounds globally. Knowledge of such microbial activities helps mankind to find various strategies to utilize agricultural natural resources in long term sustainable manner. Microorganisms are not only utilized for resource generation but also can be exploited in environmental cleanup [3-5].

In sustainable agriculture, bacteria and fungi are being utilized as biofertilizers. Plant growth promoting rhizobacteria (PGPR) play important role in soil fertility and can be used as biofertilizers that stop deteriorations of soil caused by excessive application of chemical fertilizers.

Important genera in PGPR

Azotobacter and *Rhizobium* sp. are nitrogen fixers, *Pseudomonas* spps are popular for phosphate solubilization and siderophore production but various *Bacillus* sp. are also phosphate solubilizers. *Pseudomonas* and *Bacillus* sp. not only produce auxins, vitamins and growth factors that enhance plant growth but also degrade proteins into amino acids.

Microbes as Biocontrol agents

Pseudomonas and *Bacillus* spps produce siderophores and enzymes like chitinases and proteinases that in turn help in growth inhibition of various plant pathogenic fungi. The entomopathogenic bacteria, fungi, and viruses are used as bioinsecticides ex: *Bacillus thuringiensis*, *Beauveria bassiana*, etc. Potentials of microbes are required to be explored to decrease the use of chemical fertilizers and pesticides in agriculture whose adverse effects are well known [6-8].

Microorganisms as renewable resources

Due to increased human population, civilization as well as industrial growth, there is increased demand for energy sources because natural non-renewable sources such as petroleum, oil, natural gas, coal are depleting day by day, and a day may come when future generation will have to face dangerous situation. To avoid these consequences, alternative renewable energy sources such as microorganisms can help in production of biofuels, ex: ethanol, hydrogen, and methane.

Microorganisms as Scavengers of Environment:

Change in living standards contributes increased dependence on chemicals in day to day life. These chemicals are from soaps, detergents, disinfectants, insecticides, like DDT etc., which can pollute receiving water bodies through sewage. Many of these chemicals are harmful to humans and other higher organisms and vegetation. They may be carcinogenic, neurotoxic, teratogenic some of these may persist in the environments for long period and the phenomenon is called recalcitrance. Diverse group of microorganisms can be used for bioremediation of waste water and soil with such recalcitrant and xenobiotic compounds.

Xenobiotic compounds accumulate in huge amount of biodegradable organic waste that creates a big problem. Due to increased consumerism, industrialization, various kind of solid wastes are generated daily and natural rate of biodegradation is very slow as compared to their rate of accumulation. These wastes are now-a-days treated by using inherent biodegradability of microorganisms. Microorganisms have the ability to transform various toxic hazardous wastes (such as heavy metals, radioactive nuclides, colored compound, from various types of industries such as fabric industries, pharmaceutical industries, etc.) into nontoxic one. Above all, these microorganisms play main role in human health by virtue of producing various drugs and antibiotics, vitamins, amino acids and proteins etc. Several microbial activities could help to design a new strategy of life that leads to sustainable future.

Microorganisms in Sustainable Agriculture

Agriculture is most important occupation for food production worldwide. In most of the countries, more than 70% of land is used for agriculture purpose. The farming system in agriculture requires large area of land that gives low crop and livestock. Agriculture was revolutionized due to invention of organic pesticides that protect the crops from pest population. In 1800, for the first time arsenical insecticides and mixture of Bordeaux were introduced on grapes crop. Globally agriculture is carried by continuous cropping system with high use of agrochemicals and water. Agrochemicals are like chemical pesticides and fertilizers to improve the crop yield and the expenditure on it costs about 25% of the crop output approximately [9].

Chemical pesticides pose economic problems like destruction of non-target organisms (beneficial insects, natural predators) along with environmental pollution. Another aspect of chemical pesticides is development of insect resistance. Synthetic fertilizers are made as fossil fuel byproduct and increase in fuel prices also show effect on the fertilizers price. Prolonged use of fertilizers results in the loss of soil quality and possibility of ground water contamination. Hence the conservation of agriculture is identified as best alternative to sustain the soil quality [10].

In 1904, Hiltner [10] introduced the term 'rhizosphere' for the area of soil surrounding the plant root that stimulates the bacterial growth. This zone supports the diverse microbial population colonizing the habitat. Root exudates promote the colonization of roots in rhizosphere. 30% of plant photosynthesis products are found to be released through root exudation by passive and active secretion. Passive transport releases low molecular weight compounds and the active transport secretes the high molecular weight compounds. There are physical and chemical benefits in the plant root exudations such as reduced friction, desiccation, improved soil structural stability and attracting the microorganisms. Some bacteria that inhabit the root zone also elicit exudation like *Pseudomonas aeruginosa* which stimulate the root exudation in rye grass by twenty fold. Plant root colonizing bacteria promote the growth directly or indirectly [10].

These organisms directly promote the growth by nitrogen fixation, solubilization of minerals (phosphorus), production of siderophores to solubilize the iron and produce plant growth regulators. Indirectly they promote antagonism to avoid the invasion of pathogens, parasitism, competition for nutrients and enhance disease resistance.

Several organisms of this category are capable to produce auxins, gibberellins, cytokinins, ethylene and abscisic acid. *Azospirillum*, *Agrobacterium*, *Pseudomonas* and *Erwinia* induce the auxins, cytokinins and gibberellins as well as lateral root development by root colonization of *Azospirillum*. These organisms act as biocontrol agents against the soil borne pathogens by producing antibiotics, siderophores, increase competition of nutrients for pathogen and various types of enzymes. Fluorescent pseudomonads produce phenazine derivatives as antibiotics in root zone that reduces the disease causing organisms in root zone.

Biological control of pests and their vectors replaced successfully the extensive use of chemical pesticides. Biological control is the term used for an approach that controls the insect pest by using a selected living organism. Predator, parasite or infectious organism will be the selected living organism that targets a particular pest. This approach prevents the economic damage in agriculture and environment remains safe. Nearly 15,000 insect species were identified as pests that attack the farming crops and among them 300 insects are required to control the total population. Studies of entomologists revealed that most of the insect pests are susceptible to pathogenic microorganisms that possess potential to kill these pests. Bacteria, viruses, fungi and protozoa are among the pathogenic microorganisms that attack the pest species and that can be utilized to control insects and their vectors.

Microbial Insecticides

Pathogenic microorganisms with ability to invade and kill the insect pests were commonly termed as entomopathogens. Metchnikoff [11-12] (1879) were first to introduce entomopathogens that could be used to control the pests. *Metarrhizium anisopliae*, a muscordine fungus was first documented as pathogenic microorganism that was mass produced and applied as microbial pesticide for crops like sugar beets and grains. In 1940 White and Dutky [7, 13] introduced bacterium *Bacillus popilliae* spores to control Japanese bettle.

Bacillus thuringiensis was introduced in 1960-63 as effective bacterial insecticide and today nearly 40 types of products are popular as insecticide. Studies of Balch and Bird (1944) [6, 14] and Thompson and Steinhaus [3, 15] (1950) succeeded in use of viruses against the insect pests. Baculoviruses are the common choice among the 1600 viruses to control insect species. The selected viruses were targeted against sawflies in forestry during 1950. Oil based formulations of entomopathogenic fungi were used to target the pests like aphids and white flies but these fungi were effective in highly humid locations. Protozoal species of microsporidia were found disease causing agents in insects [11, 16].

Bacterial Insecticides

Bacillus popilliae: It causes milky disease in Japanese beetle *Popillio japonica*. After ingestion, spores germinate and penetrate the alimentary canal and blood appears milky due to spores and then the larva dies. This bacterium is produced commercially by feeding or injecting in the host that multiplies in the body of host. Then it is recovered by grinding the host with talc. This dust is expected to contain 100 million spores/g. when it is ingested by host; the mortality is due to septicemia [17] [12].

Bacillus thuringiensis: It was detected in dying larva of *Bombyx mori* by Ishiwata in 1901 and Berliner in 1911 from Germany in larva of *Ephestia kuehniella* [13, 14]. In 1938 it was first used as microbial insecticide against Lepidopterous larvae with name 'sporeine' and this resulted in the development of several pesticide companies all over the world. It is pathogenic in Lepidopteran larvae and 4 insect orders. Bacterium is transmitted orally, after sporulation bacteria form a toxic crystal and ingestion of these spores causes cessation of feeding. The crystal activity depends on the pH of larval gut (pH 9-10.5) and the action of proteolytic enzymes. The crystal is a protoxin activated by enzymatic hydrolysis. The crystal protein is ecologically safe and successfully tested for controlling insects affecting cotton, cabbage, maize, sunflower, sunflower and pigeon pea [13].

It is formulated under trade names such as - Thuricide (Sandoz AG), Bakthane, Dipel (Abbott labs), Biotrol, Karab, Agritol, Bactospeine (Rhone-Poulene) in dust, liquid concentrate, spray concentrate, granules and baits formulations.

Viral insecticides

Nuclear polyhedrosis viruses (NPV): Virus is rod in shape and outer envelope with protein crystals called polyhedra. NPV is transmitted by oral ingestion of polyhedra. Ingested polyhedra dissolve, releasing virus rods into the lumen of the insect host midgut, then larval skin darkens, hemolymph becomes turbid and prior to death, infected larva usually climbs to the highest point available and then dies.

The disease of *Heliothis armigera* is visible after 2-3 days of ingestion of virus. The cuticle of infected larva in the first instar become milky white in appearance but the later infected instars turn pinkish color. The diseased larva discharges a yellowish grey fluid from their mouth. The death occurs in 3-7 days; the integument became fragile and burst liberating the liquefied body contents.

Granulosis viruses (GV): GV particles are surrounded by an envelope similar to NPV envelope. The virion is rod shaped with DNA as genetic material with inclusion body i.e. capsule or granule hence the virus is named Granulosis virus. This virus is active against rice leaf roller *Cnaphalocrocis medinalis*, castor semi looper *Achaea* *janata*, hairy cater pillar *Pericallia ricini*, cut worm *Agrotis ipsilon*, internode borer *Chilo* spp. and sugarcane shoot borer *C. infuscatellus*. The fat body of Lepidopteran larvae is the primary site of infection. GV are transmitted orally and via the egg. GV infections involving the epidermis cause liquefaction of larvae similar to NPV infections [14].

Cytoplasmic polyhedrosis viruses (CPV): Particles are not enclosed in membranes as of NPV, but contain protein crystals similar to those of NPV. CPV infect the cytoplasm of the midgut epithelium of Lepidopterous larvae. Infected larvae appear to have small bodies and large heads.

Fungal insecticides

More than 36 different genera of fungi contain species which cause insect diseases. Most fungi are transmitted from one host to another by a conidium. Conidia germinate and form a special structure which penetrates the insect cuticle. Fungus then grows in insect's body until the insect is filled with mycelia. Fungal infections depend on environmental conditions such as high humidity and temperature and high population densities. Examples: *Metarrhizium anisopliae*, *Beauveria bassiana*, *Entomophthora* spp.

Beauveria bassiana belongs to class fungi imperfecti and it is actively biological control product ($2.3x10^7$ conidia/ml of product). Upon contact with target insect, the fungal spores attach themselves to the cuticle. The spores then secrete enzymes that dissolve the cuticle. This helps the fungal spore to penetrate hyphae into the body of insect. The production and proliferation of the fungal hyphae cause the release of toxicants from the fungus into the insects. Insect death occurs due to water loss and effects of nutrient loss. *Beauveria bassiana* is popular with trade name Dispel and used at the rate of 2-4 ml/L of water is effective against coleopterous, hemipterous and lepidopterous insects [14].

Microbial inoculants

Microbial inoculants or soil inoculants are agricultural applications that use beneficial microbes (bacteria or fungi) to promote plant growth. These organisms show symbiotic relationship with crop plants where in both partners get benefit. These inoculants improve plant nutrition and promote growth by stimulation of growth regulators. Nitrogen-fixers and phosphate-solubilisers are common inoculants that increase availability of macronutrients like nitrogen and phosphorus to plant [15]. These bacteria are known as plant growth promoting rhizobacteria (PGPR).

Biofertilizers contain microorganism in combination with organic fertilizers (manure) that provide high nutrient availability to host plants due to the interactions with microorganism. These fertilizers are of following types:

1. Symbiotic nitrogen fixers (*Rhizobium* spp.) used for legume crops like groundnut, soybean, increases crop yield from 10 to 35% by adding 50-200kg N/ha. *Rhizobium* spp. is gram negative bacteria, enter legume plants through nodules in the roots and fix the atmospheric nitrogen. This bacterium improves the crop yield of inoculated crop and also benefits the subsequent crop. *Rhizobium leguminosarum*, *R. melilotii*, *R. trifoli*, *R. phaseoli*, *R. lupinii*, and *R. japonicum* are common rhizobia used as inoculants for legume plants [16].
2. Non symbiotic nitrogen fixers (*Azotobacter*, *Azospirillum*) used for cereal crops like maize, oats, sorghum, sugarcane etc. increases yield from 10 to 15% by adding 20-25kg N/ha. Application of *Azotobacter* to crop plants helps in nitrogen supply by free atmospheric nitrogen fixation, synthesis of growth promoters and antibiotics. *Azospirillum* colonizes the roots and aerial parts of host plants by associative symbiosis and it is effective with farm yard manure in sorghum and millets [17].
3. Algal biofertilizers (Cyanobacteria - *Azolla*) used for paddy crop, add 10 to 35kg N/ha; *Azolla* supplies 30-100kg N/ha. *Anabaena*, *Nostoc*, *Plectonema*, *Aulosira*, *Tolypothrix*, *Oscillatoria* etc. are used in combination as dried flakes at the rate of 10kg/ha after tenth day of paddy seedlings transplantation. *Azolla* is aquatic fern that harbors *Anabaena* in leaf as symbiont that is common as green manure in paddy crop [18].
4. Phosphate solubilizers can be used for variety of crops that improve 5 to 30% yield. *Pseudomonas*, *Bacillus* spp. are phosphate solubilizers that convert insoluble form of inorganic phosphates into soluble form that can be easily consumed by applied crop plants [19].
5. Mycorrhizae used for crops and ornamental plants, increases intake of phosphorus, zinc, sulphur and water in the host plants and thereby enhances 10 to 50% yield. The symbiotic association of fungi in plant roots is called mycorrhiza. The fungal association increases nutrient uptake in host plant from soil. Ectomycorrhiza and endomycorrhiza are two types of mycorrhiza. Ectomycorrhiza are common on roots of forest trees like eucalyptus, oak, pine etc. in this association increases phosphorus, potassium and protect from pathogens. Endomycorrhiza are found in the roots of horticultural crops like coffee, betel vine, cardamom etc. and promote phosphorus nutrition [20-21].

Siderophores

The term siderophores is derived from Greek word which means iron carriers. These are ferric ion specific chelating agents synthesized by bacteria, actinomycetes, fungi and certain algae growing in low iron stress. Siderophores are iron binding proteins with molecular weight ranging from

400 - 1500 Da. These compounds play important role in scavenging iron from the environment [22]. Most of the aerobic and facultative anaerobic microorganism synthesizes at least one siderophore. Siderophores have also found to be associated with virulence mechanisms in pathogenic microorganism of animals and plants. These compounds have applications in clinical, agricultural and environmental fields. Presently 500 siderophores are reported from different microorganisms [23]. The important groups of siderophores are:

Hydroxamate siderophores,
Catecholate (phenolates) siderophores and
Carboxylate siderophores

Hydroxamate siderophores possess three secondary hydroxamate groups and each hydroxamate groups provide two oxygen molecules that form a bidentate ligand with iron. These are produced by both bacteria and fungi [24].

Catecholate siderophores are produced by certain bacteria. The catecholate group provides two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed as in the hydroxamate siderophores [25]. The finest example for catecholate siderophore is enterobactin produced by *Escherichia coli*. Carboxylate siderophores are produced by *Rhizobium* and *Staphylococcus* strains and fungi belonging to mucorales, coordinating iron with carboxyl and hydroxyl groups.

Siderophore producing microorganisms

Some important siderophore producing bacteria includes *Escherichia coli, Salmonella, Klebsiella pneumoniae, Vibrio cholerae, Vibrio anguillarum, Aeromonas, Aerobacter aerogens, Enterobacter, Yersinia and Mycobacterium* species.

Aspergillus nidulans, A. versicolor, Penicillium chrysogenum, P. citrinum, Mucor, Rhizopus, Trametes versicolor. Ustilago sphaerogina, Saccharomyces cerevisiae, Rhodotorula minuta and Debaromyces species are siderophore producing fungi [26].
Siderophore producing actinomycetes includes *Actinomadura madurae, Nocardia asteroids, and Streptomyces griseus*. *Anabaena flos-aquae* and *Anabaena cylindrica* produce siderophores [27].

Microorganisms in Renewable energy sources

Biofuels

The limited fossil fuel increased the concern about the fuel economy and much advancement of technologies has been introduced to overcome the shortage of fuels. The biofuels are the fuel production processes which are not only renewable but are also capable of sequestering atmospheric carbon dioxide to achieve environmental and economic sustainability [28]. Biofuels are rapidly being developed in order to meet global energy demand and advanced technique has been introduced to produce biodiesel, bioethanol, biomethane and biohydrogen [29-32].

Present biofuel technologies: Biomaterials and bioenergy have long been produced from plants. The production of methane, hydrogen from various microbes camp up in the early 1950s that led to the development of usage of various bacteria, fungi and algae in the production of biofuels. First generation of biofuel production includes the plant-based biofuel production whereas the second generation is related with algae – based biofuel production. The later generation includes the usage of genetically engineered microorganisms. Fuel biotechnology deals with the production of various biofuels by utilizing microorganisms and biomass [33-36].
Biodiesel production: Biodiesel is currently produced from oil synthesized from conventional fuel crops that harvest the sun's energy and store it as chemical energy. Apart from this the second generation of biodiesel production includes the production of biodiesel from algae. Biodiesel is a diesel like substance obtained from the lipids accumulated in plants and algae or hydrocarbons synthesized by plants and algae.

Oil seed based biodiesel production uses the lipid accumulated in seeds such as sunflower, safflower, peanut, olive, palm, *Jatropha* etc.[37].
Some algae accumulate lipids in their biomass when they are grown under mineral nutrient limitation. Under normal growth conditions most algal species have limited lipid content of 10- 30% dry weight. But during nitrogen depletion the cells stop dividing and store products that result in accumulation of dry weight lipid contents to double or triple. Ex: *Botrycoccus braunii* – 25-75%, *Nannochloropsis* – 31 – 68%, *Schizochytrium* spps – 50 – 77 %, *Neochloris oleaobundans* – 35 – 54%, and *Nitschia* spps -45 – 47% [38-40].

Some plants and algae accumulates hydrocarbons in their cells and some plants belonging to family Euphorbiaceae, milk weeds - *Ascelpias* spps, *Copaifera multijuga* accumulates hydrocarbons. In addition to this some algal members of both fresh and marine water accumulate hydrocarbons [41-43].

Bioethanol: It is most widely used liquid biofuel which is produced by converting sugars directly from crops like sugarcane or sugar beets or starch from corn, wheat, potatoes or through cellulose from biomass into ethanol by fermentation. Traditional bioethanol production includes the fermentative degradation of sugary materials by *Saccharomyces cerevisiae* whereas other microbes includes *Candida* spps, *Zymomonas mobilis* etc.

Current bioethanol production is based on the usage of genetically engineered microbes on cellulose biomass to obtain ethanol. This is the third generation approach of direct cellulose fermentation to produce ethanol through metabolic engineering which may meet the global demand of biofuels. Currently genetically engineered *E. coli*, *Proteus*, *Klebsiella*, *Zymomonas*, and *Clostridia* are generally involved in bioethanol production [44].

Biohydrogen: Hydrogen is one of the biofuels produced from anaerobic microbial systems. The most common approach for converting biomass to hydrogen is bacterial fermentation process essentially a truncated version of methanogenesis. A combination of moderately acidic pH and short solids retention time suppresses methanogens and accentuates hydrogen production [45]. Anaerobic bacteria, cyanobacteria and photosynthetic algae produce hydrogen. Cyanobacteria - *Gleocapsa alphicola* under sulphur starvation shows increased hydrogen production. *Arthrospira*, *Anabaena cylindrica* are also used for hydrogen production. Microalgae - *Scenedesmus obliqus*, *Chlamydomonas reinhardtii* under controlled environment produces hydrogen [46].

Biogas: Biogas is the mixture of gases produced by microbial processes consisting methane, carbon dioxide and hydrogen. Biogas especially biomethane is produced by anaerobic digestion of biomass by methanogenic bacteria, hydrolytic fermentative bacteria and acetogenic bacteria [46].

Bioelectricity

From the times immemorial man has been depending on energy. With improvement of science and technology there is a great demand for energy expenditure as the need and use of fossil fuel has been gradually increasing. Increased utility of fossil fuels due to increased economic growth and social development has lead to a large gap between energy demands and the availability of fossil fuels. These resulted in exhaustion of fossil fuels, as energy is the only driving force over the world and are in search for alternative fossil fuels that is eco-friendly and renewable [47]. The current approaches available to energy generation are not suitable as they possess adverse effects on environment. Thus the world is in search of an approach that can produce huge amount of energy and natural carbon sources.

Microbial Fuel Cell (MFC) technology is the approach to produce electricity employing bacteria using biodegradable substrates. Waste water from brewery, sugar industry, dairy, and paper industry are among the biodegradable substrates. Exoelectrogenic microorganisms such as *Aeromonas*, *Clostridium*, *Desulfobulbus*, *Geobacter*, *Geothrix*, *Geopsychrobacter*, *Pseudomonas*, *Shewanella*, *Ochrobactrum*, *Rhodopseudomonas* and *Rhodoferax* are genera that show the ability to produce electricity by MFC technology [48-49].

MFC is a technique that uses bacteria as catalyst for converting chemical energy (organic matter) into electricity. This technique consists of anode and cathode separated by a cation specific membrane. In the anode compartment metal oxide reducing microorganisms are introduced that oxidize substrate and generate electrons and protons. External circuit is used to transfer the electrons but the protons move through the solution towards cathode, then electrons and protons combine along with oxygen and result in the formation of water molecule [50]. There are three proposed ways for transfer of electrons by microorganisms such as:

1. Use of exogenous mediators like thionine or neutral red,
2. Mediators produced by bacteria,
3. Using direct transfer of electrons from cytochromes (respiratory enzymes).

There are several advantages of MFCs compared to the current approaches used in electricity generation from organic matter, they are as follows:

1. In this technique substrate (chemical energy) is directly converted to electricity with high efficiency,
2. Operation is efficient at ambient temperature,
3. Gas treatment is not required as the microorganisms release gases that enrich carbon dioxide
4. Passive aeration of cathode do not need energy input
5. This can be widely applied in locations that lack electrical infrastructure and also satisfy the energy requirements.

Simple carbohydrates such as glucose fructose are used that gives high conversion and high efficiency compared to the complex sugars like starch, cellulose. Cellulose is preferred as substrate for MFCs as it is most abundant biopolymer available in nature. When cellulose is used as substrate in energy generation, it is first solubilized by hydrolysis into simple sugars [51-53]. Co-culture or mixed culture is performed to hydrolyze the cellulose so that it makes possible to take the simple sugars by microbial cells. Energy generation is rather difficult from cellulose or cellulosic wastes than simple sugars because cellulose is highly resistant to hydrolysis and is insoluble. Lignin in combination with cellulose is very stable for microbial or enzymatic hydrolysis [54].

Conventional methods of isolating exoelectrogenic microorganisms are based on identification of soluble or insoluble metal oxides using organisms in their respiration on agar plates, not all the bacteria that produce electricity in MFCs may use metal oxides. Hence electrochemically active strains of microorganisms are lost and it is a disadvantage. New methods are developed based on dilution to extent that enriches exoelectrogenic microorganisms on anode. Hydrogen fuel cells compared to MFCs are efficient but MFCs are preferred as it produces electricity along with waste water treatment that reduces the cost of the same [55-57].

Microorganisms in Pollution control

Biodegradation of pollutants

Environmental pollution is the resultant of wide range of compounds that are released as a consequence of industrial progress. Multiple hazardous waste sites have been generated as a result of accumulation of xenobiotics in soil and water [58]. Constituents of crude oil like nitro aromatic compounds, polycyclic aromatics and other hydrocarbons along with halogenated organic compounds in combination with large and diverse group of chemicals are responsible for environmental depletion. The removal of these xenobiotics involves physico-chemical processes like excavation, incineration and landfills that are expensive and difficult to execute. These remedial strategies that clean up contaminated sites are not effective or adequate. Microorganisms are recyclers of nature that convert toxic compounds to harmless products mostly carbon dioxide and water. Hence research is increasingly focused on methods that degrade and eliminate the pollutants by biological means.

Biodegradation is a natural cost effective process that helps in removal of xenobiotic compounds by microorganisms from environment. These detoxifying ability possessing microbes play imperative role in biogeochemical cycles of biosphere [59].

Xenobiotics contaminated sites need urgent remedial solutions and search for diverse range of bacteria that utilize these pollutants as substrates. Enzymes, metabolic routes and genes involved in microbial degradation of nitro aromatic compounds, halogenated organic compounds, polycyclic aromatics and other hydrocarbons developed the broad flexibility of microorganisms in clean environment [60]. Studies on interaction between xenobiotics and microorganisms in the environment have interconnected the disciplines of biochemical and genetic engineering.

Use of microorganisms for biological clean up of polluted sites was highlighted in 1989 in accidental oil spill in Gulf of Alaska that contaminated 2000 km coast line with spilling of approximately 41000 m^3. This method of cleaning or restoration of contaminated sites was found to be self sustaining and inexpensive [61]. Bacterial communities were characterized and their responses to pollutants, isolation of potential degraders and identification of genes involved in degradation were studied. Two broad categories were studied in detail analysis of microbial diversity in a given environment: culture dependent and culture independent (Tab-1). Contaminated environments harbor wide range of unidentified pollutant degrading microorganisms that play crucial role in biodegradation and can be assessed by culture independent techniques [62].

Pseudomonas, *Escherichia*, *Sphingobium*, *Pandoraea*, *Rhodococcus*, *Gordonia*, *Bacillus*, *Moraxella and Micrococcus* are among the aerobic bacterial genera that degrade wide range of xenobiotics. *Pelatomaculum*, *Desulfotomaculum*, *Syntrophobacter*, *Syntrophus*, *Desulfovibrio*, *Methanospirillum*, *Methanosaeta* are anaerobic bacterial genera. *Pseudomonas* Bcb 12/1 and Bcb 12/3 are found to be excellent degradation abilities with high cell viability [63]. Bacteria possess strategy for gaining energy from xenobiotic compounds under aerobic or anaerobic conditions by using ultimate electron acceptors like nitrate, sulfate and ferric ions.

Table 1: Environmental factors required for microbial degradation

Factors	*Optimum value*
Temperature (°C)	15 - 45
pH	5.5 - 8.8
Moisture (%)	25 - 28
Nutrients	Nitrogen & Phosphorus
Oxygen content	Aerobic, air-filled pore space 10%
Type of soil	Clay or silt
Heavy metals	2000ppm

Chemical structures in most commonly found in xenobiotics are benzene rings and glucosyl residues. The resonance structures of xenobiotic compounds are efficiently degraded either aerobically or anaerobically. Genes for catabolism of aromatic compounds are found in mobile genetic elements such as transposons and plasmids (Tab-2). These genetic elements are horizontally transferred that result in adaptation of bacterial genera to pollutants [64].

Table 2: *Pseudomonas* plasmids with degradative ability

Plasmid	*Target compound*
SAL	Salicylate
TOL	Xylene, Toluene
CAM	Camphor
OCT	Octane, Decane
NAH	Naphthalene
pJP2, pJP4, pJB3	2, 4-Dichlorophenoxyacetic acid (2,4-D)
pWR1, pAC25	3-Chlorobenzoic acid

Xenobiotics are synthetic organic compounds that do not occur in nature and remain foreign to living organisms. Most of these compounds are not recognized by existing degradative enzymes and will accumulate in soil and water. Accumulation of xenobiotics in soil and water is a problem of increasing importance. Herbicides, pesticides, fungicides, nematicides and insecticides are common xenobiotics. Xenobiotics are mostly substituted hydrocarbons, phenyl carbonates and other compounds. The combination of microbes effectively degrades the xenobiotics than individual organism [65]. Xenobiotics are chemicals that are not present normally in the ecosystem and synthesized by human activities.

Table 3: Common Xenobiotic classes and their sources

Xenobiotic class with examples	Sources
BTEX Ex: *Benzene,* *Toluene,* *Ethyl benzene,* *Xylene*	Chemical industries, Oil/gas working areas Railway yards Airports
Chlorinated solvents	Drycleaners,

Ex: *Trichloroethylene, Perchloroethylene*	Laundries Electrical goods manufacturers Chemical manufacture
Polychlorinated biphenyl Ex: *4-Chlorobiphenyl, 4,4-Dichlorobiphenyl*	Electrical goods manufacturers Power stations Railway yards
Chlorinated phenols Ex: Pentachlorophenol	Timber mills Landfills Municipal wastes
Polychlorinated hydrocarbons (PAHs) Ex: *Naphthalene, Anthracene, Fluorene, Pyrene*	Power stations, Oil/gas working sites, Engine works
Nitroaromatic compounds Ex: *2,4,6-Trinitrotoluene (TNT),* *Octahydrotetranitrotetrazine*	Pharmaceutical manufacturers, Dye manufacturers, Pesticide manufacturers
Pesticides Ex: *Diazinon, Parathion, 2,4-D, Glycophosphate, Carbofuran, Atrazine*	Pesticide manufacturers Timber mills Agricultural practices Landfills

This term is very often used in the context of pollutants. Generally these are metallic/organic residues or by-products that enter into environment from industrial, agricultural, domestic, municipal activities and exert adverse effects and disturbances to the ecosystem (Tab-3). These xenobiotics are degraded by various microorganisms in the process of biodegradation but some resist the processes that are termed as recalcitrant molecules. These molecules persist in the soil for 3 to more than 20 years. Polychlorinated biphenyls that carry more than seven benzene rings and four chlorine atoms are recalcitrants that resist the biodegradation under normal environmental conditions [66].

Biochemical pathways of pollutants biodegradation

There are two broad categories of biochemical pathways involved in the breakdown of xenobiotics, they are: aerobic or anaerobic. Wide range of bacterial species are found to be associated with aerobic degradation of xenobiotics very efficiently but the contaminated sites are anaerobic hence the metabolizing bacteria are commonly anoxic. Microbes metabolize these compounds with typical process known as co-metabolism, here the organism transform the compound without using it as energy source. In this process organisms require the primary substrates that will support their growth. The metabolites (enzymes) released in response to primary substrate show the activity for another substrate in significant manner and these substrates are termed as co-substrates [67].

Aerobic metabolic pathway: In carbon cycle aromatic hydrocarbons have an immense turnover due to aerobic catabolic mechanisms. The aerobes degrade by releasing oxygenases that induce elemental oxygen to activate the inert xenobiotic compound. This pathway forms the intermediates such as catechol, hydroquinone, homogentisate, procatechuate and hydroxyquinol, which further are metabolized to tri-carboxylic acid cycle intermediates and finally converted into carbon-dioxide and water. Alkane degradation by aerobic means occurs with oxidation of terminal methyl groups into carboxylic acid with an alcohol intermediate and finally it ends with β-oxidation. The enzymes like esterases followed by permeases act on the phthalate isomers degradation. 3, 4-dihydroxybenzoate is formed by the action of dioxygenases which are further processed into acetyl-coA and succinyl-coA as end products [68].

Anaerobic metabolic pathway: *Clostridia*, *Desulfobacterium*, *Desulfovibrio*, *Methanococcus*, *Methanosarcina* are found among the popular anaerobic bacteria that degrade xenobiotics compounds in anoxic conditions. In this process an alternative oxygen is used to oxidize the aromatic hydrocarbons like phenols, phthalates, benzene-toluene-ethyl benzene-xylene (BTEX). These compounds themselves act as terminal electron acceptors, support microbial growth by energy generation from oxidation of simple substrates. Reactions such as dechlorination, hydrolysis, nitro reduction and dealkylation occur during anaerobic degradation of pesticides [69].

All halogenated pesticides including aliphatic, cyclic aliphatic, aniline based phenoxy alkanote and cyclodiene types undergo reductive dechlorination in which pesticides themselves act as terminal electron acceptors and these metabolic reactions are termed as halorespiration. Anaerobic bacterium *Dehalococcus ethenogenes* dechlorinate tetra chloro ethylene to ethene by inducing novel pathway of reductive dehalogenases. Benzoyl coenzyme-A pathway is important mode of denitrifying anaerobes to degrade azo dyes, carbon tetrachloride and cyclo trimethylene trinitramine (RDX) by releasing benzoate co-A ligase that subsequently break the ring by hydratase enzyme. Methanogens like *Methanospirillum*, *Methanosaeta*, and *Syntrophobacter* degrade phthalate compounds with acetate and methane as end products [70].

Co-metabolic pathway: This concept was initiated by studies on *Pseudomonas methanica* that oxidize ethane to acetic acid, propane to propanoic acid and butane to butanoic acid. Co-metabolism parallely oxidizes co-substrate during growth on a compatible carbon and energy source. Oxidation of co-substrate do not provide energy to the degrading organism hence it needs additional carbon source. *Nocardia, Pseudomonas, Xanthomonas*, *Bacillus*, *Brevibacterium*, *Flavobacterium*, *Azotobacter*, *Vibrio*, *Achromobacter*, *Arthrobacter*, *Hydrogenomonas*, *Microbacterium, Micrococcus* and *Streptomyces* strains exhibit the phenomenon of co-metabolism [71].

In this process contaminant is degraded by an enzyme or cofactor released by microbial metabolism of another compound. Co-metabolism makes the removal process to stimulate biodegradation of contaminant at low concentrations of carbon or energy. This process could be applied as aerobic or anaerobic way on wide range of contaminants in variety of diverse environments. The term co-metabolism was first introduced by Wilson and Wilson in 1985 [59]. This approach was used in the field for more than 20 years on xenobiotics such as halogenated aliphatic and aromatic hydrocarbons, pesticides, explosives, chlorinated alkenes and PAHs [72].
Degradation of xenobiotic compounds is dependent on enzyme of oxygenases class including: methane monooxygenase, toluene dioxygenase, toluene monooxygenase and ammonia monooxygenase are strong oxidizers that are released by aerobic organisms during co-metabolism. Among all the oxygenases, methane monooxygenase is well known to degrade approximately 300 different xenobiotic compounds [73].

Hydrocarbons

Benzene, toluene, ethyl benzene and xylene are grouped as BTEX and polycyclic hydrocarbons (PAHs) are most common subsurface contaminants termed as monocyclic aromatic compounds [74]. Wide range of aerobic bacteria are available that degrade the aromatic compounds. These bacteria cleave the aromatic ring by action of oxygenases. The compound is transformed to intermediates followed by that ring is reduced and finally cleaved by hydrolysis. Denitrifiers and sulfate reducers and fermentative bacteria are anaerobic group of organisms that can utilize monocyclic aromatic hydrocarbons [75].

Aliphatic hydrocarbons: These are straight chain, branched chain and cyclic carbon groups. These are compounds from carbon number (C_8 – C_{44}) degraded by bacteria such as - *Acinetobacter*, *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Arthrobacter*, *Flavobacterium*, *Bacillus* etc [76].

Aromatic hydrocarbons: These are compounds with benzene ring in their structures and very common in the environment. These are very stable and inert in nature but many bacterial species were detected that utilize them as source of energy. Lower aromatic compounds are more susceptible than higher compounds for microbial attack. *Pseudomonas*, *Sphingomonas*, *Flavobacterium*, *Burkholderia* and *Ralstonia* were demonstrated to degrade aromatic hydrocarbons [77].

Halogenated organic compounds:

These are the largest and diverse group of environmental pollutants generated by naturally occurring biotic and abiotic processes in atmosphere [78]. Chloroaromatics, haloalkanes and chloroethenes are among the recalcitrant toxic halocarbons in environment. Low electron density and oxygenase enzyme lack ability initiates the degradation pathway of halogenated compounds. Microbial enzyme systems were detected that cleave the carbon-halogen bonds and their by fulfill the carbon source requirement or alternative electron acceptor [79].

Chlorinated or brominated alkanes and alkenes (halogenated alkanoic acids, haloalkanes, trichloroethane and ethylene dibromide) are halogenated aliphatic hydrocarbons that are released from industrial and agricultural sources [80]. These are predominant in ground water at the sites of hazardous wastes and landfill leachates. Vinyl chloride, tri and tetrachloroethylene are commonly detected in the drinking water aquifers. Ethylene dibromide is a brominated hydrocarbon used as soil fumigant and reported to pollute the aquatic environments. Soil microorganisms that are capable to synthesize dehalogenases enzyme are efficient in degrading the halogenated compounds [81].

Chlorinated phenols like dibenzofuran and dioxins are toxic compounds produced during the manufacture of pesticides, pulp and paper mills that are resistant to degradation and accumulate in soil. Pentachlorophenol are used as biocide to preserve wood, leather and textile possess toxicity and remain as recalcitrants. *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Micrococcus*, *Deinococcus*, *Alloiococcus* and *Klebsiella* are found to degrade the chlorinated compounds [82].

DDT, chlorinated polycyclic hydrocarbons, polychlorinated biphenyls and p-chlorobiphenyls are wide spread environmental contaminants. Polychlorinated biphenyls are degraded by *Alcaligenes*, *Pseudomonas* and *Klebsiella*.

Polycyclic Aromatic Hydrocarbons (PAHs)

These are the group of wide spread chemical compounds as pollutants in environment. These compounds possess acute toxicity mutagenic, teratogenic and carcinogenic effects on animals. Wood distillation, gas works, oil refineries operation, emissions of vehicles, petroleum spills, coking of coal, tobacco smoke and partial combustion of fossil fuels are important sources of polycyclic aromatic hydrocarbons. The chemical features and environmental fate of PAH molecule depends on their number of aromatic rings and the pattern of ring linkage [83]. The compounds with 5 and 6 rings such as benzo (a) pyrene (BaP) and benzo (ghi) perylene are of importance among the PAH's. Hydrophobic nature and electrochemical stability are two factors involved in the persistence of PAH in the environment. Increase in number of benzene rings also increases genotoxicity and environmental persistence [84].

Half-life of PAH molecule in soil sediment is like three-ring phenanthrene ranges from 16 to 126 days but the five-ring BaP molecule range is from 229 to >1,400 days. In soil sediments PAH molecules are subjected to natural weathering processes like photo oxidation, dissolution and adsorption [85]. Various aerobic and anaerobic microorganisms like 60 bacterial genera and 80 fungal species were found among the active hydrocarbon degraders. Bacterial species like *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, *Rhodococcus*, *Corynebacterium*, *Moraxella*, *Bacillus*, and *Micrococcus* are commonly reported PAH degraders. Aerobic biochemical pathway of PAH by bacteria begins with multicomponent enzyme system that oxidize PAH to dihydrodiol which is further processed through *ortho* or *meta* cleavage pathway [86].

*Nitroaromatic compounds***:** Solvents used for amino aromatic derivatives precursors and synthesis of dyes in industrial process, plasticizers, pharmaceuticals, pesticides and explosives are the sources of nitro aromatic compounds [87]. These compounds are readily reduced to yield potentially reactive mutagenic or carcinogenic derivatives. Nitrobenzene, dinitrotoluenes and mono- and dinitrophenols are highly toxic forms of nitro aromatic compounds. Common enzymes like mono- and dioxygenases, dehydrogenases produced by bacterial species – *Pseudomonas*, *Nocardia* and *Arthrobacter* evolved the catabolism of nitro aromatic compounds [87].

2,4,6-trinitrotoluene (TNT) is most important among the nitro aromatic compounds resultant of munitions. Anaerobic bacteria such as *Clostridia*, sulfate reducers and methanogens were found to reduce nitro groups with a co-metabolic process [88].

Bioremediation

This is one among the important technologies that protect natural resources on earth by cycling natural and environmental chemical pollutants. This process clean up the ecosystem threatening contaminated sites by breakdown of pollutants that cannot be degraded by traditional disposal methods [89].

Bioremediation can be defined as process involved in degradation of various pollutants by employing biological methods. Microorganisms, plants and various biological catalysts are employed in degradation of toxic environmental pollutants [90].

The use of microorganisms for cleanup of contaminated environment has been in practice from years together. Land fill is a traditional technique used to destroy the wastes generated that contribute as practice for cleaning contaminated sites. These landfills are the natural or artificial pits used to fill with wastes then capped and often landscaped. Chemical decomposition, incineration and biological decomposition are other traditional methods of cleaning of the wastes [91].

Bioremediation and its need: The advances in industrialization, explosive development of chemical industries and modernization of life styles caused global deterioration of environmental quality. In order to cope up the quality of environment and achieve the safe life it is necessary to clean up the contaminants and pollutants from the environment [92]. Currently microbial systems are most widely employed in bioremediation programmes to treat surface and subsurface pollutants in the ecosystem. This approach to treat the potential contaminants on the site offers benefits over other physical and chemical techniques. This is relatively cheaper than other techniques but due to lack of understanding of the behavior of microbial populations in natural environments and effect on physical, biological and chemical factors that control their activity limits the applications of bioremediation practices against the environmental pollutants. Many recent technologies are now available in order to overcome the above problems and now the bioremediation is a more promising and less expensive way for cleaning up the contaminated surface and subsurface areas [93].

Surface / Subsurface environmental pollution and bioremediation practices: Vast numbers of pollutants and waste materials have been discharged into the environment per annum. Due to industrialization, modernization, globalization, urbanization and increase in the population, enhanced the utility of natural sources of environment and at the same time increased the disposal of used and waste contaminants into the environment. These contaminants cause damage to the environment and disturb the natural ecosystem. Approximately $6x10^6$ chemical compounds have been synthesized with 1000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to the third world network reports, more than one billion pounds of contaminants are released globally in air and water. These contaminants are causing ecological problems leading to imbalances in natural ecosystem. This environmental crisis made man to overcome with proper public awareness and a safest solution is the bioremediation [94].

The surface environmental contamination is due to the disposal of industries, commercial and household wastes into the soil. Usually the contaminated sites are treated with physical, chemical processes but these processes are cost effective and sometimes it is incomplete elimination. These conventional treatment technologies simply transfer the pollutants creating a new waste such as incineration residues. The subsurface contamination is also one of the major problems that threaten the drinking water resources [95]. This is mainly due to leakages, spills, improper disposal and accidents during transport, organic compounds into the subsurface areas. Ground water is one of the most vital sources of drinking water that is also been contaminated due to the leakage of petroleum hydrocarbon from underground tanks. The natural organic subsurface products coal and crude oils are used as energy sources. Leakage of underground crude oil storage tanks and pipelines results in contamination of subsurface areas. The toxic activities of organic compounds like benzene, toluene, xylene, etc. have serious effects on human health [96]. Bioremediation practices: various bioremediation practices have been used successfully along with other cleanup methods [97]. These practices are aimed to satisfy environmental effectiveness, public, cost etc. Bioremediation practices can be grouped into two types: In-situ practices and Ex-situ practices

In- situ practices: these practices are performed at the contaminated sites avoiding the transport of contaminants. These are more convenient due to less disturbance and low in cost [98]. The In-situ bioremediation practices depend on several factors that include:

1. Type of microflora that degrade contaminants,
2. Type of contaminants,
3. Nutrient availability,
4. Environmental conditions,
5. Time required for the natural degradation of contaminants

Some of the In-situ bioremediation practices include:

a. Biostimulation
b. Bioaugmentation

Biostimulation: It involves the stimulation of numbers and activity of natural microflora the bacteria or fungi in order to degrade the contaminants. This practice includes the enhancement of conditions to favor the growth of microbes that are already present in the contaminant sites so that the contaminants are naturally degraded by the microbial flora [99].

Bioventing is one of the biostimulation practices that include the supply of low air flow to the contaminated soil by providing the oxygen required for the biodegradation of contaminants [100].

Biospraying is injection of air under pressure at the contact site which is between the soil and ground water, thereby increasing the oxygen concentrations of ground water and enhancing the rate of biodegradation of contaminants [101]. Apart from this In-situ practice includes the addition of nutrients, adjusting the environmental conditions like pH, temperature, water availability etc.

Ex-situ practices: These practices include the removal of contaminants and transporting to a specific site of degradation [102]. Specialized techniques in order to clean up of environmental sites are found at the priority in this approach. The following are the Ex-situ practices used for bioremediation:

- Land forming
- Composting
- Compost piles
- Bioreactors or biovessels

Land forming: This is a practice in which the contaminated soil is spread over an area and incubated, until the contaminants are degraded. The indigenous and exogenous microflora of superficial soil degrades the contaminants. It is the simplest form of practice generally used to dispose the industrial and municipal wastes generated [103].

Composting: This is a practice in which the contaminants are mixed with non toxic agricultural and domestic wastes and treated with microbial flora. The degraded material is used as manure or fertilizer that is commonly called as compost.

Compost piles: These are specialized artificial cells used to treat surface contaminants. These cells provide favorable environmental conditions to enhance the degradation capacity of microbial flora [104].

Bioreactors or biovessels: This includes the use of specific bioreactors. Different varieties of bioreactors are employed in order to treat the contaminants [105]. This practice provides rapid and effective degradation of contaminants but it is relatively high cost effective.

Table 4: Comparison between Bioremediation practices

Technique	*Advantages*	*Limitations*
In-situ	Noninvasive Natural attenuation process Used for soil & water treatment Cost efficient	Extended time for treatment Difficult in monitoring
Ex-situ	Can done on site Low cost	Need to control abiotic loss Mass transfer problem Extended time for treatment Limitation in bioavailability
Bioreactors	Degradation is rapid Inoculants & surfactants used effectively Enhances mass transfer	Need soil excavation Highly expensive High operating cost

Bioaugmentation

This technique was started based on the fact that inoculation of legumes with symbiotic nitrogen fixer *Rhizobium* species or free living nitrogen fixers like *Azotobacter* or *Azospirillum* increases the plant yields [106]. The application of plant growth promoting microorganisms protected plants from pathogens by antagonism [107]. Bioaugmentation is attempted with inoculation of microorganisms successfully to degrade several pesticides (atrazine, dicamba and carbofuran), chlorinated solvents, polycyclic aromatic hydrocarbons and compost piles and septic tanks [108].

There are several approaches in the bioaugmentation such as:

Cell bioaugmentation
Gene bioaugmentation
Microbial-material derived bioaugmentation
Phytoaugmentation

Cell bioaugmentation - Cells are delivered into the contaminated soils by:

1. Use of carrier materials: like biosolids, charcoal-containing soil, clay, lignite, manure and peat. In this inoculants are combined with carrier material to soil that provide a protective niche and temporary nutrition for the organism. Carrier material preserves the shelf life of inoculant in encapsulated state. Long shelf life, non-toxic nature, target cell introduction are important characteristics required in ideal carrier material [109].
2. Use of encapsulated microorganisms: Materials like acrylate copolymers, agarose, alginate, gelatin, gellan gum, carrageenan, polyurethane and polyvinyl alcohol gel are used to immobilize or encapsulate cells. Encapsulation allows microorganisms to remain in a relatively non-toxic matrix through which gases and liquids can diffuse [110]. Potential benefit of encapsulation is the creation of microsites with unique microbial community that interactively remediates a given compound. Alginate is commonly used as carrier for remediation of contaminants like chromium, cresol, nitrate, pentachlorophenol, phenanthrene, phenol, phosphate and 2,4,6-trichlorophenol [111].
3. Use of activated soil: In this method both inoculant and carrier are mixed by use of activated soil. Soil that has been previously exposed to contaminant of interest and contains developed microbe degrader populations that remove the contaminant is termed as activated soil. Presence of natural degrader population and degraders have ability to compete in the environment are potential advantages of activated soil [112].

Gene bioaugmentation: Horizontal gene transfer plays important role in microbial development and adaptation in the environment. Horizontal gene transfer occurs via naked DNA, bacteriophage or physical contact and exchange of genetic material such as plasmids or transposons between microorganisms. The microorganisms introduced in bioaugmentation do not survive in contaminants, hence the horizontal gene transfer carried in the naturally occurring indigenous microorganisms in the contaminated site. As the indigenous organisms are already adapted to survive and proliferate in the contaminated environment [113-114].

Microbial-material derived bioaugmentation: In this approach biosurfactants or enzymes are directly applied either single or in combination with a microbial inoculant. Biosurfactants are used for remediation metal and organic-contaminated material. This application either protects inoculant from metal toxicity or increases the availability of organic substrate for degradation [115].

Phytoaugmentation: as the appropriate microorganisms may not be present at a given site and the bioaugmentation becomes difficult, hence the microbial genes for remediation processes are inserted directly into the plants.

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