**Nanoenhanced Microbial Bioremediation**

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**Abstract**

Bioremediation is a type of waste management technique in which microbes such as bacteria, fungi or any living organism used in the removal of contaminated particles from polluted environment. The main principle is degrading and converting highly toxic form of pollutants to less toxic forms. To increase the efficiency of microbial bioremediation, nanotechnology is going to be merge with this. Nanotechnology is the science of small sized particles having high surface to volume ratio. In last few decades, nanoscience acting as the most emerging area of research due to its small size and shape. They have been use in pharmaceuticals, diagnostics and disease treatment. They can also be used for other purposes like drug discovery, in farming, tumor detection as well as treatment, etc. Also, nanoparticles have diverse applications in various other sections like decontaminating heavy metals from water and soil. Various microbes have been identified for the synthesis of nanoparticles such as nanocellulose. Although microbes are used for bioremediation but their efficacy for bioremediation can be induced by using nanoparticles and this can be a promising strategy for bioremediation in future. Therefore, the combination of nanotechnology with microbial based bioremediation will provide a way to develop a better bioremediation technique with much better speed and less toxicity. The present chapter provides the explicit information on nanoparticles enhanced microbial bioremediation.

**Keywords**: Nanomaterials, Bioremediation, Nanoscience

1. **INTRODUCTION**

Bioremediation is one of the branch of biotechnology that deals with the process of removal of contaminants from the environment such as toxins from soil, hazardous gases from water, etc. by the help of microorganisms.

Simply, bioremediation is made up of two words Bio means living organisms and another word is remediate which means to stop the environmental damage. Hence bioremediation stands for stopping the environmental damage by the use of living organisms.

It works on the simple principle that most of the microbes uses these contaminants for stimulating their growth and convert these harmful contaminants into small molecules of water or gases etc.

But it requires certain conditions like appropriate temperature, good amount of nutrients, water, etc. Absence of any of the condition may prolong the process of bioremediation. But there are some other way that improve the process of bioremediation such as by adding amendments like oil, water, molasses or simply air, etc. they have capacity to optimize conditions for microbes and hence can increase the rate of bioremediation.

Bioremediation exists in two ways: ex situ and in situ. In situ means at the site of contamination and ex situ means location away from contamination site.When selecting any bioremediation technique, factors such as the type of pollution, its depth and intensity, environmental conditions, etc. are taken into account [1].

This process can take several months to year depending on the concentration of contaminated particles, soil, temperature, bioremediation type if it is done in situ or ex situ. Prior to beginning a bioremediation project, performance criteria—such as temperature, pH, concentration of O2 and other non living factors—that affect the efficacy of this process are also carefully examined [1]. Furthermore, when remediation of sites polluted with pollutants other than hydrocarbons is involved, it is likely that additional remediating approaches be taken into consideration [2]. These techniques may end up being more cost-effective and efficient to deploy during repair.

1. **Exsitu bioremediation**

In order to treat the pollutants, they must first be collected from polluted places and then transported to another location. Ex situ bioremediation procedures are typically selected on the bases of the following factors: the expense of treatment, the kind and depth of the pollutants, the degree of pollution, the location of the contaminated site, and its geology. Performance standards have also been outlined, and they also influence the selection of ex situ bioremediation methods [3]. This process act as necessary if soil is too dense and nutrients are not distributed evenly and climate is too cold for microbial activity.

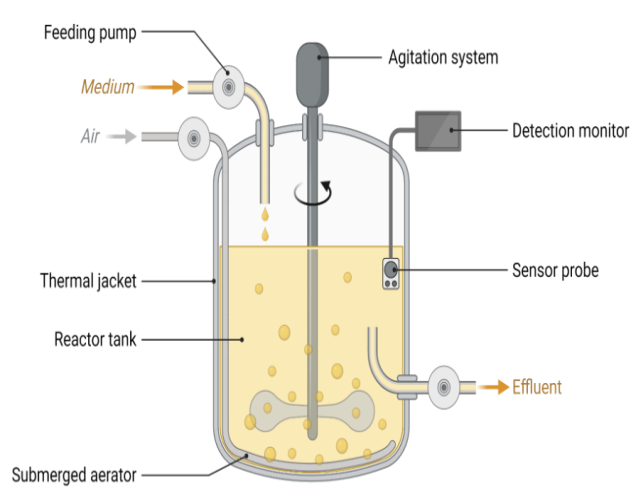
### **Biopile**

Excavated toxic soil is piled above ground in this method, which is then followed by nutrient enrichment and, occasionally, aeration to boost microbes activity. This method consists of a treatment bed, irrigation, aeration, and systems for collecting leachate and nutrients. Due to its beneficial qualities, such as cost effectiveness, which permits efficient biodegradation provided that temperature, aeration, and nutrients are properly regulated, the application of this specific ex situ technique is becoming more and more popular [4].

1. **Bioreactor**

Bioreactor is one the best technique of ex situ bioremediation which contains a vessel in which polluted materials are fed up as raw material and this raw material undergoes several biological reactions and converts into specified product. Bioreactor generally based on the principle of fermentation. It is done into several ways like batch , fed batch and in continuous way. One of the major advantage of bioreactor is that we can control the parameters like temperature, pH, substrate concentrations and aeration rate. By controlling these parameters, we can subsquently decrease the bioremediation time.

Bioreactor-based bioremediation is more efficient because it can effectively establish controlled bioaugmentation, nutrient addition, increased pollutant bioavailability, and mass transfer (contact between pollutant and microbes), which are some of the limiting factors of the bioremediation process. Additionally, it can be applied to soil or water that has been contaminated by volatile organic compounds (VOCs), such as BTEX (benzene, toluene, ethylbenzene, and xylenes).The adaptability of the design of vessel bioreactor designs maximises degradation while reducing abiotic losses[5]. It is possible to observe changes in the dynamics of the microbial population in a bioreactor containing soil slurry contaminated with crude oil over a short or long duration. This makes it simple to characterise the microbes populations involved in process of bioremediation[6,7,8].



Source by biorendor.com

Figure 1: bioreactor

### **Land farming**

This is the most simple bioremediation technique because it has low cost and less equipment requirement. This is the only technique that draws under the in situ as well as ex situ. The common thing is that the polluted soil is excavated but on the bases of treatment site, it will comes under ex situ or in situ technique.

It has been well studies in literature that if the pollutant lies less than 1 m below ground surface, bioremediation takes place without excavation, while pollutant lying above 1.7 m, there is need for the transport of soil to another site [3,9,10,11,12]. There are various conditions that increase the efficiency of bioremediation. These are aeration, nutrients like phosphorus, nitrogen, etc. and so on [12]. This technique is generally used for the remediation of hydrocarbon-polluted sites including polyaromatic hydrocarbons [13, 14]. The land farming system is adaptable to any temperature and region, and it conforms with government rules [15]. During a bioremediation operation, the building of an appropriate land possessing impermeability property reduces the risk of leakage of the pollutants into other areas [13]. This is very simple technique having minimal requirement [16]. But it has some limitations like it requires space, also microbial activites effect due to the unfavourable environmental condition and so on [16, 17]. .

1. **In situ bioremediation**

This process involves the treatment of contaminated substances at the site of pollution. There is no need of excavation hence have no risk for soil disturbance. Also these techniques are less expensive as compared to ex situ bioremediation techniques. Chlorinated solvents, dyes, heavy metals, and hydrocarbon-polluted sites have all been effectively treated with in situ bioremediation approaches [19, 20, 21, 22]. Notably, a successful in situ bioremediation depends on a number of critical environmental factors, including temperature, pH, moisture content, nutrient availability, and electron acceptor status [3].

### **Bioventing**

This technique involve aerobic degradation by delivering oxygen to in order to increase the efficiency of microbes for the effectiveness of bioremediation. The ultimate goal of bioventing is to achieve the microbial transformation of contaminants into a harmless condition by supplying nutrients and moisture to improve bioremediation [3]. Among other in situ bioremediation methods, this one has grown in favour, particularly for cleaning up sites contaminated by minor petroleum product spills [23].

One of the main point is that air flow rate does not effect the bioremediation effectiveness. This indicates that neither the rate of biodegradation nor the efficiency of pollutant biotransformation is increased by high airflow rates. This results from early air saturation on the surface, which is caused by the need for oxygen during biodegradation. However, the rate of biodegradation increased significantly as a result of reduced air injection rate. Thus, it demonstrates that one of the fundamental variables for pollutant dispersal, redistribution, and surface loss in bioventing is air injection rate.

### **Bioslurping**

In order to achieve soil and groundwater remediation via indirect oxygen provision and promotion of contaminant biodegradation, this technology combines vacuum-enhanced pumping, soil vapour extraction, and bioventing [24]. LNAPLs are recovered using this technology, which is also employed for free products. This method is also used to clean up soils that have organic chemicals that are volatile or semi-volatile polluted. Similar to how a straw sucks liquid from any vessel, the device uses a "slurp" that extends into the free product layer to draw liquids (free products and soil gas) from this layer. LNAPLs are raised to the surface by the pumping mechanism, where they separate from air and water. The system may be readily configured to function as a traditional bioventing system to finish the remediation process after all free products have been removed [20]. The method reduces the amount of groundwater produced by the operation, which reduces the cost of storage, treatment, and disposal even though it is not appropriate for remediating low-permeability soil [3]. However, excessive soil moisture reduces the rate of oxygen transfer and restricts air permeability, which in turn lowers microbial activity.

### **Biosparging**

This method is comparable to bioventing, which is the process of injecting air into the soil's surface to promote microbial development and aid in the cleanup of contaminated particles. This method involves injecting air into the saturated zone, which pushes volatile organic molecules upward and into the unsaturated zone, accelerating biodegradation. Pollutant biodegradability and soil permeability, which control pollutant bioavailability to microorganisms, are the two main determinants of biosparging efficacy [3].

### **Phytoremediation**

This technique based on the use of plant interactions such as, biological, physical, biochemical, chemical and microbiological in polluted sites to remove the toxic pollutants from the polluted sites. Depending on the pollutant type, there are several mechanisms of phytoremediation such as extraction, filtration, stabilization and volatilization and degradation. Toxic heavy metals and other elements are eliminated through extraction, transformation, and sequestration. Conversely, degradation, stabilisation, and volatilization eliminate organic pollutants like hydrocarbons; mineralization occurs when certain plants, such lucerne and willow, are utilised [25, 26]. A few things need to be considered while selecting a plant to serve as a phytoremediator. These factors include the type of root system (tap or fibrous, depending on the depth of the pollutant), biomass above ground, the toxicity of the pollutant to plants, the rate at which plants grow and adapt to the environment, site monitoring, and most importantly, the amount of time needed to reach the desired level of cleanliness. Furthermore, the plant needs to be pest- and disease-resistant [27]. In certain contaminated situations, a plant's process of removing contaminants entails three steps: uptake, which is primarily done passively; translocation, which is accomplished via xylem flow; and accumulation in the shoot [28]. The use of plant growth-promoting rhizobacteria (PGPR) is important in phytoremediation because PGPR tends to enhance biomass production and plant tolerance to heavy metals and other unfavourable soil (edaphic) conditions [29, 30].

Similar to this, bioaugmentation using endogenous rhizobacteria led to increased plant subsurface biomass, metal buildup, and improved metal removal during phytoremediation of metal-contaminated estuaries using Spartina maritima [31]. It has been documented that certain plant species are naturally able to eliminate organic and elemental contaminants from contaminated areas. Zea mays and Brachiaria mutica have also been mentioned as possible phytoremediators of locations affected by heavy metals [32, 33]. Many more plants having potential for remediation are documented in detail [26, 34, 35, 36]. Certain transgenic plants that have had genes transferred for increased phytoremediation have also been published [27].

1. **Nanobioremediation**

The most effective strategy and cutting-edge method based on the application of nanomaterials is nanoremediation. The use of nanoremediation offers novel approaches for the prompt and effective removal of pollutants from contaminated environments, enabling the handling of the great challenges of the twenty-first century, including environmental problems, crisis of pollution and so on [37, 38, 39]. The technology uses nanomaterials that have the potential to cut down on the amount of time and money needed to clean up contaminated particles [40, 41]. It also eliminates the need to treat and dispose of contaminated particles and lowers their concentration to almost zero in situ. There are various used in nanoremediation methods to break down pollutants [42, 43, 44, 45]. This is the most appropriate method than others[46, 47, 48].

1. **Tools of nanobioremediation**
2. **Nano-photocatalysis**

The majority of organic pollutants are classified as persistent organic pollutants because they are unable to break down quickly, remain for extended periods of time, eventually find their way into the food chain, and have an adverse influence on human health [49, 52]. the amount of contaminants rising daily as a result of traditional technologies' incapacity to break down persistent organic compounds. Numerous studies are being conducted in conjunction with governmental and non-governmental organisations that are committed to reducing the level of pollution by creating more intelligent and environmentally friendly methods [49, 50, 51].

One of these methods is photocatalysis, which uses extremely reactive radicals like superoxide and hydroxyl to change dangerous substances into safer ones. In the presence of sun radiation, the rate of chemical reaction can accelerate. Generally speaking, photocatalysis is a redox reaction caused by the absorption of photons, which produces valence and conduction bands. Radiation causes the generation of holes in the valence band (VB) and electrons in the conduction band (CB), which leads to the development of extremely reactive species such superoxide radicals (O2) and hydroxyl (OH). These reactive organisms destroy all water-borne microorganisms, break down waste plastics, oxidise organic pollutants, and break down air pollutants like NO2, CO, and NH3.

Because this method produces highly reactive radicals, which are potent oxidizers, it has been regarded as an effective process [44, 53]. The effective cleanup procedure for preserving the environment's health is demonstrated by the photocatalytic oxidation of various compounds like aromatic compounds, etc. [54, 55]. The last ten years have seen a significant amount of research on nanomaterial photocatalysis for environmental cleanup.

Photocatalysis works on the principle of redox reactions [56, 57].This method is thought to be a promising advanced oxidation technology (AOP) to solve the difficult issues of water disinfection, energy restoration, and air contamination. Compared to other conventional ways, photocatalysts demonstrated as a clever choice to solve pollution related because of their complete and rapid breakdown of dangerous organic materials into environment-friendly products [58, 59]. Nevertheless, electron-hole recombination could cause the efficiency to be a little lower.

1. **Role of nZVI nanoparticles in phytoremediation**

For soil repair, there are several remediation techniques available [60]. In comparison to others, phytoremediation uses plants to absorb, break down, sequester, and stabilise a variety of soil contaminants. Both the bioavailability of contaminants and the capacity of plants to absorb them determine how effective phytoremediation is. Pollutants, however, have limited biological availability. An developing technique to address these issues is the combination of nZVI and phytoremediation [61]. Gil-Dıaz and colleagues looked at how nZVI treatment affected As-polluted soil recovery [61].

The application of 10% nZVI, according to the scientists, enhanced the height as well as dry mass of barley under the influence of metal stress while also considerably reducing the Arsenic uptake through plant roots. Nonetheless, the addition of nZVI had also been shown to increase the phytotoxicity of the pollutants. In a similar vein, Cd content in plant cell walls decreased while its concentration in soluble fractions and cell organelles increased in response to nZVI. The key finding is that while high concentrations of nano zero valent iron exacerbated the oxidative damage under the influence of Cd stress, low concentrations of nano zero valent iron mitigated it. These results clarified how modest concentrations of nanoparticles can boost phytoremediation's effectiveness. In order to remediate heavy metal-contaminated locations, this study presents a promising new method that combines the use of nanotechnology and phytoremediation [60].

1. **The effect of nZVI on soil microorganism**

The largest release of engineered nanoparticles into the environment is thought to occur from remediating soil with nZVI . The increased surface area of nZVI is one of its benefits for environmental remediation; nevertheless, because of their high reactivity, they are also toxic to live microbes. Over the past ten years, a growing number of research have been conducted to explain why nZVI is hazardous to soil microorganisms [62]. ZVI affect Escherichia coli in two different ways. One is that more ferrous ions and oxygen are used in the soil as a result of nZVI oxidation [62]. However, the slow release of H2 necessary for microbiological processes caused by the presence of nZVI in soil promoted microbes growth [63]. Humic acids and organic substances are common soil constituents that may help reduce nZVI toxicity [64]. nZVI particles used for in situ cleanup are usually stabilised on the surface; the stabilisers utilised have the dual effect of increasing nZVI mobility and attenuating nZVI toxicity. By strengthening electrostatic repulsions, carboxymethylcellulose (CMC) placed on the surface of nZVI could inhibit direct contact, hence preventing death of cells. The majority of research on the mechanism of microbial toxicity indicates that exposure to nZVI causes the production of Fe2+ and ROS, which in turn causes cell structural disruption or increases member permeability [64], demonstrating the channel for Fe2+ entry into the cell.

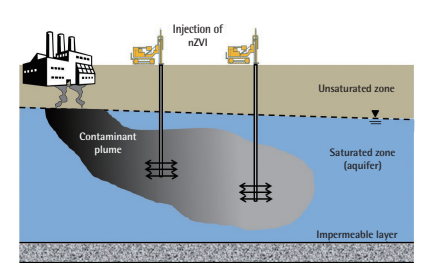
1. **Applications of nZVI**
2. **In removal of heavy metals**

As is well known, soil serves as a significant pollutant reservoir in addition to being a component of the terrestrial ecosystem. Heavy metal-induced soil pollution has become a global issue in recent decades [65]. The primary causes of metal contamination are anthropogenic activities, including mining, manufacturing, irrigation, and the application of sewage from homes or industries [66]. In a similar vein, Gil-Diaz et al. (2016) found that the application of nZVI enhanced the quantity of As in the residual fraction while decreasing the amount in the available fractions [61]. With the exception of As, nZVI effectively immobilised a number of common heavy metals found in soil, such as lead, zinc, selenium, and others [64].

1. **In removal of persistent organic pollutants**

These pollutants pose the risk to the health of humans. ZVI is thought to be a potential technique for POP reductive degradation. Persulfate activated by nZVI totally depleted recalcitrant PAHs including benzoapyrene (BaP) and anthracene (ANT); nevertheless, phenolphthalene (PHE) shown greater degradation resistance.

In addition to PAHs, the nZVI can also reduce the degradation of a number of other persistent organic pollutants, including antibiotics like ibuprofen and TCE, etc. [62].



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Figure 2: Application of nZVI in in situ bioremediation

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