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Bioremediation - An Important Tool to Conserve the Green Earth

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Abstract

Use and overuse of resources have caused its depletion which in turn is creating a havoc to the environment releasing contaminants and pollutants in the atmosphere. Hazardous chemicals and toxic substances are accumulated in the nature harming the biological diversity. Bioremediation is a process that helps degrading contaminants using biological agents like, bacteria, algae, fungi, yeast and plants. Through recombinant DNA technology, breeding line-ups and genetic modification methods, biotechnology was able enhance the natural properties of plants to make it more suitable as per the environmental conditions. Beneficial microbiological agents are used to treat contaminated culture water or waste water which utilizes contaminants as their energy sources. Use of microbes as biocontrol agent is risky; however, at critical times it was proved to be helpful.

Introduction

Use and overuse or in literal sense misuse of resources have caused depletion of natural resources which in turn is creating a havoc to the environment releasing contaminants and pollutants in the atmosphere. Hazardous chemical and toxic substances are accumulated in the nature harming biological diversity. To counteract these pollutants, scientists and researchers developed a technology called bioremediation. Bioremediation is thus defined as a phenomenon by which living beings such as micro-organisms and plants primarily degrade the pollutants and contaminants available in nature and converts them readily into harmless or less toxic forms. Bioremediation can occur on its own called as natural bioremediation or through artificial processes called as bio-stimulation or bio-augmentation.

Bio-Stimulation

Nutrient enrichment of indigenous microbial environment by adding substances that boost the ability of these microbes to break down hazardous substances.

Bioaugmentation

Direct addition of the microorganisms, usually genetically engineered microorganisms to degrade contaminants and hasten their destruction or conversion to fewer toxic forms. Bioremediation technology can be classified as: *in-situ* technology, which involves treatment of contaminations at the same site. *Ex-situ* technology, which involves movement of contaminants to other sites and are treated there using biological agents. As mentioned earlier, biological agents can be either plants or micro-organisms.

Micro-Organisms: Their Role in Bioremediation

Micro-organisms that are involved in bioremediation process mainly belong to the bacterial, algal, fungal and yeast community. Microbial mats are laminated heterotrophic and autotrophic stratified communities characteristically taken over by cyanobacteria, eukaryotic microalgae like diatoms, anoxygenic phototrophic bacteria and sulphate reducing bacteria.

Bacteria

Bacteria perform a specific metal binding mechanism to the pollutants and acts as bio remediators thereby degrading them. It is of three types:

- Intracellular accumulation (this process requires live cells),
- Complex formation on cell surface (it takes place on both live and dead cells), and
- Extracellular accumulation or precipitation (the process may require viable cells).

Potent metal bio sorbents are; *Bacillus*, *Pseudomonas*, *Streptomyces*.

Genetically Engineered Micro-Organisms

Bacteria can be genetically modified to produce certain enzymes which can metabolize industrial toxic waste components to fewer toxic forms. Some genetically modified microorganisms include recombinant PCB (Polychlorinated Biphenyl)-degrading microorganisms to convert PCBs to fewer toxic forms. *Deinococcus radiodurans* was modified to express an *Escherichia coli* enzyme which converts ionic mercury to a less toxic form and a *Pseudomonas* enzyme that can break down toluene.

1. Algae

The use of algae to remove pollutants from the environment or to convert them into harmless form is known as phyco-remediation. Algae help in monitoring metal concentration of lakes and oceans and also have the ability to degrade or accumulate toxic heavy metals and organic pollutants such as phenolics, hydrocarbons, pesticides and biphenyls from the environment. The uptake of metals by living microalgae is completed by physical adsorption onto the cell surface and as these ions are transported slowly into the cytoplasm, it is known as chemisorption.

2. Fungi

The fungal mycelia secrete various extracellular enzymes and acids which oxidizes lignin and cellulose. Since, Polyaromatic Hydrocarbons (PAHs) have a similar structure to lignin; these PAHs are degraded to form carbon dioxide.

3. Yeasts

Yeast cells help in the removal of dyes from food industry effluents by the mechanism absorption or adsorption at the cell surface. The yeast species which helps in bioremediation process and shows biodegrading properties are *Candida*, *Clavispora*, *Debaryomyces*, *Pichia*, *etc*.

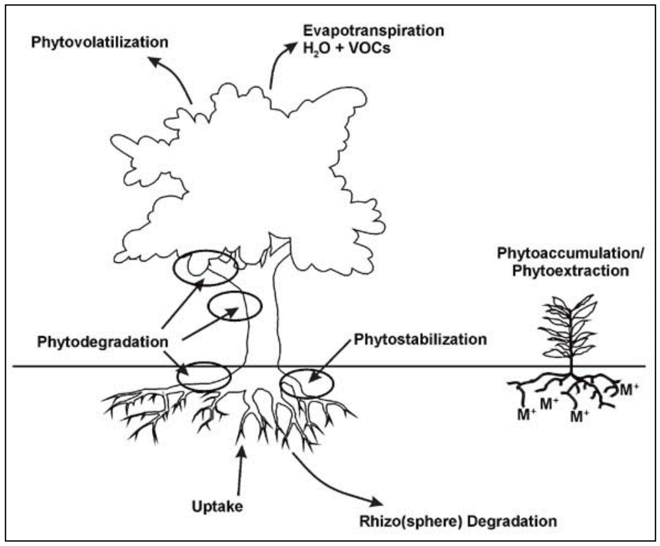

Figure 1: Types of Phytoremediation

Phyto-Remediation

Phytoremediation can be defined as use of green plants in degrading harmful contaminants in the nature. The major families which help in bioremediation include *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae* and *Scrophulariaceae*. Phytoremediation techniques are:

- *Phytoextraction*: That involves uptake of contaminants and then are translocated to different parts of plants.
- *Phyto transformation*: Breakdown of organic contaminants by metabolic processes where enzymes are used.
- *Phytovolatilization*: Harmful matters are converted and released into the atmosphere in gaseous forms.
- *Phyto stabilization*: This reduces of mobility of heavy metals in the soil and makes them harmless.
- *Rhizo filtration*: It is the decontamination of groundwater using plant roots.

Types of Plants in Remediation

- *Hyper Accumulator Plants*: These plants have genes which control the amount of metal uptake from the soil by roots and deposits the metals at other sites within the plant. Subsequently, the metals enter the plant's vascular system and get transported further to other parts of the plant and get deposited in leaf cells.

• *Non-Hyper Accumulator Plants*: These plants need addition of some chelated agents in the soil to encourage the uptake of heavy metals.

Biotechnology Applications in Bioremediation

The origin of modern biotechnology tools such as genetic engineering and breeding programmes has developed the non-native genetically modified micro-organisms to treat the chemical contaminants areas thus exhibiting enhanced degradative capabilities through bioremediation. Though advanced recombinant DNA technologies and gene cloning methods enzymatic degradations through the non-native microbes will open new vistas for further enhancing the process of bioremediation. Organization like TERI (The Energy and Research Institute) is working to increase the natural tendency of these microorganisms artificially (Jasmina *et al.*, 2019). One such product developed is Oil zapper, which is an indigenous bacterial consortium made by assembling five different strains of immobilized bacteria which were isolated from various oil contaminated sites in India and mixed with powdered corncob. It has been used for the bioremediation of oil spills as it can degrade Total Petroleum Hydrocarbon (TPH) which is present in the oil sludge and convert it to harmless carbon dioxide and water. However, bioremediation by Oil zapper technology is still ongoing research but the results are very encouraging and the cost is also 30% less than the conventional treatments.

Microbial Intervention in Aquaculture System

Microbial preparation in aquaculture can be either used for biocontrol and probiotics or to improve the quality of water. Most probiotics used as biocontrol agents in aquaculture are *Lactobacillus*, *Carnobacterium*, *Vibrio*, *Pseudomonas* or *Bacillus*. Heterotrophic probiotic bacteria when added to water can remove NH_3, H_2S and organic acids by Oxidation, Nitrification, Denitrification, Sulphur and Nitrogen Fixation. Use of microbes as biocontrol agent is risky; however, at critical times it was proved to be helpful such as eradication of weed plants.

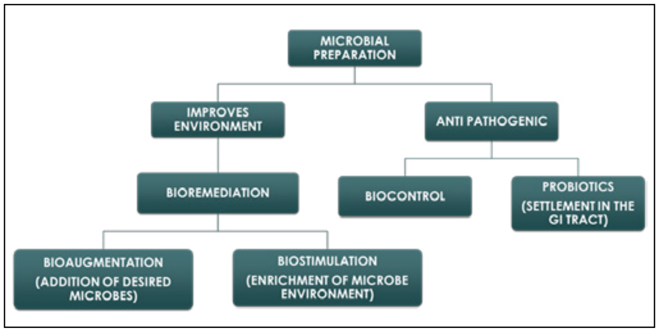

Figure 2: Flowchart for microbial preparation

Bioremediation of Aquacultural Sludge

Sludge accumulates in culture water due to large quantities of uneaten feed and organic degradation matters along with mineral sediment, airborne debris, protozoa, bacteria, fungi and residues of prophylactic and therapeutic input. Sludge determines the habitat availability of cultured animals, churn out toxic matters that can endanger the well-being of aquatic animals and, therefore, should be removed or treated. The major contributing components of aquacultural sludge are Nitrogenous Compounds, Phosphorus and Hydrogen sulfide. Ammonia, Nitrite and Nitrate constitute the nitrogenous compounds present in water bodies. Nitrite (NO_2^-) is considered toxic when present above 0.5 mg/L concentrations. Both NH_3 and NO_2^- are highly toxic to culture water. Nitrate is not considered toxic up to a concentration of 200 mg/L (Mandal *et al.*, 2011). Phosphorus accumulates as a result of uneaten feed and undigested phosphorus in feces. Dense aggregation of phosphorus in water bodies can cause eutrophication. Sulphur remains in natural water or in culture water mainly as sulfate ion. When aerobic conditions prevail, sulphur decomposes to S^{-2} and oxidizes to SO_4^{-2}. Certain anaerobe bacteria, due to insufficient oxygen, utilize the oxygen molecules in SO_4^{-2}, reducing it to Hydrogen Sulfide. Increased concentration of H_2S will disrupt respiratory functions of aquatic animals, making them susceptible to diseases.

Microbial bioremediation is the most preferred type of bioremediation as it is cost effective and able to destroy or immobilize contaminants efficiently (Gadd, 2000). Beneficial microbiological agents are used to treat contaminated water or waste water which utilizes contaminants as their energy sources. These microorganisms can be differentiated based on the source of carbon they need. Autotrophic bacteria are capable in adsorbing and transforming soluble biologically available phosphorus and nitrogen in order to proliferate which makes them good bio-remediators.

1. Bioremediation of Nitrogenous Compounds

Bacteriological nitrification and denitrification help in the removal of toxic nitrogenous compounds from aquaculture.

Ammonia-oxidizing bacteria, such as *Nitrosomonas, Nitrosovibrio, Nitrosococcus* and *Nitrospira* oxidize ammonia to nitrite during nitrification. Nitrite, is further converted to nitrate by nitrite-oxidizing bacteria of genera *Nitrobacter*, *Nitrococcus* and *Nitrospira*. Denitrification is the final phase in

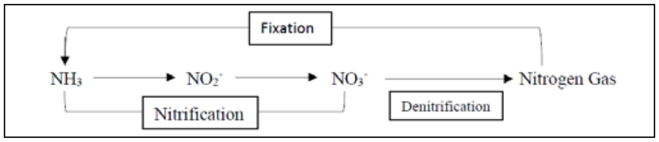

Figure 3: The Nitrogen Cycle

nitrogen cycle carried out by at least 14 genera of denitrifying bacteria some of which are: *Pseudomonas, Bacillus, Rheinheimera, Pannonibacter, Rhizobium, etc*.

2. Bioremediation of Phosphorus

Symbiotic bioremediation of phosphorus is possible using effective microorganisms (EM) and microalgae. High percentage deletion of phosphorus has been noted which is 99.15% comparable to 0.524 mg/l removal per day. In another research, it is shown that mix *Bacillus* species were able to lower phosphorus concentration in ponds by 81% (Bhatnagar and Kumari, 2013).

3. Bioremediation of Hydrogen Sulfide (H_2S)

Photosynthetic purple Sulphur bacteria are capable in decomposing organic matter, H_2S, NO_2 and other toxic components. *Chromatocyte* and *Chlorobiaceae* are the two families of photosynthetic sulphur bacteria that preferred anaerobic conditions with solar energy and sulphide to grow. *Chromatiaceae* and *Chlorobiaceae* can be mass cultured and applied as pond probiotic.

Ecological Recovery through Bioremediation

Ecological recovery is the process of assisting the recovery of an ecosystem that has been degraded, damaged and destroyed. It requires the establishment or recovery of the microbial, plant and animal communities that make a functioning ecosystem. Bio-monitors and Bio-reporters play an important role in ecological recovery. Aquatic and Pulmonated Snails are being evaluated for their suitability as bio-monitors in the recovery of oil spilled freshwater marshland. Green Florescent Protein have shown great advantage as bio-reporters which are designed to measure high concentrations of toxic chemicals.

Advantages of Bioremediation

• Biological sources can multiply and magnify in terms of initial inoculums as compared to physical and chemical means of treatment.

• Low cost and highly efficient process.

• Can be enhanced by the addition of various microorganisms to a polluted environment to promote increased rate of bioremediation.

Limitations of Bioremediation

• Limited to only biodegradable products.

• Influenced by various environmental factors.

• In aquaculture sludge, high level of contaminants limits the effectiveness of bioremediation.

• Treatment time, in bioremediation of aquacultural sludge, is typically longer than other methods.

Conclusion

Bioremediation is considered as one of the safer, cleaner, cost effective and environment friendly technology for decontaminating sites which are contaminated with wide range of pollutants. The process of bioremediation uses a number of agents such as bacteria, yeast, fungi, algae and higher plants as major tools in remedying oil spills and heavy metals present in the environment. As microorganism exhibit wide range of processes, there are still few mechanisms which are not known, therefore bioremediation is still regarded as a developing technology. Thus, there is an immediate need to for us to review and amend the existing options for environmental clean-up.

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