BIOREMEDIATION: FEATURES, STRATEGIES AND ITS CURRENT STATUS IN INDIA

NITIN SWAMY

Assistant Professor,

Department of Biotechnology, St. Aloysius' College, (Auto.), Jabalpur, Madhya-Pradesh, India

Abstract: With the advent of globalization and urbanization countries around the globe have disturbed the ecological balance in way that the earth has never seen before. Environmental pollution has been on the constant rise many folds in the past few decades owing to increased human activities on energy resources, excess use of chemicals in agricultural practices and rapid industrialization. These practices are posing various serious threats to environment and public health due to the nature of pollutants involved which are toxic in nature. These pollutants involves heavy metals, nuclear wastes, pesticides, herbicides, greenhouse gases and hydrocarbon etc. Hence, remediation of these affected sites is necessary, but the existing methods of remediation does involve chemicals which at the end again pose danger to the environment in other toxic forms. Due to this constrain in the remediation process the contaminant sites are difficult to be cleaned properly. Bioremediation has evolved as an environment friendly, reliable and cost effective method of treating contaminant sites. Bioremediation involves the use of microbial metabolism to remove these harmful pollutants from sites to make them usable again without any ill consequences on the environment. Bioremediation involves various technologies which includes biostimulation, bioaugmentation, bioaccumulation, biosorption, and phytoremediation. Bioremediation can either be carried out ex situ or in situ, depending on numerous factors, which include cost, site characteristics, nature and concentration of pollutants. This review attempts to provide descriptive information on the bioremediation, types of bioremediation and its scenario in Indian context.

Keywords: Bioremediation, Biostimulation, Bioaugmentation, Bioaccumulation, Biosorption, Phytoremediation

I. INTRODUCTION

Since the inception of the industrial revolution in the 19th century environmental pollution has grown into a global transboundary problem that affects air, water, soil and ecosystems. With exponential increases in the population, and urbanization problem of contaminated sites are increasing rapidly. Contaminated sites contains materials in or under the land that are actually or potentially hazardous to human health and the environment. Areas with a long industrial exposure are bound to be contaminated sites. Contaminated sites are formed due to various uses such as mining, industry, chemical and oil spills and waste disposal. In India there are around 35 contaminated site and about 21 sites probably contaminated sites are in Delhi alone (as reported by DPCC). Therefore the need of hour is to clean these sites as soon as possible. Hence, bioremediation has evolved as method to combat which this serious issue. Bioremediation is a technology which involve the usage of microbes and plants in cleaning up the site by utilizing the pollutant as the substrate for its metabolic cycles. It uses decomposers and green plants, or their enzymes, to improve the condition of contaminated environment. Bioremediation is a microorganism mediated transformation or degradation of contaminants into harmless or less-hazardous substances. The use of various organisms like bacteria, fungi, algae, and plants for efficient bioremediation of pollutants has been reported. The involvement of plants in the bioremediation of pollutants is called as phytoremediation. The process of phytoremediation is an evolving green technology that facilitates the removal or degradation of the toxic chemicals in soils, sediments, groundwater, surface water, and air. Nowadays Genetically modified plants are also in use for bioremediation. For instance arsenic is phytoremediated by genetically modified plants such as Arabidopsis thaliana which expresses two bacterial genes. One of these genes allows the plant to modify arsenate into arsenite and the second one binds the modified arsenite and stores it in the vacuoles. One more prominent example of genetic modified organisms being used in the bioremediation process is of Superbug. Dr. Anand Mohan Chakravarty succeeded in producing a bacteria, Superbug by genetically engineered strain of *Pseudomonas putida*. It is capable of utilizing complex chemical compounds like hydrocarbons. It is also called oil eating bug. Creation of superbug was a landmark breakthrough in bioremediation of oil spills because of it over 10 million metric tons of petro-pollutants enter the sea ecosystem each year and killing birds, shell fish, fishes, invertebrates and other sea animals and planktons. This poses more adverse effect on marine life and food chain. The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

II. BIOREMEDIATION STRATEGIES

Bioremediation can be done in-situ and ex-situ depends on whether we are treating the pollutants at the contaminated site or treating the soil of that land in laboratories.

In-Situ Bioremediation:

In situ refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit to in situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated material.

Advantages of in situ bioremediation:

1. Cost-effective, with minimal exposure to public or site personnel.
2. Sites of bioremediation remain minimally disrupted.

Disadvantages of in situ bioremediation:

1. Very time consuming process.
2. Sites are directly exposed to environmental factors (temperature, O2 supply etc.).
3. Microbial degrading ability varies seasonally.

Ex-Situ Bioremediation:

Ex situ refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.

Advantages of ex situ bioremediation:

1. Better controlled and more efficient process.
2. Process can be improved by enrichment with desired microorganisms.
3. Time required in short.

Disadvantages of ex situ bioremediation:

1. Very costly process.
2. Sites of pollution are highly disturbed.
3. There may be disposal problem after the process is complete.

Principle of bioremediation

Bioremediation is defined as the procedure whereby organic wastes are biologically degraded under controlled conditions to a harmless state, or to levels below concentration limits established by regulatory authorities. Microorganisms are workhorse in the task of contaminant destruction because they have enzymes that allow them to use environmental contaminants as a food in their metabolic pathway. The aim of bioremediation is boosting them to work by supplying optimum levels of nutrients and other chemicals essential for their metabolism in order to degrade/detoxify substances which are hazardous to environment and living things. All metabolic reactions are mediated by enzymes. Many enzymes have a remarkably wide degradation capacity due to their non-specific and specific substrate affinity. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Bioremediation technology is principally based on biodegradation. It refer to complete removal of organic toxic pollutants in to harmless or naturally occurring compounds like carbon dioxide, water, inorganic compounds which are safe for human, animal, plant and aquatic life. Numerous ways and pathways have been elucidated for the biodegradation of a wide variety of organic compounds; for instance, it is completed in the presence and absence oxygen.

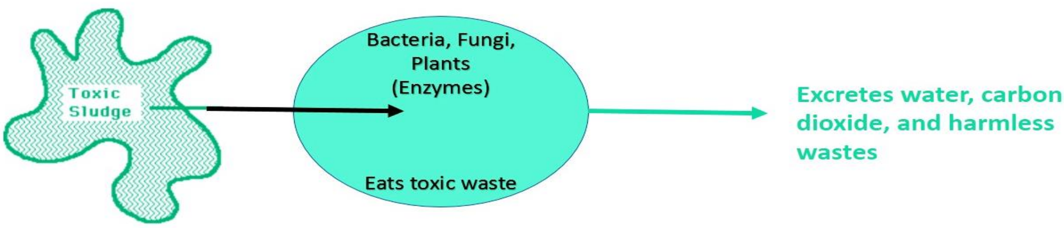

Figure 1 Principle of Bioremediation

Microbial Populations

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). Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt 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. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. Some endophytes are better suited for this, nowadays with the use of genetic engineering many customized microbes are used for effective remediation.

III. TYPES OF BIOREMEDIATION

There are various types of Bioremediation processes are in use nowadays, but here we would be discussing some of the more commonly used processes like biostimulation, bioaugumentation, bioventing, biosparging, biosorption, phytoremediation.

Biostimulation: Biostimulation is linked through the injection of specific nutrients at the site (soil/ground water) to stimulate the activity of indigenous microorganisms. It is focus with in the stimulation of indigenous or naturally existing bacteria and fungus community. Firstly, by supplying fertilizers, growth supplements and trace minerals. Secondly, by providing other environmental requirements like pH, temperature and oxygen to speed up their metabolism rate and pathway. The Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. This type of strategic path is most of the time continued in 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 basic requirement for example, energy, cell biomass and enzymes to degrade the pollutant. All of them will need nitrogen, phosphorous and carbon

Bioaugmentation: Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. It is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentation is used to ensure that the in situ microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic.

Bioventing: Bioventing is the most common in situ treatment and involves 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.

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.

Biosorption: Biosorption is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure. From a bioenvironmental systems point of view, "biosorption" is used generally to refer to sorption onto biomass or biofilm, i.e. an abiotic process. Though using biomass in environmental cleanup has been in practice for a while, scientists and engineers are hoping this phenomenon will provide an economical alternative for removing toxic heavy metals from industrial wastewater and aid in environmental remediation.

Phytoremediation: Although the application of microbe biotechnology has been successful with petroleum-based constituents, microbial digestion has met limited success for widespread residual organic and metals pollutants. Vegetation- based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water. The term "phytoremediation" is relatively new, coined in 1991. Its potential for encouraging the biodegradation of organic contaminants requires further research, although it may be a promising area for the future.

IV. FACTORS AFFECTING BIOREMEDIATION

Biological factors

A biotic factors affect the degradation of organic compounds through competition between microorganisms for limited energy sources, antagonistic interactions between microorganisms or the predation of microorganisms by protozoa and bacteriophages. The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of "catalyst" present. In this context, the amount of "catalyst" represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes(s) produced by each cell.

Environmental factors

The metabolic characteristics of the microorganisms and physicochemical properties of the targeted contaminants determine possible interaction during the process. The actual successful interaction between the two; however, depends on the environmental conditions of the site of the interaction. Microorganism growth and activity are affected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen content, lack of trained human resources in this field and Physico-chemical bioavailability of pollutants (contaminant concentration, type, solubility, chemical structure and toxicity).

Availability of nutrients

The addition of nutrients adjusts the essential nutrient balance for microbial growth and reproduction as well as having impact on the biodegradation rate and effectiveness. Nutrient balancing especially the supply of essential nutrients such as N and P can improve the biodegradation efficiency by optimizing the bacterial C: N: P ratio. To survive and continue their microbial activities microorganisms need a number of nutrients such as carbon, nitrogen, and phosphorous.

Temperature

Among the physical factors temperature is the most important one to determining the survival of microorganisms and composition of the hydrocarbons. In cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum. The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically inactive.

Concentration of oxygen

Different organisms require oxygen others also do not require oxygen based on their requirement facilitate the biodegradation rate in a better way. Biological degradation is carried out in aerobic and anaerobic condition, because oxygen is a gaseous requirement for most living organisms. The presence of oxygen in most cases can enhance hydrocarbon metabolism

Moisture content

Microorganisms require adequate water to accomplish their growth. The soil moisture content has adverse effect in biodegradation agents.

pH

pH of compound which is acidity, basicity and alkalinity nature of compound, it has its own impact on microbial metabolic activity and also increase and decrease removal process. The measurement of pH in soil could indicate the potential for microbial growth. Higher or lower pH values showed inferior results; metabolic processes are highly susceptible to even slight changes in pH. Site characterization and selection sufficient remedial investigation work must be performed prior to proposing a bioremediation remedy to adequately characterize the magnitude and extent of contamination.

Toxic compounds

When in high concentrations of toxic nature of some contaminants can create toxic effects to microorganisms and slow down decontamination. The degree and mechanisms of toxicity vary with specific toxicants, their concentration, and the exposed microorganisms. Some organic and inorganic compounds are toxic to targeted life forms.

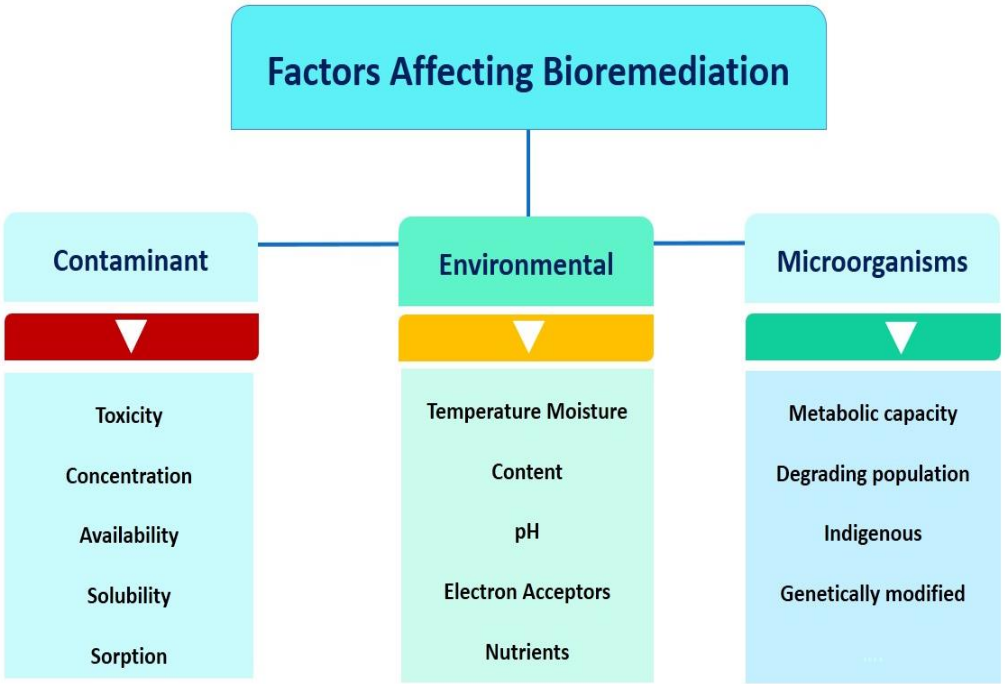

Figure 2 Factors affecting Bioremediation

V. ADVANTAGES & DISADVANTAGES

Advantage of Bioremediation

• It is a natural process, it takes a little time, as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant and increase in numbers when the contaminant is present. When the contaminant is degraded, the biodegradative population become declines. The residues for the treatment are usually harmless product including water carbon dioxide and cell biomass.

• It requires a very less effort and can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

• It is applied in a cost effective process as it lost less than the other conventional methods (technologies) that are used for clean-up of hazardous waste. Important method for the treatment of oil-contaminated sites.

• It also helps in complete destruction of the pollutants, many of the hazardous compounds can be transformed to harmless products, and this feature also eliminates the chance of future liability associated with treatment and disposal of contaminated material.

• It does not use any dangerous chemicals. Nutrients especially fertilizers added to make active and fast microbial growth. Commonly, used on lawns and gardens. Because of bioremediation change harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed.

• Simple, less labor intensive and cheap due to their natural role in the environment.

• Eco-friendly and sustainable.

The disadvantage of Bioremediation

• It is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

• There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.

• Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.

• It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.

• Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids and gases.

• It often takes longer than other treatment options, such as excavation and removal of soil or incineration.

VI. SCENARIO OF BIOREMEDIATION IN INDIA

Various government organizations and NGO's are working in this field of bioremediation, OT Biotech Limited, a joint venture between TERI and ONGC. OTBL today is providing large-scale bioremediation solutions, by application of 'OILZAPPER' for cleaning up oil spills, undertaking MEOR and WDP/PDB jobs for the prevention of paraffin deposition in oil well tubing and surface flow lines, to oil companies in India and abroad, especially in the Middle East. An example of OTBLs exceptional work was displayed in June 2008 when there was an accidental oil spill near city of Gujarat (Western India) due to crude oil trunk line rapture. Crude oil was spread in large area in farm land. OTBL had carried out the excavation of 14694 m3 of oil contaminated soil and transported the same to a secured bioremediation pit fitted with HDPE liners and after excavation the excavated site was refilled with good fertile agriculture soil.

After dumping of oil soaked soil in secured bioremediation pit, Oilzapper (crude oil degrading bacterial consortium) was applied for degradation of TPH in oil contaminated soil. Oilzapper is a consortium of crude oil degrading bacterial consortium developed by assemble of four species of oil degrading bacteria which could degrade different fraction of TPH. Oilzapper was produced in bulk and converted into powder form. After application of Oilzapper (74.5 tonnes) nutrient recipe was also sprayed on oil soaked soil and then tilling of oil soaked soil was done at regular intervals. Samples were collected from bioremediation site and tested in the laboratory to monitor the rate of degradation of TPH in oil soaked soil. Ex situ bioremediation of oil soaked soil by application of Oilzapper (oil degrading microbes) is shown in Figure 3.

After completion of bioremediation (TPH reduced to 5000 ppm), toxicity of bioremediated soil was done in laboratory approved by Ministry of Environment and Forest, Government of India. After fish toxicity test bioremediated soil is used in green belt development. The result of bioremediation of oil contaminated soil is given at zero day (at time of start of bioremediation job) oil content in oil soaked soil was 14.50% and it reduced to 7.31% after two months of initiation of bioremediation. Oil content in oil soaked soil was further reduced to 3.12% after three month and 1.70% after 3.5 months. After 4 months oil content is same soil was reduced to 0.58% (5800 ppm) as shown in Figure 4. Degradation of different fraction of TPH was also monitored. As shown in Figure 5 that most of alkane fraction were degraded in oil soaked soil in four months. Similarly aromatic fraction was also degraded in soaked soil in four months as shown in Figure 6. After testing of level of oil content in oil soaked soil at bioremediation sites, fish toxicity of oil soaked soil was also tested. The fish toxicity test result revealed that in control soil (where only tilling was done) fish could not survive while in bioremediated soil fishes survived.

Figure 3 OTBL bioremediation at North Kadi, Gujarat

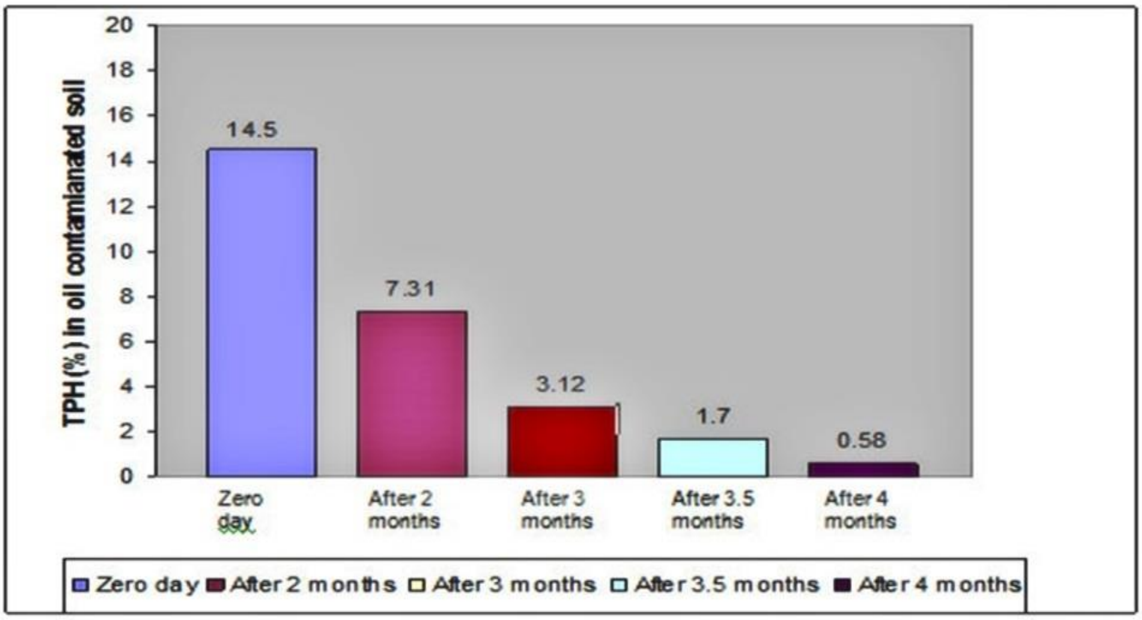

Figure 4 TPH (%) in the oil contaminated soil undertaken for bioremediation at secured bioremediation site situated at North Kadi, Gujarat.

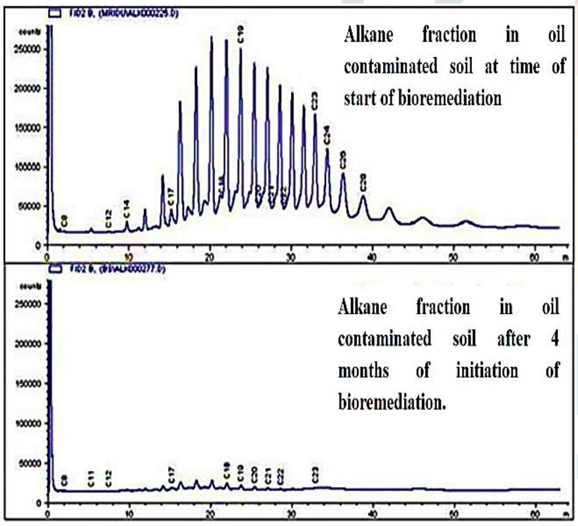

Figure 5 GC fingerprinting indicating the biodegradation of Alkane fraction of TPH in oil contaminated soil

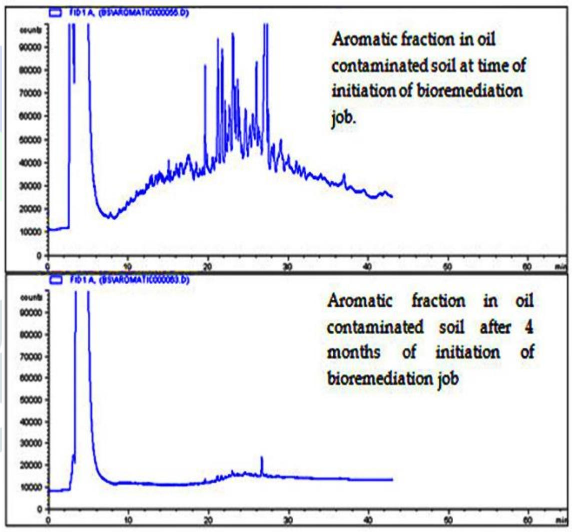

Figure 6 GC fingerprinting indicating the biodegradation of aromatic fraction of TPH in oil contaminated soil

This study revealed that bioremediation of oil spill, oil soaked soil is mot environment friendly and cost effective as compared to other treatment methods. Bioremediation of oil spill in large area is possible. OTBL has been providing services on Bioremediation of oil spill sites on turnkey basis to major oil companies in India. OTBL has developed site specific oil degrading bacteria for bioremediation process. With application of KT Oilzapper and specific nutrient recipe, oil contaminated site was completely clean and after that same site was restored and converted into green cover.

Gujarat Institute of Desert Ecology (GUIDE), was set up in 1995 with the objectives like rehabilitation of degraded soils to increase production of bio-mass and for restoration of degraded Eco-systems in Kachchh region. This institution has done a lot in fulfilling its objectives. GUIDE has worked extensively in the Kachchh and Saurashtra region, are the main regions in Gujarat, which are bestowed with rich mineral resources. Gujarat Mineral Development Corporation (GMDC) is engaged in tapping major mineral resources especially lignite in Panandhro and Mata-Na-Math in western Kachchh region. Soil destruction due to mining results in a permanent reduction of soil productivity.

GUIDE is assessing and reclaiming mined out sites, and other degraded lands need thorough understanding of several factors governing growth. Thus, restoration following advanced technologies becomes imperative. Thus, GUIDE is exploring the potential of both plant species and microbial diversity following ex-situ experiments and in-situ trials to restore such degraded lands. All such interventions have helped in developing ground cover and reducing salinity ingression in the region. With its vast experience in restoration ecology, GUIDE now looks forward to expand its expertise at national level.

Centre for Science and Environment (CSE) is a public interest research and advocacy organization based in New Delhi. It has accomplished many projects in bioremediation of land. Some of its notable work are Anoxic Bioremediation in Kushak Drain, New Delhi in which Bioremediation on Kushak Drain has been carried out on 2.8 km stretch from S.P. Marg to Satya Sadan. New Delhi Municipal Corporation (NDMC) took an initiative to treat open drain by Anaerobic Bioremediation technology. In the beginning, whole of the drain was inoculated with Persnickety@713. One dosing station was established at the starting point of the drain and bacteria medium was dosed at high concentrations initially. Half of the dosing solution was introduced at the dosing station and remaining half was used to inoculate the bacterial product throughout the drain. Once the bacterial strain entered the regeneration phase dosing quantity was reduced and maintained till the end of the project. Results was increase in Dissolve Oxygen: 72% approximately, BOD reduction: 77% approximately, TSS reduction: 80% approximately. Other notable examples include Anoxic Bioremediation in Hauz Khaz Lake, New Delhi and Bioremedaition in Amarnath Shrine, J &K.

These are few prominent examples were government and NGO's are working to create an impact but the need of hour is that government should take preventive measures and encourage various bioremediation methods to fight this demon called as environment pollution.

VI. CONCLUSION

Bioremediation provides a technique for cleaning up pollution by enhancing the natural biodegradation processes. So by developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting field trials of new bioremediation techniques which are cost effective, and dedicating sites which are set aside for long term research purpose, these opportunities offer potential for significant advances. There is no doubt that bioremediation is in the process of paving a way to greener pastures. European countries like Germany, Netherlands and United States of America are the world leaders in the implementation of these bioremediation process and when it comes to India still a lot has to be done when it comes to adopting these techniques. Government of India should take appropriate actions to make sure that bioremediation is use across the country in reduction of environmental pollution. Although Ministry of Environment and forest has taken many initiatives to make these contaminant sites greener but still these steps are just few drops in ocean. So, bioremediation is a greener and cost effective method to make these sites pollutants free. Government should collaborate with research NGO's and institutes to make a bigger impact as these environment pollutants are very harmful for the ecological balance.

REFERENCES

[1] M. Vidali, 2001 "Bioremediation. An overview," Pure and Applied Chemistry, vol. 73, no. 7, pp. 1163–1172.
[2] M. Leung, 2004"Bioremediation: techniques for cleaning up a mess," Journal of Biotechnology, vol. 2, pp. 18–22.
[3] Vidali M., 2001. Bioremediation: An overview. Pure Applied Chemistry, 73:1163-1172.
[4] Lee TH, Byun IG, Kim YO, Hwang IS, Park TJ, 2006. Monitoring biodegradation of diesel fuel in bioventing processes using in situ respiration rate. Water Science and Technology, 53(4-5):263-72.
[5] Sutar Harekrushna and Das Chandan Kumar, 2012.A Review on: Bioremediation" International Journal of Research in Chemistry and Environment Vol. 2 Issue 1 January 2012(13-21).
[6] Endeshaw Abatenh, Birhanu Gizaw, Zerihun Tsegaye, Misganaw Wassie, "Application of microorganisms in bioremediation-" Journal of Environmental Microbiology Vol.1 No.1 December 2017.
[7] Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) "The Role of Microorganisms in Bioremediation- A Review". Open J Environ Biol 2(1): 038-046.
[8] Retrieved on June 23, 2019 from http://www.otbl.co.in/Case-Studies-meor.php.
[9] Retrieved on June 25, 2019 https://gujaratdesertecology.com/restoration-ecology-bioremediation-phytomining/.
[10] Retrieved on June 25, 2019 https://www.cseindia.org/.