ECO-BIOTECHNOLOGIES

ACADEMIC COURSE **ADVANCES IN BIOREMEDIATION**

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LO 1: INTRODUCTION TO REMEDIATION TECHNOLOGY

1. Introduction to remediation technology

Our environment is contaminated by chemical compounds harmful to all biological systems. Uncontrolled industrial development, population growth, urbanization, higher demand for food, salinization of agricultural lands and release of hazardous chemicals are the major contributors of today's environmental pollution problems. There is an international need for environmental friendly practices and a growing demand for restoration of contaminated sites.

1.1. Terms in Remediation

Pollution is the discharge of a toxic or contaminating substance that is likely to have an adverse effect on the natural environment or life. **Contaminant** makes a place or a substance (such as water, air, or food) no longer suitable for use. **Remediation** restores contaminated site to return them to their natural state. **Environmental remediation** is the removal of pollution or contaminants from soil, water (both ground water and surface water) and air. These waste products are removed for the protection of human health, as well as to restore the environment. These cleaned up sites can also be used for urban development. Environmental remediation is highly regulated and subject to an array of legal requirements, which are generally based on assessments of human health and environmental risks. Remediation projects can range from large, expensive projects, on which a great deal of effort is spent to clean up contaminated sites, to smaller, less costly projects, such as cleaning up a highway accident in which oil is spilled. Remediation projects usually begin with a site assessment to determine the costs of the project, as well as the technology that would be the most appropriate for the particular site.

Environmental remediation is carried out on various environmental media, including soil (topsoil, subsoil and sediment), water (groundwater and surface water) and air. **Soil contamination** can result from chemical spills, industrial activity, and the use of certain fertilizers and pesticides. Soil contamination is caused by many of the same factors that cause groundwater contamination. **Water contamination** may be the result of industrial practices (mining or drilling for natural gas and oil) and release of pollutants directly into the water or by runoff from the ground. **Air contamination** is caused by any substance that people introduce (greenhouse gases such as carbon dioxide (CO_2), methane, sulfur dioxide (SO_2) and chlorofluorocarbons) or natural causes (forest fires, volcanic eruptions, wind erosion, pollen dispersal, evaporation of organic compounds, and natural radioactivity) into the atmosphere.

Soil remediation refers to strategies that are used to purify and revitalize the soil. **Water remediation** is the process of removing contaminants from water. Often, the soil and groundwater are contaminated from the same source (chemical spills, industrial activity, and the use of certain fertilizers and pesticides) and both must be remediated at the same time. **Air remediation** is the process of removing the presence of pollutants from the air.

The available remediation technologies for contaminated environment are mainly divided into two groups such as *in situ* and *ex situ*. ***In situ* technology** involves treating the contaminated material at the site. ***Ex situ*** technology is a remediation option where the contaminant is removed from its original location and cleaned on-site or off-site. Various remediation technologies can be used to remove contaminants from the environment. The methods used at a particular site depend on the type and extent of the pollution, as well as the characteristics of the site itself. There are many different remediation methods, and new technologies are regularly being developed.

1.2. Classes of Remediation

The remediation can be divided into two methods such as conventional and biological.

Conventional remediation methods are:

- Excavation and dredging
- Soil vapor extraction
- Solidification and stabilization
- Soil washing
- Air sparging
- Pump and treat
- Chemical oxidation
- Incineration

Biological remediation method is:

- Bioremediation

1.2.1. Conventional Remediation Methods

1.2.1.1. Excavation and dredging

This technique involves removing contaminated soil or other materials from a polluted site. The most common form of soil remediation process is **excavation**. This can be as simple as hauling the soil away and replacing it with uncontaminated soil, or it might involve more complex processes such as aeration. This depends on the contaminant.

Dredging is the physical removal of contaminated sediments from freshwater or marine water in order to reduce risks to human health and the environment. Dredging depends on environmental conditions (the hardness and quantity of material to be dredged, site exposure, the method of disposal, etc.), contaminant types and the degree of contamination of sediments. Releases of contaminants associated with dredging can occur in particulate, dissolved or volatile fractions, each characterised by differing transport and/or exposure pathways. Different types of dredging equipment and techniques are employed to achieve the required project outcomes in the most efficient way.

1.2.1.2. Soil vapor extraction

Soil vapor extraction (SVE) technology uses vacuum pumps to create a movement of air in the soil matrix that remove the contaminant, which is distributed through all the soil phases (Grasso, 1993). The extracted vapours are then treated and discharged to the atmosphere or re-injected into the subsurface if permitted. SVE is more commonly used remedial approach for removal of contamination from the soils when excavation is not feasible due to presence of some physical barriers (building, tree, etc.), or where the extent of soil contamination is extensive. The contaminanted soil and groundwater can be purified at the same time by SVE technique.

1.2.1.3. Solidification or stabilization

This technique involves mixing of a contaminated media with a specific binder in order to convert hazardous constituents into less soluble, mobile or toxic forms by incorporating waste into a solidified matrix. This technology is currently being used to treat a wide variety of wastes from different industries, such as sludge from the tannery and from the wastewater treatment plants of electroplating and metal finishing industries. These wastes usually contain heavy metals, organics and soluble salts.

1.2.1.4. Soil washing

This process refers to *ex situ* or *in situ* techniques that employ physical and/or chemical procedures to extract metals contaminants from soils. **Soil washing** procedure is mostly suitable for granular soils with less clay content and contaminated with inorganic pollutants (heavy metal). Soil washing is one of the few permanent treatment alternatives to remove metal contaminants from soils. If the contaminants are organic, it is necessary to remove organic pollutants and then certain solvents or surfactants are used as washing agents.

1.2.1.5. Air sparging

In this technique air is injected through a contaminanted site. This injected air helps to flush (bubble) the contaminants up into the unsaturated zone where a vapor extraction system is usually implemented in conjunction with air sparging to remove the generated vapor phase contamination. **Air Sparging** is an *in situ* remedial method that is used to reduce lighter molecule constituents (benzene, ethylbenzene, toluene and xylene) from petroleum products like gasoline from the saturated soil zone. Air sparging is a cost-effective, time-efficient system for the remediation of volatile and/or biodegradable contaminants.

1.2.1.6. Pump and treat

This technique involves pumping contaminated ground water out of the ground and purifying it before returning it to the ground. Besides, **pump and treat** (PAT) is able to provide a vital alternative with fast response time, high reliability and adaptability. PAT has been widely used in groundwater remediation and aquatic restoration due to its excellence in pollutants removal and contaminants migration control.

1.2.1.7. Chemical oxidation

Chemical oxidation aims to mineralize the pollutants to CO_2, water (H_2O) and inorganics, or at least to transform them into harmless or biodegradable products. Chemical oxidization process has been used for decades in the waste-water industry for the treatment of carbon-containing compounds, such as petroleum hydrocarbons and chlorinated solvents, and numerous other contaminants.

1.2.1.8. Incineration

Incineration is a thermal treatment (high temperature) process for destroying hazardous waste materials. Incineration of waste materials converts the waste into ash, flue gas, and heat. The ash is mostly formed by the inorganic constituents of the waste, and may take the form of solid lumps or particulate carried by the flue gas. The flue gases must be cleaned of gaseous and particulate pollutants before they are dispersed into the atmosphere.

1.2.2. Biological Remediation Method

1.2.2.1. Bioremediation

Bioremediation is the use of organisms to remove pollutants from the soil, water and air. This is done either by treating contaminated materials at the site or by removing contaminated materials that are then treated elsewhere. Different organisms (bacteria, fungi, algae and plant) are used to remove contaminants and are usually uniquely suited for certain types of chemicals.

2. Hazardous environmental contaminants

A hazardous contaminant is a substance or energy introduced into the environment that has undesired effects, or adversely affects the usefulness of a resource. Environmental contaminants may cause long or short term damage by changing the growth of plant or animal species or by interfering with human amenities, comfort, health, or property values. Some hazardous contaminants are biodegradable and therefore will not persist in the environment in the long term. Contaminants with high persistence in the environment have to be cleaned or remediated to eliminate their potential risks to sustained environmental health. Major sources of contaminants polluting soil, water, and air are:

2.1. Fossil Fuels (Petroleum, Natural Gas and Coal)

Coal, oil and gas are three major forms of fossil fuels non-renewable sources of energy formed from the organic remains of plants and animals buried under earth for millions of years. Fossil fuels consist largely of carbon and hydrogen. The burning process actually is chemical reactions with oxygen in the air. For the most part, the carbon combines with oxygen (O) to form CO_2, and the hydrogen (H) combines with oxygen to form water vapor. The released CO_2 is the cause of the greenhouse effect.

Coal is a solid combustible material consisting of organic matter and minor amounts of inorganic materials. There are basically four types of coal, each of which varies in terms of its heating value, chemical composition, ash content, and geological origin. The four types of coal are anthracite, bituminous, sub-bituminous, and lignite. Many hazardous pollutants are released when coal is burned. Major pollutants are: SO_2, nitrogen oxides (NOx), carbon monoxide (CO), particulates, hydrocarbons, ozone (O_3), volatile organic compounds, toxic metals (cadmium (Cd), arsenic (As), nickel (Ni), chromium (Cr), and beryllium (Be). As coal is burned, several pollutants linked to the environmental problems of acid rain, urban ozone, and global climate changes are released. Noncombustible mineral content of coal is partitioned into bottom ash and fly ash. Flue gases are also released from the combustion of this fossil fuel.

Bottom ash and boiler slag are composed principally of silica, alumina, and iron (Fe), with smaller content of calcium (Ca), magnesium (Mg), sulphates, and other compounds. Due to the inherent salt and heavy metal content and in some cases low pH, this material may exhibit corrosive and toxic properties. Elements showing enrichment in bottom ash or slag include Barium (Ba), Be, Cobalt (Co), Manganese (Mn), Cesium (Cs), Copper (Cu), Nickel (Ni), Strontium (Sr), Tantalum (Ta), Vanadium (V), Tungsten (W), Europium (Eu), Hafnium (Hf), and Zirconium (Zr) concentrated in part by density segregation effects. Oxides of slicon (SiO), aluminum (Al), Fe, and Ca comprise more than 90% of the mineral component of typical fly ash. Mg, potassium (K), sodium (Na), titanium (Ti), and sulphur (S) are minor contituents. They account for about 8% of the mineral component, while trace constituents such as As, Cd, lead (Pb), mercury (Hg), and selenium (Se) together make up less than 1% of the total composition of coal. Flue gases from coal combustion consist of mainly uncombusted nitrogen, carbon dioxide, and water wapor.

The discovery of crude oil, in the 19th century, produced an inexpensive liquid fuel source that aided in the industrialization of the world and the improvement of living standards. The petrochemical industry is a major source of hazardous organic wastes, produced during the manufacture or use of hazardous substances. The recovery, transportation, and storage of raw oil or petrochemicals are major sources of hazardous wastes, often produced as the consequence of technological accidents. Sea water and fresh water pollution due to oil and oil-product spills, underground or soil pollution due to land spills or leakage from pipelines or tanks, and air pollution due to incineration of oil or oil sludge, are major cases of environmental pollution. Gasoline is the main product in the petrochemical industry and consist of ~70% aliphatic linear and branched hydrocarbons, and 30% aromatic hydrocarbons, including xylenes, toluene, di- and tri-methylbenzenes, ethylbenzenes, benzene, and others. Other pure bulk chemicals used for chemical synthesis include formaldehyde, methanol, acetic acid, ethylene, polyethylenes, ethylene glycol, polyethylene glycols, propylene, propylene glycol, polypropylene glycols, and such aromatic hydrocarbons as benzene, toluene, xylenes, styrene, aniline, phthalates, naphthalene, and others.

Natural Gas is a combustible mixture of gaseous hydrocarbons that accumulates in porous sedimentary rocks, especially those yielding petroleum. Natural gas is primarily composed of methane, but also contains ethane, propane, butane and heavier hydrocarbons. It also contains small amounts of nitrogen (N), CO_2, hydrogen sulphide (H_2S) and trace amounts of water. The most toxic components (propane and butane) in the mixture are present in small amounts. The leakage of methane, a potent global warming gas, during the drilling and extraction of natural gas from wells and its transportation in pipelines is an important environmental hazard. It burns cleaner than other fossil fuels. The combustion of natural gas produces negligible amounts of S, Hg, and particulates.

2.2. Industrial Waste

Industrial wastes are identified as harmful wastes due to their hazardous inorganic and organic constituents. These wastes exhibit common features of hazardous wastes such as harming human health or vital activity of plants and animals (acute and chronic toxicity, carcinogenicity, teratogenicity, pathogenicity, etc.), reducing biodiversity of ecosystems, flammability, corrosive activity, ability to explode, and so on. Hazardous industrial wastes include oil-polluted soil and sludges, hydroxide sludges, acidic and alkaline solutions, sulfur-containing wastes, paint sludges, halogantaed organic solvents, nonhaloganeted organic solvents, galvanic wastes, salt sludges, pesticide-containing wastes, explosives, and waste waters and gas emissions containing harmful substance. Secondary wastes are produced from the collection, treatment, incineration, or disposal of hazardous industrial wastes, such as sludges, sediments, effluents, leachats, and air emissions. These secondary wastes may also cause soil, water, and air pollution.

2.3. Municipal Waste (Solid Waste and Sewage)

Solid waste (trash or garbage) is generally produced by individuals and include unneeded or broken personal items, spoiled food, paper, garden plants, plastics, metals, textiles, etc. Municipal solid waste may contain substances potentially harmful to human health and the environment.

Sewage is a highly complex mixture of wastes, usually dominated by fecal materials (organic matter) but also containing toxic chemicals (ions of metals, pesticides, other toxic chemicals, etc.) that have been dumped into the disposal system by industries and home owners.

2.4. Agricultural Waste

Agricultural waste is generally produced by various agricultural operations such as manure (poultry houses and slaughterhouses) and other wastes from farms (harvest waste, fertilizer run- off from fields, pesticides that enter into water, air or soils, and salt and silt drained from fields. According to Conserve Energy Future, agricultural pollution is a primary source of pollution in water and lakes, as chemicals and toxins make their way into groundwater upon settling at the bottoms of these large water bodies. The accumulation of these substances ultimately leads to health issues such as blue baby syndrome and neurological ailments. Agricultural waste can also negatively affect aquatic animals and plants because fertilizers, manure, ammonia and waste make their way into local environments and disrupt ecosystem processes.

2.5. Pesticides

They are the toxic chemical substances which are released intentionally into our environment to kill living organisms (insects, herbs, fungus, bacteria, etc.). Pesticides control or kill organism through a variety of mechanism, including the inhibition of biological processes such as photosynthesis, mitosis, cell division, enzyme function, growth; interference with the synthesis of pigments, proteins or deoxyribonucleic acid (DNA); destruction of cell membranes; or the promotion of uncontrolled growth. The use of pesticide is as old as human civilization. The first intentional use of a pesticide is 2500BC by Sumerians. They used the sulfur compounds to kill insects. Pesticides cause contamination in the environment mainly through air, soil, water and wildlife. Pesticides not only kill target pests but also damage many other organisms that are also present in the air, soil and water. Therefore, it decreases biodiversity. Safe alternate methods like development of relatively cheaper biopesticide should be encouraged.

2.5.1. Insecticides

They are toxic chemicals designed to be deliberately released into the environment to kill insects. They can also cause negative effect on the ecosystem. Insecticides easily contaminate the air, soil and water by spraying.

2.5.2. Herbicides

These chemicals are used to kill undesirable plants or "weeds". Most of them will kill all the plants they touch, while some of them are designed to target one species. It is well known that herbicide application can pose a risk for the vegetation surrounding an arable field. Herbicides can cause environmental contamination such as air, soil and water. These herbicides can enter aquatic ecosystems as a result of terrestrial runoff, and to a lesser extent, of direct application and aerial spraying. Microbial communities in freshwater ecosystems are not directly targeted, but these communities are exposed to herbicides and can be directly or indirectly affected by these compounds.

2.5.3. Fungicides

These chemicals are used to kill fungi. Using fungicides causes air, water and soil pollutions. Fungicides get in to environment by agricultural or hospital usage. For example, areas of intensive agriculture, aquatic environments and etc. are exposed to agricultural pesticides, which are regularly detected in surface waters in these areas. As another example, after application of azole fungicides to treat fungal infection in human, these chemicals may reach to the receiving environment via direct or indirect discharge of wastewaters, thus posing potential risks to non-target organisms. But, most of fungicides have relatively low mammalian toxicities, and except for carbamates such as benomyl, a relatively narrow spectrum of toxicity to soil-inhabiting and aquatic organisms. Their greatest environmental impact is toxicity to soil and water microorganisms.

2.5.4. Bactericides

This chemical agent prevents the formation of bacteria. Bactericides are often used as coatings and corrosion inhibitors in oil wells and connecting pipelines in intermediate storage to the refinery for crude oil. These chemicals are increasingly added to antimicrobial surfaces and other products to kill bacteria or inhibit their growth. These are also intensively used in animal husbandry and in veterinary medicines for the purposes of caring for and rearing food-producing animals. Another important use of bactericides is in cooling tower systems to control the growth of some harmful bacteria such as *Legionella* that might otherwise be released from cooling towers in aerosols.

2.6. Heavy and Non Heavy Metals

Heavy and non-heavy metals occur naturally in the environment from pedogenetic processes (erosion, volcanic activities, etc.) and anthropogenic activities (mining, refining ores, tanneries, batteries, paper industries, pesticides and fertilizer industries, etc.). These metals essentially become contaminants in the soil and water environment because of their excess generation by natural and man-made activities. These pollutants are of specific concern due to their toxicity, bio-accumulation tendency and persistency in nature for long periods of time. The main heavy and non-heavy metal contaminants are:

Antimony occurs naturally in the ground and is often used in the flame retardant industry. It is also used in ceramics, glass, batteries, fireworks and explosives. Drinking water gets contaminanted through natural weathering of rock, industrial production, municipal waste disposal or manufacturing processes.

Arsenic (As) is a semi-metal element which is odorless and tasteless. Agricultural and industrial practices cause arsenic contamination in drinking water.

Asbestos is naturally occurring mineral. Six minerals (chrysotile, crocidolite, anthophyllite, tremolite, actinolite and amosite) have been characterized as asbestos. It is used in the production of cements, floor tiles, paper products, paint, and caulking; in transportation-related applications; and in the production of textiles and plastics. Most of the asbestos pollute the environment by extensive opencast mining, generating enormous amount of mine waste and host rocks.

Barium (Ba) occurs naturally in some aquifers that serve as sources of ground water. The major uses of barium are in oil and gas drilling muds, automotive paints, bricks, tiles and jet fuels. It generally pollutes drinking water after disolving from naturally occurring minerals in the ground and unconventional drilling practices.

Beryllium (Be) occurs naturally in the ground and is often used in electrical equipment and mechanical industries. It generally gets into water from run-off during mining operations, discharge from processing plants and improper waste disposal from industrial applications.

Boron (B) Boron is a naturally occurring element. In nature it is found combined with oxygen and other natural elements forming several different compounds called borates. Anthropogenic boron contamination in aquifers has been attributed to leaking septic systems and borate mining.

Cadmium (Cd) is a contaminant in the metals used to galvanize pipe. Corrosion of galvanized pipes or improper waste disposal causes water pollution. The other source of Cd is PVC-window frames, plastics and plating on steel.

Chromium (Cr) occurs naturally in the ground. It is often used in the electroplating of metals. It generally gets into water from run-off during old mining operations and improper waste disposal from industrial application (plating, metallurgy, pigments, and leather tanning.

Chlorine (Cl) is naturally occurring substance. It gets in to the groundwater mostly by human activities (road salt, fertilizers, industry waste or sewage).

Cyanide (CN) is used in electroplating, steel processing, plastics, synthetic fabrics, metal recovery processes and fertilizer products. Improper waste disposal causes water pollution.

Copper (Cu) is found naturally in sand stones and in minerals such as malachite and chalcopyrite. Increased levels of Cu are due to uses in fertilizers, building materials, rayon manufacture, pesticide sprays, agricultural and municipal wastes and industrial emissions.

Fluoride (F) occurs naturally in some water supplies. Improper waste disposal and mining activities cause water pollution.

Lead (Pb) occurs naturally (weathering of parent rocks and ore deposits) and man-made (mining, industrial emission; smelting, waste water irrigation and application of fertilizers) sources. Mining activities and industrial activities (plastics, finishing tools, cathode ray tubes, ceramics, solders, pieces of lead flashing and other minor product, steel and cable reclamation) cause environmental pollution.

Mercury (Hg) occurs naturally in the environment and can be found in metallic, inorganic, and organic forms. It is used in metal processing industries, medicinal, cosmetic, and spiritual purposes. It usually contaminates the water as a result of improper waste disposal.

Nickel (Ni) occurs naturally in the ground. It is generally used in electroplating, stainless steel and alloy products. Mining, industrial application (nickel plating, colored ceramics, batteries, furnaces used to make alloys or from power plants and trash incinerators) and refining operations (trash cause environment pollution.

Nitrate (NO_3) occurs naturally in soil and water. It is used in fertilizer and is found in sewage and wastes from human and/or farm animals and generally gets into drinking water from those activities.

Selenium (Se) is generally found in food and soils. It is widely used in electronics, TV cameras, computer cores, photocopy operations, the manufacture of glass, chemicals, drugs, and as a fungicide and a feed additive. Mining and retorting activities can cause environment contamination.

Silver (Ag) occurs naturally in elemental form and as various ores. Ag is generally used in industry, photographic chemicals, water distillation equipment, mirrors, silver plating equipment, special batteries, table cutlery, jewelery, dental medical and scientific equipment including amalgams. The main source of silver contamination of environment (air, soil and water) is by natural and anthropogenic sources (photographic developing solutions that photofinishers discard directly to sewers).

Sodium (Na) occurs naturally in food and drinking water. Drinking water contributes only a small fraction to the overall Na intake. The main sources of Na contamination are near coastal areas, windborne sea spray and domestic, commercial and industrial discharges. In general, sodium salts are not acutely toxic substances because of the efficiency with which mature kidneys excrete sodium. High concentrations of Na tend to increase the corrosive action of water, give it unpleasant taste, and tend to hamper the operation of ion exchange softeners in the removal of hardness.

3. Characterization of a contaminated site

Industrialization and extraction of natural resources cause environmental pollution in the World. Large amount of toxic (more than 450 million kilograms) waste materials (containing heavy metal) are disposed into the environment (soil, water and air) every day. These contaminants are causing ecological problem in all living organisms. Decontamination of the polluted environment is an essential issue to maintain ecological balance. Extensive work has been devoted to the development of remediation techniques by developed countries.

Therefore, it is very important to assess and characterize the contaminated sites for:

- Determining concentration and spatial distribution of harmful pollutants under consideration.
- Determining the extent of site remediation (zonation) based on which the suitable remediation technique is selected.
- For assessing environmental and human health risk due to contamination.

More specific questions have to be answer for site characterization and assessment:

- What is the source of contaminants?
- What is the type and physical form of contaminants?
- Spatial and depth wise extent of contamination
- Whether the contaminants are stationery or movable?
- If they are movable, identification of the significant pathways
- Identification of the potential receptors of contaminants

A suitable remediation method has to be selected based on the toxicity level of contaminants and the risk they pose to the environment. The remediation does not aim for entire decontamination. The major focus is to bring the contamination level well below the regulatory toxic limit. The sustainability of remediation technologies is extremely important to analyze the long-term effect to avoid any potential problems in future.

In order to select the appropriate remediation techniques, the following steps need to be followed.

Identify Information Gaps. A desk study should be undertaken to decide if enough information exists to carry out a satisfactory risk assessment to a required degree of confidence. If not, the objectives of a further investigation need to be defined.

Site Safety. The potentially hazardous nature of the site will require consideration from the outset in order to identify any safety measures needed to protect personnel or the environment.

Nature of the Investigation. Once the objectives of a further investigation have been established, a decision on the nature of the investigation necessary to obtain suitable data must be made.

Sampling Locations and Depths. The locations and depths at which samples are to be collected and the number of locations required must be considered.

Chemical Analyses. The specification of what analyses should be carried out on the samples obtained during the investigation will be determined through research on the history of the site and consideration of the conditions encountered on-site.

Sampling Methods. The methods by which samples are to be obtained, preserved, and transported to the chemical testing laboratory require consideration.

Consultants and responsible parties often agonize over which technology will be the most cost effective, yet still meet the cleanup goals. Cost is a key factor in the selection of a remediation system because securing and budgeting funding is a serious problem that requires innovative uses of technologies to address and resolve the contamination issue. Often a single remediation technology is unable to adequately remediate a site's contaminated soil and/or groundwater to below acceptable levels.

Currently used environmental cleaning applications mainly involve conventional remediation techniques. However, advancements in biosciences and biotechnology allow development of highly effective new and sustainable remediation techniques utilizing biological agents (bioremediation) in cleaning polluted environment and maintaining a healthy ecological balance. It is expected that most of the conventional environmental cleaning techniques will be replaced successfully by sustainable bioremediation techniques in near future. Bioremediation has many advantageous features such as permanence, low cost (60-90% less than other technologies), low maintenance, no eyesore, capability of impacting source zones, decreasing site clean-up time, and full degradation of contaminant.

4. Bioremediation

Today environmental pollution is a universal problem because of uncontrolled population growth and fast industrialization and urbanization in the world. Contaminated environment needs to be cleaned by appropriate remediation techniques for ecological balance. Currently used environmental cleaning applications mainly involve conventional remediation techniques. New advancements in biosciences and biotechnology allow development of highly effective new and sustainable remediation techniques. It is expected that most of the conventional environmental cleaning techniques will be replaced successfully by sustainable bioremediation techniques in near future.

4.1. Criteria for Bioremediation Strategies

There are critical factors that should be considered when evaluating the use of bioremediation for site cleanup. These factors are described below under separate headings.

4.1.1. Magnitude, toxicity and mobility of contaminants

The proper investigation and characterization for contaminanted sites have to be done as follows:

- Horizontal and vertical extent of contamination
- Nature of contaminants at the site
- The likely mobility of contaminants in the future

4.1.2. Geophysical, geochemical and biological characteristics of the contaminated site

Soil structure contains different textures ranging from low to high contents of sand, silt and clay. A granular and well-structured soil can facilitate effective delivery of air, water and nutrients to the microorganisms for *in situ* bioremediation.

Moisture content (water) is the primary factor in determining the dielectric constant of soil and other mediums. Soil moisture content generally ranges from 25 to 28 %.

pH (power of hydrogen) ranges from 5.5–8.0, which is the optimum range for the growth of microbes and to destroy the contaminants.

Temperature ranges from 15–45^{o}C. Temperature affects biochemical reaction rates and the rates are double for each 10^{o}C rise in temperature.

Oxygen is mainly used for the initial breakdown of the hydrocarbon in the contaminated sites. The amount of available oxygen will determine whether the bioremediation is carried out under aerobic or anaerobic condition.

Microbial diversity exists in the contaminated site such as *Pseudomonas*, *Aeromonas*, *Flavobacteria*, *Chlorobacteria*, *Corynebacteria*, *Acinetobacter*, *Mycobacteria*, *Streptomyces*, *Bacilli*, *Arthrobacter*, *Aeromonas*, *Cyanobacteria*, and etc.

Macrobenthos diversity is consortium of aquatic plants such as *Eichornia crassipes*, *Salvinia molesta*, *Ceratophyllum demersum* and is consortium of aquatic animals such as *Anodonta woodiana* and *Limnodrilus hoffmeisteri* which have high potential to degradation of turbidity, biochemical oxygen demand, chemical oxygen demand, ammonia, nitrite and nitrate in domestic wastewater.

4.1.3. Proximity of human and environmental receptors

Whether or not the bioremediation is suitable for cleaning the environment for a site is dependent on whether the rate and extent of contaminant degradation is sufficient to maintain low risks to human or environmental receptors.

4.1.4. Degradability of contaminants

The biological degradation of a compound is generally high if the compound occurs naturally in the environment such as petroleum hydrocarbons. In contrast, synthetic compounds with a high molecular weight (complex ring structures and halogen substituents) degrade more slowly than simpler straight chain hydrocarbons.

4.1.5. Planned site use

In order to decide whether bioremediation is a suitable cleanup method for a site depends on whether the rate and extent of contaminant degradation is sufficient to reduce risks to acceptable levels.

4.1.6. Ability to properly monitor

The environmental factors include chemical and physical characteristics that influence the bioavailability of contaminants, the availability of other nutrients, the activity of biological processes (temperature and pH, for example), and characteristics of the contaminants with respect to how they interact with the site's geochemical and geological characteristics.

4.1.7. Research and technical aspects

Although there are a number of contaminants that are biodegradable, including petroleum hydrocarbons, alcohols and solvents, many widely used industrial chemicals such as polychlorinated biphenyls (PCBs), pesticides, coal tars, chlorinated solvents, and polynuclear aromatic hydrocarbons are not degraded so readily. So, more intensive research is needed, but funding for this kind of basic research is diminishing. Unlike the conventional treatment technologies, bioremediation technique must be tailored specifically to each polluted site. Each waste site has unique characteristics, and thus requires individual attention. As yet, official criteria for evaluating the success or failure of particular strategy have not been established.

4.1.8. Human resources

Because bioremediation is a new technology, there is a lack of trained human resources in this field. A successful bioremediation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, hydrogeology, soil science and project management. Universities do not offer qualifications in bioremediation engineering and such combined expertize can be acquired only through experience and training on the job.

4.1.9. Degradability of contaminants

Unlike other industries, bioremediation does not result in the production of high value-added products. Thus, venture capital has been slow to invest in the technology and, as a consequence, commercial activity in research and development has lagged far behind other industrial sectors. As bioremediation is considered innovative technology, clients and regulatory agencies often scrutinize bioremediation more closely than conventional technologies. Consequently, tighter restrictions and performance standards are frequently imposed on bioremediation than on other remediation technologies. This can ultimately lead to a greater risk from a liability standpoint if the bioremediation program does not accomplish the predetermined goals.

4.2. Bioremediation Technologies

Bioremediation technologies can be grouped into five techniques: *in situ*, *ex situ*, bioreactor, natural attenuation, and phytoremediation. These techniques do primarily one of two things: they either remove the contaminants from the substratum (decontamination or cleanup techniques) or reduce the risk posed by the contaminants by reducing exposure (stabilization techniques).

4.2.1. *In situ* Bioremediation Techniques

Bioremediation technologies that are used "in place" without removal of the contaminated matrix from air, water and soil. The advantages and disadvantages of these techniques are given in Table 1. Commonly used *in situ* treatments are:

4.2.1.1. Bioventing

Bioventing involves supplying air and nutrients through wells to contaminated soil to stimulate the local bacteria. If the contamination is deep under the surface, this technique can be used for environmental cleaning. Bioventing supply low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere.

4.2.1.2. Biodegradation

Biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate indigenously occurring multiple organisms to degrade organic contaminants. Generally, this technique can be used for contaminated soil and groundwater.

4.2.1.3. Biosparging

This method is one of the *in situ* remediation technologies that use local microorganisms to biodegrade organic ingredient in the saturated site. In order to increase the biological activity of the naturally occurring microorganisms, air and nutrients are injected into the saturated zone. Biosparging is used to clean groundwater and soil contaminated with petroleum constituents. The ease and low cost of installing small diameter air injection points allows considerable flexibility in the design and construction of the system.

4.2.1.4. Bioaugmentation

This method involves the addition of microorganisms naturally occuring or exogenous to the contaminated sites. This method is especially useful for contaminated soil with inorganic compounds.

4.2.1.5. Biostimulation

Biostimulation is a type of natural remediation that can improve pollutant degradation by optimizing conditions such as aeration, addition of nutrients, pH and temperature control. This method can be considered as an appropriate remediation technique for petroleum pollutant's removal in soil and requires the evaluation of both the intrinsic degradation capacities of the autochthonous microflora and the environmental parameters involved in the kinetics of the *in situ* process.

Table 1. Advantages and disadvantages of *in situ* techniques

Advantages	Disadvantages
• No need to excavate & transport soils - typically less expensive • Can treat a large volume of soil at once • Causes less contaminants to be released than *ex situ* techniques • Creates less dust • Most effective if the soil is permeable sandy soil (uncompacted)	• Least effective in clays/highly layered subsurface environments - oxygen cannot be evenly distributed throughout the treatment area • May be slower to reach cleanup goal (if less easily degradable contaminant, requires years) • May be more difficult to manage (than *ex situ* techniques)

	• The seasonal variation of microbial activity exists • The sites are directly exposed to environmental factors like temperature, oxygen supply etc. • Problematic application of treatment additives like nutrients, surfactants, oxygen etc. • It is a very tedious and time consuming process.

4.2.2. *Ex Situ* Bioremediation Techniques

Ex situ technologies are remediation options where the affected soil (excavation) and water (pumping) are removed from their original location and cleaned on-site or off-site. The most important *ex situ* bioremediation treatment processes include landfarming, biopiles, and composting. The advantages and disadvantages of these techniques are given in Table 2.

4.2.2.1. Land farming

These technique applications are the easiest and most effective method for petroleum contaminated soil. The main aim is to stimulate natural biodegradative organisms and to facilitate their aerobic degradation of pollutants. Land farming received much attention as a disposal alternative technique because it has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities. However, the practice is limited to the treatment of superficial 10–35 cm of soil.

4.2.2.2. Composting

Composting method is the process of degrading organic wastes by microorganisms under thermorphilic condition (40-65°C). This method has been applied to soils and biosolids contaminated with petroleum hydrocarbons, solvents, chlorophenols, pesticides, herbicides, PAHs, and nitro-aromatic explosives.

4.2.2.3. Biopiles

Biopile is a bioremediation technology in which land farming and composting are used as a hybrid system. This technique, which is a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization is used for treatment of surface contamination with petroleum hydrocarbons. Biopiles have been considered as a feasible, cost effective and less destructive remediation technique for petroleum contaminated soils.

Table 2. Advantages and disadvantages of *ex situ* techniques

Advantages	Disadvantages
• *Ex situ* techniques can be faster, easier to control, and used to treat a wider range of contaminants and soil types than *in situ* techniques. • There is more certainty about the uniformity of treatment because of the ability to homogenize, screen, and continuously mix the soil.	• They require excavation of soils, leading to increased costs and engineering for equipment. • More risk of material handling/worker exposure conditions. • Usually requires treatment of the contaminated soil before and, sometimes, after the actual bioremediation step.

4.2.3. Bioreactors

It is a large fermentation chamber for growing organisms (bacteria or yeast) that are used in the biotechnological production of substances such as pharmaceuticals. It is also used for the conversion of harmful waste to less harmful substances. This technique is used to remediate contaminated soil and water with fuel hydrocarbons and organics. Bioreactor has two phases (dry and slurry). The slurry phase bioremediation is a relatively more rapid process compared to the dry phase. In a slurry phase bioreactor, contaminated soil is combined with water and other additives in a large chamber. In contrast to slurry phase, dry phase bioreactor does not include extra water. The advantages and disadvantages of this technique are given in Table 3.

Table 3. Advantages and disadvantages of bioreactor technique.

Advantages	Disadvantages
• Relatively rapid treatment • Reduced pellet formation • Increased slurry homogenization • Increased bioavailability	• Soil-water separation can become a problem. • There is a need for wastewater treatment after the soil is dewatered.

4.2.4. Natural Attenuation

The natural attenuation is a process to reduce the mass, toxicity, mobility, volume, or concentration of contaminants (organic and inorganic) in soil, groundwater and surface water. These processes are grouped as physical, chemical and biological. Physical phenomena are advection, dispersion, dilution, diffusion, volatilization and sorption/desorption. Chemical processes are ion exchange, complexation and abiotic transformation. Biological processes are aerobic and anaerobic biodegradation, plant and animal uptake. Natural attenuation is a cost-effective remediation technology. The advantages and disadvantages of this technique are given in Table 4.

In order to use natural attenuation as a cleaning process, sites must meet one or more of the following criteria:

- It must be located in an area with little risk to human health or to the environment.
- The contaminated soil or groundwater must be located an adequate distance from potential receptors.
- There must be evidence that natural attenuation is actually occurring at the site.
- High permeability speeds contaminant spread, low permeability slows the breakdown. Ideally, natural attenuation works best in soils whose permeability ranks somewhere between high and low.

Table 4. Advantages and disadvantages of natural attenuation technique

Advantages	Disadvantages
• *In situ destruction* - no wastes generated and no cross-media transfer • Little risk to human health or to the environment • The most toxic and mobile contaminants usually biodegrade most quickly and reliably • Non-intrusive • Cost-effective	• Time frames may be as long as remediation by groundwater extraction and treatment • Long-term monitoring • Aquifer heterogeneity complicates site characterization (not unique to natural attenuation) • Intermediates of biodegradation may be more toxic than the original contaminants

• Easily combined with other remedies • No down time due to equipment failures	• Occasionally more expensive than other remedies, especially pump and treat (due to monitoring requirements)

4.2.5. Phytoremediation

Phytoremediation is a bioremediation technique that uses various types of plants and associated microorganisms to remove, transfer, stabilise, and destroy contaminants in the soil, sludge, sediments, wastewater, groundwater and air. Plants are able to indicate, exclude, accumulate, and hyperaccumulate or metabolise toxic inorganic or organic substances. Thereby they contribute significantly to the fate of chemicals, and they can be used to remove unwanted compounds from the biosphere. Phytoremediation offers an environmentally friendly, cost effective, and carbon neutral approach for the cleanup of toxic pollutants from the environment. The advantages and disadvantages of this technique are given in Table 5.

Phytoremediation mechanisms involve phyto-extraction, phyto-stabilization, phyto-volatilization, phyto-degradation, phyto-accumulation, rhizofiltration, rhizosphere biodegradation, and hydraulic control. These mechanisms are shown in Figure 1.

4.2.5.1. Phyto-extraction

Phyto-extraction uses plants or algae to remove contaminants from soils, sediments or water. In this mechanism, plants remove heavy metals or radionuclides from soil through their root system and accumulate them in the root or transport them up into shoot or leaves. Plant may continue to remove contaminants until it is harvested. After harvest, if the contaminated soil does not reach significant cleaning up level, the growth/harvest cycle must usually be repeated through several crops to achieve a significant cleanup.

The main advantage of phyto-extraction is being environmental friendly. It does not cause any harm to soil quality. Another benefit of phyto-extraction is that it is less expensive than any other cleanup process. As this process is controlled by plant, it takes more time than any traditional soil cleanup process.

4.2.5.2. Phyto-stabilization

In this mechanism, the plants immobilize the contaminated chemical compounds from soil and water. Contaminants are taken up by roots and precipitated in the rhizosphere. This prevents the contaminant getting into food chain.

4.2.5.3. Phyto-volatilization

In this mechanism, water containing organic contaminants is taken up by plants, and the contaminants are released into the air through by their leaves. The contaminant may become modified along the way, as the water travels along the plant's vascular system from the roots to the leaves, whereby the contaminants evaporate or volatilize into the air surrounding the plant.

4.2.5.4. Phyto-degradation

In this mechanism, contaminants (organic) are metabolized and destroyed within plant tissues. These smaller pollutant molecules may then be used as metabolites by the plant as it grows, thus becoming incorporated into the plant tissues.

4.2.5.5. Phyto-accumulation

In this mechanism, contaminants along with other nutrients and water are taken up by plant roots. This contaminant mass is not destroyed but accumulate in the plant shoots and leaves.

4.2.5.6. Rhizofiltration

This mechanism uses both terrestrial and aquatic plants to absorb, concentrate and precipitate metal from contaminanted water (surface or groundwater).

4.2.5.7. Rhizosphere biodegradation (Rhizodegration)

In this mechanism, plants relief common substances through their roots, supplying nutrients to microorganisms in the soil. So the microorganisms enhance biological degradation. For instance, plant root exudates carbohydrate sources (sugars, alcohols, and organic acids) for the soil microflora. These compounds enhance microbial growth and activity, and also they act as chemotactic signals for microbes.

4.2.5.8. Hydraulic control

In this mechanism, contaminated ground water is remediated by trees.

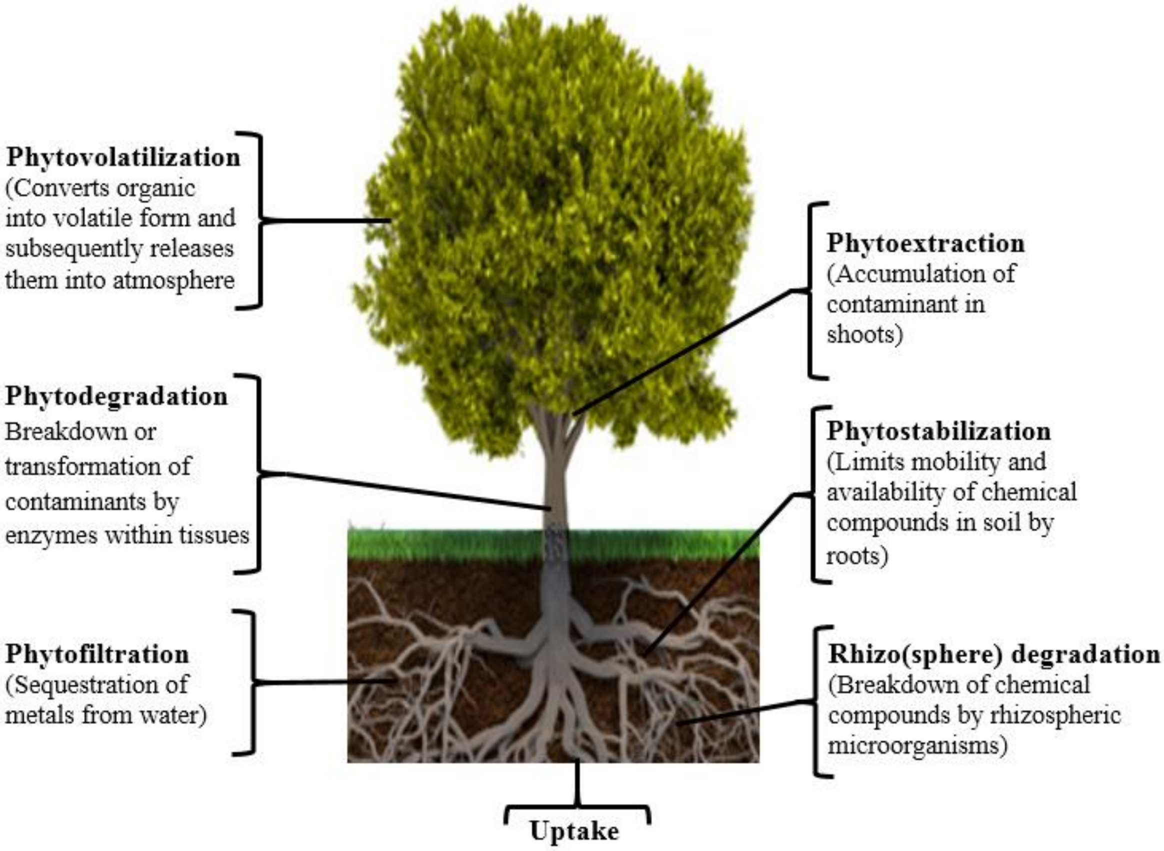

Figure 1. Phytoremediation mechanisms

Table 5. Advantages and disadvantages of phytoremediation technique

Advantages	Disadvantages
• Phytoremediation is less expensive than the old "pump and treat" method for the treatment of contaminated water. • Phytoremediation is also much less expensive than digging out the contaminated site. • Up to 95% of TCE present in water could be removed by simply planting trees and letting them grow. • Phytoremediation takes no maintenance once instituted.	• Phytoremediation is limited to sites with lower contaminant concentrations (USEPA, 1996). • Phytoremediation is restricted to sites with contamination as deep as the roots of the plants being used. • The food chain could be adversely affected by the degradation of chemicals. • The air could be contaminated by the burning of leaves or limbs of plants containing dangerous chemicals.

• Since phytoremediation uses plants, it is aesthetically pleasing. • After plants are introduced, wildlife is able to flourish at the once uninhabitable site. • Solar energy is used to drive the cleansing activity.	

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LO 2: BIOREMEDIATION OF HAZARDOUS POLLUTANTS

1. Bioremediation of hazardous pollutants

In the last two centuries, a series of technological advances provided significant benefits to human health, food production, housing, comfort, transport, and tourism. These human activities demand the development of new chemicals, materials and enormous quantities of energy, exploit natural resources and create large amounts of human made waste polluting the environment. Pollution of the soil, water, and air originates from a variety of sources and continues to be a major issue all around the world. Due to release of enormous quantities of organic and inorganic compounds as a result of the burning fossil fuels, mining activities, industrial and domestic wastes (sewage sludge/wastewater) and unhygienic approach of rapidly growing population, the environment has suffered multiple detrimental effects. Pollutants released from human activities are linked to ecological disasters such as acid rains and global warming. Contaminated ecosystems are causing impacts on plants, microorganisms, aquatic organisms and life support functions such as immobilization, mineralization and nitrification that is ultimately affecting human health as well as the health of the ecosystem. Therefore, the present situation demands urgent action to restore the proper functioning of biogeochemical cycles, which is the driving force behind life on our planet. Biogeochemical cycles are driven by the metabolic activity of microbial communities able to prevent pollutants from reaching the biosphere.

The remediation of a polluted environment can be attempted through conventional or biological measures. The selection and application of remedial procedures depend on the type and extend of the contamination. Scientists are reaching on a consensus to decrease the release of pollutants and to ameliorate their effects by bioremediation. The bioremediation methods are considered as efficient, environmental friendly and cost-effective alternatives to physicochemical treatment technologies for remediation of contaminated ecosystems. Advantageous features of bioremediation over conventional remediation methods include:

- Elimination rather than transfer of the contaminants to another medium
- Minimizing exposure of workers to the contaminants
- Requiring low energy
- Possible reduction in the duration of the remediation process
- Lower cost

- Less or no harm to ecosystem

1.1. Bioremediation of Ecosystems Contaminated With Heavy Metals

All metals, in spite of whether they are essential or non-essential, can exhibit toxic effects at elevated concentrations. Once a pollutant finds entry into a living organism, it may exhibit an injurious action. The effect of the pollutant is therefore a function of its concentration at the site of its action. Metal toxicity becomes more severe in acidic medium, nutrient-deficient ecosystem and poor physical conditions.

The remediation can be attempted through conventional remedial measures such as land filling and leaching, excavation and burial or soil washing. An extensive use of solid waste landfills for disposal of municipal and industrial wastes as well as inappropriate use of agro-chemicals has generated a huge amount of leachate causing groundwater pollution, and the potential for groundwater contamination by leachate has necessitated for the invention of novel engineering designs for landfills. Remediation of heavy metals-polluted ecosystems could be carried out using physicochemical processes such as ion exchange, precipitation, reverse osmosis, evaporation and chemical reduction. However, due to problems such as membrane fouling, high costs, high energy requirement and low removal efficiency, these processes show little relevance in industries. In general, technical applicability, cost-effectiveness and plant simplicity are the key factors in selecting the most suitable treatment method to remove heavy metals (such as Cu, Ar, Pb and Zn) and cyanide from contaminated ecosystem. However, the latest technologies like photocatalytic reduction, surfactant-based membranes, liquid membranes and surface complexation are more efficient for heavy metals removal from contaminated ecosystems.

1.1.1. Microbe-Based Clean Up System (Microbial Bioremediation)

Microorganisms uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). The microbial cell walls, which mainly consist of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups. Among various microbe-mediated methods, the biosorption process seems to be more feasible for large scale application compared to the bioaccumulation process, because microbes will require addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen demand or chemical oxygen demand in the waste. Further, it is very difficult to maintain a healthy population of microorganisms due to heavy metal toxicity and other environmental factors.

Fungi of the genera *Penicillium, Aspergillus* and *Rhizopus* are potential microbial agents for the removal of heavy metals from aqueous solutions. Endophytic bacteria that are known to be beneficial to plants also enhance the ability of host plants accumulating higher levels of heavy metals.

Microorganisms are ubiquitous in heavy metal-contaminated environments and can easily convert heavy metals into non-toxic forms. In bioremediation processes, microorganisms mineralize the organic contaminants to end-products such as CO_2 and H_2O, or to metabolic intermediates which are used as primary substrates for cell growth. Different mechanisms of bioremediation are known, including biosorption, metal-microbe interactions, bioaccumulation, biomineralisation, biotransformation and bioleaching. Microorganisms are capable of dissolving metals and reducing or oxidizing transition metals. Different methods by which microbes restore the environment are oxidizing, binding, immobilizing, volatizing and transformation of heavy metals. Bioremediation can be made successful in a particular location by the designer microbe approach, and by understanding the mechanism controlling growth and activity of microorganisms in the contaminated sites, their metabolic capabilities and their response to environmental changes.

1.1.1.1. Microbial bioremediation by adsorption

Heavy metals can be biosorbed by microbes at binding sites present in cellular structure without the involvement of energy. Among the various reactive compounds associated with bacterial cell walls, the extracellular polymeric substances (high-molecular weight compounds secreted into their environment) are of particular importance and are well known to have significant effects on acid-base properties and metal adsorption. Secreted extracellular polymeric substances have a great ability to complex heavy metals through various mechanisms including proton exchange and micro-precipitation of metals.

1.1.1.2. Microbial bioremediation by physio-bio-chemical mechanism

Biosorption is the process which involves higher affinity of a biosorbent towards sorbate (metal ions), continued until equilibrium is established between the two components. *Saccharomyces cerevisiae* acts as a biosorbent for the removal of Zn (II) and Cd (II) through the ion exchange mechanism. *Cunninghamella elegans* is a promising sorbent against heavy metals released by textile wastewater. Fungi are potential biocatalysts to access heavy metals and transform them into less toxic compounds. Some fungi such as *Klebsiella oxytoca, Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp., *Pleurotus pulmonarius, Botryosphaeria rhodina* have metal binding potential. Pb (II) contaminated soils can be remediated by fungal species like *A.*

parasitica and *Cephalosporium aphidicola* with biosorption process. Hg resistant fungi (*Hymenoscyphus ericae, Neocosmospora vasinfecta* and *Verticillium terrestre*) were able to biotransform a Hg (II) state to a nontoxic state. Many of the contaminants are hydrophobic, and they are taken up by microbes through the secretion of some biosurfactant and direct cell-contaminant association. Biosurfactants form stronger ionic bonds with metals and form complexes before being desorbed from soil matrix to water phase due to low interfacial tension. Bioremediation may also involve aerobic or anaerobic microbial activities. Aerobic degradation often involves introduction of oxygen atoms into the reactions mediated by monooxygenases, dioxygenases, hydroxylases, oxidative dehalogenases, or chemically reactive oxygen atoms generated by enzymes such as ligninases or peroxidases. Anaerobic degradations of contaminants involve initial activation reactions followed by oxidative catabolism mediated by anoxic electron acceptors. The immobilization technique is used to reduce the mobilization of heavy metals from contaminated sites by changing the physical or chemical state of the toxic metals. Solidification treatment involves mixing of chemical agents at the contaminated sites or precipitation of hydroxides. In the contaminated sites, microbes mobilize the heavy metals by leaching, chelation, methylation and redox tansformation of toxic metals. It is not possible to destroy heavy metals completely, but the process transforms their oxidation state or organic complex, making them water-soluble, precipitated and less toxic. In the bioremediation of contaminated environments, microbes use heavy metals and trace elements as terminal electron acceptors or reduce them through the detoxification mechanism. Microorganisms remove heavy metals through the mechanisms which they employ to derive energy from metals redox reactions, to deal with toxic metal through enzymatic and non-enzymatic processes. Two main mechanisms for development of resistance in bacteria are detoxification (transformation of the toxic metal state and making it unavailable) and active efflux pumping of the toxic metal from cells. The basic redox (oxidation and reduction) reaction takes place in the soil between toxic metals and microorganisms; microorganisms act as an oxidizing agent for heavy metals and cause them to lose electrons, which are accepted by alternative electron acceptors (nitrate, sulphate and ferric oxides).

In aerobic conditions, oxygen acts as an electron acceptor, while in anaerobic conditions microbes oxidize organic contaminants by reducing electron acceptors. The microorganism takes energy for growth by oxidizing the organic compound with Fe (III) or Mn (IV) as an electron acceptor. Anaerobic degradation of organic contamination is stimulated with the higher availability of Fe (III) for microbial reduction. Biodegradation of chlorines from contaminants takes place through reductive dechlorination, where contaminants as chlorinated solvents act as electron acceptors in respiration. Microorganisms reduce the state of metals and change their solubility, like the *Geobacter* (anaerobic respiration bacterial species found in anaerobic conditions in soils and aquatic sediment), and reduce the Uranium soluble state (U6+) to insoluble state (U4+). Different defense systems (exclusion, compartmentalization, complex formation and synthesis of binding protein and peptides) reduce the stress developed by toxic metals. These metal binding protein transcription factors are known to mediate in hormone and redox signaling process in the context of toxic metal (Cd, Zn, Hg, Cu, Au, Ag, Co, Ni and Bi) exposure.

1.1.2. Phytoremediation of Heavy Metals

Phytoremediation is an eco-friendly *in situ* remediation technology driven by solar energy. Plants and associated microorganisms can be used for removal of heavy metals partially or completely remediate selected contaminants from soil, sludge, sediments, wastewater and ground water. In the phytoremediation of heavy metals, the initial step is phytoextraction, the uptake of heavy metal contaminants from soil or water by plant roots and their translocation to and accumulation in biomass. Translocation of metals to shoots is an important biochemical process and is desirable in an effective phytoextraction. The next important process of phytoremediation is phytofiltration, which includes rhizofiltration, blastofiltration or caulofiltration. In this, the metals are absorbed or adsorbed and thus their movement in soil and underground water is minimized. In addition to the above process, phytostabilization or phytoimmobilization reduces the mobility and bioavailability of metals in the environment. Plants perform the immobilization of heavy metals in soils by sorption through roots, precipitation, complex formation or metal valence reduction in the rhizosphere. Some of the heavy metals such as Hg and Se, absorbed by plants from polluted soils, get converted into volatile forms and subsequently released into the atmosphere by phytovolatilization process. This process does not remove the metals completely but rather transfers them from one medium (soil or water) to another (atmosphere) from which they can reenter soil and water.

Removal of heavy metals through phytoremediation, especially hyperaccumulators to degrade and detoxify contaminants receives wide attention due to its efficacy and cost efficiency. The criteria used for hyperaccumulation varies according the metal. Hyperaccumulator plants exhibit higher heavy metal tolerance and accumulating abilities compared to other plants. Difficulty in finding heavy metal hyperaccumulators, slow growth and lower biomass yield limit the use of hyperaccumulators. This makes the process time-consuming and therefore not feasible for rapidly contaminated sites or sewage treatments. Rhizospheric microorganisms such as Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria, playing important roles in plant growth and/or metal tolerance via different mechanisms, are beneficial for the design of a phytoremediation plan to select appropriate multifunctional microbial combinations from the rhizosphere. It is likely that remediation role of rhizosphere is the main part of phytoremediation and removal of contaminants is achieved by the combined activity of plants and microorganisms. The main reason for the enhanced removal of metals in the rhizosphere is likely the increase in the number and metabolic activities of microorganisms. In the rhizospheric degradation process, the metal toxicity to plants can be reduced by the use of plant growth-promoting bacteria, free-living soil microorganisms that exert beneficial effects on plant growth. In this process, plants can stimulate microbial activity about 10–100 times by the secretion of exudates which contain carbohydrates, amino acids, flavonoids etc. In return, the rhizosphere bacteria facilitate the generation of larger roots helping to enhance plant survival.

1.2. Bioremediation of Ecosystems Contaminated With Organic Pollutants

Crude petroleum and its refined products are the major sources of organic contaminants polluting the ecosystems. Petroleum is mainly composed by three hydrocarbon (paraffin, naphthenes, and aromatic) fractions. Each petroleum fraction is usually composed by hundreds of different hydrocarbon molecules rather than a defined composition. Thus, fractions are dissimilar in terms of volatility, bioavailability, toxicity, degradability, and persistence. Spills are difficult to avoid during the petroleum processing and delivery. This complex array of compounds presents a tremendous challenge for designing effective bioremediation strategies. Once petroleum hydrocarbons reach an environment, damage can be the result of several causes. Primary biological impact is due to the blocking effect of oil layer to water, nutrients, O_2, and light access. Cytotoxic and mutagenic effects of hydrocarbons lead to long-term pollution consequences. A more bioavailable toxic compound not only shows increased noxious effects but also has higher accessibility for biodegradation. In contrast, strongly adsorbed fraction is less toxic but more difficult to take care of. This general rule is relevant for designing biological strategies for the cleanup of polluted soils or sediments because petroleum hydrocarbons tend to tightly adsorb to these matrices.

Selection of an appropriate strategy for remediation relies on the physicochemical properties of the polluted matrix and on the degree and age of the spill. The aim of bioremediation is to overcome the limiting factors that slow down biodegradation rates. Bioremediation of organic compounds can be accomplished either by *in situ* or *ex situ* treatments. During *in situ* applications, the organic contamination is treated at the site. The *ex situ* technologies involve the transport of the polluted soil to a place where a suitable treatment system can be engineered for the removal of organic contaminants. For petroleum hydrocarbons, four scenarios may arise:

1. The excess of carbon source due to hydrocarbon input results in limitation of other nutrients. Addition of nitrogen and phosphorus can restore the balance and increase biodegradation rates.
2. Insufficient oxygen availability decreases biodegradation rates. Air injection or simple stirring can overcome oxygen limitation during aerobic hydrocarbon degradation.
3. Low bioavailability of hydrocarbons. Addition of environmentally friendly surfactants (such as those non-toxic and biodegradable ones produced by microorganisms or plants) can improve solubility and thus bioavailability of hydrocarbons.
4. Non-efficient catabolic machinery from native microbial communities. Addittion of pure culture or microbial consortium hydrocarbon-degrading microorganisms can enhance degradation rates.

1.2.1. Microbe-Based Bioremediation of Organic Pollutants

Main *in situ* remediation strategies for hydrocarbon polluted soils are biostimulation, bioaugmentation, and bioventing.

Biostimulation process involves the enhancement of native microorganisms' metabolism by management of environmental factors and nutrients. The addition of native or exogenous hydrocarbon-degrading microorganisms, when native microbial communities lack the desired catabolic capabilities is necessary in order to achieve bioaugmentation.In order to enhance aerobic metabolism of organic compounds, a network of slotted pipes are used to deliver air (by either passively or by forced aeration) by bioventing.

The most common *ex situ* bioremediation technologies for oil-polluted soil treatments are biopiles, composting, and landfarming. The aim is to speed up hydrocarbon degradation by adding low-cost nutrients and oxygen.

In landfarming process, controlled spreading of organic waste on the soil surface allows native microorganisms to aerobically degrade organic pollutants. It is one of the most commonly used technologies for the remediation of petroleum hydrocarbon contaminated soil. Landfarming treatment involves treating a flat layer of contained, contaminated soil (up to 1.0 m in thickness) by applying nutrients and aerating the soil through periodic tilling to promote the biodegradation and volatilization of petroleum hydrocarbons. Treatment strategies vary for landfarms and can be tailored according to site-specific characteristics including climate, location, soil type and temperature. Nutrient amendments, pH buffers and bulking agents may be applied to stimulate aeration of co-substrates, microbial metabolism or bacterial inoculations and can significantly increase the efficiency of bioremediation.

Biopiles and windrow composting involve the mixing of polluted soil with organic material as a bulking agent. This mixture promotes microbial activity by improving soil texture, aeration, and moisture maintenance. Main difference between both strategies is the aeration methodology. Composts are aerated by turning the soil/bulking agent mixture periodically with a modified windrow turner, whereas in biopiles a pipe network delivers air. Biopiles and windrows have been successfully used for the remediation of a wide range of contaminants.

1.2.2. Phytoremediation of Organic Pollutants

Phytoremediation is based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms, such as transpiration, photosynthesis, metabolism, and mineral nutrition. Plants act as solar-driven pumping and filtering systems as they take up contaminants through their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered, or volatilized. Phytoremediation relies on plant roots that encourage microbial activity through the release of metabolic products and improved aeration, subsequently facilitating the biodegradation of petroleum hydrocarbons through microbial degradation pathways or co-metabolism. The effectiveness of phytoremediation varies depending on the concentration of petroleum hydrocarbons, depth of contamination, climatic conditions and soil moisture characteristics at a site, all of which influence the growing potential of plants. Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances; roots can stabilize and bind substances on their external surfaces, and when they interact with microorganisms in the rhizosphere. Uptaken substances may be transported, stored, converted, and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules.

1.3. Recent Strategies for Bioremediation

The use of certain genetically modified organism (microorganisms and plant) (GMO) to clean up the contaminated environment (air, soil and water) is a new strategy for bioremediation. This biotecnological technique had first mentioned in early 1990s. Scientists currently use genetic engineering approach to increase the organism ability to metabolize specific chemicals such as hydrocarbons and pesticides. This biotechnological technique has been studied intensively to improve the degradation of hazardous waste under laboratory condition. The genetically engineered organisms have higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions. Genetic modification technology has been used for microorganism and plant for an advanced bioremediation method.

1.3.1. Genetically Engineered Microorganisms for Bioremediation

The control and optimization of microbial bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors. Some researchers assert that using genetically modified microorganism (GMM) for bioremediation is more effective than using indigenous microorganism for bioremediation.

1.3.2. Genetically Engineered Plants for Bioremediation

In nature, over 450 plant species (grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, water hyacinth, etc.) are able to accumulate metals (Zn, Ni, Mn, Cu, Co, and Cd), metalloids (As), and nonmetals (Se) in their body. Furthermore, combining the biotechnology techniques with phytoremediation is more effective remediation method. Genetically modified plant (GMP) approaches has been used successfully for the last two decades.

2. Future prospects

Recombinant DNA technology allows production of character-specific efficient plants and microorganisms for bioremediation of soil, water and activated sludge by exhibiting enhanced degrading capabilities against a wide range of chemical contaminants. Genetically engineered organism can withstand adverse stressful situations and can be used as a bioremediators under various and complex environmental conditions.

Genetic engineering has led to the development of “microbial biosensors” to measure the degree of contamination in contaminated sites quickly and accurately. Various biosensors have been designed to evaluate heavy metal concentrations like Hg, Cd, Ni, Cu and As. Genetic engineering of endophytes and rhizospheric bacteria for plant-associated degradation of pollutants in soil is considered to be one of the most promising new technologies for remediation of metal contaminated sites. Bacteria like *Escherichia coli* and *Moreaxella* sp.

expressing a phytochelatin have been shown to accumulate 25 times more Cd or Hg than the wild-type strains.

The main constraint of phytoremediation technology is the accumulation of pollutants or their metabolites in plant tissues, which shortens plant life and releases contaminants into the atmosphere via volatilization. This problem can be minimized by manipulation of metal tolerance, accumulation and degradation potential of plants against various inorganic pollutants. The bacterial genes responsible for metal degradation can be introduced to plants to allow degradation of metals within the plant tissues. Application of genetically engineered plant-based bioremediation for various heavy metals pollutants is in the forefront due to its eco-friendliness and reduced health hazards compared to physico-chemical based strategies, which are less eco-friendly and more hazardous to human health. Various microbial genes can be harnessed in the transgenic plant for detoxification and accumulation of inorganic contaminants. The metal-detoxifying chelators such as metallothineins and phytochetains can confer resistance to the plant by enhancing uptake, transport and accumulation of various heavy metals. Fast-growing as well as high-biomass-yielding plants like poplar, willow and Jatropa could be used for both phytoremediation and energy production. Among the fast-growing and high-biomass-yielding plants, poplar is the most commonly studied because of its rapid growth rate and potential to produce high biomass within a short period of time (5–8 years). Many of the poplar hybrid varieties have been genetically modified with microbial catabolic genes and specific transporters for increased remediation. For example, mercuric reductase and γ-glutamylcysteine synthetase genes showed increased resistance to Hg and Cd and Cu, respectively, through accumulation of higher concentrations of these metals. Plants engineered with multiple genes will facilitate complete degradation of pollutants to ensure that the harvested biomass can be utilized completely for additional benefits.

Engineered bioremediation strategies involve either the addition of growth stimulators (electron acceptors/donors) to the rhizosphere for reduction of heavy metals or addition of nutrients to the contaminated soil for enhancement of microbial growth and bioremediation properties of microorganisms or genetically modified plants. Many engineered bacteria with heavy metal reduction capacity through the expression of improved enzymes like chromate and uranyl reductase were applied in a specific rhizosphere to perform a specific function. Similarly, genetically modified plants are also known to produce specific compounds which may support the rhizospheric transformation of heavy metals.

The main drawbacks of phytoremediation technology are storage and accumulation of pollutants in the plant materials and the remediation process slowing down and often becoming inadequate when the contaminated site has multiple pollutants. The appropriate solution to these problems is to combine the microbe-plant symbiosis within the plant rhizosphere or to introduce microbes as endophytes to allow degradation of pollutants within the plant tissues. The microbial population in the rhizosphere is much higher than present in vegetation-less soil, and this is due to the facilitation provided by the plants through release of substances that are nutrients for microorganisms. This approach has been evaluated under laboratory conditions, and if it succeeds in field conditions, this technology could facilitate accelerated removal of pollutants, which in turn will support high plant biomass production for bioenergy. The major strategies for implementing bioremediation processes include biostimulation and bioaugmentation approaches guided by specific microbes in combination with plants.

Apart from the above discussed strategies, the remediation of heavy metals and trace elements can be achieved by nanotechnology. Nanoparticles enhancing microbial activity to remove toxic pollutants is called “nanobioremediation”. Nano-based technologies not only reduce the costs of cleaning up contaminated sites at a large scale, but also reduce the process time as well. “Bionanotechnology” or “nanotechnology through biotechnology” is the bio-fabrication of nano-objects or bifunctional macromolecules used as tools to construct or manipulate nano-objects. Wide physiological diversity, small size, genetic manipulability and controlled culturability make microbial cells ideal producers of nanostructures ranging from natural products, such as polymers and magnetosomes, to engineered proteins or protein constructs, such as virus-like proteins and tailored metal particles. This innovative technique would be a promising tool to address the escalating problem of heavy metal as well as organic contaminants in the environment.

3. Conclusions

Environmental pollution and global warming are real. Human activities make significant contributions to these problems. These problems affect people all around the world. People should start acting responsible towards the environment. Remediation of a waste is much harder than creating the waste. Therefore, it is better to prevent waste than to treat or clean up waste after it is formed.

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