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BIOREMEDIATION OF WASTEWATER USING MICROBIAL TECHNOLOGIES

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Abstract

Bioremediation is an innovative and most promising technology arising in today's world to decrease the pollutants with the help of microbes which neutralizes the harmful pollutants from contaminated sites. The environment comprises of complex variables which includes air, water, and land. The positive correlation made by the environment is responsible for the existence of humans along with other living creatures as plants, animals, and microbes. The technological advancement in industrial societies has led to severe environmental pollution of air, soil, and water, which make the indispensable part of human life. The increasing population, urbanization and rapid industrialization in the current world becoming challenging to the groundwater resources management in developing countries. Therefore, these factors have ended up in generation of huge amounts of solid waste in various toxic forms which ultimately pollute the entire ecosystem. Bioremediation is an innovative and promising technology in which organisms or microbes are used to remove or neutralize harmful pollutants from contaminated sites including water and soil. It requires a technique to select organisms capable of uptake or release some enzymes which can degrade these pollutants. Using biological materials, it is more effective than the traditional strategies because bioremediation strategies can be used directly at the site of contaminant without the need to transfer contaminant materials. In the bioremediation process, bacteria alone, consortia, or combination of bacteria and algae, Fungi etc. can be used for effective biodegradation.

Key words: *Bioremediation, Pollutants, Heavy metals, Bacteria, Fungi, Algae, Microbial Consortia.*

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Introduction

Environmental contamination by heavy metals from anthropogenic and industrial activities has caused considerable irreparable damage to aquatic ecosystems. Sources which are responsible for these activities include the mining and smelting of ores, effluent from storage batteries and automobile exhaust, and the manufacturing and inadequate use of fertilizers, pesticides, and many others. The metals and metalloids that contaminate waters and are most commonly found in the environment include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel. These metals are the subject of concern due to their high toxicity. Apart from being hazardous to human health, they also have an adverse effect on the fauna and flora, and they are not biodegradable in nature. Thus, there is a need to seek new approaches in developing treatments to minimize or even eliminate metals present in the environment.

Several different physicochemical and biological processes are commonly employed to remove heavy metals from industrial wastewaters before their discharge into the environment [1]. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, osmosis, evaporation, and sorption are not vary much beneficial these days as they are cost-effective, and some of them are not environmentally friendly [2, 3]. On the other hand, bioremediation processes show promising results for the removal of metals, even when present in very low concentrations where physicochemical removal methods fail to operate. Furthermore, this is an eco-compatible and economically feasible option. The bioremediation strategy is based on the high metal binding capacity of biological agents, which can remove heavy metals from contaminated sites with high efficiency. Keeping these points in mind, microorganisms can be considered as a biological tool for metal removal because they can be used to concentrate, remove, and recover heavy metals from contaminated aquatic environments [4]. Several studies have been conducted using microorganisms for the uptake of heavy metals in polluted waters as an alternative strategy to conventional treatments [5–7]. Bioremediation by microorganisms is very useful due to the action of microorganisms on pollutants even when they are present in very dilute solutions, and they can also adapt to extreme conditions. Although the mechanisms associated with metal biosorption by microorganisms are still not well understood, studies show that they play an important role in the uptake of metals and that this action involves accumulation or resistance.

Factors Affecting Microbial Bioremediation

Bioremediation is involved in degrading, removing, altering, immobilizing, or detoxifying various toxic chemicals and physical wastes from the environment through the action of bacteria, fungi and plants. Microorganisms are involved through their enzymatic pathways act as biocatalysts and facilitate the progress of biochemical reactions that degrade the desired pollutant. Microorganisms are act against the pollutants only when they have access to a variety of materials compounds to help them generate energy and nutrients to build more cells. Bioremediation includes all those processes and actions that take place in order to return the natural environment altered by contaminants to its original condition [8]. It primarily uses microorganisms, fungi, green plants, or their enzymes to degrade and transform environmental contaminants into harmless or less toxic forms. It uses relatively low- cost, low-technology techniques, which generally have a high public acceptance [9].

The controlling and optimizing of bioremediation processes is a complex system due to many factors. These factors are included here: the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

Biological factors

Biotic factors affect the degradation of organic compounds through competition between microorganisms for limited carbon 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 number of enzymes(s) produced by each cell. The major biological factors are included here: mutation, horizontal gene transfer, enzyme activity, interaction (competition, succession, and predation), its own growth until critical biomass is reached, population size and composition [10,11].

Environmental factors

The metabolic characteristics of the microorganisms and physicochemical properties of the targeted contaminants determine possible interaction during the process. Microorganism growth and activity are affected by pH, temperature, moisture, 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). This above listed factors are determined kinetics of degradation [10,12]. Biodegradation can occur under a wide range of pH; however, a pH of 6.5 to 8.5 is generally optimal for biodegradation in most aquatic systems [13]. Most environmental factors are listed below.

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 [14,15]. Biodegradation in aquatic environment is limited by the availability of nutrients [16].

Temperature

Among the physical factors temperature is the most important for the survival of microorganisms and composition of the hydrocarbons [17]. Biological enzymes participated in the degradation pathway have an optimum temperature and will not have the same metabolic turnover for every temperature. Moreover, the degradation process for specific compound needs specific temperature. Temperature speeds up or slow down the bioremediation process because of highly influenced microbial physiological properties. The rate of microbial activities increases with temperature to its maximum level at an optimum temperature. It became decline suddenly with further increase or decrease in temperature and eventually stop after reaching a specific temperature [18].

Concentration of oxygen

Different organisms have oxygen requirements as per their action mechanism. 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 [19].

Moisture content

Microorganisms require adequate water to accomplish their growth.

pH

pH of compound having acidity, basicity, and alkalinity in nature, has its own impact on microbial metabolic activity. The increase and decrease in pH may also affect the contaminants removal process. Higher or lower pH values showed inferior results; metabolic processes are highly susceptible to even slight changes in pH [20].

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.

Metal ions

Metals are important in small amount for bacteria and fungus, but its high quantity inhibit the metabolic activity of the cells, that directly or indirectly have impact on rate of degradation.

Toxic compounds

Toxic compounds are toxic for the microbial population and can also slow down the degradation process. 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 [10].

Bioremediation Process

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or levels below concentration limits established by regulatory authorities. The detoxification and rehabilitation of contaminated water with the use of microbes has emerged as the most safe, easy, and effective technology. The native water and soil microorganisms have been explored for their ability to remove or detoxify toxic products released due to human activities in the environment viz. mining of ores, oil and gas extraction, pesticides, pigments, plastic, organic solvents, fuel, and industrial processes [8] Studies have been carried out to promote the use of modified microbes designed specially to increase sensitivity towards toxic metals. Biological removal includes the use of microorganisms (fungi, algae, and bacteria) for heavy metal treatment from wastewater [21]. However, introduction of heavy metals into the water causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms at relatively low concentrations [22]. The modification of the microbial make up is mainly brought about by exerting an inhibitory action through blockage of essential functional groups, displacement of essential metal ions or modification of active conformations of biological molecules [23,24].

Microorganisms are omnipresent that dominate in heavy metal-contaminated water and can easily convert heavy metals into non-toxic forms. In bioremediation processes, microorganisms mineralize the organic contaminants to end-products such as carbon dioxide and water, or to metabolic intermediates which are used as primary substrates for cell growth. Microorganisms are capable of two-way defense viz. production of degradative enzymes for the target pollutants as well as resistance to relevant heavy metals. Different mechanisms of bioremediation are known, including biosorption, metal-microbe interactions, bioaccumulation, biomineralization, biotransformation and bioleaching. Microorganisms remove the heavy metals from soil by using chemicals for their growth and development. They are capable of dissolving metals and reducing or oxidizing transition metals. Different methods by which microbes restore the environment are oxidizing, binding, immobilizing, volatizing, and transformation of heavy metals. Bioremediation can be made successful in a particular location by the designer microbe approach, and by understanding the mechanism controlling growth and activity of microorganisms in the contaminated sites, their metabolic capabilities, and their response to environmental changes. Bacteria, Fungi, and algae are known for bioremediation of toxic hazardous materials including heavy metals [25]. Since microorganisms have developed various strategies for their survival in heavy metal- polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization, which can be exploited for bioremediation either by *ex situ* or *in situ* methods [26-29]. A global survey to examine the use of bioremediation technologies for addressing the environmental problems was carried out by Elekwachi [30]. The developed economies made higher use of low- cost *in situ* bioremediation technologies such as monitored natural attenuation, while their developing counterparts appeared to focus on occasionally more expensive *ex situ* technologies. There are many reports about biodegradation and bioremediation strategies being utilized by bacterial species [31-34].

Bacterial species that performed efficiency in pollutants degradation that are *Pseudomonas, Actinobacter, Acaligenes, Arthrobacter, Bacillius, Berijerinckia, Flavobacterium, Methylosinus, Mycococcus, Nitrosomonas, Nocardia, Serratia and Xanthofacter, Exiguobacterium, Geobacter* and *Mycobacterium* as listed by many researchers [25]. Many bacteria, such as *Azotobacter, Actinomycetes* and *Pseudomonas* could synthesize different substances to capture Fe^{2+} which they require for their metabolic activity and bio-sorption [35]. *Geobacter metallireducens* is a Fe (III) reducing organisms that can oxidize a variety of aromatic pollutants (benzeneand naphthalene) and eliminate or decrease uranium (a radioactive waste) from drainage water in mining operations and from contaminated groundwater [36]. *Exiguobacterium aurantiacum* has showed capability for phenol degradation and PAHs in laboratory applying a batch culture when provided with pure compounds as a sole source for life [37] Microorganisms have the ability to uptake heavy metals from contaminated water [38].

Algae also play an important role in the bioremediation of wastewater and decontaminate pollutants such as organic and inorganic compounds. Algae can be used in the free form or immobilized form for bioremediation of toxic heavy metals. Recently, El-Sheekh [39] studied the ability of two species of green unicellular microalgae namely *Chlorella vulgaris* (freshwater algae) and *Chlorella salina* (marine alga) to bio-remediate different types of polluted water and they found that algae were able to reduce pH, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, ammonia, phosphate, sulphate, calcium, magnesium, sodium, potassium, heavy metals (Zn, Cu, Mn, Ni, Co, Fe and Cr) and the number of total coli form bacteria from different mixtures of water samples. Various algal species such as *Chlorella vulgaris, Rhizoclonium hieroglyphicum* and mixed algae culture (*Microspora* sp., *Navicula* sp., *Lyngbya* sp., *Cladophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp.) have proved efficiency in waste waters treatment [39].

Bioremediation is occurred naturally and encouraged with in addition of living things and fertilizers. Bioremediation technology is principally based on biodegradation. It refers 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 [40]. Numerous mechanisms 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.

Advantage of Bioremediation

- It is a natural process, it takes a little time, as an acceptable waste treatment process for contaminated materials. 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 and the potential threats to human health and the environment that can arise during transportation.
- It is applied as a cost-effective process due to less cost than the other conventional methods (technologies) that are used for clean-up of hazardous waste [41].
- 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. Because of bioremediation change harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed [42].
- Simple, less labor intensive and cheap due to their natural role in the environment. Eco-friendly and sustainable [43].
- Nonintrusive, potentially allowing for continued site use. Relative ease of implementation [44].
- Effective way of remediating natural ecosystem from a number of contaminates and act as environment friendly options [45].

Disadvantage of Bioremediation

- It is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- 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.
- Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of “clean”, evaluating performance of bioremediation is difficult.

Heavy metals cannot be destroyed biologically (“no degradation”, changes occur in the nuclear structure of the element), but only transformed from one oxidation state or organic complex to another. Besides, bacteria are also efficient in heavy metals bioremediation.

Microorganism uptake heavy metals actively by bioaccumulation andpassively by adsorption [46]. The microbial cell walls, which mainly consist of polysaccharides, lipids, and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups [47]. Among various microbe-mediated methods, the biosorption process seems to be more feasible for large scale application compared to the bioaccumulation process, because microbes will require addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen demand or chemical oxygen demand in the waste.

Types of bioremediations

There are different types of treatment technologies under bioremediation processes. The basic bioremediation methods are:

Biostimulation

This kind of strategic is linked through the injection of specific nutrients at the site (soil/ground water) to stimulate the activity of indigenous microorganisms. It is totally focused 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 [7,44]. The Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes [5].

Bioattenuation [Natural attenuation]

Bioattenuation or natural attenuation is the eradication of pollutant concentrations from surrounding. It may be carried out with in biological processes (aerobic and anaerobic biodegradation, plant, and animal uptake), physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption), and chemical reactions (ion exchange, complexation, abiotic transformation) [48].

Bioaugmentation

The addition of pollutant degrading microorganisms (natural/exotic/ engineered) to augment the biodegradative capacity of indigenous microbial populations on the contaminated area, these processes are known as bioaugmentation. In order to rapidly increasing the natural microorganism population growth and enhance degradation that preferentially feed on the contaminants site. Microbes are collected from the remediation site, separately cultured, genetically modified and returned to the site. It is used to ensure that the *in-situ* microorganisms can totally remove and alter these contaminants to ethylene and chloride, which are non-toxic [49].

Genetically Engineered Microorganisms (GEMs)

These are the microorganisms whose genetic material has been already changed by applying genetic engineering techniques inspired by natural or artificial genetic exchange between microorganisms and comes under the recombinant DNA technology. Genetic engineering has been improved the utilization and elimination of hazardous unwanted wastes under laboratory conditions by creating genetically modified organisms [50]. Genetically engineered microorganisms (GEMs) have shown potential for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of chemical contaminants. In GEMs four activities / strategies were studied listed further: (1) modification of enzyme specificity and affinity, (2) pathway construction and regulation, (3) bioprocess development, monitoring, and control, (4) bioaffinity, bioreporter sensor applications for chemical sensing, toxicity reduction, and end point analysis. Therefore, GEMs can be used effectively for biodegradation purpose and will lead to represent/indicate a research frontier with broad implications in the future time [51].

Bioventing

Bioventing is involved in venting of oxygen through soil to stimulate growth of natural or introduced bacteria and fungus in the soil by providing oxygen to existing soil microorganisms; indeed, it is functional in aerobically degradable compounds. Bioventing uses low air flow rates to provide only enough oxygen to sustain microbial activity. Effective bioremediation of petroleum contaminated soil using bioventing has been proved by many researcher [52,53].

Biopiles

Biopiles is a way of excavated soil contaminated with aerobically remediable hydrocarbons, can be treated in "biopiles". Biopiles (also known as biocells, bioheaps, biomounds, and compost Piles) are used to reduce concentrations of petroleum pollutants in excavated soils during the time of biodegradation [54-55].

Conclusion

Biodegradation is very fruitful and ecofriendly method used for remediating, cleaning, managing, and recovering the polluted environment through microbial activity. The speed of unwanted waste substances degradation is determined in competition within biological agents, inadequate supply with essential nutrient, uncomfortable external abiotic conditions (aeration, moisture, pH, and temperature), and low bioavailability of the pollutant. Bioremediation has been used in different sites globally within varying degrees of success. Mainly, the advantages are greater than that of disadvantages which is evident by the data provided by the researchers working on the several levels of pollutants and their microbial bioremediation. A lot of studies reported different species from the explored sites and used them as effective control mechanism. Although, much of the studies has been carried out on the effective sites and needed to be explored on commercial levels.

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