

**AN OVERVIEW ON: BIOREMEDEATION**N. T. Rangari*¹, T. M. Kalyankar³, P. K. Puranik², S. M. Kalmegh¹ and S. R. Chaudhari¹¹Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, Maharashtra, India²Government College of Pharmacy, Aurangabad, Marathwada, India³School of Pharmacy, SRTM University, Nanded, Marathwada, India**Corresponding author e-mail:** nalanda_rangari@rediffmail.com**ABSTRACT**

Bioremediation is the transformation or degradation of contaminants into non-hazardous or less hazardous chemicals. This technology includes biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site.

Keywords: Bioremediation, Phytoremediation, Rhizoremediation**INTRODUCTION**

Bioremediation can be described as a process that involves microorganisms or their enzymes to return an environment that was altered by containments to its original study used to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria. In order for the microbes to eliminate the harmful chemicals the right temperature, nutrients and amount of oxygen must be present in the soil and groundwater. These conditions allow the microbes to grow and multiply so they can eliminate the entire contaminant. If the microbes are not in the correct condition, they may grow to slowly, die or even create more harmful chemicals. Bioremediation is an area of study under biotechnology.

A basic purpose of bioremediation is that it is used to clean up the environment such eliminating harmful chemicals by using natural biological processes such as living organisms, an environment has microorganisms that work to protect itself but these organisms die when there is a large amount of contamination. Bioremediation helps to recover the

population of microorganisms as well as to keep them alive so they can kill whatever is attacking. Bacteria is used in the process to keep the microorganisms alive known as bioaugmentation. Bioremediation is the use of microorganism metabolism to remove pollutants.

Technologies can be generally classified as in-situ or ex-situ. In-situ bioremediation involves treating the contaminated material at the site, while ex situ involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation technologies are phytoremediation bioventing, bioleaching, landfarming, bioreactor, composting, and bioaugmentation. Bioremediation consists of using living organisms to reduce or eliminate toxic pollutants. These organisms may be naturally occurring or laboratory cultivated. These organisms either eat up the contaminants or assimilate within them all harmful compounds from the surrounding area, thereby, rendering the region virtually contaminant-free. Importantly, bioremediation can also be used in conjunction with wide range of traditional physical and chemical technologies to enhance their efficacy.^[1]

STEPS IN BIOREMEDIATION

First Step: The first analysis, step, compliance requires examination of the contaminated site in light of the regulations that govern it and the actions that need to be taken. Minimum remediation goals based on the level at which contamination is considered unacceptable may be set.

Second Step: The second stage is site characterization, is one of the more challenging and difficult aspects of a bioremediation project. Site evaluation involves analysis of the physical characteristics of the site, the source of pollutants, the nature of the contaminated soil or water, and the distribution and concentration of contaminants.

In addition, the type and volume of material to be treated have to be evaluated in light of the growth and biological activity of microorganisms either known to exist or capable of degrading the pollutants if introduced there.

The approach that is usually taken is to initiate a 'flask essay' under controlled laboratory conditions. A pollutant compound is added to a small, contained sample of soil, sediment, or water gathered from the field site under examination. The flask is inoculated with the selected microorganisms, nutrient levels, and aeration, along with other parameters, is adjusted, and the evolution of various metabolites is measured. If a radiolabeled parent compound is used, then the rate of production of radioactive CO₂ can give direct mineralization rates for organic compounds.^{[2], [3]}

Third Step: In the third stage is the feasibility of a possible bioremediation approach which usually involving assays of pollutant degradation carried out under controlled laboratory conditions, has to be determined.

It is at this stage that the suitability of a bioremediation approach falls under severe scrutiny. Time considerations are often involved in determining whether or not the bioremediation approach even if cost effective is suitable for the situation or problem at hand.

Fourth Step: The fourth stage in a bioremediation project is the process of remediation itself, which may involve multiple steps, such as pretreatment, or anaerobic followed by aerobic treatment of contaminated soil or material, as discussed above. Monitoring systems are employed to evaluate the progress and extent of contaminant degradation or conversion.

Fifth Stage: The last stage of a bioremediation project involves final monitoring of the site for the presence of contaminants and closure of the project.

TECHNOLOGY OF BIOREMEDIATION

Living organisms or just their enzymes can be used to accomplish this task. Lignin is not one compound, but, a group of polyaromatic chemicals that harden wood. It is normally difficult to extract from pulp and ends up a pollutant once it is removed. Most bioremediation technology is designed to remove a pollutant once it is generated or released into the environment although some types of bioremediation remove chemicals before they become pollutants. Technologies using bioremediation treatment include bioaugmentation, biofilters, bioreactors, biostimulation, bioventing, composting and landfarming.^[4]

Biofilters: The removal of organic gases by passing air through compost or soil containing microorganisms capable of degrading the gases. It has been used to remove volatile organic compounds from air.

Bioreactors: The treatment of a contaminated substance in a large tank containing organisms or enzymes. Bioreactors are commonly used to remove toxic pollutants from solid waste and soil.

Biostimulation: The use of nutrients or substrates to stimulate the naturally occurring organisms that can perform bioremediation. Fertilizer and growth supplements are the common stimulant. The presence of small amounts of the pollutant can also act as a stimulant by turning on for the bioremediation enzymes.

Bioventing: This is similar to biostimulation. It involves the venting of oxygen through soil to stimulate the growth or natural and introduced bioremediation organisms. This is used predominantly for soils contaminated with petroleum products.

Composting: This involves mixing contaminated materials with compost containing bioremediation organisms. The mixture incubates under aerobic and warm conditions. The resultant compost can be used as a soil augmentation or be placed in a sanitary landfill.

Landfarming: The use of farming tilling and soil amendment techniques to encourage the growth of bioremediation organisms in a contaminated area. It

has been used successfully to remove large petroleum spills in soil. These technologies are classified as either in situ or ex situ. In situ technologies are the ones commonly seen in the media. They involve the use of organisms or enzymes to remove pollutants in the location that is polluted. Ex situ technologies involve the removal of the contaminated material where it can be treated using bioremediation.

Bioaugmentation: This is a general term describing the addition of organisms or enzymes to a material to remove unwanted chemicals. Bioaugmentation is used to remove byproducts from raw materials and potential pollutants from waste. Bacteria are the most common bioaugmentation organisms. Many applications are accomplished using vegetation to remove excess nutrients, metals and pathogenic bacteria. Waste water from human and agricultural effluent is cleaned this way using wetland plants.

Terminology of Bioremediation: The choice of bioremediation technologies used for site remediation is often dictated by whether it is possible or practical to physically manipulate the contaminated materials. If the contaminated materials can be removed and treated ex situ in a cost-effective manner, then this is often the approach selected because it offers the advantages of better process control, more rapid rates of degradation, and greater minimization of risk.^[5]

Intrinsic Bioremediation: Often bioremediation can be accomplished without human intervention by microorganisms that are naturally found in the contaminated matrix. For this approach to be used, it is usually necessary for the rate of contaminant degradation to exceed the rate of contaminant migration.

Knowledge of the following key site characteristics are required to evaluate the likely success of intrinsic remediation; the bioavailability of contaminants, levels of nutrients, the presence of minerals to buffer the pH of the matrix, adequate levels of electron acceptors (either oxygen, nitrate, ferric iron, or sulfate), and site specific contamination migration rates.

Engineered Bioremediation: In some cases, it may be desirable to construct engineered systems to supply nutrients, electron acceptors or other materials that enhance the rate or extent of contaminant degradation. This is desirable when the rate of contaminant migration, the location of environmental receptors or other liability issues dictate that steps be taken to optimize the rate of contaminant degradation in order to mitigate contaminant migration.

Combination of Technologies: Non biological treatment technologies or source removal may be used to reduce the total amount of contaminant present at the site before, or concurrent with, bioremediation. For example, excessively contaminated soils may be excavated at the source of contamination, volatile contaminants may be vacuum extracted, or undissolved pools of contaminants may be pumped from aquifers. Bioremediation technologies are used “in place” without removal of the contaminated matrix. Both intrinsic and engineered bioremediation technologies can be used in situ. Bioremediation technologies require removal of the contaminated matrix by excavation so it can be manipulated in some way through the use of slurry reactors, composting, biopiles, or other technologies.

TYPES OF BIOREMEDIATION

Biotransformation: Is the alteration of contaminant molecules into less or nonhazardous Molecules.

Biodegradation: Is the breakdown of organic substances in smaller organic or inorganic molecules.

Mineralization: Is the complete biodegradation of organic materials into inorganic constituents such as CO₂. These three classifications of bioremediation can occur either in situ (at the site of contamination) or ex situ (contaminant taken out of the site of contamination and treated elsewhere). There are advantages and disadvantages to both in situ and ex situ strategies. Ex situ strategies removes the contaminants and places them in a contained environment. This contained environment allows for easier monitoring and maintaining of conditions and progress, thus making the actual bioremediation process faster.^[6]

EX-SITU BIOREMEDIATION:

Ex-situ bioremediation is bioremediation taking place after the contaminated soil is excavated and removed. It may be expensive, mostly due to the cost of digging and hauling the soil. It disrupts the site and requires a good deal of room. Ex-situ bioremediation is, however, usually far more successful than in-situ bioremediation. The act of excavation breaks up the natural soil packing and dramatically increases permeability and porosity. Air is naturally added as the soil is churned up, and most of the effects of soil conditions and chemistry are controllable. This alone is usually enough to greatly stimulate microbial action. It is bioremediation after the contaminated soil removed. It may be expensive, mostly due to the cost of digging and hauling the soil. It disrupts the site and requires a good deal of room. Ex-situ

bioremediation is, however, hauling the soil. It disrupts the site and requires a good deal of room. Ex situ treatments involve the physical removal of the contaminated matrix to controlled and contained reactors, compost heaps or lagoons. Ex-situ bioremediation involves excavation of the contaminated soil and treating in a treatment plant located on the site or away from the site. This approach can be faster, easier to control, and used to treat a wider range of contaminants and soil types than in-situ approach.^[7]

Slurry-phase bioremediation:

Contaminated soil is combined with water and other additives in a large tank called a 'bioreactor' and mixed to keep the microorganisms which are already present in the soil in contact with the contaminants in the soil. Nutrients and oxygen are added, and conditions in the bioreactor are controlled to create the optimum environment for the microorganisms to degrade the contaminants.

Upon completion of the treatment, the water is removed from the solids, which are disposed of or treated further if they still contain pollutants. Slurry-phase biological treatment can be a relatively rapid process compared to other biological treatment processes, particularly for contaminated clays. The success of the process is highly dependent on the specific soil and chemical properties of the contaminated material. This technology is particularly useful where rapid remediation is a high priority.

In slurry-phase bioremediation, the contaminated soil is mixed with water to create slurry. The slurry is aerated, and the contaminants are aerobically biodegraded. The treatment can take place on-site, or the soils can be removed and transported to a remote location for treatment. The process generally takes place in a tank or vessel, but can also take place in a lagoon process. The Contaminated soil is excavated and then screened to remove large particles and debris. A specific volume of soil is mixed with water, nutrients, and microorganisms. The resulting slurry pH may be adjusted, if necessary.

Solid-phase bioremediation: In the cases that treats soils in above-ground treatment areas equipped with collection systems to prevent any contaminant from escaping the treatment. Moisture, heat, nutrients, or oxygen are controlled to enhance biodegradation for the application of this treatment. Solid-phase systems are relatively simple to operate and maintain, require a large amount of space, and cleanups require more time to complete than with slurry-phase processes.

Solid-phase soil treatment processes include landfarming, soil biopiles, and composting.^[8]

Biological Treatment: Biological treatment is a process whereby contaminants in soil, sediments, sludge or groundwater are transformed or degraded into innocuous substances such as carbon dioxide, water, fatty acids and biomass, through the action of microbial metabolism. Biological processes are typically implemented at low cost. Contaminants can be destroyed and often little to no residual treatment is required. However, the process requires more time and it is difficult, in general, to determine whether contaminants have been completely destroyed. Additionally, microbes may often be sensitive to toxins or highly concentrated contaminants in the soil.

Biopiles: Biopiles, also known as biocells or biomounds are engineered systems in which excavated soils are combined with soil amendments, formed into compost piles and enclosed for treatment. They are commonly provided with an air distribution system by blowers or vacuum pumps. Several properties of the process such as nutrients and oxygen can be controlled in order to enhance the remediation procedure. This technology is generally used to reduce concentrations of petroleum constituents in excavated soils.

Bioreactors: A bio-reactor is a generic term for an engineered system in which contaminants are degraded, in a specific media, with microorganisms. This technique also referred to as slurry phase bioremediation, varies considerably in its operating conditions. The principal emphasis is on stimulating the biological degradation rate by choosing the optimum temperature, pollutant concentration, degree of aeration and other factors. Typically, the soil is mixed with water and any prescribed additives and placed in a batch reactor vessel. This slurry is kept at controlled operating conditions, with oxygen and nutrients supplied as required, until the remediation is complete.

Composting: Composting is a controlled biological process by which organic contaminants in the soil are converted by microorganisms, under both aerobic and anaerobic conditions, to innocuous and stabilized by-products. Soils are excavated and mixed with bulking agents and organic amendments such as wood chips and plant wastes. Typically, thermophilic conditions (54 to 65 °C) must be maintained to properly compost contaminated soil. Proper conditions of oxygen and moisture help to achieve maximum degradation efficiency.^[9]

Landfarming: Landfarming, also known as land treatment or land application, is an above-ground remediation technology for soils. It reduces concentrations of contaminants through biodegradation. In the ex-situ process, the contaminated soil is first excavated, mixed with soil amendments such as soil bulking agents and nutrients and then tilled into the earth. The soil is spread over an area and periodically turned to improve aeration. Turning the soil also avoids the disadvantages of having heterogeneous degradation. Soil conditions are controlled to optimize the rate of contaminant degradation. The enhanced microbial activity results in degradation of adsorbed petroleum product constituents through microbial respiration. The petroleum industry has used landfarming for many years. This technique is also applicable in in-situ interventions with a different technological setup.^[10]

PHYSICAL AND CHEMICAL TREATMENT

Physical and chemical treatment uses the physical and chemical properties of the contaminants or of the contaminated medium to destroy, separate, or contain the contamination. In the physical processes the phase transfer of pollutants is induced. In the chemical processes the chemical structure of the pollutants is changed by means of chemical reactions to produce less toxic or better separable compounds from the solid matrix. These treatments are typically cost effective and can be completed in short time periods.

Chemical extraction: Chemical extraction is a process that separates contaminants from soils, thereby reducing the volume of the contaminant that must be treated. The two major chemical extraction processes, which are based on the type of contaminants present in the soil, are

Acid extraction: uses acids to extract contaminants from soils. Heavy metals are potentially suitable for recovery. Clean soils are dewatered and mixed with lime and fertilizer to neutralize any residual acid.

Solvent extraction: uses solvents to remove metals and mixtures of metal and organic compounds. Soil is removed and treated.

Physical separation is generally used before chemical extraction, on the assumption that the major part of the contamination is on the smaller particles.

Dehalogenation: Dehalogenation, also known as dechlorination, is a technology in which the chlorine in organic compounds is displaced by hydrogen or a reducing radical containing a hydrogen donor. This

process is achieved by either the replacement of the halogen molecules or the decomposition and partial volatilization of the contaminants. Contaminated soil is screened, processed with a crusher, mixed with chemical reagents and the mixture is heated in a reactor.

There are two dehalogenation processes:

Base-Catalyzed Decomposition: Where contaminated soil is screened, processed with a crusher and pug mill and mixed with sodium bicarbonate. The mixture is heated to above 330°C (630 °F) in a reactor to partially decompose and volatilize the contaminants. The volatilized contaminants are captured, condensed and treated separately.

Glycolate or Alkaline Polyethylene Glycol: Process in which an alkaline polyethylene glycol reagent is used. In this process, the reaction causes the polyethylene glycol to dehalogenate to form glycol ether and/or a hydroxylated compound and an alkali metal salt, which are water-soluble byproducts.^{[11], [12]}

Separation: Separation techniques reduce the volume of contaminated soil through physical and chemical processes by selectively removing the portion containing the contaminants. There are several types of separation techniques.

Gravity Separation: It is used to separate solids from soil, based on the density difference between contaminants and soil e.g., when the metal-contaminated soil is suspended in water, denser materials such as metals sink and are removed

Sieving or Physical Separation: It is based on separation according to size of the particles.

Magnetic Separation: It is used to separate slightly magnetic particles from soil. All uranium and plutonium compounds are slightly magnetic while most soil is nonmagnetic.

Chemical Leaching processes: It uses weak acids such as acetic acid to dissolve and wash the metals from the soil. The metals recovered by the process can possibly be recycled.

Solar detoxification: Solar detoxification, otherwise known as Photolysis, is an emerging remedial technology which is used for the destruction of a wide range of hazardous organic chemicals in soil and or water by photocatalytic oxidation or direct thermal decomposition. In this process, vacuum

extraction is used to remove contaminants from soils. After condensation, contaminants are mixed with a semiconductor catalyst and fed through a reactor illuminated by sunlight or exposed to ultraviolet radiation from electric lamps. The light activates the catalyst and this result in the generation of radicals which oxidize the contaminants into non-toxic byproducts such as water, carbon dioxide and inorganic salts.

Chemical reduction or oxidation: Chemical reduction or oxidation, also known as redox reactions, converts hazardous contaminants to non-hazardous or less toxic compounds that are more stable, less mobile and or inert. Redox reactions involve the transfer of electrons from one compound to another. One compound is oxidized and the other is reduced. It is a short to medium term technology.

Soil washing: Soil washing is a technique in which contaminants fine soil particles are separated from bulk soil in an aqueous-based system on the basis of particle size. Contaminants are removed from the soil in one of two ways. By dissolving or suspending them in the wash solution. By concentrating them into a smaller volume of soil through particle size separation, gravity separation and attrition scrubbing. The concept of reducing soil contamination through the use of particle size separation is based on the finding that most organic and inorganic contaminants tend to bind, either chemically or physically, to clay, silt and organic soil particles. Most silt and clay are stuck to larger particles like sand and gravel. Washing separates the small particles from the large ones by breaking adhesive bonds. The separated material is smaller in volume and is more easily disposed of.

Solidification or stabilization: In ex-situ Solidification or Stabilisation contaminants are physically bound or enclosed within a low-permeability mass, or chemical reactions are induced between a stabilizing agent and contaminants to reduce their mobility. Ex situ, however, typically requires disposal of the resultant materials. This technique is also applicable in in-situ interventions with a different technological setup.

Soil vapours extraction: Soil Vapour Extraction, also known as Soil Venting, is a technology which in principle is similar to in-situ SVE. However, in the ex-situ process, the contaminated soil is excavated. A vacuum is applied to a network of aboveground piping to encourage volatilization of organics from the excavated media. The soil piles may be covered with a geomembrane to prevent volatile emissions and to prevent the soil from becoming saturated by

precipitation. The process includes a system for handling off-gases.

Thermal Treatment: Thermal treatments offer quick cleanup times but are typically the most costly treatment group. This difference, however, is less in ex-situ applications than in in-situ applications. Cost is driven by energy and equipment costs and is both capital and Operation & Maintenance intensive. Thermal processes use heat to increase the volatility, to burn, decompose, destroy or melt the contaminants. Cleaning soil with thermal methods may take only a few months or several years. The time it takes depends on three major factors that vary from site to site, type and amounts of chemicals present, size and depth of the polluted area type of soil and conditions present.

Hot Gas Decontamination: Hot gas decontamination is essentially a low temperature thermal desorption process. The process raises the temperature of the contaminated soil to approximately 260 °C for a specified period of time by exposing it to hot gases, volatilizing the contaminants, and destroying them in an afterburner. This technology can be used to decontaminate equipment and structures that have been contaminated with explosive residues.

Open Burning: Open burning, also known as open detonation, is a technique used to destroy excess, obsolete, or unserviceable munitions and explosive materials. These materials are destroyed by self-sustained combustion, which is ignited by an external source. An auxiliary fuel may be added to initiate and sustain the combustion of materials. In the past, open burning generally occurred in the surface of the land or in pits. Recently, burn trays and blast boxes have been used to control and contain resulting emissions.

Thermal desorption: Thermal desorption is a physical separation process in which water and organic contaminants in contaminated soil are volatilized by heating the soil to moderately high temperatures (100° - 550°C). A carrier gas or vacuum system transports the volatilized water and organics to the gas treatment system. The bed temperatures and residence times designed into these systems volatilize selected contaminants but not degrade them. Based on the operating temperature of the desorber, thermal desorption processes can be categorized into two groups. High temperature thermal desorption contaminants are heated to 320 to 560°C. Low temperature thermal desorption contaminants are heated to between 90 and 320 °C. Two common thermal desorption designs are the rotary dryer and thermal screw. Rotary dryers are

horizontal cylinders that can be indirect- or direct-fired. The dryer is normally inclined and rotated. For the thermal screw units, screw conveyors or hollow augers are used to transport the medium through an enclosed trough. Hot oil or steam circulates through the auger to indirectly heat the medium. All thermal desorption systems require treatment of the off-gas to remove particulates and contaminants. Particulates are removed by conventional particulate removal equipment, such as wet scrubbers or fabric filters.^[13]

Plasma arc: Plasma arc technology is a pyrolysis process. It uses a plasma arc device to create extremely high temperatures (10000°C or even higher) for destruction of toxic substances in contaminated soil. In plasma arc treatment an electric current is directed through a low-pressure gas stream to create a thermal plasma field. Plasma arc fields can reach 5000 to 15000 °C. Energy is transferred to contaminants exposed to the plasma and contaminants are then atomized, ionized, pyrolysed and finally destroyed as they interact with the decaying plasma species.

Incineration: Incineration is a technology which uses high temperatures, 850° - 1200°C and oxygen, to volatilize and combust different kinds of hazardous contaminants. Auxiliary fuels are used to initiate and sustain combustion. Proper incinerator design and operation are essential to ensuring adequate destruction of undesirable combustion gases. A properly operated incinerator can meet the stringent requirements for all gaseous emissions. Air pollution-control systems are employed to remove particulates and to neutralize and remove acids. Incineration is different from other thermal technologies in that it oxidizes bulk quantities of contaminants that may be in liquid and solid phase. Four common incinerator types are rotary kiln, liquid injection, fluidized bed and infrared kiln.

Pyrolysis: Pyrolysis, also known as plasma pyrolysis, is the thermal degradation of organic species in the absence of oxygen or other reactant gases. In practice, as it is not possible to achieve a completely oxygen-free atmosphere, actual pyrolytic systems are operated with less than stoichiometric quantities of oxygen.

This thermal technology is a form of incineration at operating temperatures above 430 °C. Organic materials are transformed into gases, small quantities of liquid and a solid residue (coke) containing carbon and ash. The off-gases may also be treated in a secondary thermal oxidation unit. Particulate removal equipment is also required. Conventional thermal

treatment methods such as rotary kiln, rotary hearth furnace or fluidized bed furnace are used for waste pyrolysis.

Vitrification: This ex-situ technique is much like in situ vitrification, except that it is done inside a chamber. Heating devices include plasma torches and electric arc furnaces. With plasma torch technology, contaminated soil is fed into a rotating hearth; the contaminants and molten material are held against the side by centrifugal force. During the rotation, the contaminants move through plasma generated by a stationary torch.

To remove the molten material from the furnace, the hearth's rotation slows and the slag flows through a bottom opening. Effluent gases are generally kept in a separate container where high temperatures combust/oxidize the contents. The arc furnace contains carbon electrodes, cooled sidewalls, a continuous feed system, and an off-gas treatment system. In this process, contaminated soil is fed into a chamber where it is heated to temperatures greater than 1500°C. The melt exits the vitrification unit and cools to form a glassy solid that immobilizes inorganics.

IN-SITU BIOREMEDIATION:

In-situ refers to in-place bioremediation, without excavation. In-situ bioremediation is far more complex, difficult to control, and less successful than exsitu methods. Many additives and bioremediation systems are on the market, but success usually depend so site conditions, and it is extremely difficult or impossible on many sites for a delivery system to reach all affected areas, particularly in fine-grained or highly stratified soils. Natural attenuation, as mentioned above, is usually occurring at all sites, and is often the main limiting factor in contaminant plume movement.

If natural attenuation is the sole method of remediation at a site. In-situ refers to in-place bioremediation, without excavation. In situ bioremediation is far more complex, difficult to control, and less successful than ex-situ methods. Many additives and bioremediation systems are on the market, but success usually depends on site conditions, and it is extremely difficult or impossible on any sites for a delivery system to reach all affected areas, particularly in fine-grained or highly stratified soils. In-situ Bioremediation treats the contaminated soil or groundwater in the location in which is found. In this technology oxygen and occasionally nutrients are pumped under pressure into the soil through

wells. The nutrients are spread on the surface to infiltrate into the contaminated area of material or the saturated zone.^[14]

Natural attenuation: As mentioned above, is usually occurring at all sites, and is often the main limiting factor in contaminant plume movement. In-situ bioremediation is far more complex, difficult to control, and less successful than ex-situ methods. Many additives and bioremediation systems are on the market, but success usually depends on site conditions, and it is extremely difficult or impossible on many sites for a delivery system to reach all affected areas, particularly in fine-grained or highly stratified soils. Extra monitoring requirements are usually necessary to ensure that remediation is progressing. Natural attenuation, as mentioned above, is usually occurring at all sites, and is often the main limiting factor in contaminant plume movement. Due to inherent site conditions soil heterogeneity. Permeability, pH, temperature, oxygen levels, other soil and groundwater chemistry, and type and amount of contamination); natural attenuation may be exceedingly slow. Active in-situ bioremediation methods are attempts to overcome one or more of the limiting factors.

Biostimulation: Is the addition of something in an attempt to increase the activity of the naturally occurring microbial population? This can range from the addition of nutrients to the increase of oxygen. This category includes bio-venting, the pumping of air through the soil and/or groundwater to increase oxygen. It also includes chemical additives such as oxygen, and nitrogen or fertilizer additions. Some forms of biostimulation work quite well. All are quite dependent on site conditions none can be applied successfully at every site. Corrective action plans must be complete and detailed as to the product, amounts, application methods, monitoring, reapplication needs and final testing.

Bioaugmentation: Is the adding of additional microbial cultures? Unless a site has been completely sterilized, there are usually microbial cultures already in place, with a complex interaction of species. The activities of one species may provide nutrients for another. Some species often live on others, or their waste streams. Some species will degrade hydrocarbons much better at higher contaminant levels, while other microbes function at lower levels. A whole suite of cultures is usually critical species can dramatically slow site remediation. It is difficult to add microbial cultures. Microbes injected into a well rarely extend beyond the well sand pack, a foot or so into the formation. Adding extra microbes from

the outside, even from cultures taken from the site, overburden the site population and change species proportions.

FACTORS USE IN BIOREMEDIATION:

Biological factors: The biological factors are primarily concerned with the numbers of specific kinds of microorganisms present and the expression and activity of metabolic enzymes, in other words, the amount of 'catalyst' present.

Environmental factors: The environmental factors include chemical and physical characteristics that influence the bioavailability of contaminants, the availability of other nutrients, the activity of biological processes, characteristics of the contaminants with respect to how they interact with the sites geochemical.^{[15], [16]}

BIOINFORMATICS APPLIED IN BIOREMEDIATION:

Bioremediation of a contaminated site typically works in one of two ways, to enhance the growth of whatever pollution eating microbes might already be living at the contaminated site. Specialized microbes are added to degrade the contaminants. The fields of Biodegradation and Bioremediation offer many interesting and unexplored possibilities from the bioinformatics point of view. They need to integrate a huge amount of data from different sources: chemical structure and reactivity of the organic compounds, sequence, structure and function of proteins comparative genomics, environmental biology etc.

Bioinformatics: Bioinformatics is the combination of biology and information technology. It is the branch of science that deals with the computer based analysis of large biological data sets. Bioinformatics incorporates the development to store and search data and of statistical tools and algorithms to analyze and determine relationships between biological data sets, such as macromolecular sequences, structures, expression profiles and biochemical pathways. Bioinformatics is the focus on cellular and molecular levels of biology. Biology and computers are becoming close cousins who are mutually respecting, helping and influencing each other and synergistically merging more than ever. The huge data from biology mainly in the form of DNA, RNA and protein sequences is putting heavy demand on computers and computational scientists. Bioinformatics has taken on a new glittering by entering in the field of Bioremediation. Bioinformatics is the application of computer sciences and related technology to the industries for

using the huge available database for computational biology.

Bioremediation using Proteomics: The cellular expression of proteins in an organism varies with environmental conditions. The changes in physiological response may occur due to the organism's adaptive responses to different external stimuli, such as the presence of toxic chemicals in the environment. The advent of proteomics has allowed an extensive examination of global changes in the composition or abundance of proteins, as well as identification of key proteins involved in the response of microorganisms in a given physiological state. A number of reports have described sets of proteins that are up- or down-regulated in response important to remove from the environment. In situ and ex situ bioremediation of has been partially achieved using natural and genetically engineered microorganisms. Using a proteomics approach, the physiological changes in an organism during bioremediation provide further insight into bioremediation-related genes and their regulation.

Bioremediation using Genomics: Non-molecular techniques: at present, most applied microbiological investigations of bioremediation processes make use of the 'treatability study' in which samples of the contaminated environment are incubated in the laboratory and the rates of contaminant degradation or immobilization are documented. Such studies provide an estimate of the potential metabolic activity of the microorganisms that are responsible for bioremediation, or why particular amendments that can be evaluated for engineered bioremediation applications do or do not stimulate activity. When bioremediation processes are researched in more detail, attempts are generally made to isolate the organisms responsible.

Genomics and Proteomics in Bioremediation: The growing demands of genomics and proteomics for the analysis of gene and protein function from a global bioremediation perspective are enhancing the need for microarray-based assays enormously. In the past, protein microarray technology has been successfully implicated for the identification, quantification and functional analysis of protein in basic and applied proteome research.

Other than the DNA chip, a large variety of protein-microarray based approaches have already been verified that this technology is capable of filling the gap between transcriptomics and proteomics. The availability of bacterial genomes relevant to biodegradation in recent years has allowed the

feasibility to study the complex interactions between cellular reactions from a genomic and proteomic level. A quantitative understanding of how cells function requires every gene and protein to be placed in their dynamic context, which entails the integrated consideration of many interacting components.^{[17], [18]}

LIMITATIONS OF BIOREMEDIATION:

There are several limitations to bioremediation. One major limitation has to do with the nature of the organisms. The removal of pollutants by organisms is not a benevolent gesture. Rather, it is a strategy for survival. Most bioremediation organisms do their job under environmental conditions that suit their needs. Consequently, some type of environmental modification is needed to encourage the organisms to degrade or take up the pollutant at an acceptable rate.

In many instances the organism must be presented with low levels of the pollutant over a period of time. This induces the organism to produce the metabolic pathways needed to digest the pollutant. When using bacteria and fungi, it is usually necessary to add fertilizer or oxygen to the material containing the pollutant. This can be disruptive to other organisms when done in situ. In situations where simple compounds and metals are being taken up it is likely that these pollutants are at toxic levels for the organisms. Overall, the organisms do not always live as well on the pollutant diet as on other nutrients found more commonly in their environment. This is problematical when doing in situ remediation. Neither the government nor industry wants to spend large amounts of money to clean up pollution.

Industry in particular likes to keep costs down. Bioremediation is generally very costly, is labor intensive, and can take several months for the remediation to achieve acceptable levels. Air bioremediation in particular is very inefficient, considering the volume of polluted air generated by industry. Another problem is that both ex- situ and in situ technologies can cause environmental disruption beyond the damage done by the pollution. The long-term effects of introducing naturally occurring non-native bioremediation organisms into an area are not fully understood. The impact of genetically altered bioremediation organisms is even less understood.^{[19], [20]} There is much misinformation about bioremediation and a general lack of experience with the actual process of bioremediation in most industries. Bioremediation is often underestimated and neglected by industry as an alternative to pollution control. Despite the obvious economic and environmental benefits of bioremediation, industries

tend to maintain the status quo of waste hauling and disposal practices which have not remedied the pollution. Traditional industry pollution control practices tend to favor the transport and relocation of waste as opposed to the transformation of waste into harmless by products available at large is questionable as many businesses have entered the industry with the objective of repackaging and remarketing existing products originally designed for a different purpose. Due to these factors, evaluating the actual costs of biotreatment can be challenging. Although bioremediation is comparatively much less expensive than traditional legal methods of remediation the pricing of bioremediation products can range dramatically.

CONCLUSION

Bioremediation is the use of biological treatments, for the cleanup of hazardous chemicals in the environment. This review focuses exclusively both on microbiological bioremediation and phytoremediation technologies that have proven successful. At present, employing the biochemical abilities of microorganisms is the most popular strategy for the biological treatment of contaminated soils and waters. This biological strategy is dependent on the catabolic activities of the indigenous microflora, optimizing the conditions in situ for growth and Ex situ treatments involve the physical removal of the contaminated matrix to controlled and contained reactors, compost heaps or lagoons. The acceptance of bioremediation as a viable cleanup strategy, however, in many cases also

depends on cost i.e. cannot be more expensive than existing chemical and physical treatments. The thorny issue of the cost of a remediation strategy is highlighted. It indicates that the bioremediation strategies listed are competitive in terms of cost as well as in terms of the impact on the contaminated matrices. Bioremediation is far less expensive than other technologies that are often used to clean up hazardous waste. Many techniques of dispersal, collection, removal, landfill disposal and incineration simply dilute or sequester the contaminants or transfer them to another environmental medium. In contrast, bioremediation can be regarded as a more effective and environmentally friendly strategy since it results in the partial or complete biotransformation of organo-xenobiotics to microbial biomass and stable, innocuous end products. Bioremediation helps in cleaning up of ground water, soils, lagoons, sludges, and process-waste streams. Bioremediation may rely on either indigenous microbes or exogenous microbes. Industries generate waste during processing of coal and oil and also nuclear energy production. The wastes generated are buried and as a result contaminants have migrated through the soil into groundwater supplies Pesticides and fertilizers used in agriculture also have contaminated the ground water. The specific bioremediation technology used is determined by several factors, for instance, the type of microorganisms present, the site conditions, and concentration and toxicity of contaminant chemicals. Different microorganisms degrade different types of compounds and survive under different conditions.

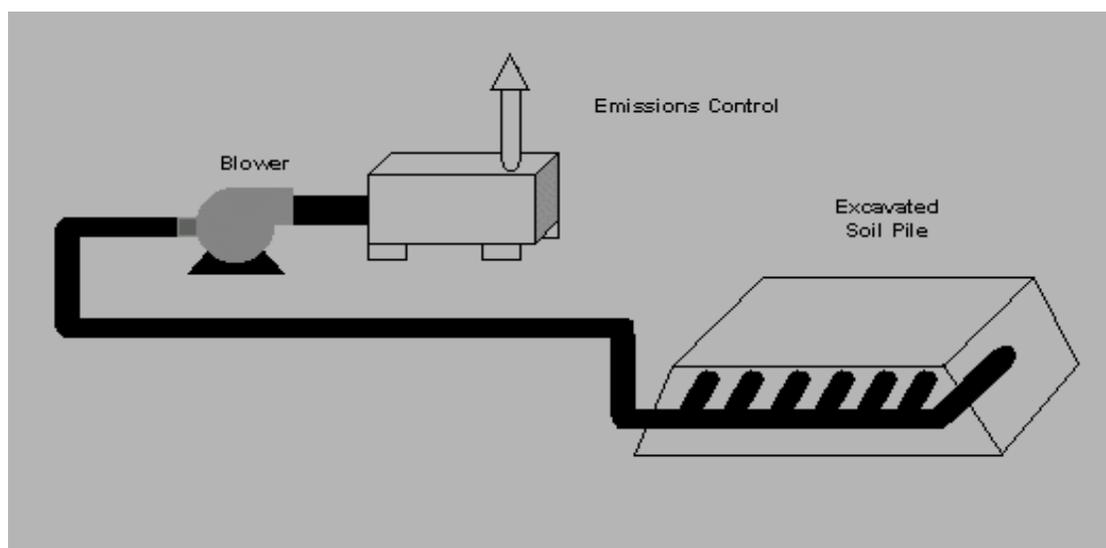


FIGURE 1: EX-SITU BIOREMEDIATION

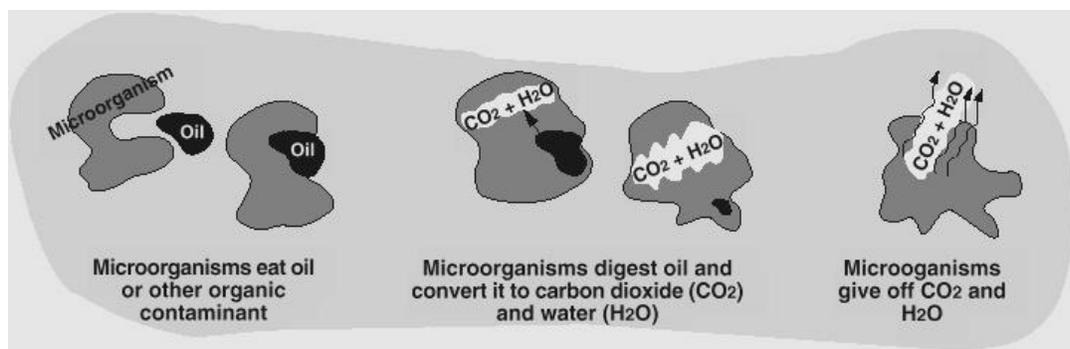


Figure 2: Schematic Diagram of Aerobic Biodegradation in Soil.

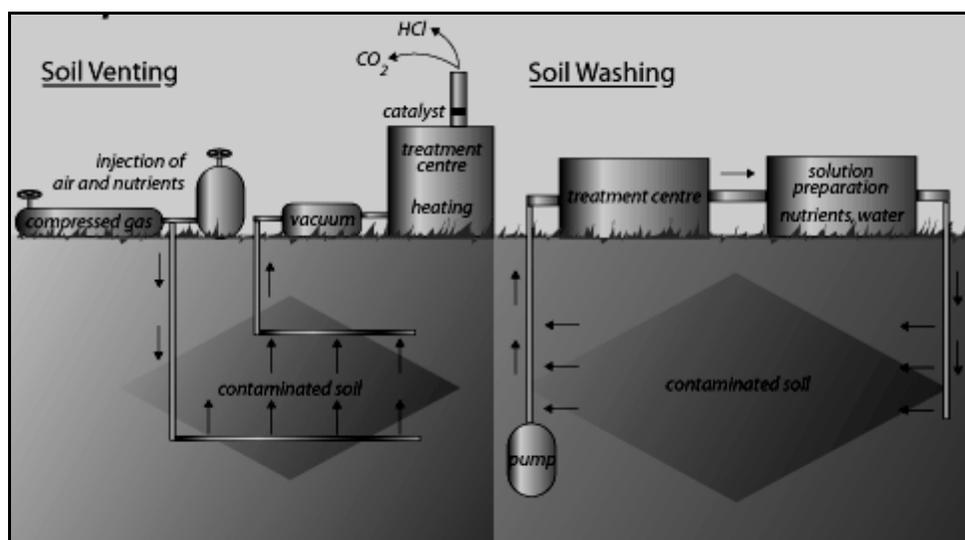


Figure 3: In-situ bioremediation.

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