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A Review: Efficacy of Microorganisms in Bioremediation

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Abstract

The world today is facing the problem of environmental pollution. The term “Bioremediation” refers to a biological mechanism of recycling wastes to a form which other organisms can use. To overcome this, microorganisms serve as a potent alternative. Owing to an amazing metabolic activity, microorganisms survive almost all environmental conditions. Microbes can be used in bioremediation of environmental pollutants as they exhibit a vast nutritional capacity. The eradication, degradation, detoxification and

immobilization of various physical and chemical wastes can be done by bioremediation involving microorganisms. The degradation and transformation of pollutants (hydrocarbons, oil, heavy metal, pesticides, dye’s) is carried out enzymatically. The rate of degradation is determined by two factors; biotic and abiotic. Methods such as biostimulation, bioaugmentation, bioventing, biopiles and bioattenuation are common worldwide and as per their specificities have advantages and disadvantages as well.

Keywords: Bioremediation, Microorganisms, Biodegradation, Biostimulation, Bioaugmentation, Bioventing

Introduction

Microorganisms are widely distributed on the biosphere because of their metabolic ability is very impressive and they can easily grow in a wide range of environmental conditions. The nutritional versatility of microorganisms can also be exploited for biodegradation of pollutants. This kind of process is termed as bioremediation. It is continued through based on the ability of certain microorganisms to convert, modify and utilize toxic pollutants in order to obtaining energy and biomass production in the process ^[1].

Instead of simply collecting the pollutant and storing it, bioremediation is a microbiological well organized procedural activity which is applied to break down or transform contaminants to less toxic or non-toxic elemental and compound forms. Bioremediators are biological agents used for bioremediation in order to clean up contaminated sites. Bacteria, Archaea and Fungi are typical prime bioremediators ^[2]. The application of bioremediation as a biotechnological process involving microorganisms for solving and removing dangers of many pollutants through biodegradation from the environment. Bioremediation and biodegradation terms are more interchangeable words. Microorganisms are act as a significant pollutant removal tool in soil, water, and sediments; mostly due to their advantage over other remediation procedural protocols. Microorganisms are restoring the original natural surroundings and preventing further pollution ^[3]. The aim of review to express current trend the application/role of microorganisms on bioremediation and to contribute relevant background which is identified gaps in this thematic area. Presently, it is hot research area because microorganism are eco-friendly and promising valuable genetic material to solve environmental threats.

Factors Affecting Microbial Bioremediation

Bioremediation is involved in degrading, removing, altering, immobilizing, or detoxifying various 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. The efficiency of bioremediation depends on many factors; including, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms ^[4]. The reason for rate of degradation is affected due to bacteria and pollutants do not contact

each other. In addition to this, microbes and pollutants are not uniformly spread in the environment. 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 Factor

Biotic factors are affecting 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 amount of enzymes(s) produced by each cell. The expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation. Furthermore, the extent to contaminant metabolism specific enzymes must be participated and their “affinity” for the contaminant and also the availability of the contaminant is largely needed. 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^[5, 6].

Environmental Factors

The metabolic characteristics of the microorganisms and physicochemical properties of the targeted contaminants determine possible interaction during the process. The actual successful interaction between the two; however, depends on the environmental conditions of the site of the interaction. Microorganism growth and activity are affected by pH, temperature, moisture, soil structure, 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 determine kinetics of degradation^[5, 7]. 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 and terrestrial systems. Moisture influences the rate of contaminant metabolism because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems^[8]. 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. In small concentrations the

extent of hydrocarbon degradation also limit. The addition of an appropriate quantity of nutrients is a favorable strategy for increasing the metabolic activity of microorganisms and thus the biodegradation rate in cold environments^[9, 10].

Biodegradation in aquatic environment is limited by the availability of nutrients^[11]. Similar to the nutritional needs of other organisms, oil-eating microbes also require nutrients for optimal growth and development. These nutrients are available in the natural environment but occur in low quantities^[12].

Temperature

Among the physical factors temperature is the most important one to determining the survival of microorganisms and composition of the hydrocarbons^[13]. In cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum. The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically inactive^[12, 14]. Biological enzymes are 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 need specific temperature. Temperature also speed up or slow down bioremediation process because highly influence microbial physiological properties. The rate of microbial activities increases with temperature, and reaches 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.

Concentration of Oxygen

Different organisms require oxygen others also do not require oxygen based on their requirement facilitate the biodegradation rate in a better way. 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^[12].

Moisture Content

Microorganisms require adequate water to accomplish their growth. The soil moisture content have adverse effect in biodegradation agents.

pH

pH of compound which is acidity, basicity and alkalinity nature of compound, it has its own impact on microbial metabolic activity and also increase and decrease removal process. The measurement of pH in soil could indicate the potential for microbial growth^[15]. Higher or lower pH values showed inferior results; metabolic processes are highly susceptible to even slight changes in pH^[16].

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. This work should at a minimum encompass the following factors: fully determine the horizontal and vertical extent of contamination, list the parameters and locations to be

sample and the rationale for their choice, describe the methods to be used for sample acquisition and analysis to be performed.

Metal ions

Metals are important in small amount for bacteria and fungus, but in high quantity inhibit the metabolic activity of the cells. Metal compounds have direct and indirect impact on rate of degradation.

Toxic Compounds

When in high concentrations of toxic nature of some contaminants, can create toxic effects to microorganisms and slow down decontamination. 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^[5].

Principle of Bioremediation

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities. Microorganisms are suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as a food. The aim of bioremediation is encouraging them to work by supplying optimum levels of nutrients and other chemicals essential for their metabolism in order to degrade/detoxify substances which is hazardous to environment and living things. All metabolic reactions are mediated by enzymes. These belong to the groups of oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases. Many enzymes have a remarkably wide degradation capacity due to their non-specific and specific substrate affinity. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate^[17].

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^[18]. 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.

The Advantage of Bioremediation

- It is a natural process, it takes a little time, as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant and increase in numbers when the contaminant is present. When the contaminant is degraded, the biodegradative population become declines. 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. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
- It is applied in a cost-effective process as it lost less than the other conventional methods (technologies) that are used for clean-up of hazardous waste. Important method for the treatment of oil-contaminated sites^[19].
- 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. Commonly, used on lawns and gardens. Because of bioremediation change harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed^[20].
- Simple, less labor intensive and cheap due to their natural role in the environment.
- Eco-friendly and sustainable^[21].
- Contaminants are destroyed, not simply transferred to different environmental media.
- Nonintrusive, potentially allowing for continued site use.
- Relative ease of implementation^[17].
- Effective way of remediating natural ecosystem from a number contaminates and act as environment friendly options^[22].

The Disadvantage of Bioremediation

- It 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 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.

Bacteria are also efficient in heavy metals bioremediation. Microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various

mechanisms, such as adsorption, uptake, methylation, oxidation and reduction. Microorganism's uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). Microbial methylation plays an important role in heavy metals bioremediation, because methylated compounds are frequently volatile. For example, Mercury, Hg (II) can be biomethylated by a number of different bacterial species *Alcaligenes faecalis*, *Bacillus pumilus*, *Bacillus sp.*, *P. aeruginosa* and *Brevibacterium iodinium* to gaseous methyl mercury [23].

Types of Bioremediation

There are different types of treatment technologies or techniques under bioremediation processes. The basic bioremediation methods are: Bio-stimulation, attenuation, augmentation, venting and piles.

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 focus 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, 17]. The Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. This type of strategic path is most of the time continued in the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the basic requirement for example, energy, cell biomass and enzymes to degrade the pollutant. All of them will need nitrogen, phosphorous and carbon [5].

Bioattenuation (Natural Attenuation)

Bioattenuation or natural attenuation is the eradication of pollutant concentrations from surrounding. It is carried out with in biological processes it maybe includes (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). Terms such as intrinsic remediation or biotransformation are included within the more general natural attenuation definition [24].

When the environment is polluted with chemicals, nature can work in four ways to clean up [25]. 1. Tiny bugs or microbes that live in soil and groundwater use some chemicals for food. When they completely digest the chemicals, they can change them into water and harmless gases. 2. Chemicals can stick or sorb to soil, which holds them in place. This does not clean up the chemicals, but it can keep them from polluting groundwater and leaving the site. 3. As pollution moves through soil and groundwater, it can mix with clean water. This reduces or dilutes the pollution. 4. Some chemicals, like oil and solvents, can evaporate, which means they change from liquids to gases within the soil. If these gases escape to the air at the ground surface, sunlight may destroy them. If the natural attenuation is not quick enough or complete enough, bioremediation will be enhanced by either bio stimulation or bioaugmentation.

Bioaugmentation

It is one of the mechanism of biodegradation. The addition of pollutant degrading microorganisms (natural/exotic/engineered) to augment the biodegradative capacity of indigenous microbial populations on the contaminated area these processes 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. For convince, all essential microorganisms are found in their sites where soil and groundwater are contaminated with chlorinated ethenes, such as in tetra chloroethylene and trichloroethylene. 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 [26].

Bioaugmentation is the process of adding engineered microbes in a system which act as abioremediators in order to quickly and totally eliminate complex pollutants. Moreover, genetically modified microorganisms showing and proving that can increase the degradative efficiency of a wide range of environmental pollutant. Because of having diverse metabolic profile to change into less complex and harmless end products [27-29]. Natural species are not fast enough to break down certain compounds so to facilitate must be genetically modified through DNA manipulation; genetically engineered microbes act as break down pollutants much faster than the natural species and highly compete with the indigenous species, predators and also various abiotic factors. Genetically engineered microorganisms have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a broad coverage of chemical and physical pollutants [30, 31].

Genetically Engineered Microorganisms (GEMs)

Genetically engineered microorganism is a microorganism whose genetic material has been already changed by applying genetic engineering techniques inspired by natural otherwise artificial genetic exchange between microorganisms. These kind of artistic work and a scientific procedure is mainly termed as recombinant DNA technology. Genetic engineering has been improved the utilization and elimination of hazardous unwanted wastes under laboratory conditions by creating genetically modified organisms [32]. Recombinant living organisms currently able to insert the appropriate gene for a production of particular enzyme which can degrade various pollutants [33].

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. Recently, a number of opportunities forward for improving degradative performance using genetic engineering strategies. For example, rate-limiting steps in known metabolic pathways can be genetically manipulated to yield increased degradation rates, or completely new metabolic pathways can be incorporated into bacterial strains for the degradation of previously recalcitrant compounds. In GEMs four activities / strategies to be done these are: (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. Essential genes of bacteria are carried on a single chromosome but genes specifying enzymes required for the catabolism of some of these unusual substrates may be carried on plasmids. Plasmids have been implicated in the catabolism. Therefore, GEMs can be used effectively for biodegradation purpose and leads to represent/indicate a research frontier with broad implications in the future time [34].

Advantage of GEMs in bioremediation: The major function is speed up the recovery of waste polluted sites, increase substrate degradation, displays a high catalytic or utilization capacity with a small amount of cell mass, create safe and purified environmental conditions by decontamination or neutralizing any harmful substances.

Disadvantage of GEMs in bioremediation: The major drawbacks are never carried out in traditional procedure, in some case the death of cells are happened, having challenge associated with their release in the surrounding, In a particular level it showed that delay of growth and substrate degradation, seasonal variation and other abiotic factor fluctuation have direct and indirect impact and relationship on microbial activity; finally, introduced foreign modified strain to the system leads to unreacted and cause unmeasurable adverse effect on the natural structural and functional microorganism's community composition and occurrence.

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 airflow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil. Effective bioremediation of petroleum contaminated soil using bioventing has been proved by many researcher [35, 36].

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