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Microbial Enzyme for Environmental Remediation

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ABSTRACT: This article is about the benefits one can attain from the use of microbes for a cleaner environment. The enzymes that the microbes have in them help in the breakdown process. The closer is the protein chain the stronger is the functioning of the microbe. The microbe feed on the organic substrate or the inorganic substrate. These enzymes are different from the ones found in plants and animals as these enzymes found in microbes are more stable and mobile and can be easily transmitted.
Microbial remediation is the method of use of microorganisms for degrading the toxins that are present. This method can range from the use of chemical method where in the DNA of the microbe is altered so that they can feed on the complex pollutant or by introducing gene code by natural method. The microbes release oxygen, destroying the harmful toxins thus only giving back to the environment. Nowadays they are being used for various purposes in industrial application. The more favourable condition the more does these microorganisms grow freely and increase in number thus processing things at a high rate.
KEYWORDS: Microbes, Enzymes, Genetic engineering, genetically altered, DNA, microorganism, Toxins, alkalinity, Bio attenuation, Bioremediation, Bio-augmentation and pollutants.

I. INTRODUCTION

Quality of life still remains the most disputed issues in the geopolitics scenario. The major objective of human rights was also to provide a quality of life and not which merely amounts to living life like that of an animal existence. The quality of life is intrinsically associated with the quality of environment. With the increasing technological development what has increased is the more indiscriminate use of hazardous substances and materials that adversely impact the environment. (1) The continuous abuse and contamination with pesticides in case of soil and other xenobiotics has come up as major source of pollutants. The origin of these pollutants is endless like that of using cheaper and primitive source of energy like coal and wood for cooking, using of chemical weapons production. These pollutants affect the health of humans, animals. The degrading condition of the environment has led to an ecological disbalance. (2)

Poverty is also a factor that adds on to the use of unclean sources of energy. A recent study conducted by the Blacksmith Institute of New York[1] presented a dirty 30 summary matrix study wherein they highlighted the 30 most polluted countries where the major link for the cause of pollution was found out to be poverty. (3)

Owing to these factors there was a major need to discover effective technologies for the restoration of these polluted sites. One of the easiest and the most cost-effective way of restoring the quality and removing the potential pollutants from the soil and the hydrosphere is the use of enzymes. The distinguishing factor that has been recognised in the bioagents is their ability to transform the harmful pollutants and can restore the original balance. Bioremediation is the use of microorganisms in the restoration or the transformation of the contaminants into non-hazardous substance. This mainly involves use of bacteria, algae, fungi. Even plants are used in this process and this technology has emerged in the restoration of the groundwater quality and the removal of toxic chemicals from soil. (4)

Different ways have been implanted for the restoration of these polluted sites but the two most commonly used one are: engineering and biological process. The first one is mostly founded on physical and chemical methods while the latter focuses on the use of bioagents.

Several methodologies have been applied for the remediation of polluted systems and many of them, when implemented in the target sites, have led to successful results. Two basic strategies have been utilized: engineering and biological ones. (5) Engineering strategies are basically founded on physical and chemical methods, whereas biological

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strategies require the involvement of biological agents (6)

USE OF ENZYMES FOR DEGRADATION MECHANSIM

Enzymes are nothing but large biomolecules responsible for a series of chemical reactions. These are protein molecules and act as biological catalysts. They extract energy from the energy yielding biochemical reactions, to assist the transfer of electrons from a reduced organic donor to another chemical compound. (7) They are often used in the process of bioremediation and are considered as bio remediators.

The International Union of Biochemistry and Molecular Biology has framed a nomenclature for enzymes, the EC numbers; that is each enzyme is described by four number preceded by EC which stands for "Enzyme Commission". In the present system the enzymes use the name of the substrate or the type of the reaction and is ended with “ase” example. Maltose. The use of enzyme mostly pertains to the acceleration of the reaction. (8)

The enzymes are mostly a chain of amino acids. The functioning of an enzyme depends mostly on the way the amino acids are concentrated and arranged. When this arrangement is weak, they may be broken by adverse environmental or external conditions like acidity,alkalinity, salinity and temperature. Once broken these enzymes become non-functional.

Most enzyme contain a protein and a non-protein part known as the cofactor. The protein part of the enzymes isknown 'apoenzyme', while the non-protein part is known as the cofactor. Together the apoenzyme and cofactors are known as the 'holoenzyme’. (9)

They are three dimensional in structure and they use both organic and inorganic compounds thus assisting the process. The structure of the enzyme can further be divided – the one that is continuously responsible for the catalytic action is the active site. (10)

One of the major sources of enzymes are microorganisms. They remain a better source than enzymes from plants and animals because of their mobility and also because of their stable nature. It is easier to mutate and manipulate the genes of the bacterial cells thus aiding the process of enzyme production. They can also be cultured in a large number in a short time. (11)

Usually most of these microorganisms find in difficult to adapt under harsh circumstances and would only be effective where environmental factors favoured but after several stages of mutation these microorganisms can now function under extreme conditions such as temperature, alkalinity, salinity, ph. he microorganisms are effective because they can tolerate higher concentration of pollutants. Their growth also increases with the use of soil pollutant. (12)

The process of microbial remediation uses multiple methods for enzymes production. This mostly depends upon the site. Enzymes mostly work as catalysts. The most beneficial use of these enzymes is that they can either narrow up or broaden their specificity and can be applied to varied compounds in a mixture. The use of enzymatic protein remains a good alternative to overcome the disadvantages related to the use of microorganisms. (13)

The use of enzyme always remains a better alternative than the use of traditional method for bioremediation. The best thing about enzymes is that they can used under adverse condition and their functioning is not inhibited by the usual microbial metabolic inhibitors. (14)

They act efficiently at low pollutant concentrations and as well as work under the presence of predators. At a later stage the enzymes develop the capability to remediate the compounds that add to the toxicity in the environment. The sustainability of the working of enzyme remains an established concept and needs no questioning.

The recent example demonstrated by of the structure and methods of immobilization of biomolecule named lactase has been found to be beneficial for the decontamination of pollutants. (15)

ENZYMES FOR ORGANIC SUBSTRATE:

Oxidoreductases – These helps catalyse the oxidation- reduction reactions. These microbes take energy from energy giving biochemical reactions and help in the transfer of electrons from donor to acceptor in the reaction by utilizing FAD/NADH/NADPH. They play a major role when it comes to increasing the compound’s reactivity with water (16)

During this reaction the containments are oxidised to less toxic compounds. This process is mostly used by bacteria, fungi in the detoxification of compounds in soil. They participate in the modification of various xenobiotics present in the soil. A lot of them help in the reduction of the radioactive material present in soluble form to an insoluble form. This process produces lots of energy and heat. The genus of Fabaceae, gramine belong to this family which take part in the oxidative degradation of soil containments. (17)

Hydrolases – These enzymes are used for the remediation of aquatic ecosystem. With the

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increase of industries, the damage to river bodies has also increased. Hydrolases also known as hydrolytic enzymes help in disrupting the chemical bond and lower the toxicity of these bonds (18)

They are useful in anaerobic environment and actively participate in the process of alcoholysis. The different enzymes like analyses, lipases is being used in various industries such as biomedical science and chemical industries.They catalyze the hydrolysis reactions of carbohydrates, proteins and esters. (19)

FACTORS AFFECTING BIOREMEDIATION:

Microorganisms are catalyst that help in detoxing, decomposing and breaking down the waste be it chemical or physical. These microbes help in the process with the help of enzymes that act as catalyst and pave path for the initiation of these processes and degrade the desired pollutant. But this breakdown process can only happen when these microbes have access to the nutrients and the favourable condition for the growth (20).

A lot of microorganisms become non-functional if the pollutant is too toxic or the conditions are too unfavourable. The rate of biodegradation depends upon the capacity of these organisms to build more and more cells. The degree of efficiency is depended upon the following factors categorising them into chemical, environmental and biological. The chemical factor depends upon the type of pollutants. (21)

The addition of pollutant plays a major role as these microbes feed on the chemical. But not all types of microbes can feed on all type of pollutant. If a particular site, there exist which do not feed on that type of pollutant they have to engineered genetically so that they can feed on those pollutant and release nitrogen, carbon thus helping in the multiplication of the microbes. The rate of biodegradation also gets affected when the bacteria and the pollutant are not in contact with each other. Thus, if there is absence of a microbial population that is capable of degrading the pollutants that might act as a hindrance in the process. (22)

The surrounding environment plays a huge role and adds on to being a factor that can either be a favourable situation or an unfavourable situation. These may range from the temperature, the alkalinity, salinity of the soil. These factors mostly affect the metabolic feature of the microbes and the physiochemical nature. For the successful functioning a favourable relation with these factors is something that is much needed. These factors have been discussed in detail below: (23)

1. Ph- Ph of a compound determines a lot of properties about that depend ranging from their alkalinity,basicity, acidity. The ph. of a compound has a major influence on the microbial metabolic process/ the optimum ph. is said to range from 6.5 to 8.5 for bioremediation in aquatic or territorial systems. The moisture factor is a major determinant in affecting the rate of metabolism because it is the moisture which determines the what material can be made soluble as the osmotic pressure. If the Ph is too high or too low it might not give the desired result and might disrupt thegrowth. (24)
2. Availability of nutrients – To make sure the microbes act as catalysts it is important that they get the right amount of nutrient. The amount of nutrient help in the multiplication of these microbes especially the supply of nitrogen and phosphorus thus creating the optimal carbon – nitrogen- phosphorus ratio. If these nutrients are present in small quantity the extent of hydrocarbon breakdown also gets limited. This is the reason why in a cold environment it is difficult for the microbes to work as efficiently, also in aquatic environment because of the lack of nutrient the efficiency of the microbes gets limited. (25)
3. Temperature - For the composition of hydrocarbon, temperature remains the most important constituent. It can be seen in cold environment where oil spill occurs these microbes' cells shut down their functioning due to extremely low temperature which freezes the cytoplasm, which makes these microbes inactive. The slow decomposition by the natural phenomenon isn't an alternative. The pressure on these microbes at such low temperature might not give as good results as that in normal condition. Temperature also influences their metabolic activities and thus altering their intrinsic functions (26)
4. Concentration of oxygen – not all organisms require oxygen in the same quantity, as oxygen is mostly a compound these microbes release in the breakdown process the amount of oxygen required mainly depends whether the degradation is being carried out in the aerobic or anaerobic condition. Oxygen aids in the metabolism (27)

Coming on to the last factor that affects the growth and that is the biological factor. These factors get important when there has to be a degradation of an organic compound by creating a competition between the microbes for the limited amount of carbon that is there or by the creation of

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threat by the protozoa and the bacteriophages. The amount of catalyst present is also a major factor when it comes on determining whether the rate of metabolism gets increased or decreased. The extent of their capability to breakdown these contaminants also depend upon the affinity these microbes have towards the contaminant. Thus, various factors like: population size, mutation, composition play an essential role. (28)

Bioremediation (Aim And Techniques)

Bioremediation is the method by which organic wastes are treated from being harmful toxic substance to their non- toxic state with the help of microorganism. These microorganisms have enzymes that feed on these organic wastes and turn them as food. Thus, bioremediation aims at improving the condition of the environment with the help of these microorganism. Increase in microorganisms would increase the process of detoxification by supplying optimum nutrients. There are different techniques that have used for this process. (29)

When it comes to highly toxic heavy metals these microorganisms are highly effective as they breakdown these heavy metals from oxidised form to complex form. They have developed themselves in such a way that they are protect themselves against the methylation, reduction from these heavy metals. There are different techniques that are used. Some rapidly used one are termed below (30)

1) Biostimulation- To initiate the activity of the microorganisms, what is done in this strategy is that these microorganisms are chained by injecting various essential nutrients in the soil and water. The focus is to provide the microbes with the basic essentialities that are required for their functioning namely,nitrogen, oxygen and phosphorus. The main attention is towards the activation of the naturally existing bacteria in the soil and groundwater. This activation is done by various methods: Firstly, when it comes to injection in soil it can be done by supplying fertilisers, important minerals and supplements, secondly by adding a bit of pollutant so that the microbes can breakdown these pollutants into oxygen and improve the functioning. (31)
2) Bioattenuation(Natural Attenuation) – It is the using of natural biological phenomenon to get rid of the pollutant concentrations in the surrounding. These processes can range from physical reaction (namelyabsorption) to the chemical reaction. It may include the use of aerobic, anaerobic and plant use. The environment uses its own intrinsic method to cleanse its system. In an environment full of pollutants, the microbes and the bacteria that are present in it feed on these toxins as a source of food and convert these pollutants thus releasing oxygen and other harmless gases. They also convert it into water. The chemicals after the first stage do not get completely absorbed, they stick to the soil thus not getting mixed with the groundwater and transporting to other area. There are certain pollutants which after the first stage can change their forms and these toxins evaporate from the soil and water, in that case these gases escape to the air surface at the ground level and sunlight destroys them. (32)
3) Bioaugementation – The addition of microorganisms and these microorganisms can be genetically modified to increase the biodegerative quality of the natural microbial population at the polluted site. This usually happens where there are complex pollutants, the microbes from those remediation sites are collected, separated, cultured then are modified are finally put back in the site. (33)

They are bioremediators and functions in increasing the degradation of a wide range of pollutants. Because of this diverse metabolic profile, they tend to break these pollutants into harmless end products. The natural specifies are not efficient enough to break down these pollutants thus their microbes need to be genetically altered using the mode of DNA manipulation, these altered microbes then function than the indigenous species. (34)

4) Immobilised Microbial enzyme- Generally the impediment that comes in the process of the proper functioning of the enzyme is their stability in the highly toxic environmental condition or the normal environmental condition that these microbes are not suitable to function in. Thus, in this process the enzymes are immobilised so that they can sustain the unfavourable conditions. They are being used a lot these days for ex – the conversion of horseradish peroxidase (HRP) onto calcium-alginate beads. This was done with the help of the crosslinking catalyst – glutaraldehyde to breakdown the synthetic dyes. The other example can be the immobilisation of laccasse enzyeme purified from fungal Pleurotus nebrodensis for the treatment of industrial wastewater. Anthracene concentration is said to aid the immobilisation of the laccasse. In case of reactive dyes, the immobilised enzymes are maintained at 85% and after several cycles with a decolourisation

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efficiency at about 85% to 90%. Laccasse used to immobilise metal organic structure having peroxidase magnetic functions helped further in the breakdown of methylene blue and crystal violet dyes. The using of method of absorption in the immobilisation of HRP onto Polyamide 6 electrospun fibre (HRP-PA6) has been used to treat Malachite Green dye and RB5 dye with a 70 % efficiency.

5) Genetic Engineering Approach – This method is also known as recombinant DNA Technology. The varied need of the environmental condition has led to the modification in the DNA of these microbes and cloning so that the complex pollutants can be broken-down easily. This can be done either through recombining DNA of these organisms or by natural genetic exchange. Essential enzymes Their use can be wide ranged – from treating soil to groundwater, industrial use and others. They can used for biodegradation process. The process can be executed in four steps namely: by modifying the enzyme until a specificity is attained 2) regulation 3) the development, controlling and the monitoring of the growth 4) using the bio affinity reporter for noting whether they can used for further analysis – like toxicity reduction. (35)

Because of their increased use in industries the use of enzymes in environmental application is restricted. Researchers have brought new study to increase their use in industrial application, this is by way of using engineered microbes to increase the yield of enzyme production. This can be done in two methods: the trial to take the natural form of gene in vitro and the introduction of such gene into the host cell. Mostly in such cases laccasse enzymes is used. This enzyme is mostly a gene from Fusarium oxysporum cDNA and is used for cloning. The other laccasse gene named as FoLacc5 related to 99 percent cloned laccasse gene. Research on other enzyems like that of cloned yacK gene form E. coli K12 is induced in CueO host. Indigo carmine dye is used in the detoxification and the change of Ganoderma. In several cases the pichia pistoris when modified with the manganese peroxidise is used to breakdown the red dye. The advantages of using this method is needless. Some of the important one being:

- They help in increasing the speed of recovery of the polluted land as they help in biodegradation and they act as catalyst and help in the breakdown of harmful substances and neutralising toxins and creating a purified environment

II. CONCLUSION

The most eco-friendly and the cheapest method present for the remediation of the environment is the use of microorganisms. The best part about relying on this method is that they further do not lead to any toxic side effects as they breakdown the chemicals and other toxins to release gases or products that are not toxins and are not harmful for the environment. These microbes feed on the toxins and with the help of the enzymes digest the chemicals. These can function in situ and ex situ. In case of in situ treatment the metabolic function of the bacteria is improved with the introduction of a new gene code by the recombination of the DNA. However, the performance of bioremediation may be affected by a lot of factors namely – the degree of the toxicity of the compound, the nature of the pollutant. If it is a heavy complex pollutant then the process may be staggered also depends upon the microbiology of the place for where it is needed and also the limitation that comes with the groundwater or the soil and also whether there are enough electron receptors or not.

Several methodologies have been applied for the remediation of polluted systems and many of them, when implemented in the target sites, have led to successful results. Two basic strategies have been utilized: engineering and biological ones Engineering strategies are basically founded on physical and chemical methods, whereas biological strategies require the involvement of biological agents

A great amount of work depends upon the understanding of the biology of these microorganisms and require a lot of understanding. The TCP Discovering TCP-degrading enzymes and chlorpyrifos degrading enzymes in the cow rumen microbiome is an instance for this claim. With the release of such pollutants, it also important to understand the various needs that the microbes also need that influences the rate at which these organisms would interact with the environment.

These factors get important when there has to be a degradation of an organic compound by creating a competition between the microbes for the limited amount of carbon that is there or by the creation of threat by the protozoa and the bacteriophages. The amount of catalyst present is also a major factor when it comes on determining whether the rate of metabolism gets increased or decreased. The extent of their capability to breakdown these contaminants also depend upon the affinity these microbes have towards the contaminant. Thus, various factors like: population size, mutation, composition play an essential role.

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