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Application of Indigenous Biofilm Forming Bacterial Strain in Bioremediation of Heavy Metals

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ABSTRACT: Contamination of the environment by heavy metals has posed as a serious risk to not only the environment but is also a serious health risk to humans because of their long-term effects and involvement in the food chains. These heavy metals interfere with the cellular function of microorganisms, plants, animals and humans leaving all the living organisms at a health risk. To solve the problem of heavy metal pollution several methods are being implemented but the most convenient method is bioremediation where the microorganisms are being used for the transformation of heavy metals into lesser toxic forms. The major drawbacks faced during the bioremediation are the slower rate and inability to control the process in natural environment, but due to the growing concern of the world towards the problem of heavy metal pollution, the removal of heavy metals by bioremediation is considered as the long term effective and the logical method by being less expensive and effective when compared to other methods. Presence of different heavy metals in a single environment can be combated by genetic manipulation of microorganism and making them compatible for the remediation of different heavy metals by a single strain of microorganism. Based on the site of implementation of the bioremediation can be classified into two types in-situ bioremediation and ex-situ bioremediation. Microorganism have different mechanisms by which they can either recover heavy metals from the nearby surrounding or utilize them in their cellular functions or convert them into other forms which are non-toxic to any organisms.

Keywords: Biofilm, Bioremediation, Heavy metals, Pollution, Toxicity

INTRODUCTION

Recent advancements in technology and industrialization have increased the burden of the environment by releasing pollutants, harmful toxic wastes, heavy metals etc., which have been causing serious damages to the environment. Heavy metal pollution is one of the types of pollution which has received less attention from the humans but has posed a serious threat because of the hazardous impacts even in small concentrations. Heavy metal pollution occurs mainly form industries such as mining, tannery, dyeing, electroplating; sewage and waste water treatment plants. Waste water released from electroplating industry is composed of heavy metals such as Nickel and Chromium and their levels have even surpassed the permissible limits. Wastes from tannery industry contains Copper, Chromium, Iron, Manganese and Zinc. Various researches have shown that the water released from these industries into water bodies have heavy metal concentration beyond permissible limit of drinking or irrigation purposes making it unfit for utilization by humans. Using water which contains heavy metals for the irrigation in cultivable land have resulted in bioaccumulation of heavy metal in the plants, their products and food chains associated with them (Hullebusch 2017).

The Comprehensive Environment Response Compensation and Liability Act (CERCLA) of USA has stated the maximum tolerable heavy metal concentration as 0.01, 0.05, 0.002, 0.001 and 0.015 for Arsenic, Cadmium, Mercury, Silver, Chromium and Lead respectively (Tchounwou *et al.,* 2012). The Indian Standards for Heavy Metals has established soil standard metal concentration as 300-600, 250-500, 75-250, 135-270, 3-6 mg/kg for Zinc, Lead, Nickel, Copper and Cadmium respectively. Indirect heavy metal pollution is caused by contaminated surface of soils or rain water and ground water (Leah *et al.,* 2015). Rivers are the largest source for fresh water and even they are contaminated by pollutants (Kanan *et al.,* 2006). The exposure chances to these heavy metals is increasing consistently with the expanding use of technology in various industrial, domestic and agricultural sectors. The effects of heavy metals largely depend on their chemical nature like inorganic arsenic is easily absorbed by the cells and there they interfere with the cellular reactions to a larger extent as compared to their organic forms because of the poorer cellular absorption in organic forms. Heavy metals attach themselves to the binding sites of proteins thus removing the original metal leading to toxicity and cellular malfunction.

Different exposure rates have been seen in case of heavy metals including dermal, inhalation and ingestion (Cooper *et al.,* 2009, Bissen *et al.,* 2003).

There have been several specially designed processes for the removal of heavy metals from water bodies and soils, which are classified into three categories 1) Physical (Super critical fluid extraction, Electrodialysis, Filtration and Precipitation), 2) Chemical (Ion exchange, Electrochemical Technique) and 3) Biological (Bioremediation). The application of these treatment techniques depends upon nature of the Heavy metal. The physical and chemical methods both have certain advantages and disadvantages. The application of the physical and chemical methods is limited on smaller scale and requires artificial conditions where the contaminated soil or water is transferred to a facility and then they are treated for the removal of heavy metals. Apart from these limitations physical and chemical methods are said to produce by-products which are further processed, making these methods time consuming and requiring extra labour, further leading to extra costs. The biological method of heavy metal involves the usage of microorganisms to treat the heavy metals under natural conditions or artificial conditions. The biological method of heavy metal remediation is more beneficial when compared with the other two methods because of the utilization of the natural components to remove the heavy metals as they do not produce any waste by-product, more efficiency, less involvement of human, and its application in both natural environment and artificial conditions (Shem *et al.,* 1993). Bioremediation process uses either naturally present or intentionally added microorganisms to absorb and degrade the target contaminants and clean a polluted area. Bioremediation methods may involve bacteria, fungi or plants depending upon the region of pollution and the type of contaminant. Various number of studies have shown using algae, bacteria and fungi as bio absorbents (Allard *et al.,* 1997, Segun *et al.,* 2017).

A. Sources of Heavy Metal Pollutants in the Environment

Plants do not take up the naturally occurring heavy metals as they exist in insoluble forms like minerals, precipitation or complex structures. The greater adsorption capacity of naturally occurring heavy metals makes them unavailable for uptake by the plants. Natural processes like volcanic eruptions, meteorites, erosions, heavy metals weathering are the reasons for the presence of heavy metals in the environment. Anthropogenic sources include battery manufacturing, alloy production, atmospheric deposition, biosolids, manufacturing of explosives, coating, improper piling of solid industrial wastes, pesticides, fertilizers, leather tanning, irrigation by sewage water, smelting, electroplating industries, dyes, steel industries, textiles and wood preservation (Cvjetko *et al.,* 2010, Wuana *et al.,* 2011).

Accumulation of heavy metals in the food web is influenced by factors like sources of pollutants, concentration of metals in soil and its properties, degree of uptake by the plants and degree of absorption by the animals. The Geochemical cycle of heavy metals results in the build-up of heavy metals in the environment risking all the organisms due to their presence above the levels permitted. Routes of heavy metal introduction into the environment includes parent material weathering, geochemical cycle alteration by humans, ingestion of soil, transfer of heavy metals from parent location to various location, high concentration of heavy metal discharge from industries into the ecosystem (Gadd *et al.,* 2010, D' amore *et al.,* 2019).

Mining and Ore processing have adversely affected the environment by destructing and altering the ecosystem causing biodiversity loss and pollutants aggregation in the environment. The recovery of ecosystem after mining and other processes could take time up to several decades and the large quantities of dump produced are often left abandoned without properly treating the waste. These abandoned sites contaminate the soil and water bodies through the accumulation of particles and thus creating a need to treat these areas before they are discharged into the environment (Andersson *et al.,* 2019). Environmental, Anthropological Sources, Exposure Routes, Effects, Mechanism of Toxicity of Heavy Metals are tabulated in Table 1.

B. Toxic Effects of Heavy Metals

Physiological adaptations of organisms and their nutritional status, tropical interactions have a major role in the toxicity. When heavy metals get absorbed into the body they are distributed among the various tissues in the body and they tend to persist in bones, Kidneys and Liver for long time and affects the cellular functions and cellular organelles (Jaishankar *et al.,* 2014). Toxicity of heavy metals increases as the environment becomes more acidic and deficient in nutrients. Heavy metals at acidic pH form free radicals because extra protons are present for the saturation of binding sites of metals. The surface of the adsorbent is charged positively which decreases the attraction capacity between the adsorbent and metal cations. This increases the bioavailability of heavy metals and thus increasing the toxicity towards plants and microorganisms. Metal ions at higher pH (basic) replaces the protons and forms various species complexes like hydroxo-metal complexes. Small changes in pH can be used to control the bioavailability and solubility of heavy metals (Olanirin *et al.,* 2013). In soil containing low quantity of organic matter, contamination by heavy metals is higher.

Table 1: Environmental, Anthropological Sources, Exposure Routes, Effects, Mechanism of Toxicity of Heavy Metals.

Toxic Heavy Metals	Environmental Source	Anthropogenic Source	Exposure Routes	Effects	Mechanism of Toxicity	References
As	Released from volcanoes	Combustion of coal, pesticides, wood preservatives	Ingestion through food, Inhalation of contaminated dust	Carcinogenic, cardiovascular disorders, diabetes, neurobehavioral disorders	Biomethylation of As leading to As(III) formation which is carcinogen, As(V) substitutes phosphates leading to failed DNA repair, cellular inhibition	Tchounwou *et al.*, 2012
Be	Volcanic dust	Coal combustion, oil combustion	Contaminated air, ingestion of contaminated food, water, direct contact with alloys, industries	Allergies, heart diseases, lung disease, cancer, chromosomal abbreviation, mutation	Inhaled or ingested particles get deposited in lungs and other organs and are dissolved in blood stream	Cooper *et al.*, 2009
Cd	Earths crust, water, leafy vegetables.	Battery production, fertilizers, pesticides, welding, fossil fuels combustion	Contaminated food ingestion, smoking, occupational exposure	Lung and stomach cancer, renal injury, chromosomal damage, multi-organ failure, osteoporosis	DNA damage leading to inhibition of protein and nucleic acid synthesis, repair mechanisms blocked, nephrotoxicity	Jaishankar *et al.*, 2014
Cu	Excessive quantities of cu in any environment leads to toxicity	Polishing, mines, paints, electroplating industry, printing machineries	Acidic foods, cooking food in uncoated copper utensil, ingestion of water containing excessive copper	Abdominal pain, vomiting, diarrhoea, liver and kidney failure, metabolic disorders	Overloading of homeostasis system with copper ions, excessive copper ions replace the iron cofactor in Iron-Sulphur cluster proteins	Jaishankar *et al.*, 2014
Ni	Dust from weathering of soil and rocks, forest fires, volcano ashes	Electroplating industries, burning of fossil fuels, porcelain enamelling	Dust inhalation, oral ingestion of contaminated food and water	Cardiovascular diseases, dermatitis, cancer lung and nasal cancer	Reduction of mitochondrial DNA which eventually leads to destruction of mitochondrial DNA	Singh *et al.*, 2011
Pb	Earth's crust	Batteries, soldered metal, X-Ray shields, paints, glass manufacturing, fossil fuel combustion, ammunition	Contaminated food and water ingestion, inhaling lead dust	System toxicity, anemia, multi-organ failure	Mimics Ca inhibiting metabolism and Ca cycling in body, interrupts tumor suppressor proteins	Jaishankar *et al.*, 2014
Sb	Soil erosion, volcanic eruption	Coal combustion, smelting, mining	Contaminated water, acidic fruit juices containing antimony oxide dissolved from the glaze of containers	Inhibits enzyme activities, cancer, cardiovascular, liver and respiratory diseases Reduced growth rate and inhibition of chlorophyll synthesis in plants	Antimony binds to the sulfhydryl groups and increases oxidative stress leading to the inactivation of various key enzymes	Cooper *et al.*, 2009
Se	Water bodies, soil drainage	Coal combustion, metal smelting and refining industries, mines	Inhaling dust containing selenium, consumption of foods irrigated with water containing selenium	Endocrine system dysfunction, liver damage, natural killer cells activity impairment	Induces oxidative stress and forms malformed selenoproteins	Xu *et al.*, 2020
Cr	Present in all environment	Processing metal, dyeing industry,	Ingestion of contaminated	Dermatitis, kidney damage, asthma,	Chromosomal aberrations, DNA strand break	Tchounwou *et al.*, 2012

	segments in lower concentrations	metallurgy, wood preservation	food and water, dermal	allergies, respiratory tract cancer		
Tl	Present in terrestrial elements in low concentration	Fossil fuel combustion, cement factories, oil refineries and metal smelting	Direct skin contact, ingestion of contaminated foods or inhalation	Burning feet syndrome, coma, convulsions, ataxia, hallucination, gastroenteritis, affect homeostasis	Impairs glutathione metabolism, oxidative stress and disruption of potassium regulated homeostasis	Cvjetko *et al.,* 2010
Hg	Present in water, soil and air	Electrical industries, dentistry, mining, paints	Dermal, ingestion or inhalation	Crosses blood-brain and placental barrier, accumulation kidneys, liver. Neurotoxicity, gastrointestinal toxicity, nephrotoxicity	Forms covalent bonds with proteins and disables antioxidants.	Sharma *et al.,* 2018

Organic matter strongly influences the capacity of cation exchange, capacity of buffer and also holds the heavy metals, because of this the metals present in the organic soil along with a combination of heavy metals show lower mobility and lower bioavailability towards the plants and microorganism, than the metals available in the mineral soil (Olaniran *et al.,* 2013, Jaishankar *et al.,* 2014, Haimi 2003).

A list of bacteria with an ability to bioremediate heavy metals by forming biofilm is given in Table 2. Temperature also has an important role in adsorption capacity of heavy metals. Increase in temperature results in increased adsorbate diffusion rate across the external boundary layer and internal pores of adsorbate particles because of the decrease in the liquid viscosity as the temperature increases. The stability of metal ion species is also affected by the changes in temperature.

Table 2: Bacteria able to Perform Bioremediation of Heavy Metals by Forming Biofilm.

Heavy metal	Microorganism	References
Arsenic	*Pseudomonas flourescens (AK1)*	Prithvirajsingh *et al.*, 2001
	Pseudomonas aeruginosa (AK2)	Tariq *et al.*, 2019
Beryllium	*Marine Pseudomonas*	Rajendra *et al.*, 1999
Mercury	*Alcaligenes faecalis*	Gupta *et al.*, 2015
	Bacillus pumilus	Mahler *et al.*, 1986
	Bacillus cereus	Kannan *et al.*, 2006
	Pseudomonas aeruginosa	Babiker *et al.*, 2020
Cadmium	*Bacillus licheniformis*	Syed *et al.*, 2015
	Bacillus laterosporu	Rajeswari *et al.*, 2013
Copper	*Acidithiobacillus*	Samal *et al.*, 2013
	Cupriavidus metallidurans	Luis *et al.*, 2011
Nickel	*Escherichia coli (AS21)*	Gupta *et al.*, 2015
	Escherichia coli (AS17b)	Gupta *et al.*, 2015
	Mycobacterium chlorophenolicum (AS33)	Babiker *et al.*, 2020
Lead	*Bacillus cereus (RPb5-3)*	Natarajan *et al.*, 2008
Antimony	*Agrobacterium tumefaciens A5 strain*	Li *et al.*, 2016
Chromium	*Ochrobactrum anthropi*	Tandon *et al.*, 2020
	Cellulomonas marina ES6 and WS01 strain	Focardi *et al.*, 2013
	Lysinibacillus sphaericus	Huang *et al.*, 2016
	Microbacterium oleivorans	Sarkar *et al.*, 2016
Thallium	*Ferroplasma acidiphilum*	Zhang *et al.*, 2014
	Leptospirillum ferrooxidans	Liu *et al.*, 2019
	Metallibacterium schefflera	Azibuike *et al.*, 2016
	Acidithiobacillus thiooxidans	Azibuike *et al.*, 2016
	Ferrovum myxofacien	Azibuike *et al.*, 2016
	Sulfuriferula plumbiphila	Eswayah *et al.*, 2016
Selenium	*Shewanella oneidensis*	Eswayah *et al.*, 2016
	Pseudomonas stutzeri	Tomei *et al.*, 1994
	Desulfovibrio desulphuricans,	Li *et al.*, 2014
	Rhodopseudomonas palustris	Li *et al.*, 2014
	Azospirillum brasilense	Tugarova *et al.*, 2014

Factors like cell wall configuration, sites of biosorption and chemical moieties ionization influences the stability of the microorganisms. In a study conducted it was observed that the sorption capacity of lead increased from 0.596 to 0.728 mg/g, when the temperature was increased from 25 to 45°C.

Metal toxicity disrupts the structure and functions of enzyme by the binding of thiol and protein group, or by the replacement of cofactors in prosthetic group of enzymes. A common case of metal toxicity can be seen in case of Lead and Mercury exposure, which causes the development of autoimmunity in humans causing diseases like Rheumatoid Arthritis, various circulatory system disorders, nervous system disorders, Kidney diseases and damages the foetal brain in humans. Being exposed to lead and mercury in children leads to reduced intelligence, increased cardiovascular disease risk and impaired development (Babiker *et al.,* 2020). Cadmium which is a carcinogenic and mutagenic metal disrupts the endocrine system, damages fragile bones, lungs, and influences regulation of calcium in the biological systems. Chromium toxicity leads to loss of hairs, diarrhoea, vomiting, nausea and headaches (Mahler *et al.,* 1986).

The heavy metal build-up in plants disrupts several metabolic functions involving physiological processes and biochemical processes, destruction of cellular organelles lesions, chlorosis, delay in germination, inhibition of respiration and photosynthesis, disruption of enzymatic activities, oxidation stress, premature falling of leaves, reduction in biomasses and crop yields, stunted growth, senescence eventually leading to the death of the plants.

In microorganism effects of heavy metal toxicity affects size of population, diversity, activity and also disrupts genetic structure. Heavy metals affect the morphological structure, cellular metabolism and growth by interfering and altering the nucleic acid structure which leads to cellular membrane disruption, causing disturbance in functions of cells, enzymatic activity inhibition, inhibition of oxidative phosphorylation leading to lipid peroxidation, alteration in osmotic balance and denaturation of proteins.

C. Comparison Between the Physical-Chemical and Biological Methods

The physical and chemical methods of remediation of Heavy metals includes ion exchange, chemical precipitation, reverse osmosis, oxidation and reduction, ultra-filtration, adsorption and electrodialysis, but these methods have limitations such as – large quantity of sludge is generated, operating conditions of these methods are very sensitive and small variation affects their ability, lower efficiency and expensiveness. Although there are many techniques available for the treatment of heavy metals, but they do not meet required standards of an ideal treatment which should be acceptable, suitable and applicable to the local environment where the treatment is required and should also meet the established standards of Maximum Contaminant Level (MCL). The benefits of physico-chemical treatments over the Biological methods are the ability to accommodate variable input loads with seasonal flows and discharge system and the treatment plants can be modified as per the requirement, but these benefits are outweighed because of a number of drawbacks faced such as the higher costs of operation, consumption of energy, sludge maintenance costs, treatment plant installation costs (Barakat 2010). Biological methods can be applied to all the components of the environment – air, water and soil and involves not only the use of microorganisms but also uses algae, fungi and plants. The biological methods can be both active (energy dependent) and passive (non-energy dependent). Microorganisms can be directly introduced into the polluted site or an artificially designed bioreactor where the microorganisms are cultured under artificially controlled conditions to treat the heavy metals. The biological methods used for the treatment includes biotransformation, bioleaching, phytoremediation (growing plants able to remediate heavy metals in contaminated areas), biomineralization, biosorption etc. The biological methods are not as limited as the physico-chemical methods and the end products after the treatment of the heavy metals does not require much human attention as they are either utilized completely or converted to other non-toxic forms which can reside in the environment without any harmful effect to the ecosystem.

D. Quorum Sensing and Biofilm Formation

Microorganism have been known to perform a high degree of coordinated multicellular behaviour for the formation of biofilm. Biofilm is an aggregate of microorganisms where the microorganism's cells are adhered to a surface or to each other. These cells are lodged in a self-made matrix made up of a substance known as Extracellular Polymeric Substance (EPS). EPS contains larger quantities of polysaccharides and lesser quantities of proteins and DNA forming a matrix where the cells of bacteria get lodged. Bacteria living in the same community without any physical contact secrete smaller amounts of extra cellular molecules which interacts with each other. Bacteria are capable of regulating various physiological processes and activities in a group by a procedure known as Quorum Sensing, according to which the bacterial cells are capable of producing, detecting and responding to minute diffusible signal molecules. The cell-cell communications by Quorum Sensing have shown important involvement in several microbial infections. The various characteristics controlled by quorum sensing includes 1) attachment to the surface, 2) production of extracellular polymer, 3) synthesis of biosurfactant, 4) competence, 5) sporulation, 6) bioluminescence 7) virulence factor secretion. Quorum sensing functioning involves secreting and detecting the autoinducer molecules which piles up inside the cell. The EPS is advantageous to the secreting strains of bacteria as they push the descendant bacterial cells to the areas with higher nutrient availability and suffocates remaining other neighbouring cells are incapable of producing EPS. Different bacterial cells behave differently to the cell density threshold limit. *Pseudomonas aeruginosa* activates EPS production at higher cellular density whereas *Vibrio cholerae* terminates the secretion of EPS after reaching the higher cell density quorum sensing threshold (Lukasz *et al.*, 2013).

The Acyl-Homoserine Lactone (acyl-HSL) system in Gram-negative bacteria and peptide based signalling system in Gram-positive bacteria are said to be the most in detail described quorum sensing systems in bacteria. Apart from these two systems there is AI-2 system whose study has been limited and is seen to be present in both gram-positive bacteria and gram-negative bacteria. Acyl HSL system involves a single enzyme for the signal synthesis from cellular metabolites. This signal belongs to the family of LuxI, named after lux system of *Vibrio fischeri*. In this system an acylated homoserine lactone acts as a signal which diffuses across the cellular membrane. The acyl chain varies in length, degree and substitution type. As the signal level builds up it leads increase in local cell density, causing the interaction of the signal with the cytoplasmic DNA binding receptor protein. This LuxR homolog-signal complex controls the expression of genes regulating the quorum sensing. In peptide-based signalling production of smaller, linear or cyclic peptides which are translated as large pro-peptides within the cells occurs, which are processed further during the secretion. These signals are incapable of being identified inside the cell, but a protein sensor which is bound to the membrane, belonging to a two-component signal transduction family interacts with peptides. Then the sensor bound to the peptide activates an associated response regulator which regulates the quorum sensing regulated gene expression. The AI - 2 quorum sensing system is seen in both gram-negative and gram-positive bacteria and is involved in interspecies communication. In this system furanosyl borate diester is the extracellular signalling molecule whose production is controlled by luxS gene product. This signalling mechanism has been not been explained completely and has been studied in *Vibrio harveyi* (Zhao *et al.*, 2014).

Biofilm formation mechanism:

Quorum sensing is involved in the biofilm formation for several species. The formation of biofilm involves a series of steps:

1. Attachment: The bacterium attaches itself to a substratum. The nature of substratum also affects adherence along with several microbial factors. In cyclic peptide dependent accessory gene regulator (agr) quorum sensing supresses various surface adhesins which regulate the association with matrix and includes fibrinogen along with fibronectin-binding proteins.

2. Maturation: The organization of mature biofilm varies form uniform, homogenous biofilms to thoroughly organized biofilms, integrated with empty spaces along with cellular towers enclosed into the extra cellular matrix. The factors affecting the biofilm structural organization are motility, EPS production and Rhamnolipids production.

3. Aggregation and Dispersion: Cell aggregates have been observed in Industrial environment like waste water treatment plant and natural environment like marine snow. The dispersal process helps the bacteria to settle onto newer surfaces and reinitiates biofilm production with the help of quorum sensing. In conditions where there is over crowding of microorganisms and limited resources are present, quorum sensing becomes the ultimate way for communication between the cells to form a biofilm (Grujic *et al.*, 2017). An illustration of biofilm formation is given in Fig. 1.

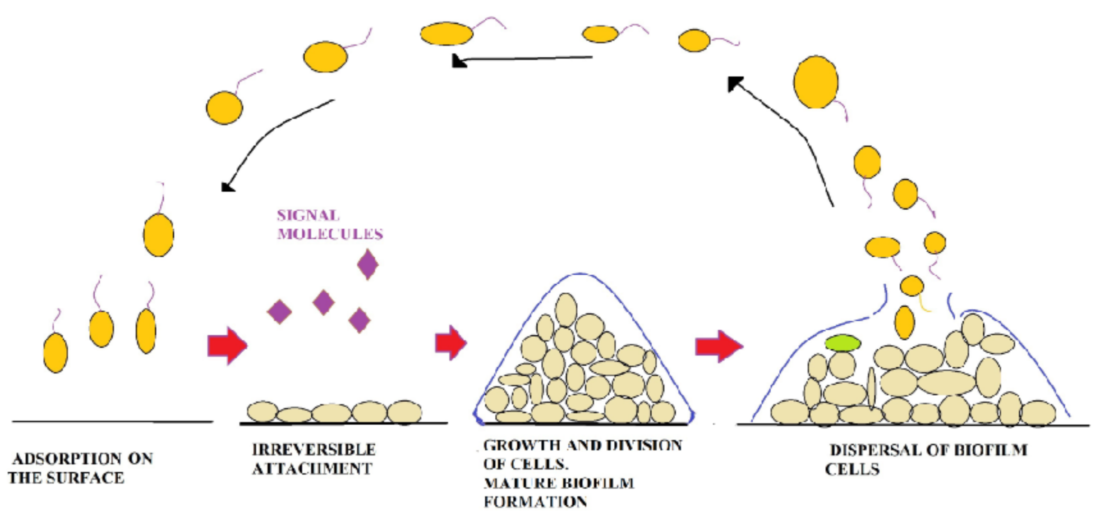

Fig. 1. Diagram Showing the Process of Biofilm Formation.

E. Bioremediation

Biofilm are microbial clusters which are attached to the substratum and are capable of growing in nearly any environment which has moisture, available nutrients and a surface for attachment. The presence of heavy metals in our environment either from natural sources (volcanoes, earth's crust, etc.) and man-made sources (industries, mining, etc.) has led to the possibility of environmental problems which could last for longer periods and bioremediation is an evolving technology for the clean-up of the contaminated environmental sites. The conventional methods to remove heavy metal from a solution generally involves removal of heavy metals by adsorption process, chemical oxidation or chemical reduction, precipitation, ion exchange, evaporative recovery, electro-chemical techniques, sludge filtration and reverse osmosis. But these techniques are rather expensive and less practical and few of these techniques are unsuccessful when the concentration of the metal is less than 100 mg/L in solution. High solubility of most heavy metals also challenges their removal from solution. Reverse Osmosis method utilizes membranes but has certain drawbacks involving slower and ineffective removal, impure sludge generated which requires higher costs, energy involvement, cautious disposal and membrane blockage (Kumar *et al.,* 2018, Selvi *et al.,* 2019).

Bioremediation utilizes microorganisms in restoring the natural conditions of the surrounding by removing toxic metals wastes with cost effectiveness and long-term environmental benefits. Bioremediation functions either in natural manner or can be further improved by adding nutrients, electron acceptors and various extra factors. Detoxification of heavy metals is done by valence transformation mechanisms of metals whose individual valence state varies in their toxicity. Metal toxicity is affected by the concentration of the bio-available metal concentration, in comparison with the concentration of total soluble metal. In Mercury resistant bacteria, Organomercurial lyase enzyme converts methyl mercury to Hg(II), which is 100 folds less toxic than methyl mercury. Detoxification mechanism generally involves metal binding, volatilization and vacuole compartmentalization. Chelators which bind to heavy metals and facilitates absorption and transportation of the metal ions are involved in metal binding. In volatilization metal ions are converted into volatile states which is possible only in case of Selenium and Mercury (Kapahi *et al.,* 2009). Various methods of bioremediation is illustrated as a flowchart in Fig. 2.

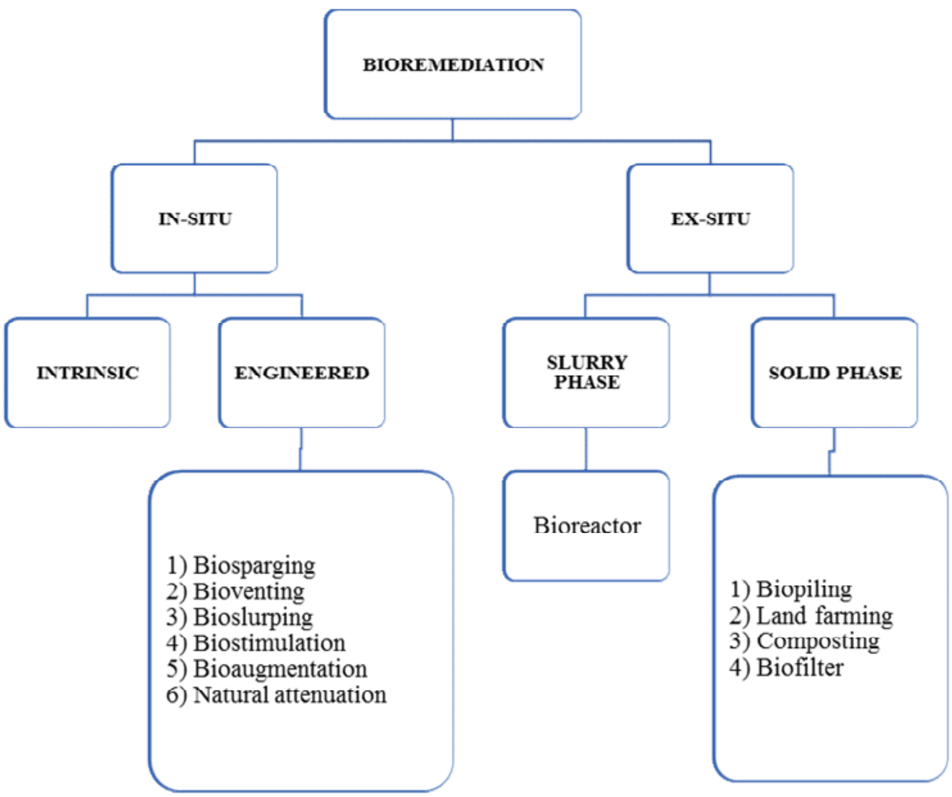

Fig. 2. Flowchart showing different types of Bioremediation.

Based on the methods of transportation and pollutants removal bioremediation techniques are classified into two types 1) In situ and 2) Ex situ. Further these are classified into various types based on their method of removal and the components required by them.

In situ techniques: Biosparging involves injecting air to enhance the microbial activity of the indigenous microbes. In Bioventing air and nutrients are supplied to stimulate the natural biodegradation and provides lower air flow rates to supply oxygen only enough to assist activities of microbes. Bio slurping combines bioventing with increased free product recovery with the help of vacuum to separate contaminants. In Bio stimulation environment is modified by the addition of electron acceptors and nutrients which optimizes the growth and microbial activity in a natural population. Bioaugmentation is introduction of exclusive genetically modified microorganisms to target the specific pollutants to speed up the rate of degradation. Natural attenuation involves no external factors like nutrients, the natural microorganism reproduces by themselves and reduces the pollutants concentration by themselves.

Ex situ Techniques: In Bioreactors microorganisms performs biological reaction in a tank, providing favourable conditions for indigenous microorganisms to decompose pollutants. Biopiles involves excavation of soil and mixing with soil amendments (materials added to improve water retention, permeability, drainage, aeration and water filtration). Land farming involves tilting of topsoil and addition of water and nutrients to it. Composting involves decomposition of organic wastes by microbes in high nutrients and aerobic conditions. It is a combination of landfarming and composting. In Biofiltration pollutants and composts are trapped and exposed to aerobic microorganisms leading to their degradation.

Bioaccumulation, Biosorption, Biomineralization and Biotransformation are the methods utilized by microbes in environment contaminated by heavy metals for bioremediation. The toxicity of pollutants and adverse environmental conditions contributes to the ineffectiveness to maintain healthy microorganism's population. The choice organism should be able to develop resistance towards metal and microorganism can be indigenous to the contaminated environment or could be isolated from another habitat. The understanding of metabolic pathways has helped in microbial isolation, improving survival rates of microorganisms and stability in the environment has helped to manipulate the metal adsorption capacity of microorganisms (Huang et al., 2016). The Peptidoglycan and Polysaccharides present in the cell walls serves as an active binding site for the uptake of heavy metals. Redox reactions carried out by the microorganisms helps in the metal mobilization and immobilization from the environment. Adsorption is the ability of microorganism to adhere ions and molecules onto their surface to form a surface complex. Majority of molecules possesses functional groups like -OH, -SH and -COOH onto their surface. The microbial cells can develop resistance towards heavy metals by excreting heavy metal chelating substances. Other resistance mechanism involves metal ions binding to intracellular molecules (mitochondria, metallothionein or vacuole) resulting in changes in metal ion distribution. Interaction between molecules and metal ions occurs through metals associated with cell walls, intracellular interaction, metal siderophores, extracellular polymeric reaction, extracellular metal mobilization or metal immobilization and metal volatilization (Azubuike *et al.,* 2016).

The other factors influencing the bioremediation of various metals includes bioavailability of metals in microorganism, oxygen, pollutants concentration, moisture, electron acceptors, osmotic pressure, redox potential, pH, composition of soil, temperature activity and water activity. In soil the bioavailability of metals is further affected by factors like buffer capacity, ion exchange, mineral content in soil, metal oxides and organic matter (Dixit *et al.,* 2015).

Chromium, Calcium, Cobalt, Manganese, Magnesium, Iron, Potassium, Nickel, Sodium and Zinc are micronutrients which uses redox process to stabilize molecules by electrostatic interactions (Tandon *et al.,* 2020). Various metals like Aluminium, Silver, Cadmium, Gold, Mercury, Lead are non-essential and does not have any biological role and they are toxic to the microorganisms. Heavy metal ions can form complex compounds inside the cells leading to toxicity in microbes. Metals like Hg, Cd and Ag binds to Sulfhydryl group of enzymes which is essential to the metabolism of microorganism and inhibits the activity of sensitive enzymes. The problem of heavy metal uptake mechanism is solved by microorganism by two methods. First method is instigated by Chemiosmotic gradient across the bacterial cytoplasmic membrane. The other method is slower and involves ATP hydrolysis and occurs only at special situation like cell starvation.

Higher levels of essential or non-essential metals than the required amounts are capable of causing damage to the cell membranes and disruption of specificity in enzyme, interfering with the cellular functions and damaging the DNA structure. Higher concentration of heavy metals imposes oxidative stress on microbes because of which microbes are forced to develop properties such as metal-ion homeostasis factors and resistance towards metals. The metal resistance mechanism involves permeability barrier exclusion, intracellular sequestration and extracellular sequestration, actively transporting efflux pumps, reduced sensitivity of cellular targets towards metals and detoxification of enzymes (Dutta *et al.,* 2018).

CONCLUSION AND FUTURE SCOPE

Microorganisms are known to be ubiquitous in the biosphere and their presence can be beneficial or harmful to the environment. There is no naturally occurring compound which cannot be degraded by some microbe. This property of microorganisms can be utilized to degrade the contaminants and pollutants which are causing harm to any ecosystem and its residents proving that bioremediation can be a powerful tool for the clean-up of these sites. Heavy metals are naturally present elements which have higher atomic weights. Heavy metals have various utilization in industries, domestic, medical field, agriculture and technology and this has led to the existence of heavy metals in the ecosystem which has raised concerns because of their potential adverse effects on the health of humans and environment. Heavy metals are considered hazardous to the ecosystem because of their three characteristics: persistence, bioaccumulation and toxicity. There have been various traditional methods for the removal of these pollutants but they have shown various limitations and Bioremediation has been known to be cost effective and eco-friendly technique by using biological agents. The interaction between the metals and microorganism impacts the growth, colonization and the biofilm formation for the bioremediation of heavy metals. Biofilm mediated bioremediation have been particularly useful in the heavy metal's removal form soil samples and water samples. Bioremediation is also being performed on larger scale by the help of bioreactors. With being a natural process and nearly no side effects, quick turn-around time which makes the polluted soil and polluted water useful and minimum equipment requirement, bioremediation is the most effective way to remove heavy metals from the environment.

FUTURE PROSPECTIVE

Further research in the field of bioremediation will help in the better understanding of the microbe metal interaction. Biostatistical and bioinformatics research conducted on the microorganism will be useful in evaluating the nature of an microorganism and its interaction with the surrounding. Research in the field of Proteomics, Genomics and Metabolomics concerned with the microorganisms and the study of their genes and enzymes would be helpful in producing genetically modified organism which will have greater potential of remediation and their capabilities could be adjusted according to the environment and the type of pollutant to obtain even better results.

Conflict of Interest: The authors declare no conflict of interest.

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