Natural Biotechnology for Environmental Clean-up

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Abstract **- The unprecedented population increase and industrial development during the 20th century has not only increased conventional solid and liquid waste pollutants to critical levels but also produced a range of previously unknown pollution problems for which society was unprepared. The growth of the world population, the development of various industries, and the use of fertilizers and pesticides in modern agriculture has overloaded not only the water resources but also the atmosphere and the soil with pollutants .**

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Introduction

Even though we can travel to the Moon, send robots to Mars, make super computers and clone organisms we still have dificulties to clean the water we use. In many parts of the world the availability of water is a crucial issue, and even more so, clean water. Environmental pollution is the most horrible ecological crisis that man is facing today. Pollution is a global threat to the environment and it becomes a scare word of today's world. The rapid growth of human populations fuelled by technological developments in health and agriculture has led to a rapid increase in environmental pollution. The unprecedented population increase and industrial development during the 20th century has not only increased conventional solid and liquid waste pollutants to critical levels but also produced a range of previously unknown pollution problems for which society was unprepared. The growth of the world population, the development of various industries, and the use of fertilizers and pesticides in modern agriculture has overloaded not only the water resources but also the atmosphere and the soil with pollutants . In the last few decades the handling of wastewater appeared to be one of the most important. The degradation of the environment due to the discharge of polluting wastewater from industrial sources is a real problem in several countries. This situation is even worse in developing countries like India where little or no treatment is carried out before the discharge . In spite of the many steps taken to maintain and improve the quality of surface and groundwater, the quantities of wastewater generated by these industries continue to increase and municipalities and industries are confronted with an urgent need to develop safe and feasible alternative practices for wastewater management Bioremediation is a pollution-control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms.

The natural processes such as crude oil formation, soil formation, waste disposal, nitrogen fixation, biological pest control, pharmaceutical production, dispersal of fruits and pollination are all accomplished by the enormous biodiversity available worldwide . The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling and transport of hazardous material. Additionally, it is very dificult and increasingly expensive to find new landfill sites for the final disposal of material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

Three primary ingredients for bioremediation are: 1) presence of a contaminant, 2) an electron acceptor, and 3) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved. Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic chemicals such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such contaminants into less toxic forms. Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance.

Bio remedial approach

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". Technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere). Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth of the pollution-eating microbes within the medium (bio stimulation). Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals, and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes.

Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as Cd & Pb are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil. Bone char has been shown to bioremediate small amounts of Cd, Cu & Zn.

Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a remediation; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more accepted than other technologies. Most remediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules.

Inimitability for remediation

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 decolouration mechanisms

Many processes were employed to remove dye molecules from industry effluents and the treatment methods can be divided into the following categories:

Physical methods: Physical methods such as adsorption ion exchange and membrane filtration were employed in the removal of dyes. The main disadvantages of these physical methods were they simply transfer the dye molecules to another phase rather than destroying them and they were effective only when the effluent volume is small .

By adsorption: Adsorption is the transfer of solute dye molecule at the interface between two immiscible phases in contact with one another. The removal of colour from dye industrial effluents by the adsorption process using granular activated carbon has emerged as a practical and economical approach .

By Ion Exchange: Removal of Anions and Cations from dye industry effluent can be carried out by Ion exchange method by passing the waste water through the beds of ion exchange resins where some undesirable cations or anions of waste water get exchanged for sodium or hydrogen ions of the resin.

By membrane filtration

Reverse osmosis (RO) and electro dialysis are the important examples of membrane filtration technology. The contribution of reverse osmosis in removing this high salt concentration is of great. This RO reject can be reused again in the process. For reactive dyeing on cotton, the presence of electrolytes in the waste water causes an increase in the hydrolyzed dye affinity making it difficult to extract. The total dissolved solids from waste water were removed by reverse osmosis. Though it is suitable for removing ions and larger species from dye bath effluents with high efficiency, it possesses some disadvantages like clogging of the membrane by dyes after long usage and high capital cost.

Chemical methods

Chemical methods such as chemical oxidation , electrochemical degradation , and ozonation were employed in dye removal effectively. A variety of oxidizing agents were used to decolorize wastes by oxidation techniques effectively. Among that sodium hypochlorite decolorizes dye bath efficiently. Even though it is a low cost technique, it forms absorbable toxic organic halides. Ozone on decomposition generates oxygen and free radicals. The main disadvantage of this technique is that it requires an effective sludge producing pretreatment.

Electrochemical method

The requirement of chemicals and the temperature to carry the electro chemical reaction is less than those of other equivalent non-electrochemical treatment. It can also prevent the production of unwanted side products. But, if suspended or colloidal solids were high in concentration in the waste water, they slow down the electrochemical reaction. Therefore, those materials need to be sufficiently removed before electrochemical oxidation.

Biological methods

Bioaccumulation and biosorption are the two main technologies in biological process for of dye bearing industrial effluents. They possess good potential to replace conventional methods for the treatment of dyes industry effluents . Biological process can be carried out in situ at the contaminated site, these are usually environmentally benign i.e., no secondary pollution and they were cost effective. These are the principle advantages of biological technologies for the treatment of dye industry effluents. Hence in recent years, research attention has been focused greatly on biological methods for the treatment of effluents . The disadvantage of this degradation process is that it suffers from low degradation efficiency or even no degradation for some dyes and practical difficulty in continuous process.

Microbial Population for Bioremediation Processes

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 excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or 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.

Granular sludge

Even though anaerobic azo dye reduction could be readily achieved with different microorganisms, there is no strain reported so far that is able to decolourise a broad range of azo dyes. Therefore, the use of a specific strain or enzymes for reductive decolourisation does not make much sense in treating textile wastewater, which containing many kinds of dyes. The use of mixed cultures, such as anaerobic granular sludge, which is composed of stable microbial pellets with a high activity, is probably a more logical alternative. However, a little is known about the microbiological aspects of the reductive decolourisation of azo dyes with anaerobic consortia, commonly found in wastewater treatment plants, although the applicability of the cost-effective high-rate anaerobic reactors for azo dye reduction has been well demonstrated.

Non-biological colour removal

While advanced oxidation processes (AOPs) have been studied extensively both for recalcitrant wastewater in general and dye wastewater in particular, their commercialization has yet not been realized because of certain barriers . These processes are costly and complex also at the present level of their development . Additional impediment exists in the treatment of dye wastewater with higher concentration of dyes, as AOPs are only effective for wastewater with very low concentrations of organic dyes.

Factors that Control Microbial Dye Decolouration

Microorganisms are sensitive to the presence of chemical substances, such as dyes, high salinity, variations in pH and high content of organic compounds . For bioremediation processes, the most useful microorganisms are those isolated from textile industry-contaminated environments, including soil, effluents and sludge from wastewater treatment plants, because they are adapted to grow in extreme conditions . The bio decolouration process is dependent on the following factors: the azo dye structure, carbon and nitrogen sources, salinity, pH, temperature, dye concentration and the presence or absence of oxygen.

Effects of the azo dye structure

It has been observed that the enzymatic degradation of the dye is highly influenced by its structure . Recent studies have revealed that the enzymatic activity is induced by the presence of dyes in such a way that this activity is significantly higher at the end of the decolouration process.

Influence of carbon and nitrogen sources in the decolouration process

Carbon and nitrogen sources have an important influence on the extent of decolouration using microorganisms. Carbon sources have two purposes: as sources of carbon and energy for the growth and survival of the microorganisms and as electron donors, which are necessary for the breakage of the azo bond . Carbon sources are accepted differently by different microorganisms and have an important effect on the extent of decolouration.

Influence of salinity, dye concentration, pH, temperature and oxygen in the decolouration process

The operation conditions affect the efficiency of microorganisms to decolourate azo dyes, such as the presence of salts, concentration of the dyes, pH, temperature and oxygen. Generally, a sodium concentration above 3000 ppm causes moderate inhibition of most bacterial activities [80]; thus, azo dye removal efficiencies under saline conditions decrease. However, there are examples of halo tolerant microorganisms that are able to decolourate azo dyes in the presence of salts. The dye concentration has effects on microbial azo dye decolouration.

Bioremediation Strategies

Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water). Bioaugmentation techniques involve the addition of microorganisms with the ability to degrade pollutants.

Genetic engineering approaches

The use of genetic engineering to create organisms specifically designed for bioremediation has great potential. The bacterium *Deinococcus radiodurans* (the most radio resistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste. Most commonly, the process is misunderstood. The microbes are ever-present in any given context generally referred to as “normal microbial flora”. During bioremediation (biodegradation) processes, fertilizers/nutrients supplementation is introduced to the environments, in efforts to maximize growth and production potential. Common misbelieve is that microbes are transported and dispersed into an unadulterated environment. *Micoremediation* is a form of bioremediation in which fungi are used to decontaminate the area. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by mycelium.

Advantages of Bioremediation

Bioremediation is natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminants increase in numbers when the contaminant is present; when the contaminant is degraded, the bio degradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water and cell biomass. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products.

Disadvantages of Bioremediation

Bioremediation 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 successes 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 Bioremediation often takes longer time than other treatment options, such as excavation and removal of soil or incineration.
- Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of "clean", evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments.

Conclusion

The emerging of recent studies in molecular biology and ecology offers opportunities for more efficient biological processes to detoxify contaminants. Notable accomplishments of these studies include the clean-up of polluted water and land areas. Bioremediation is far less expensive than other technologies that are often used to clean up hazardous waste. Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, bio stimulation, and Bioagumentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behaviour of soil microorganisms that are indigenous to soil. Bio stimulation also utilizes indigenous microbial populations to remediate contaminated soils. Bio stimulation consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Bioagumentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms. There are a number of cost or efficiency advantages to bioremediation which can be employed in areas that are inaccessible without excavation

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