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THE USE OF MICROBES IN RESTORATION OF DETERIORATED ENVIRONMENT

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ABSTRACT: *Bioremediation is a national goal for restoration and cleanup of contaminated land and water bodies. Microorganisms such as bacteria, fungi and algae can be enhanced to remove pollutants from the environment, as this organism obtain both energy and tissue building materials when they feed on wide variety of compound found in environmental contaminants. These microbes are often isolated from the nature or have been modified through genetic engineering to break down and convert heavy compounds to harmless organic compound. Growing microbes in the laboratory require maximum concentration and should not be considered as safe.*

KEYWORDS: Bioremediation, Cleanup, Contamination, Environment, Micro Organisms

INTRODUCTION

The increasingly obvious effects of pollution of the biosphere and aquatic have continued to attract concern of both scientist and public worldwide. In recent years there has been an increased in emission of industrial effluents containing toxics chemicals, hydrocarbon and heavy metals. The ultimate fate of these pollutants including partitioning into various aquatic environmental compartments (water, suspended solids, sediments and biota) and factors such as; concentration, dilution, water solubility, biogeochemical processes taking place, adsorption to soils, suspended particulates and sediments, lipophilicity, and bioaccumulation in living organisms (Khan and Sinha, 1996). These pollutants drain into the river, which is often the source of drinking water for another town downstream. Municipal water treatment facilities in most of the developing countries, at present, are not equipped to remove traces of pollutants, especially pesticides, hydrocarbon and heavy metals, consequently exposing every consumer to unknown quantities of pollutants in the water they consume. Also, pollution is increasing important fact that determines the health and distribution of wildlife and biodiversity. It has been recognized that the persistence and bioaccumulative tendency of these substances, their metabolites and residues in the environment make them not to remain where they are applied but instead partition between the major environmental compartments in accordance with their physico-chemical properties and may thereby become transported several kilometres from the point of their original release. Such environmental distribution may lead to exposure of living organisms including man

Oil spills devastate ecosystems and the atmosphere by tainting water sources, releasing toxic vapours, and causing irreversible contamination to aquatic and terrestrial habitats (Semple.,1998). Consumers are not directly affected by the detrimentally impacted by the spill via depletion of their crucial food, energy, and water sources; this depletion results in a domino effect of mortality throughout the food web and transforms healthy ecosystems into dead zones (Semple *et al*.,1998). Moreover, some hydrocarbons are semi-volatile; they evaporate into the air and create heavy vapors that reside near the ground, causing hazardous

conditions that may subsist for decades. During the Deepwater Horizon spill, the Natural Resources Defense Council reported that citizens of Louisiana suffered from nausea, vomiting, headaches, and labored breathing caused by heavy oil vapors (NOAA, 1999). Other hydrocarbons are carcinogenic and irritating to the skin and airways; with prolonged contact, people can develop acute health problems (Hurtig, 2002; San Sebastian *et al*., 2001).

Surface finishing industries, discharging a variety of toxic metals such as Cd, Cu, Ni, Co, Zn and Pb into the environment. Eventually, build-up of dangerous concentrations of toxic metals, pesticides in fish and in grains and vegetables grown in contaminated soils is most alarming due to harmful effects of these contaminants on human health (Jasper, 1989). It is well known that these pollutants can be extremely toxic as they damage nerves, liver and bones, and also block functional groups of vital enzymes (Jastrow,1998). Metals like Nickel are listed as a possible human carcinogen and associated with reproductive problems and birth defects.

Various technologies exist that enable the detoxification/deactivation and removal of toxic compounds from the water and soil, mostly based on physicochemical extraction method. They are costly and completely destroy soil microorganisms. High reagent requirement and unpredictable metal ion removal are some other disadvantages associated with such techniques. Further, strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution. These disadvantages can become more pronounced and further aggravate the process cost in case of contaminated ground waters, mine tailings effluent and other industrial wastewaters due to voluminous effluents containing complexing organic matter and low contamination.

Three prominent methods have been utilized for oil spill cleanup: mechanical pumping, combustion, and bacterial bioremediation. When the Deepwater Horizon spill escalated, the oil company British Petroleum (BP) used oil skimmers to remove and treat crude oil-contaminated water from the Gulf of Mexico. However, this method accumulates large energy and production expenditures. Later, the combustion of buoyant oil was used but this method produced dangerous fumes (Aigner *et al.*, 2010). Considering these factors, the needs to develop more effective and cheaper technologies becomes apparent. Conventional physico-chemical metal-bearing waste waters may not always prove successful due to the high costs of processing effluents of high volume and low contamination or because the treated water does not meet certain legal standards, e.g. it contains complexing organic matter. Biotechnological approaches can succeed in those areas and are designed to cover such niches as described above. Microbial communities are highly diverse and capable of conducting an extensive range of metabolic activities (Fliermans and Balkwill, 1989). Irrespective of depth or geological formation, subsurface microorganisms carry out the entire major nutrient cycling, i.e., carbon, sulfur, nitrogen, manganese, iron and phosphorus. Although each geological formation appears to have its own microbial structure, sandy formations that are highly permeable to air or water flow have higher microbial activity. They have proven capability to take up pollutants from aqueous solutions, especially when the concentrations in the effluent range from less than 1 to about 20 mg/l (Brierley, 1990). Besides, flexibility to handle the range of physico-chemical parameters in effluents, selectivity to remove only the desired pollutants and the cost-effectiveness are some added advantages of biological cleanup techniques.

As discussed above, many a times, biosorption alone may not suffice for effective pollutants remediation. Under such situation, application of active and growing cells might be a better option due to their ability of self-replenishment, continuous metabolic uptake of pollutants after physical adsorption, and the potential for optimization through development of resistant species and cell surface modification (Wilde and Benemann, 1993; Sandau *et al*., 1996). Considering metals that usually diffused into the cells during detoxification get bound to intracellular proteins or chelatins before being incorporated into vacuoles and other intracellular sites. These processes are often irreversible and ensure less risk of metal releasing back to the environment (Fomina, 2005). In contrast to conventional chemo-physical and biosorptive methods, employment of active microorganisms may allow development of a single stage process for removal of most of the pollutants present in industrial effluents. Growing cells have unlimited capacities to cleave organo-metallic complexes, degrade organic compounds, as well as take up other inorganic ions such as ammonium, nitrate and phosphate. Further, dissolved and fine-dispersed metallic elements can also be removed via immobilization. Yet, there are significant practical limitations to biouptake by living cell systems such as sensitivity of the system to extremes pH, high metal/salt concentration and requirement of external metabolic energy (Aksu and Donmez, 2006). However, such challenges can be met via strain selection and exploitation of organic wastes as carbon substrates. The isolation and selection of non-selective and resistant strains shall be a crucial aspect to overcome the prime constraint of employing living cell systems. Incidentally, resistant cells are expected to bind substantially more toxicants, which in turn is a prerequisite for enhanced bioprecipitation/intracellular accumulation and development of an efficient process. Instead of depending upon single species, a better approach could be towards designing a consortium of strains having high toxicant biosorption, bioaccumulation and bioprecipitation capacities

Several studies on application of growing microbial cells for metal scavenging have been reported. However, in toxic metal removal applications, it is important to ensure that the growing cells can maintain a constant removal capacity after multiple bio accumulation–desorption cycles, and a suitable method is required to optimize the essential operating conditions. The situation demands a multi-prong approach including strain isolation, cell development and process development in order to make the ultimate process technically and economically viable.

The aim of this paper is to analyze the prospects of using microbes that are environmentally friendly (bacteria, fungi, and algae) to achieve healthy environment.

Bacteria

The application of environmental biotechnology as a successful remediation tool depends on the ability to stimulate or enhance specific activity of indigenous or introduced microorganisms. The challenge has been to enhance the activity of these microorganisms and develop means to bring the contaminant into direct contact with the organisms to achieve optimal bioremediation. The strain of bacteria (methanotrophic bacteria) has a ubiquitous distribution in the environment and the use of natural gas or methane with other nutrients to stimulate their bioremediation activities through methane monooxygenase had been successful as a remediation option. These two features allow for a relatively efficient, inexpensive, and safe means to manipulate the environment to accelerate bioremediation.

It is now well recognized that trichloroethylene (TCE) and some chlorinated alipthatic compounds that can be degraded by a diversity of bacteria including *methanotrophic bacteria* (Little et *al*.,1988), selected methanogens (Bouwer and McCarty, 1984), and species of *Pseudomonas* (*P. cepacia, P. mendocina and P. putida*) capable of also degrading aromatic compounds (Nelson et *al*., 1988). Ensley (1991) had demonstrated a linkage between TCE degradation and aromatic metabolism in *P. cepacia* G4, *P. mendocina* and *P. putida*. Ensign *et al*., (1991) reported that pure cultures of *Xanthobacter* spp. metabolized TCE with the utilization of propylene as a carbon and energy source presumably using the enzyme alkene monooxygenase. Fliermans, *et al* ., (1988) and Bowman *et al*., (1993) have shown that enrichments for methanotrophs in subsurface samples collected from the Savannah River Site in South Carolina stimulate the microbial degradation and complete mineralization of TCE and other chlorinated aliphatic compounds both in the laboratory and *in situ*. Propane utilizers or propanotrophs that also exhibit non-specific oxidase activity may also be used for bioremediation. Where mixtures of chlorinated aliphatic hydrocarbons including 1,1,1-trichloroethane, are present propane may be the stimulant of choice using air-sparging technology (Tovanabootr and Semprini, 1998). However, methanotrophs are optimal in bioremediation when TCE is the primary contaminant of concern. While sediment methanotrophic bacteria can be efficient in degrading TCE from contaminated groundwater (Bowman *et al*., 1993), certain methanotrophs are more efficient at TCE degradation than others (Koh, *et al*., 1993). It has been suggested that mixed populations are more efficient in TCE degradation (Uchiyama *et al.,* 1992)

Fungi

Fungi are known to be able to accumulate significant amounts of heavy metals varying from a few percent to 20% of dry mass (Tobin *et al*., 1984; Gadd, 1993), suggesting that microbial biomass may affect the mobility of metals in the soil system. According to the calculations by Söderström (1979), the surface of interaction between fungi and soil is up to 0.14 m^2 in 1 g of soil. They can remove metals from the wastes both by metabolism dependent (bioaccumulation) or independent (biosorption) processes (Gadd, 1993). Fungi enhance root absorption up to 47-fold in an area (Smith and Read, 1997) and provides nutrients and water otherwise not accessible for plants (Cui and Nobel, 1992; George *et al*., 1992; Nadian *et al*., 1997) and facilitate the establishment and survival of vegetation under stress conditions (Jasper *et al.,* 1989; Smith *et al*., 1998). For instance, mycorrhizal fungus stabilize the tailing material with the net of hyphae and improve its structure, as they produce substances that bind soil particles, leading to the formation of soil aggregates (Thomas *et al*., 1993, Wright and Upadhyaya, 1996, Jastrow *et al.,* 1998). Fungi can remove metals from the wastes both by metabolism dependent (bioaccumulation) or independent (biosorption) processes (Gadd, 1993). The components of the fungal cell wall can be very efficient in binding heavy metals due to the presence of free amino, hydroxyl, carboxyl and other groups (Gadd, 1993). Therefore, saprobic fungi can be commercially grown in bulk culture and their either live or dead biomass used as biosorbents for heavy metals and other contaminants (Morley and Gadd, 1995; Kapoor and Virarghavan, 1995; Mullen *et al.,* 1992; Fomina et al., 2005). Similar phenomena occur in ectomycorrhizal (Denny and Wilkins, 1987; Turnau and Dexheimer, 1995), ericoid (Bradley *et al*., 1982) and arbuscular mycorrhizal fungi (Joner *et al.,* 2000; Gonzales-Chavez *et al*., 2002, 2004). Some of these fungi can also precipitate heavy metals outside the mycelium by producing various organic acids or enzymes such as the acid phosphatase (Turnau and Dexheimer, 1995) or pigments, which additionally prevents the migration of metals.

Algal

Algae are capable of living in habitats where few other organisms could survive, an attribute that give them an edge over other bioremediants. They can live in virtually any habitat in which there is sunlight and adequate moisture. Algae are found in freshwater lakes, ponds, streams, swamps, on moist soil, wood, and throughout the sunlit zones of the marine environment, as well as on the snowfields and glaciers. This diversity in algal characteristics plays significant roles in algae's ability to grow, adapt, and reproduce in different habitats. These enhance their usefulness in bioremediation. The main issue of concern in algae bioremediation is to ascertain a definite or best oxidation pathway that algae use to degrade pollutants such as hydrocarbons and then clone the cDNA for the specific enzyme that produces the best oxidation results. Once cloned, the gene can be over-expressed in algae via a proper over-expression vector through transfection. Also, genetic fortification would allow the enzyme to oxidize crude oil without an algal medium, eliminating the need for containment methods. The concept of algae bioremediation has much potential that requires holistic investigation. Algae, especially *Coccochloris elabens* can efficiently degrade "heavy" crude oils and live in high "heavy" crude oil conditions, abilities that are necessary for crude oil bioremediation (Xiuqi *et al*.,2013). Another alga that are very significant in bioremediation are; *Volvox aureus* and Scenedesmus *obliquus.*

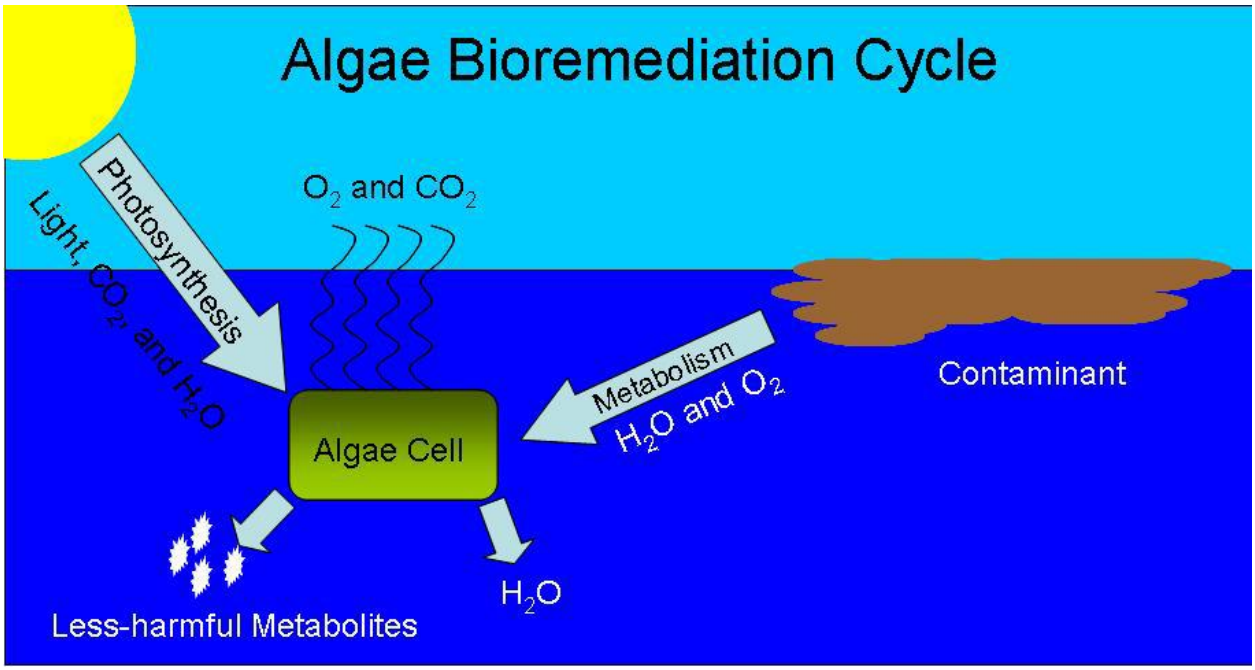

Figure 1: Algae Bioremediation. Metabolism of the contaminants through algae produces CO_2, H_2O, and less-harmful components. In turn the algae are able to use the CO_2, H_2O, and less-harmful components as nutrition necessary for photosynthesis and sustainability.

***Source**: Cui and Nobel,1992.*

GROWING OF THESE MICROBES

Bacteria

Significantly higher numbers of methanotrophic bacteria inhabit rhizosphere soils and on roots of *Lespedeza cuneata* (a legume) and *Pinus taeda* (Loblolly Pine). It was previously demonstrated that rhizosphere soils from these two types of plants showed higher rates of ^{14}C-TCE mineralization compared to non-vegetated soils (Brigmon *et al*., 1999). For successful multiplication of bacteria, they must be supplied with necessary nutrients for optimum production. Methane is the only nutrient source to the microorganisms; sufficient quantities must be supplied resulting in high flow rates through the bioreactors. Because of the large gas demand relative to the poor solubility of methane and oxygen, this mass transfer can present a design challenge. In addition, pure oxygen and methane can potentially form explosive mixtures so appropriate handling methods are necessary. The media size or design used should be tailored to the velocity of flow. Bioreactors with methanotrophic bacteria have been successfully maintained with both mixed cultures (Alvarez et a.,2000) as well as pure cultures (Uchiyama *et al*., 1992)

As with all microorganisms, maintenance of methanotrophs in a bioreactor or culture requires specific nutrient conditions. Growth factors that can influence methanotrophic activity are methane concentrations, copper concentration, nitrogen source (NO_3, NH_4^+), oxygen supply, pH, temperature, and the origin of the culture or inoculum used. While methanotrophs have been isolated in culture conditions containing 50% methane (Wise *et al*., 2000), optimal conditions for TCE degradation in methanotrophic bioreactors are between 4 and 20 % (Strandberg *et al.*, 1989). There is possibility of growing;

i. a mixture of oil-eating bacteria and are effective at degrading a variety of oils
ii. a mixture of pesticide-eating bacteria that efficiently at degrades different pesticides
iii. a mixture of metal-eating bacteria that have the propensity of eliminating different metals
iv. a mixture of bacteria that is not selective in contaminants degradation

Methodological Skills in Isolating and Culturing Bacteria

Bacteria had been successfully isolated in the laboratory. However, field experiments using these organisms have not been that successful in oil spill region because of poor design and implementation of a successful bioremediation program (bioremediation need thoroughly assessment of a site for suitability and to optimize conditions to achieve a satisfactory result).

Strain of bacteria can be collected from soil samples. The soil sample must be aseptically and passed through a sieve to remove large pieces of debris and vegetation. The bacterium isolated by plating dilutions of soils in saline solution on nutrient agar and incubated. The number of colonies counted and distinct colonies sub cultured. Biochemical tests can be performed on isolated colonies. For water analysis, a serial dilution of the water and the diluents inoculated on the growth media. Filter the water using a sterile Watman filter paper. The filter paper aseptically transferred to a nutrient agar media and incubated overnight; Sub cultured all the identified colonies onto a fresh media using the streak plate method. Once isolated, there would be colonies and then carry out biochemical testing to identify the

organism. After identifying the present of microbes in the site samples, the environmental requirements for them to thrive must be artificially supplied to maintain a desire level of activity. The ultimate consideration is if and when the targeted cleanup goal can be achieved. Oxygen requires was achieve through the use of an aeration system, oxygen being the electron acceptor of choice for the systems used. Moisture must be added to the treatment area by spraying. This must be done carefully to avoid excess water, because it can leach contaminants into the ground water or decrease the amount of air in the subsurface pores. Methane and other nutrients must be supplied in sufficient quantities to enhance the nutritional requirement of the microbes. This maintains the effectiveness of the bioremediation system. The nutrients were added in soluble form through the system used for moisture maintenance except nitrogen, which was added in the form of gaseous ammonia.

It is important to note that microbial transformation of organic contaminants normally occurs because the organisms can use the contaminants for their own growth and reproduction. Organic contaminants serve two purposes for the organisms: they provide a source of carbon, which is one of the basic building blocks of new cell constituents, and they provide electrons, which the organisms can extract to obtain energy. Hence, by providing basic environmental needs, microbes thrive successfully and the cleanup was successful.

Fungi

Fungi divide asexually, sexually or by both processes. They reproduce by means of spores. Fungi that reproduce by asexual spores and sexual pores are called perfect fungi and the fungi that reproduce only by asexual spores are called imperfect fungi (deuteromycetes). Most fungi are saprophytes, securing their nutrients from dead organic materials. They are chemo organo heterotrophs and use organic compounds as a source of carbon, electrons, and energy. Fungi grow best in dark, moist habitats, but they are found wherever organic material is available. Example of fungus that could be used as bioremediants is *Mycorrhizal* species (Fig.2)

Isolation, culture, and microscopic examination of molds require the use of suitable selective media and special microscopic slide techniques. A variety of media are available for the primary inoculation and recovery of fungi from clinical specimens. No one specific medium or combination of media is adequate for all specimens. Media must be carefully selected based on specimen type and fungal suspected agents. Media is dispensed into containers such as 25 x 150 mm screw cap tubes or 100 mm Petri dishes. Petri plates offer the advantage of a large surface area for isolation and dilution of inhibitory substances in the specimens, but must be poured thick with at least 25 ml of medium to resist dehydration during incubation. Because plates are vented, they are more likely to become contaminated during incubation. When molds are collected from the environment, the Sabouraud's agar is most frequently used. It is a simple medium constituting 4% glucose, 1% peptone, and 2% agar-agar. The pH of the medium is then adjusted to 5.6 to inhibit the growth of other bacteria.

Plates may be placed in gas permeable bags or sealed with gas permeable tape to offset this disadvantage. Each Petri plate must be labeled on the bottom, and the lid at least must be taped at two points to prevent accidental opening of the plate. All inoculated media should be read every 2 days following incubation and twice weekly thereafter. Plates must be opened only within a biological safety cabinet to prevent contamination of the plate and exposure of personnel to potentially dangerous fungi. Media in tubes have a smaller surface area but offer maximum safety and resistance to dehydration and contamination. If the specimen is from a

contaminated site, it is important to include media that contain inhibitory substances such as chloramphenicol, gentamicin, or cycloheximide. Chloramphenicol or gentamicin will inhibit most bacterial contaminants, while cycloheximide inhibits most saprobic moulds. It is important to remember that cycloheximide may also inhibit opportunistic fungi such as some species of *Aspergillus, Fusarium, Scopulariopsis, Pseudallescheria, zygomycetes,* some dematiacious fungi, and yeasts such as *Cryptococcus neoformans* and some *Candidas* species. Antibacterial agents may inhibit the growth of aerobic actinomycetes like *Nocardia* species. It is important to use media with and without inhibitory agents. Specimens from normally sterile sites can be inoculated to media without inhibitory substances

Algae

Algal cultures are essential when conducting bioremediation, assessment of zooplankton food preferences, and determination of algal life histories. Algal cultures may be "unialgal," which means they contain only one kind of alga, usually a clonal population (but which may contain bacteria, fungi, or protozoa), or "axenic," meaning that they contain only one alga and no bacteria, fungi or protozoa.

The four major techniques for obtaining unialgal isolates are; streaking, spraying, serial dilution, and single-cell isolations. Streaking and spraying are useful for single-celled, colonial, or filamentous algae that will grow on an agar surface; cultures of some flagellates, such as *Chlamydomonas* and *Cryptomonas* may also be obtained by these procedures. Many flagellates, however, as well as other types of algae must be isolated by single-organism isolations or serial-dilution techniques. A particularly effective means of obtaining unialgal cultures is isolation of zoospores immediately after they have been released from parental cell walls, but before they stop swimming and attached to a surface. Recently-released zoospores are devoid of contaminants, unlike the surfaces of most algal cells. Filaments can be grabbed with a slightly curved pipette tip and dragged through soft agar (less than 1%) to remove contaminants. It is best to begin with young branches or filament tips which have not yet been extensively epiphytized.

Antibiotics can be added to the growth medium to discourage growth of contaminating cyanobacteria and other bacteria. Addition of germanium dioxide will inhibit growth of diatoms.

Axenic cultures can be obtained by treating isolated algae to an extensive washing procedure, and/or with one or more antibiotics. Resistant stages such as zygotes or akinetes can be treated with bleach to kill epiphytes planted on agar for germination. It is usually necessary to try several different concentrations of bleach and times of exposure to find a treatment that will kill epiphytes without harming the alga.

Steps Planned and Methodology for Field Remediation Investigation

Site Location: The site must be assessed for its suitability and to optimize conditions to achieve a satisfactory result. Will the environmental conditions permit microbial growth and activity or involving manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate?

Factors to be Considered

The control and optimization of 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 (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

Microbial population Investigation: Indigenous microbes from a contaminated area and those isolated from elsewhere must be taken to the contaminated site (bioaugmentation) using appropriate media. The microbes must be treated to enhance their adaptation and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream.

Example of bacteria that can be grown aerobically includes *Pseudomonas* species, *Alcaligenes* species, *Sphingomonas* species, *Rhodococcus* species and *Mycobacterium* species. Also, anaerobical bacteria such as *Bacillus* species and *Clostridium* species for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and Chloroform are quite promising.

Bio stimulation: This involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon. Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells.

Environmental requirements

Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have been also isolated in extreme conditions; most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it is possible to reduce the pH by adding lime. Temperature affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells die. Plastic covering would be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms, and irrigation will be needed to achieve the optimal moisture level. The amount of available oxygen will determine whether the system is aerobic or anaerobic. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorinated compounds are degraded only in anaerobic ones. The soil would be tilled to increase the oxygen amount in the soil. Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter should be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for in situ clean-up techniques. Optimum environmental conditions required for microbial activity for the degradation of contaminants are: Soil moisture (25–28%), Soil pH(5.5–8.8) , Temperature (°C) 15–45,Type of soil (Low clay or silt content)

Bioremediation Strategies

Considering a generally large subsurface micro biota, there is considerable interest for the prospect of degrading hazardous contaminants *in situ* by stimulating selective bacterial populations (biostimulation) or by the addition of organisms to contaminated sites (bioaugmentation). Stimulation of an indigenous population of methanotrophs by methane is likely to enrich for species that are well adapted to their environment, whereas the deliberate addition of more microorganisms into such an environment may be compromised since the introduced organisms are not as likely to be able to compete. The two techniques are;

In situ techniques: *In situ* bioremediation means that the environmental restoration of contaminated sediments is not moved from the site or groundwater is not pumped and treated at the surface. When an *in-situ* bioremediation technology is employed the relocation and transport of materials may be avoided. This makes *in situ* bioremediation, where applicable, a highly attractive technology for remediation because contaminants are removed in place, not simply moved to another location or volatilized (Khelmelenia *et al.,* 1996). Evaluation, characterization, and utilization of microbial communities associated with *in situ* bioremediation of subsurface and groundwater contamination is a technological necessity for environmental restoration and assessment. Industrial and government nuclear production and waste management facilities have generated a significant quantity of organic wastes. These wastes have found their way into the vadose zones and groundwater resulting in unacceptable environmental impacts. The adaptability and manageability of indigenous microorganisms make them ideal for the remediation of hazardous environmental wastes under a diverse range of habitats. Public relations are enhanced because much of the action occurs with minimum above ground activity and equipment.

Techniques to be implored in in situ bioremediation

In situ treatment would be limited by the depth of the soil that can be effectively treated that will maximize diffusion rate (30 cm into the soil)

The most important land treatments are;

i. **Bioventing**. This will involve supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs 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. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

ii. **In situ biodegradation**. This involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

iii. **Biosparging**. Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and there by increases the

contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

iv. **Bioaugmentation**. Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit: 1) nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and 2) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

Ex- Situ Bioremediation: This involves using bioreactor.

Bioreactors: This is a large tank for growing micro organisms. A microorganism that, through its biochemical reactions, can produce medically or commercially useful materials, e.g. yeast producing beer by fermentation or genetically modified bacteria producing insulin. Its usage has been successful to an extent, for instance, methanotrophic bacteria have been used as a means of complete removal of contaminants. Bioremediation with methanotrophic bacteria in bioreactors has been tested with free, immobilized, and attached cells. Studies using methanotrophs for TCE removal have been carried out in bioreactors with bacteria attached to carbon (Niedzielski *et al*., 1989), diatomaceous earth (Strandberg *et al*., 1989), glass (Phelps *et al*., 1991), and ceramic packing material (Brigmon *et al*., 1995). Both aquatic and air bioreactors utilizing methanotrophic bacteria have been developed to remove TCE.It universal effectiveness has not successful.

In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pretreatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

CONCLUSION

Bioremediation is an emerging technology in the cleanup of contaminated soil and water. Microbes such as bacteria, fungi, and algae are promising and had shown to be very effective in stabilizing the biosphere. A carefully choosing microbe effectively remove complex organic compound without causing damage to the environment. These microbes can be optimized in-situ or ex-situ through genetic engineering to achieve the desire objective. Growing microbes in the laboratory require maximum concentration and should not be considered as safe and stable

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