**Review of Bioremediation techniques and current practices**

PUSHPA K.L1 AND K L PRAKASH2

Department of Environmental Science, Bangalore University, Bangalore-560056

**Abstract**

A summary of the development of bioremediation techniques is presented in this chapter. The significant advantages and limitations of bioremediation are also elucidated. Based on its application, all the bioremediation methods have advantages and disadvantages. Bioremediation is the process which involves the microorganisms to transform the pollutant into less toxic or non-toxic. In this process, microorganisms are beneficial to remediate the polluted environment and many microbes like aerobes, anaerobes, and fungi are also involved in the bioremediation. Bioremediation is a very effective management tool to remediate contaminated sites. This biological treatment system has various applications, like cleaning contaminated water, soil, sludge, and waste streams.The key to successful bioremediation resides in continuing to develop the scientific and engineering work that provides the real bases for both science and technology and its evaluation.

**Keywords:** Bioremediation, Toxins, Microbes, contaminant, Clean-up, Bioventing

**Introduction**

Urbanization, industrialization, and population pressure on natural resources are causing significant stress to the global environment (Asha Juwarkar *et al*., 2010). Enormous amounts of pollutants such as polychlorinated biphenyl compounds (PCBs), hydrocarbons, dyes, pesticides, esters, heavy metals, petroleum products and nitrogen-containing chemicals persist in the environment released from various industrial and agricultural resources. The accumulation of these toxic and carcinogenic chemicals becomes hazardous to the environment and also to flora and fauna living in the environment (Babita Sharma *et al.*, 2018). The pollutant's difficult biodegradability is considered as its duration of contamination. Around 10% of all wastes were safely disposed of, as per the estimation made by the Environmental Protection Agency (EPA)(Reddy and Mathew 2001). The traditional remediation method involves removing soil from a contaminated site to a landfill (‘dig and dump’), which is not a sustainable solution. In the meantime, it only transfers the contaminant from one place to another. It poses a considerable risk due to requirements for excavation, handling and transporting hazardous materials (Meghana Raj *et al*.,2014).

A technique called bioremediation uses the metabolic power of microorganisms to purify contaminated areas. This technique can be carried out in a non-sterile open environment containing various microorganisms, one of the most important characteristics (Kazuya Watanabe, 2001). The bioremediation technique provides a safe and economical alternative to the disposal in waste dump sites and to commonly used physico-chemical strategies (Dietmer H Piper and Walter Reinkae, 2000). The term Bioremediation has been made into two words: “bios” means life and to living organisms; to remediate means to solve the problem. “Bioremediation” uses living microorganisms to degrade environmental pollution (Sasikumar and Papinazath, 2003). To reduce (degrade, detoxify, mineralize, or transform) the concentration of contaminants to a harmless condition, a biological mechanism involved in bioremediation depends upon the nature of the pollutants. Agrochemicals, dyes, greenhouse gases, heavy metals, hydrocarbons, chlorinated compounds, plastic, nuclear waste, and sewage are just a few examples of the pollutants that can be removed (Christopher *et al*., 2016). Environmental pollution such as soil, groundwater and surface water can be resolved by bioremediation or phytoremediation using living organisms and green plants(Ravindra Singh *et al*., 2014).

In the Bioremediation process, biological agents, mainly microorganisms like yeast, fungi or bacteria, are employed to remediate the contaminated soil and water (Strong and Burgess, 2008). This technology depends on promoting the growth of specific microflora indigenous to the contaminated sites that can perform desired activities. Establishing microflora can promote growth by adding nutrients, adding terminal electron acceptors or controlling moisture and temperature conditions (Agarwal, 1988). Bioremediation involves biotransformation and biodegradation by transforming contaminants into non-hazardous or less hazardous chemicals. During this, the microbes metabolize the harmful chemicals to produce by-products like methane, water, carbon dioxide, and biomass. Biotransformation is the microbial-aided alteration of the molecule or structure of inorganic compounds into environmentally friendly compounds, while Biodegradation is the breakdown of organic or bioaccumulation (Ravindra Singh *et al*., 2014).

**Types of Bioremediations**

There are mainly two ways bioremediation is initiated based on the places where wastes are removed, namely *Ex-situ* bioremediation and *In-situ* bioremediation. Ex-situ technologies are the treatments that remove contaminants at a separate treatment facility. In-situ bioremediation technologies involve the decontamination of pollutants in their place of origin (Tomatado and Vasavo 2001). The nature of microorganisms involved in these technologies could be aerobic, anaerobic, or both, depending on the nature of the contaminant and microorganisms. The commonly used bioremediation technologies are summarized in Table 1.

Table 1: Summary of bioremediation treatment technologies and the target contaminants (Meghana Raj *et al*., 2014)

|  |  |  |
| --- | --- | --- |
| **Technology** | **Matrix** | **Target contaminants** |
| Bioaugmentation | Soil, Sludge, groundwater | Benzene,toluene,ethylbenzene,Xylene(BTEX);petroleum hydrocarbons;pesticides;solvents |
| Biostimulation | Soil, sludge, groundwater | BTEX petroleum hydrocarbons, pesticides, solvents  |
| Bioventing  | Soil | Petroleum hydrocarbons, nonchlorinated hydrocarbons, pesticides |
| Intrinsic bioremediation | Soil and groundwater | Fuel hydrocarbons (BTEX), solvents |
| Bioreactors: Slurry based  | Soil, sludge, groundwater | Explosives (TNT), hydrocarbons (BTEX), Polyaromatic hydrocarbons (PAHs) pesticides, wood preservatives |
| Land farming | Soil, sludge, sediment | Total petroleum hydrocarbons (TPH), pentachlorophenol (PCP) pesticides |
| Composting | Soil, sludge, sediment | Explosives hydrocarbons (PAH), pesticides, PCP |
| Fungal remediation (white-rot fungus) | Soil | Chlorinated aromatic hydrocarbons, polychlorinated dibenzo pi dioxins explosives (TNT) hydrocarbons (PAHs), pesticides |

**Effecting factors for bioremediation**

Bioremediation process control and optimisation involves a complicated system with numerous variables. The presence of a microbial population that can break down the pollutants, the accessibility of contaminants to the microbial population, and environmental conditions (viz, soil type, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) are among these considerations.

**Table 2: Showing factors of bioremediation (Shilpi Sharma, 2012)**

|  |  |
| --- | --- |
| **Factors** | **Condition required** |
| Microorganisms | Aerobic or Anaerobic |
| Natural Biological processes of microorganism | Catabolism and Anabolism |
| Environmental Factors | Temperature, pH, Oxygen content, Electron acceptor/donor |
| Nutrients | Carbon, Nitrogen, Oxygen, etc. |
| Soil Moisture | 25-28% of water holding capacity |
| Type of soil | Low clay or silt content |

**Microorganisms**

Microorganisms can be isolated from any environmental conditions as they can adapt and grow in desert conditions, sub-zero temperatures, extreme heat, water with excess oxygen, and anaerobic conditions, with hazardous compounds or on any waste stream. Microbes and other biological systems require energy and a carbon source to degrade or remediate environmental hazards.

Microorganisms used for bioremediation can be grouped into

* **Aerobic:** Aerobic bacteria are recognized for their degradative abilities in the presence of oxygen. The contaminants like pesticides and hydrocarbons are used as carbon and energy sources to degrade them. ***Examples****: Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*.
* **Anaerobic**: These anaerobic bacteria are recognized for their degradative activities without oxygen. Anaerobic bacteria employed to bioremediate polychlorinated biphenyls (PCBs) in river sediments, dechlorinate the solvent trichloroethylene (TCE), and chloroform are gaining more and more attention.
* **Ligninolytic fungi**: White rot fungus and Phanaerochaete chrysosporiumcan degrade a diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, corn cobs or sawdust.
* **Methylotrophs:** Methane is used by aerobic bacteria to produce carbon and energy. Methane monooxygenase, the first enzyme in the pathway for aerobic degradation, is active against a wide variety of substances, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

**Environmental factors**

* **Nutrients –** The most crucial element for all living things is carbon, which is also required in higher concentrations than other elements. It makes up around 95% of the weight in addition to hydrogen, oxygen, and nitrogen. The type of bioremediation depends on the concentration of soil contaminants. Phosphorous and sulphur contribute 70% of the remainder. Carbon to nitrogen nutritional requirements are 10:1, while carbon to phosphorous nutritional requirements are 30:1.(Shilpi sharma,2012).

**Table 2 Shows the Environmental Conditions**

|  |  |  |
| --- | --- | --- |
| **Environmental Factor** | **Optimum conditions** | **The condition required for microbial activity** |
| Available soil moisture | 25-85% water holding capacity | 25-28% of water holding capacity |
| Oxygen | >0.2 mg/L DO, >10% air-filled pore space for aerobic degradation | Aerobic, minimum air-filled porespace of 10% |
| Redox potential | Eh > 50 mV | --- |
| Nutrients | C:N:P = 120:10:1 molar ratio | N and P for microbial growth |
| pH | 6.5-8.0 | 5.5 to 8.5 |
| Temperature | 20-30 ºC | 15-45ºC |
| Contaminants | Hydrocarbon 5-10% of dry weight of soil | Not too toxic |
| Heavy metals | 700ppm | Total content 2000ppm |

**Soil**

* **High concentrations of contaminants (roughly 5% or more**): The soil is agitated in a purifying water solution containing an interface active agent and then separated from the oils. After that, bioremediation is started to clean the soil efficiently. At the experimental stage, bioremediation alone has turned contaminated soil into soil suited for landscaping, and work is continuing to make this process even more efficient and effective.
* **Low concentrations of contaminants:** Soils with low concentrations can be treated using bioremediation alone. It takes about 6 months to a year to purify soil containing two Percent heavy oils, but at a concentration of 0.8 Percent, the job can be done in only about one to two months. This environmentally friendly method makes recycling and reusing soil possible without much effort (Shilpi Sharma,2012).

**In-situ bioremediation Techniques**

In-situ bioremediation techniques involve treating the pollutants at the site of pollution. It does not require any excavation. In-situ techniques are cost-effective compared to ex-situ bioremediation techniques due to no extra excavation costs. The primary concern is the cost of design and on-site installation of sophisticated equipment to improve microbial activities during bioremediation (Christopher *et al*., 2016). Some bioremediation techniques (viz., bioventing, biosparging and phytoremediation) might be enhanced, while others (viz., Intrinsic bioremediation or natural attenuation) might proceed without any enhancement. In-situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals and hydrocarbon-polluted sites. The essential environmental factors that must be met for in-situ bioremediation to succeed are the electron acceptor's status, moisture content, nutrient availability, pH, and temperature (Philp and Atlas, 2005).

**Intrinsic Bioremediation**

Intrinsic Bioremediation is also known as natural attenuation or Bioattenuation. Removing pollutants from the environment is known as bioattenuation or natural attenuation. It is carried out within biological processes that may include (viz., Aerobic and anaerobic biodegradation, plant and animal uptake). Numerous physical, chemical, and biological mechanisms are used in the intrinsic bioremediation processes to lessen the bulk, toxicity, mobility, volume, or concentration of contaminants (USEPA 1999).In this bioremediation technique, aerobic and anaerobic biodegradation, dilution, sorption, volatilization decay, chemical or biological stabilization and transformation of contaminants are involved in this bioremediation technique (Endeshaw *et* *al.*, 2014). In this technique, time consumption is a challenging factor. However, no other techniques are available can be used for on-site remediation.

**Bioventing**

In the promising new method known as "bioventing," oxygen is supplied to the site to encourage in-situ aerobic biodegradation of pollutants by microorganisms. Common groundwater pollutants like BTEX compounds are easily degradable by aerobic microorganisms. Hence, adding oxygen to contaminated aquifers to stimulate aerobic degradation is the most commonly used technology (Meghanaraj *et al*., 2014). Low airflow rates are used in bioventing to deliver just enough oxygen to support microbial activity. The most typical method of supplying oxygen is direct air injection into soil pollution that has remained after using wells. Adsorbed fuel residuals and volatile compounds are biodegraded as vapours move slowly through biologically active soil (Shilpi Sharma, 2012:Wen-Wei Li,2015). Soils contaminated with petroleum hydrocarbons, nonchlorinated solvents, pesticides, wood preservatives and other organic contaminants are remediated by using bioventing.

**Biostimulation**

Biostimulation is the injection of specific nutrients at the site to stimulate the indigenous microorganisms. Firstly, there is a need to supply fertilizers, growth supplements and trace minerals. There is a need to provide other environmental factors such as pH, temperature and oxygen to enhance the metabolic rate and their pathway. The addition of nutrients and oxygen can continue most of the time in this type of strategic path. These nutrients are building blocks of life and allow microbes to create the basic requirements like cell biomass, energy, and enzymes to degrade the pollutants (Endeshaw *et al*., 2014)

**Bioaugmentation**

It is one of the biodegradation mechanisms which involve the addition of pollutant-degrading microorganisms (natural/exotic/engineered) to augment the biodegradable capacity of the indigenous microorganism population in the contaminated area. This process is known as Bioaugmentation. Microbes are taken from the remediation site, cultivated separately, genetically altered, and then released back onto the site. It is also necessary to ensure that the in-situ microorganisms can remove and alter chlorinated ethane, such as tetrachloroethylene and trichloroethylene, from soil and groundwater to ethylene and chloride, which is non-toxic (Niu *etal*.,2009). The technique of adding engineered microbes to a system that functions as bioremediation to wholly and swiftly remove complex contaminants is known as bioaugmentation. The ability of genetically modified microbes to bioremediate soil, groundwater, and activated sludge has been demonstrated. These organisms have exhibited improved degradative properties for various chemical and physical contaminants.

**Ex-situ Bioremediation**

**Composting**

Organic wastes are broken down by microorganisms during the composting process, usually at high temperatures. Composting temperatures typically range from 550 to 650C. The higher temperatures result from heat generated by microorganisms as they break down the organic waste **(**Shilpi sharma.,2012). This stage is essential for maintaining the right temperature, moisture content (by irrigation), and oxygenation (by turning the windrows). The various designs used in composting include (1) aerated static piles, in which compost piles are aerated using blowers or vacuum pumps; (2) in-vessel composting with mechanical agitation, in which compost is placed in a reactor vessel and mixed and aerated; and (3) windrow composting, which is a more economical method and involves placing compost in long piles known as windrows and periodically mixing with mobile equipment (Meghanaraj et al., 2014).

**Land farming**

Land farming is a straightforward method in which polluted soil is excavated, deposited on a bed that has been prepared, and repeatedly tilled until pollutants are broken down. The intention is to promote the aerobic breakdown of pollutants by local biodegradative bacteria. Typically, the practice is restricted to treating the top 10-35 cm of soil. Land farming has drawn much attention as a disposal alternative since it has the potential to lower monitoring and maintenance costs and clean-up obligations.

**Biopiles**

Excavating soil contaminated with hydrocarbons that can be treated in "biopiles" is a method known as "biopiles". When biodegrading, biopiles (such as biocells, bioheaps, biomounds, and compost piles) lower the levels of petroleum pollutants in excavated soils. This method involves piping and pumps that either drive air into the biopile under positive pressure or draw air through the pile under negative pressure to provide the biopile system with air . The breakdown of adsorbed petroleum pollutants results from microbial respiration, which increases microbial activity(Emami *etal*.,2012) .

**Bioreactors (Slurry based)**

The use of slurry or aqueous reactors for the ex-situ treatment of polluted soil and water pumped up from a plume. In reactors, bioremediation entails treating contaminated water or solid material (soil, sediment, or sludge) through a designed containment system. A slurry bioreactor is a containment vessel and device used to produce a three-phase (solid, liquid, and gas) mixing condition to speed up the bioremediation of water-soluble and soil-bound pollutants. The water slurry of the contaminated soil and biomass (typically native microorganisms) is capable of degrading target contaminants.

In general, a bioreactor system's pace and degree of biodegradation are more relevant than in-situ or solid-phase systems because the enclosed environment is easier to regulate, control and predict. Reactor systems have various drawbacks despite their advantages. Before being added to a bioreactor, the contaminated soil needs to be pre-treated (such as by excavation), or the contaminant can be physically or chemically removed from the soil.

**Fungal Remediation**

Fungal metabolism has been employed for the decontamination of several organic contaminants, especially hydrocarbons. One group of fungi, particularly white-rot fungus (Phanerochaete chrysosporium), can degrade various organic contaminants, including PCBs, PAHs, and explosives. Lignin peroxidases, the enzymes these fungi produce, are responsible for this extensive biodegradative ability. The ability of white-rot fungi to degrade chlorinated hydrocarbons, PAHs, PCBs, polychlorinated(p)dioxins, pesticides (lindane and DDT), and some azodyes has been demonstrated. Also, white-rot fungi have been shown to degrade PAHs such as benzo(a)pyrene, pyrene, fluorene, and phenanthrene, but degradation is favoured under nitrogen-limited conditions at low pH.

**Advantages of Bioremediations**

* As an appropriate waste treatment technique for polluted material like soil, it is a natural process that takes some time. When a contaminant is present, microbes can break down it and multiply. The biodegradative population decreases as the pollutant degrades. Water, carbon dioxide, and cell biomass are examples of the residues for the treatment that are typically unharmful byproducts.
* It requires minimal effort and can often be carried out on-site without causing a significant disruption of everyday activities. It also eliminates the need to transport quantities of waste off-site, besides possible threats to the environment and humans during transportation.
* With regulatory encouragement, it has greater public acceptance as it can eliminate waste forever with no long-term liability.
* It is applied cost-effectively as it lost less than the other conventional methods (technologies) used to clean up hazardous waste—an essential method for treating oil-contaminated sites.
* It also helps in the destruction of the pollutants. Many of the hazardous compounds can be transformed into harmless products, and this feature also eliminates the chance of future liability associated with the treatment and disposal of contaminated material.
* It does not use any dangerous chemicals. Nutrients, especially fertilizers, are added to make active and fast microbial growth and are commonly used on lawns and gardens. Because bioremediation changes harmful chemicals into water and harmless gases, the harmful chemicals are destroyed.
* Simple, less labour intensive and cheap due to their natural role in the environment.
* It is eco-friendly, sustainable, and can be combined with other physical and chemical treatments.
* Nonintrusive, potentially allowing for continued site use.
* The relative ease of implementation.

**Limitations of Bioremediations**

* It is limited to only biodegradable pollutants and organic compounds. Not all contaminants are susceptible to rapid and complete degradation.
* Some worry that biodegradation can be more hazardous or persistent than the parent chemical.
* Essential site criteria for success in many biological processes include isolating metabolically competent microbial populations, knowing the appropriate ambient conditions and nutrient requirements for their growth, and types of pollutants.
* Extrapolating from bench and pilot-scale investigations to full-scale field operations is challenging.
* Because pollutants might exist as solids, liquids, or gases, substantial research is needed to develop the best bioremediation techniques for sites with many contaminants that are disproportionally distributed.
* It frequently requires more time than alternative treatment methods, including excavation and soil removal or incineration.

**Conclusion**

One of the promising and alluring options for cleaning up the polluted environment using microorganisms is bioremediation. The presence of a microbial population that can break down pollutants, the accessibility of contaminants to the microbial population, and environmental conditions are the key influences on the bioremediation process. The new insights into process optimization, validation, and impact on the ecosystem achieved by the innovative molecular microbiological techniques will make bioremediation a more reliable and safer technology.

**References**

1. Godleads Omokhagbor Adams, Prekeyi Tawari Fufeyin, Samson Eruke Okoro, and Igelenyah Ehinomen, “Bioremediation, Biostimulation and Bioaugmentation: A Review.” International Journal of Environmental Bioremediation & Biodegradation, vol. 3, no. 1 (2015): 28-39. doi: 10.12691/ijebb-3-1-5.
2. Babita Sharma,Arun kumar Dangi., and Pratyoosh Shukla. (2018). Contemporary enzyme-based technologies for bioremediation: a review. *Journal of Environmental Management*, *210*, 10-22.
3. Asha Juwarkar A., Sanjeev Singh K., and Ackmez Mudhoo,(2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and bio/technology*, *9*, 215-288.
4. Sharma, B., Dangi, A. K., & Shukla, P. (2018). Contemporary enzyme-based technologies for bioremediation: a review. *Journal of Environmental Management*, *210*, 10-22.
5. Wen-Wei Li., and Han-Qing Yu (2015). Electro-assisted groundwater bioremediation: Fundamentals, challenges and future perspectives. *Bioresource Technology*, *196*, 677-684.
6. Tomatada Iwamoto.,and Masao Nasu, (2001). Current bioremediation practice and perspective. *Journal of bioscience and bioengineering*, *92*(1), 1-8.
7. Kensa, V. M. (2011). Bioremediation-an overview. *Journal of Industrial Pollution Control*, *27*(2), 161-168.
8. Kazuya Watanabe,(2001). Microorganisms relevant to bioremediation. *Current opinion in biotechnology*, *12*(3), 237-241.
9. Francis Chapelle, H. (1999). Bioremediation of petroleum hydrocarbon‐contaminated groundwater: the perspectives of history and hydrology. *Groundwater*, *37*(1), 122-132.
10. Endeshaw Abatenh.,Birhanu Gizaw,Zerihun Tsegaye.,and Misganaw Wassie.,(2017). The role of microorganisms in bioremediation-A review. *Open Journal of Environmental Biology*, *2*(1), 038-046.
11. Dietmar Pieper.,and Walter Reineke.,(2000). Engineering bacteria for bioremediation. *Current opinion in biotechnology*, *11*(3), 262-270.
12. Christopher Chibueze Azubuik..,Chioma Blaise Chikere.,and Gideon Chijioke Okpokwasili. (2016). Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, *32*, 1-18.
13. Boopathy, R. (2000). Factors limiting bioremediation technologies. *Bioresource Technology*, *74*(1), 63-67.
14. Anna Dzionek.,Danuta Wojcieszyńska. and Urszula Guzik. (2016). Natural carriers in bioremediation: A review. *Electronic Journal of Biotechnology*, *23*, 28-36.
15. Ruchita Dixit., Wasiullah.,Deepthi Malaviya.,Kupuswamy Pandiyan.,Udai Singh.,Asha Sahu. and Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*, *7*(2), 2189-2212.
16. Mary Kensa, V. (2011). Bioremediation-an overview. *Journal of Industrial Pollution Control*, *27*(2), 161-168.
17. Sasikumar CS, Papinazath T (2003) Environmental management: bioremediation of polluted environment. In: Bunch MJ, Suresh VM, Kumaran TV (eds) Proceedings of the third international conference on environment and health, Chennai, India, 15–17 Dec 2003. Department of Geography, University of Madras and Faculty of Environmental Studies, York University, Chennai, pp 465–469
18. Reddy CA, Mathew Z (2001) Bioremediation potential of white rot fungi. In: Gadd GM (ed) Fungi in bioremediation. Cambridge University Press, Cambridge
19. Strong PJ, Burgess JE. 2008. Treatment methods for wine-related and distillery wastewaters review.Bioremedy Journal, 12: 70-87
20. Agarwal SK. 1998. Environmental Biotechnology (1st ed). 267-289, APH Publishing Corporation, New Delhi, India
21. Singh, R. (2014). Microorganism as a tool of bioremediation technology for cleaning environment: a review. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *4*(1), 1.
22. 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.
23. Niu GL, Zhang JJ, Zhao S, Liu H, Boon N, et al. (2009) Bioaugmentation of a 4- chloronitrobenzene contaminated soil with Pseudomonas putida ZWL73. Environmental Pollution 57: 763-771. (<https://goo.gl/ZQbwgC>)
24. Meghana Raj.M., Venkateswarlu. K and Naidu.R.Bioremediation., Encyclopaedia of Toxicology, Volume (1), pp 485-489.
25. Ravindra Singh, Pushpendra Singh and Rajesh Verma (2014). Microorganism as a tool of bioremediation technology for cleaning environment: a review. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *4*(1), 1.
26. Iwamoto, T., & Nasu, M. (2001). Current bioremediation practice and perspective. *Journal of bioscience and bioengineering*, *92*(1), 1-8.
27. Philp JC, Atlas RM (2005) Bioremediation of contaminated soils and aquifers. In: Atlas RM, Philp JC (eds) Bioremediation: applied microbial solutions for real-world environmental cleanup. American Society for Microbiology (ASM) Press, Washington, pp 139–236
28. Shilpi Sharma., (2012). Bioremediation: features, strategies and applications. *Asian Journal of Pharmacy and Life Science*, *2231*, 4423.
29. Emami S, Pourbabaee AA, Alikhani HA (2012) Bioremediation Principles and Techniques on Petroleum Hydrocarbon Contaminated Soil.Technical Journal of Engineering and Applied Sciences 2: 320-323. **Link:**https://goo.gl/2iCBqU