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Review Article

BIOREMEDIATION- THE NATURE'S WAY

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ABSTRACT

Bioremediation is the field of science that is gaining the crucial importance for the time being regarding the sustainability and survival of the mankind. It is the best of the processes for detoxification and removal of the hazardous substances that are accumulating in the environment in the course of development of our life-style. Bioremediation focuses on the principles on which our nature works. It is the process by which microorganisms like microbes, bacteria or fungi etc. degrade complex organic compounds into simpler ones. Microorganisms use the organic compounds for increasing their biomass or as part of their respiration process and in the way degrade the complex contaminants into non-toxic simpler fragments. Understanding of the underlying mechanism of functioning of microorganisms and their application in the process of remediating action are the two key factors of bioremediation. Bioremediation is nature's way of cleaning up. It is ecological as well as cost effective. The process of bioremediation have been successfully used in petrochemical plants, chemical plants, refineries, food processing plants, marine barges, truck washes, wood treating plants and in various waste management applications. It is considered worldwide as the best technique for treatment of hazardous wastes in an environment friendly manner and the way for our future sustainability. This review focuses on the basic understanding of the process of bioremediation and the different pathways with mechanistic outline.

Keywords: Bioremediation, CSM, Aerobic pathway, Anaerobic pathway, Cometabolic bioremediation, *In situ* process, *Ex situ* process

1. INTRODUCTION

As far as sustainability is concerned, the present scenario of the earth alarms us towards a total extinction of the living world in the near future in a very fast way. The human civilization is really facing a big challenge for its existence. We are threatened but are not able to cut down our practices that are incurred upon us with the progress of civilization. Together with the load of increasing population, the mother earth is really striving to meet the demand of more and more energy, more food grains, more shelters, more transportations and everything more than needed previously. The progress of human civilization is really tracked with the introduction of thousands and thousands of newer compounds each and every year for use on the earth. Till date, only in the developed countries, there are stringent regulations regarding use of newer chemicals and that commenced only some decades ego. But for the rest of the world, the scenario is all together different. Particularly in the poor and developing countries, newer chemicals are introduced in the market only for high business benefit without any regulations. This in turn is affecting the balance, the equilibrium, because the globe is really only one. This exhaustive production in all sectors leads to an accumulation of the substances that are hazardous to the environment. Substances are considered as hazardous if it posses any one of the four characteristics: ignitability, corrosivity, reactivity and toxicity. The term 'hazardous' is actually related to 'quantity', such that it's accumulation can bear a threat to the ecosystem. Regarding use of Petroleum, there occurs accumulation of various complex hydrocarbons. Industry drains heavy metal ion slurry, agricultural sector drains pesticides and so on. Now, what is the sink for all those disposals? It is nowhere, but our own land, river and air, we are disposing our 'garbage of living' to our nature and at the same time we are using the nature as our next subsequent resource. So with every bit of disposal, we are affecting our future resource towards downward quality. This is the farthest side of sustainability. So what is the way out? There are two ways; first one is to cut down our demands, lower our standard of living, go in the backward arrow of civilization - that is impossible. The other way is to opt for processes that can detoxify the hazardous substances. Hazardous substances can be treated by chemical, thermal and physical methods. Chemical methods involve ion exchange, precipitation, oxidation-reduction, and neutralization. But at the end, all of them results in end products, that are minimally toxic too, so overall only the volume of toxicity is reduced at the cost of energy and money. The prominent thermal method is high-temperature incineration that leads to severe air-pollution. Physical methods involve processing of contaminated waters at evaporation plants that also demands energy and also leaves toxic sediments and air pollutants. Another process is solidification, which is performed by encapsulating the waste in concrete, asphalt, or plastic. Encapsulation leads to a solid mass. Waste can also be mixed up with lime, fly ash, and water to form solid cement like products. For radioactive wastes, there are glass-immobilzation techniques. But whatever be the best of technology, there is a serious threat of leaching which if occurs, will certainly ruin the scenario to greater extent. Land disposal is the final way out, two basic methods being landfilling and underground injection, but how much land to spare for and how to stop the spreading of toxic substances to adjacent lands– these are two very pertinent questions. So, all the on-going processes to address the challenge of detoxifying the hazardous substances have a potential impact on the environment, also they need high energy input as well as costly. We really need some other way out.

There is really another way and the ultimate way – to follow the nature in which it works. The nature has a ubiquitous mechanism of recycling, by which it converts the disposals into resources in a magnificent way. The nature does this by means of the numerous microorganisms that are present everywhere in the environment. These microorganisms are the key tools of nature towards recycling. The first task of recycling is 'biodegradation'. The nature always tries to tackle any adverse condition up to its potential, that is the nature has its own mechanism to reduce the deteriorating effect from accumulation of hazardous substances by converting them into non- hazardous by means of biodegradation, but once again nature has its own limit. The present day scenario of accumulation of hazardous substances is beyond the capacity of nature, the load is increasing monumentally day by day and humans till date have not find any way out of converting the hazardous substances into non-hazardous by expending energy which creates a zero impact overall. So the way remains, to help nature in the way it does the work, the work of remedy, the work of healing. Microorganisms are able to decompose most, but not all of the substances that are considered as hazardous and in the process of decomposition they ultimately form non-hazardous substances and most importantly the pathway is a zero-impact pathway that means the processes do not generate any hazardous substance in addition to the non- hazardous end product. But the microorganisms do the task very specifically, that is a particular microorganism or group of microorganisms respond only to a particular substance or a particular group of substances. That is really very vital and it necessitates a vast domain of research to know the specificity between microorganisms and substances and also the conditions that maximizes the efficacy of the processes. Once the queries are all furnished, we can deploy the specific microorganism or group of microorganisms in the accumulation site of that particular substance or a particular group of substances, maintaining the conditions and the process will ultimately lead to get rid of the harmful impact of the particular hazardous substance or group of such substances. That is also a remedial process to detoxify our environment in the pathway of nature. This is the central idea of bioremediation [1, 2], the way nature works.

2. PRINCIPLE OF BIOREMEDIATION

Bioremediation is the use of biological processes to return the environment to its original state [3, 4]. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities [5]. So bioremediation is the way to degrade hazardous substances to less toxic level by use of living organisms, primarily microorganisms. Bacteria, fungi, plants etc can be employed for the purpose. The microorganisms break down hazardous substances by using them either as energy source or sometimes cometabolizing them with an energy source. In principle, bioremediation is associated with the production of energy in a redox-type reaction within the microbial cells. The reactions comprise respiration and other biological functions necessary for cell maintenance and reproduction. In simple view point, a substance to be biodegraded, there needs a specific or group of specific microorganisms; they will feed on the substance and will grow in population. Therefore a delivery system capable of supplying an energy source (electron donor), an electron acceptor, and nutrients, is essential for effective operation. Usually, electron acceptors and nutrients are the two most important components of any delivery system. The degradation pathway is always multistep and complex, also different microorganisms can take part in the ultimate degradation pathway of a single substance whereas a microorganism can use the first phase converted product of a different microorganism, as its metabolite. Microorganisms had been evolved in millions of years. Some microorganisms are indigenous to some places for a particular substance that means the microorganisms can recognize the substances or contaminants. But now-a-days a number of newer chemical compounds are thrown into the nature, many of these are not familiar to the microorganisms and that's why the native microorganisms are not able to degrade them. For this reason, there occurs ample research to genetically modify the microorganisms according to the structure of the contaminants for generating the potential of degradation [6]. For effective bioremediation, the first and foremost task is the growth and activity of the microorganisms. So manipulations of environmental conditions are very much essential to control the process of bioremediation and its rate of action. The rate of the degradation is a crucial point for any bioremediation method to be potentially effective.

3. CONTROLLING FACTORS OF BIOREMEDIATION

3.1. Contaminant concentrations

It prominently control microbial activity. If the concentrations are high enough, the contaminants may leave toxic effects on the microorganisms present and the living cells may be damaged, resulting to failure of growth of microorganisms. On the reverse, low contaminant concentration may hinder induction of degradation enzymes within the microorganisms, the situation results to inefficient functioning. Therefore the contaminant concentration should be judiciously measured before operational set up for optimal result.

3.2. Contaminant bioavailability

Contaminant bioavailability is vital in the sense that it is directly related to fruitful bioremediation process [7]. The contaminants' state of aggregations vary from site to site, they may be sorbed strongly on solids, sequestered by bulky molecules, diffused deeply in micro pores of soil or sediments or may be present in non-aqueous phase liquid (NAPL) form. In these cases, contaminants are only poorly accessible by the microorganisms. So, bioavailability for microbial reactions is lower despite the concentration being high. The contaminant may also be hydrophobic, just like petroleum derived products and therefore they adsorb strongly in soil with high organic matter content. In situations like this, contaminants are required to be brought in states where the maximal proximity with the microorganisms can be achieved, that the contaminants should be in bioavailable form for degradation to take place [5].

3.3. Site characteristics

Site characteristics have a profound influence on the efficacy of any bioremediation strategy [3]. They include pH, site matrix, temperature, water content, nutrient availability, and redox potential. All these factors are considered under 'Site environmental conditions'. The site environmental conditions conjointly dictate the rate and efficiency of the bioremediation pathway for a particular site. Table 1, provided by M. Vidali, serve as a general guideline relating to environmental conditions for potential oil biodegradation [1].

Table 1: Environmental conditions for potential oil biodegradation [1]

Parameters	Condition required for microbial activity	Optimum value for oil degradation
Soil moisture	25–28% of water holding capacity	30–90%
Soil pH	5.5–8.8	6.5–8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10–40%
Nutrient content	N and p for microbial growth	C:N:P = 100:10:1
Temperature (˚C)	15–45	20–30
Contaminants	Not too toxic	Hydrocarbon 5–10% of dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

3.4. pH

pH directly influence the solubility and biological availability of nutrients, metals, and other constituents. Microorganisms can survive only within a delicate pH range. To achieve typical bacterial growth the pH of the medium should preferentially be maintained between 6 to 8.

3.5. Nutrients

Nutrients are vital for microbial cell growth and division. Nutrients serve as the building block of life and they help the microorganisms to produce necessary enzymes that are able to break down the contaminant structure. Carbon is the first and foremost element of life and thus needed in greater amount than other elements. About 95% weight of a cell is composed with carbon, hydrogen, oxygen and nitrogen. Sulfur and phosphorous are the next important elements for cell functioning. Appropriate amounts of trace nutrients are also required for microbial growth. The nutritional prerequisite of carbon to nitrogen ratio should be approximately 10:1, and carbon to phosphorous be 30:1.

3.6. Water content

It is another vital index of site environmental conditions. The amount of moisture is much essential for bacterial cell growth and functioning. Moisture content controls the diffusibility of nutrients from micro pores of the site-matrix to baceterial cell-wall and also the transportation of the nutrients through the cell wall to the core of bacterial cell. Water content also controls the solubility of oxygen in the matrix. Too much wet matrix decreases the bioavailability of oxygen and thus slows down aerobic process.

3.7. Redox Potential

Redox Potential and amount of oxygen in the medium shifts the biodegradation pathway between aerobic and anaerobic extremes. Oxygen content being less, anaerobic process becomes dominant which slows the degradation process and also produces less energy in comparison to aerobic conditions and thus less desirable. Different types of of electron acceptors such as nitrate, sulfate, manganese oxides and iron oxides, if present in the medium, influence the redox potential and thus influence the delivery system for effective bioremediation process.

3.8. Site matrix

It actually describes everything about the particular site, whether it is land, sedimental disposal, wet body or river bed, whatever it may be. There are different phases, complexly developed over time in each site. How much the site is prone for growth and functioning of the microorganisms, depends on the geochemical conditions of the site as well as on distribution, diffusibility and bio-availability of the nutrients in the matrix. The permeability of different zones of the matrix governs the ambient level of moisture and oxygen content in the matrix.

3.9. Temperature

It is another parameter, which governs the rate of the entire process overall. The metabolism processes within the microorganisms are all enzymatic reactions and these reactions are influenced by temperature to the extent that every 10°C rise in temperature approximately doubles the rate of the reaction. Temperature also helps to mobilize the sorbed nutrients and increase their bioavailability to microorganisms. However, microorganisms are not the type of species that can survive a broad temperature range. Most of the bacterias found in soils are mesophiles that can maintain their activity only when the temperature range is moderate, likely within 25°C to 45°C. Some bacterias are found near hot springs, they are thermophiles that mean they can survive in hot conditions, typically between 60°C to 122°C. For them, the rising of temperature increases the diffusibility of nutrients, which in turn increases the cell functioning to a faster rate.

4. CONCEPTUAL SITE MODEL (CSM)

CSM is the next important part after key factors of bioremediation have been covered. The first step for any bioremediation program is to set up a conceptual site model (CSM) to estimate the potential for applying bioremediation at a site [8]. The CSM considers the nature and extent of contamination and site characteristics via parametric characterization of site hydrogeology, geochemistry along with oxidation-reduction conditions, biodegradation potential related to contaminant fate and transport and availability of receptor and exposure pathways. After a CSM having been constructed and refined, a vivid characterization of the existing microbial community as well as the characteristics essential for the functioning of an appropriate microbial community in case of exogenic microorganisms being required, are thoroughly worked out. It is followed by the viability studies involving treatability, examination of soil comparability together with the structure and function of the microbial community to make certain that undesirable reactions with the contaminants or their degradation products are mostly prevented. Finally the application set up with the ins and outs of the process control parameters and cost factor are analyzed. Once all these steps are complete, the bioremediation process can be implemented at the site with essentially continuous monitoring.

5. MECHANISTIC PATHWAYS

Mechanistically bioremediation can occur via two pathways. They are direct pathway and indirect pathway. Direct pathway involves the direct action of the microorganisms on the contaminant, whereas the indirect pathway, which is referred as Co-metabolic bioremediation occurs via the breakdown of a contaminant by an enzyme or cofactor that is formed during microbial metabolism of a different compound. Again both the direct and indirect pathway can occur either in aerobic condition or anerobic condition [9]. However the scenario is not rigid at all. Many a times more than a single pathway work simultaneously in a site depending on the types of contaminants and microorganisms present in the site and the site environmental conditions. Table 2 provides representative examples of bioremediation by microorganisms and the corresponding contaminants where they act upon.

5.1. Aerobic Bioremediation (Direct)

When the condition is aerobic and there is ample supply of appropriate nutrients, microorganisms can break down many organic contaminants to carbon dioxide, water, and microbial cell mass. Oxygen is used as the electron acceptor in aerobic bioremediation [10]. Aerobic metabolism is more likely to occur and can be useful for hydrocarbons and other organic compounds, mainly petroleum hydrocarbons and many fuel oxygenates (e.g., methyl tertiary-butyl ether [MTBE]). Many organisms have the potential of degrading hydrocarbons using oxygen as the electron acceptor and the hydrocarbons as the source of carbon and energy. The ionic form of metals may also be changed in aerobic technologies. For a site containing mixed metal and organic wastes, it is very important to judge whether the oxidized forms of the metal species (such as arsenic) will be environmentally benign, before application of the bioremediation strategy.

Aerobic bioremediation is mostly effective for degradation of mid-weight hydrocarbon contaminants, because the lighter products, being volatile, do not stick in the matrix for longer time and for high molecular weight products it takes too long a time for effective degradation. Aerobic oxidation always occur naturally in adequate conditions, however oxygen, which is generally considered to be the prime growth-controlling factor for hydrocarbon-degrading bacteria, is found to be depleted in zones that have hydrocarbon contamination. In situations like this, supplementary source of oxygen should be provided to the site. Enhanced aerobic bioremediation technologies address primarily on increasing oxygen levels and can accelerate biodegradation potentially by several orders over naturally-occurring, non-stimulated rates. The stoichiometric ratio for effective aerobic process is estimated to be 3:1 mole ratio of oxygen to hydrocarbon. The efficacy of aerobic bioremediation solely relies on the ability of oxygen delivery to the proximity of hydrocarbon-degrading microorganisms. So there should be a balance between the oxygen source in the site-matrix, the rate of oxygen conversion in bio-available form and the transportation of that bio-available oxygen through the subsurface to the proximity of microorganisms.

5.2. Anaerobic Bioremediation (Direct)

Under anaerobic conditions, microorganisms break down organic contaminants to methane, lesser amount of carbon dioxide, and trace amounts of hydrogen gas [11]. Anaerobic metabolism comprises different processes like fermentation, methanogenesis, denitrification, sulfate- and iron-reducing activities, and reductive dechlorination. According to the type of contaminants, one or more of these activities may take place. In the anaerobic metabolism process, nitrate, sulfate, carbon dioxide, oxidized metals, or organic compounds, like chlorinated hydrocarbons, may function the role of electron acceptor. Fermentation of organic substrates indirectly supplies the quantity of hydrogen, needed for the reaction.

Generally, anaerobic conditions are utilised to degrade highly halogenated contaminants, although some petroleum hydrocarbons may be potentially biodegraded in anaerobic conditions. The halogenated compounds, e.g. chlorinated solvent such as tetrachloroethene (TCE), 1,1,1-trichloroethane (TCA), chloroform (CF), trichloroethene (TCE), carbon tetrachloride (CT), and methylene chloride or their degradation products dichloroethene (DCE), vinyl chloride (VC), dichloroethane (DCA), and chloroethane serve the role of the electron acceptor whereas hydrogen functions as the direct electron donor. Chlorinated solvents are stable and distribute in multiple phases depending on their release to the site and site conditions. They remain in a vapor phase in unsaturated soils, dissolved phase in groundwater, and also as non-aqueous phase liquids (NAPL) in the subsurface. Most of the chlorinated solvents have higher density compared to water and they are hydrophobic.

In anaerobic biodegradation process of chlorinated compounds, the chloride ions are generally observed to be removed sequentially as in the degradation of tetrachloroethene (perchloroethene or PCE) to trichloroethene (TCE) to cis- dichloroethene (DCE) and finally to ethene via vinyl chloride(VC) [12]. In each step hydrogen, the electron donar, is oxidized to form hydrogen chloride, while the chlorinated ethenes get reduced. Hydrogen is typically the most important electron donor for anaerobic dechlorination.

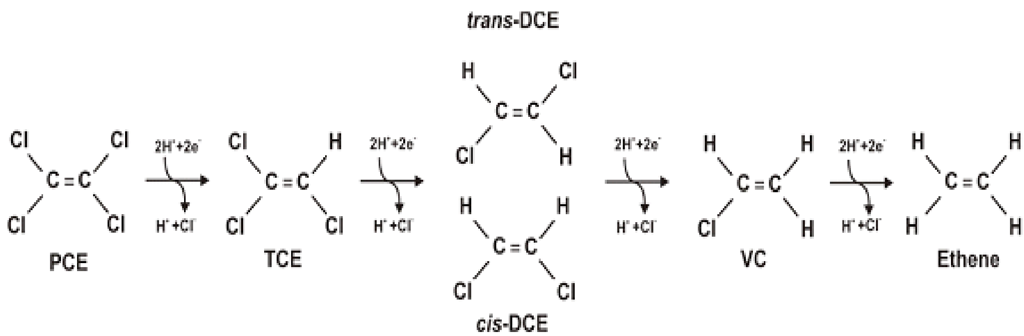

Fig. 1: Dechlorination of PCE [12]

5.3. Cometabolic Aerobic and Anaerobic Bioremediation

Some microorganisms have the potential to degrade contaminants while oxidizing or reducing other compounds (metabolites) for energy and carbon. Cometabolic bioremediation is referred to the degradation of a contaminant by an enzyme or cofactor that is formed during microbial metabolism of another compound [13]. The type of bioremediation is highly desirable as it ensures that only the microbes with the potential to degrade the contaminant of concern should only be stimulated. One important aspect of cometabolic bioremediation is that it can be operative even when the contaminant concentration is very low, because in case of low contaminant concentration direct aerobic or aerobic pathway cease to be operative unless there is some energy or carbon benefit of the microorganism out of the contaminant concentration. Cometabolic bioremediation may be aerobic as well as anaerobic. In aerobic cometabolic bioremediation, the process is oxidation and the contaminant gets oxidized by an enzyme or cofactor formed during microbial metabolism of another compound with oxygen. A variety of substances like methane, ethane, ethene, propane, butane, aromatic hydrocarbons (such as toluene and phenol), and ammonia have been found to play the role of electron donors in cometabolic aerobic oxidation. In anaerobic cometabolic bioremediation, the picture is all the different, the contaminant gets reduced by an enzyme or cofactor formed during microbial metabolism of another compound in an environment devoid of oxygen. In case of cometabolic anaerobic reduction, the substances like methanol, acetate, glucose, lactate, sulfate or pyruvate can act as substrates. It may be pointed out that, in cases of cometabolic bioremediation, biodegradation of the contaminant does not provide any energy or growth benefit to the microorganism mediating the reaction, compared to direct bioremediation as stated earlier. However, there is one point of concern, that in some cases there are chances that some intermediate product may be formed during cometabolic bioremediation that may inhibit the microbial metabolism process.

6. BIOREMEDIATION STRATEGIES

Bioremediation, on a whole, can be described as the application of biological processes to degrade, break down, modify, transform, and/or essentially remove contaminants or impairments of quality from soil and water either completely or partially, such that the evil effect can be minimized. Strategically, bioremediation can be broadly classified under two heads *in situ* processes and *ex situ* processes. *In situ* processes involve the treatment of contaminants at the site of accumulation, whereas if the contaminants are excavated from its parent site of accumulation and transported to some other site for bioremediation, it is considered as *ex situ* process [14].

6.1. *In situ* Bioremediation

In situ bioremediation methods are potentially advantageous as they do not involve excavation or extraction of the contaminated soil or water and therefore very cost effective. The site is also minimally disrupted, thus the amount of dust created is less. Another important aspect of *in situ* bioremediation is that treatment of soil and groundwater can be continued simultaneously. However, this process also has some drawbacks, the main of them being the time-period, it is really a time-consuming method compared to other bioremediation techniques. Being a relatively long-term process, there occur seasonal variations in microorganism population and activity, which are not always very easy to handle out and equally it is quite difficult to predict the effect of remediation of contaminated sites. *In situ* bioremediation can generally be classified under three subdivisions: Natural attenuation, biostimulation and bioaugmentation. If the *in situ* bioremediation is carried out by indigenous microorganisms, the processes are either natural attenuation or biostimulation and it is bioaugmentation when the microorganisms are exogenic.

6.1.1. Natural attenuation

It is the process of bioremediation that occurs naturally without human intervention. It is happening during course of time long before the scientific community was able to actually figure it out and it goes on its own pace that needs nothing other than monitoring. This natural attenuation solely depends on natural conditions and functioning of the microorganisms that are indigenous to the matrix, whether it is land or water.

6.1.2. Biostimulation

It also uses indigenous microorganisms to remediate and it is nothing but the process of natural attenuation with human additives to increase the remediating effect potentially. Whenever any one or more of the components of a delivery system, namely an energy source (electron donor), an electron acceptor (phosphorus, nitrogen, oxygen, carbon), or nutrients (biogas slurry, manure, spent mushroom compost, rice straw and corncob etc) are provided on a supplementary basis from outside to the indigenous microorganisms of a site, the process is called biostimulation [3]. Biosparging and bioventing are the two common forms of biostimulation.

6.1.2.1. Biosparging

It is an improvised in-situ remediation technology that utilizes indigenous microorganisms to biodegrade organic constituents in the saturated zone [15]. In biosparging, air (or oxygen) and nutrients (if needed) are injected into the saturated zone of water column to increase the biological activity of the indigenous microorganisms. Biosparging is effectively used to reduce concentrations of petroleum constituents that are dissolved in groundwater, adsorbed to soil below the water table, and within the capillary fringe or hazardous hydrocarbons that are mixed in underground storage tank.

6.1.2.2. Bioventing

Bioventing is also an *in situ* remediation technology, however it is the process of aeration of the unsaturated vadose zone to stimulate aerobic biodegradation [16]. Bioventing enhances the activity of indigenous bacteria and simulates the natural in situ biodegradation of hydrocarbons in soil by inducing air or oxygen flow directly into the unsaturated zone of sub-surface of soil and, if necessary, by adding nutrients. It is different from biosparging in the way that, oxygen and nutrients are streamed direcly into unsaturated zone of dry soil, rather than in saturated zone of liquid body or moist zones of soil or sole water body as in biosparging. During bioventing, oxygen may be supplied through direct air injection into residual contamination in soil. Bioventing primarily assists in the degradation of adsorbed fuel residuals, but also assists in the degradation of volatile organic compounds (VOCs) as vapors move slowly through biologically active soil.

6.1.3. Bioaugmentation

It is the process of bioremediation whenever the microorganisms are not indigenous to the site of remediation [17]. Here exogenic microorganisms that are sourced outside of the site are introduced to the site for the purpose of detoxification. Situations may arise when some indigenous microorganisms, not capable of breaking down a contaminant, are genetically modified in the laboratory and brought back to the original site for the process of bioremediation. This also falls under bioaugmentation.

6.2. *Ex situ* Bioremediation

Ex situ methods are the processes where the contaminants are treated at some places other than their parent sites of accumulation [18]. It is a totally monitored process, thus very much effective and rapid also. However, it is quite costly, because it needs excavation or extraction of the contaminants and transportation to newer site and thus bears the threat of dispersion of contaminants during transport. Different ex situ procedures have been innovated according to the phase of the contaminant matrix. Contaminants in liquid phases can be treated in constructed wetlands while for semi-solid or solid wastes, bioremediation is carried out in slurry bioreactors. For solid contaminations, the biodegradation runs through land farming, composting and biopiles.

6.2.1. Constructed wetland (CW)

It is a man-made wetland for treatment of municipal or industrial wastewater, grey water or storm water over flow [19]. It may also be planned for land recovery after mining, or as a lessening step for natural habitats ruined for land development. Constructed wetlands are monitored systems that utilize natural functions of soil, plants and organisms for treatment of wastewater. They are designed according to the type of wastewater. They function to treat both centralized and on-site wastewater. If the waste water contains high amount of suspended solids or soluble organic matter, as may be revealed through measurement of BOD and COD values, primary treatment is suggested before introduction of wastewater into constructed wetlands. The main purpose of constructed wetlands is to act as a biofilter, just similar to natural wetlands. Constructed wetlands have two main types, subsurface flow constructed wetlands and surface flow constructed wetlands. They function to remove suspended solids, organic matter and not essentially the pathogens (*i.e.,* bacteria, viruses, protozoan and helminths), although some extent of pathogen removal is expected and subsurface wetland provide greater ability in this aspect than surface wetlands. Physical, chemical, and biological processes play their roles simultaneously in wetlands for removal of contaminants from wastewater. An understanding of these processes is very much essential for designing wetland systems, as well as for understanding the fate of chemicals once they are introduced the wetland.

6.2.2. Bioreactor

It refers to any manufactured device or system that supports a biologically active environment. In the context of bioremediation, bioreactors are the *ex situ* innovations where the contaminated soil or water are introduced into bioreactors, which provide the environment to grow and functioning of the microorganisms, already installed depending on the type of contaminants [18]. The process can either be aerobic or anaerobic. According to the mode of operation, bioreactors may be categorized in three classes as batch, fed batch or continuous. Organisms that grow in bioreactors may be installed in liquid medium or may be adhered to the surface of a solid medium. Organisms introduced via submerged cultures may be in suspended state or immobilized state. Suspension bioreactors can generally employ a vast variety of organisms, as no special attachment surfaces is needed and also operate substantially than immobilized cultures, with the drawback of sequential removal of the microorganisms with the effluent in case of continuously operated process. This difficulty can be overcome in case of immobilized culture that adheres to a solid surface. The solid surface can be packed bed, fibrous bed, membrane or newly devised moving media.

6.2.3. Slurry bioreactor

Slurry bioreactor is really one step ahead of simple bioreactor [20]. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three phase (solid, liquid, and gas) mixing condition to hasten the biodegradation of soil-bound and water-soluble contamination as a water slurry of the contaminated soil, sediment, or sludge and biomass (usually indigenous bacteria) capable of degrading targeted contaminants [21]. The pathway provides enhanced process control and greatly accelerates the rates of biodegradation of contaminants through better physical contact between pollutants and microorganisms as well as improvised distribution of nutrients, gases (air, oxygen), and other materials for support of biological process. Contaminated soil characterization is a must prerequisite before introduction of the soil in slurry bioreactor. The characterization involves quantification of parameters regarding particle size distribution, texture/composition (silt, clay, sand), soil nutrients (nitrogen, phosphorous), pH, cation exchange capacity (CEC), speciation of metals and total organic carbon. Slurry bioreactors are one of the most efficient technologies that are used in the bioremediation of contaminated soils mainly because the process is rapid and totally controllable.

6.2.4. Landfarming

It is one of the most widely used soil bioremediation technologies [22]. Landfarming, generally known as land treatment or land application, is a top-soil or surface-land remediation technology which diminishes the concentrations of petroleum disposals by means of biodegradation [23]. In this technology excavated contaminated soils are spread in a thin layer on the ground surface and stimulation of aerobic microbial activity within the soils is achieved through aeration and/or the supplements of minerals, nutrients and moisture. The enhanced microbial activity plays the role in degradation of the adsorbed petroleum product constituents through microbial respiration. For contaminated soils that are shallow, preferably within about three feet below ground surface, effective stimulation of microbial activity can be attained without excavating the soils, but excavation is the way out if contaminant adsorption exists to soil-depth of nearly five feet or greater. It is a relatively simple technology, bears less cost, but applies only when the contaminant concentration is not severe and the contaminants are quite easy to biodegrade. It is very much possible to reduce the volume of residues in landfills.

6.2.5. Compost bioremediation

A new compost technology, named as compost bioremediation [24], is currently being employed to reinstate contaminated soils, manage odors, dealing with stormwater and breaking down of volatile organic compounds (VOCs). Compost bioremediation addresses to the use of a biological system of micro-organisms in a mature, cured compost to sequester or break down contaminants in water or soil by means of thermophilic bacteria under aerobic conditions. Microorganisms eat up contaminants in soils, ground and surface waters and air. The contaminants are metabolized and in the way transformed into humus and inert byproducts like carbon dioxide, water, and salts. Compost bioremediation has been proved to be effective in degrading or modifying different types of contaminants including chlorinated and nonchlorinated hydrocarbons, heavy metals, wood-preserving chemicals, pesticides, bleaching solvents, petroleum products and explosives. Compost especially used for bioremediation purpose is termed as “tailored” or “designed” compost as it is specifically made for the treatment of specific contaminants at specific sites. In compost bioremediation, biodegradation of solid contaminants occurs through the process of oxidation and hydrolysis, the optimum temperature ranging between 40°C to 70°C for the growth of microorganisms involved in composting. This method is eco-friendly with simple protocols and permits the control of high volumes of waste and ends up with the total mineralization of pollutants.

6.2.6. Biopiles

Bioplies are more sophisticated systems of composting, although more expensive but allows more efficient control of the process [18, 25]. A bio-pile is a bioremediation technology where excavated or extracted soils are mixed up with soil amendments to form the compost piles and treated. The basic bio-pile system is composed of a treatment bed, an aeration system, an irrigation/nutrient system and a leachate collection system. Moisture, heat, nutrients, oxygen, and pH are monitored to enhance biodegradation. An irrigation/nutrient system is provided through underground streaming for passage of air and nutrients through the soil. Soil piles can be high up to twenty feet. This technology has been mainly used for remediation of petroleum-contaminated soil.

7. MERITS AND DEMERITS OF BIOREMEDIATION

The merits of bioremediation can be summarized on the basis of principles, strategies and outcomes. First of all, it is an eco-friendly process, producing minimum impact to the environment. Being a natural process or improvised natural process, it is cost effective. Bioremediation is noninvasive as well as nonintrusive and thus suitable for potential long term use at the site. Apart from in situ processes, they can operate quite rapidly and strategically they are easy to implement. Intensive workman load is not required. After completion of the process, the site can be adjusted to its original state and thus serves the master key towards sustainability.

Regarding demerits, the first point is that it is not applicable for all hazardous substances, the scope limits for only those substances that are biodegradable. So for majority of heavy metal ion contaminants, radioactive wastes, most types of plastics etc, bioremediation has essentially zero potential. There also remain threats that in some cases the products of biodegradation may be harmful, so elaborate study is required, but that is very crucial in the laboratory to set up conditions approaching to that of a natural contaminated site. Except for in situ processes, there is limited scope of large scale implementation regarding the very high size of contaminated zones in general and severity of contamination. Performance based site specific regulatory criteria are not always very easy to prepare, so for each new site, everything needs to be started from the beginning-that posses a retardation regarding implementation.

Table 2: Representative examples of bioremediation by microorganisms and the corresponding contaminants where they act upon

Microorganisms	Microorganism Type	Contaminants	Reference
P. alcaligenes P. mendocina and P. putida, P. veronii, Achromobacter, Flavobacterium, Acinetobacter	Bacteria	Petrol and diesel polycyclic aromatic Hydrocarbons,Toluene	[26, 27]
Bacillus cereus A	Bacteria	Diesel oil	[28]
Bacillus spp. ETL-2012	Bacteria	HKG212 Textile Dye (Remazol Black B)	[29-31]
Pseudomonas aeruginosa, *Bacillus pumilus*	Bacteria	Sulfonated di-azo dye Reactive Red HE8B Remazol Navy Blue dye	[32]
Penicillium chrysogenum	Fungi	Monocyclic aromatic hydro carbons, benzene, toluene, ethyl benzene and xylene, phenol compounds	[33]
Phanerochaete sordida	Fungi	Polychlorinated dibenzofurans	[34]
Aspergillus niger	Fungi	Polychlorinated biphenols	[35]
Candida viswanathii	Yeast	Phenanthrene, benzopyrene	[36]
Monoraphidium braunii	Algae	Bisphenol	[37]
Scenedesmus quadricauda	Algae	Fungicides (dimethomorph and pyrimethanil) and herbicide (isoproturon)	[38]
Chlamydomonas reinhardtii	Algae	Herbicide (prometryne)	[39]

8. CONCLUSION

With some underlying demerits, bioremediation is still the most fruitful sustainable route towards remediation. There is an avalanche of research activity around the globe to cater the need for bringing more and more contaminant types under the scope of bioremediation. Genetically modified microorganisms are fabricated, improvisations regarding optimization of potential functionality are implemented– these enable an extension of the site volume and more control over seasonal variations is achieved. Number of site implementations is increasing greatly and diversified contaminated zones are being tackled. This is a really promising picture. The overall paradigm also generates a potential boost in the economy. Standing in the first lap of the twenty first century, we can be optimistic to have a world quite remediated from the present condition through the course of bioremediation in near future to leave a habitat for our next generations to live a better life.

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