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Review Article

BIOREMEDIATION OF HEAVY METALS USING SPIRULINA

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ABSTRACT

Environmental pollution is a result of Industrial revolution. Environmental pollution effects quality of life and environmental ecosystem. Since decades, many attempts are being done to reduce environmental pollution. Among these attempts Bioremediation is one of the good remedial techniques. In the Bioremediation technique microorganisms are used to reduce the toxicity of harmful wastes and heavy metals from contaminated wastes water. Bioremediation has provided problem solving opportunities in the field of solid waste by detoxifying wastes. Due to its cost effectiveness and environmental impact offers attractive and more conventional clean-up technologies. In this regard researchers are carried out to study the effect of bioremediation by using various cyanobacteria species in reducing the toxicity. At this juncture this review glimpses the studies done in relation to removal of heavy metals in waste water by using Bioremediation techniques.

***Keywords**: Bioremediation, Heavy Metals, Cyanobacteria, Environmental Pollution*

INTRODUCTION

Rapid economic development is not possible without the growth and expansion of industrial sector. In acquiring the raw material and at the time of release of the waste materials, industries alter the environmental conditions detrimentally. Several industries have heavy metals as their major component, and their release in the surroundings is a matter of great concern today (Nriagu and Pacyna, 1988). The dangers that linger with the high concentration of heavy metals in the soil and water around industrial plants cannot be overlooked. The decontamination of the polluted soil and water has been a challenge for a long time. With several limitations associated with traditional physicochemical methods for decontaminating the polluted sites, there has been shift towards biological means to counter this problem. This is the reason the use of microorganisms for the recovery of metals from waste streams (Macaskie *et al.*, 1987), and the employment of plants for landfill application (Watanabe, 1997), is achieving growing attention. Lower cost and higher efficiency at low metal concentrations make these methods very attractive in comparison to physicochemical methods for heavy metal removal (Gadd *et al.*, 1992). In biological techniques of heavy metal's decontamination, the application of microorganisms is easier and practically feasible. There are several reports concluding that the metal-binding capacities of several biomasses including marine algae (Centikaya *et al.*, 1999; Doshi *et al.*, 2006), fungi (Barros *et al.*, 2003; Volesk, 1986), and yeast (Clemens *et al.*, 1999) are very high. Thus the understanding of the mechanism of decontamination by microorganisms will be useful in identifying the potential microbes to be screened for this purpose. In the review we attempt to summarize the mechanism used by the microbes in general for the binding of heavy metals, and report the information available about *Spirulina platensis* in this regard.

Principles of Bioremediation

Environmental biotechnology is not a new field, composting and wastewater treatments are familiar examples of old environmental biotechnologies. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas.

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 by definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants (Rani *et al*., 2007) to degrade or detoxify substances hazardous to human health and/or environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated sites. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation the process is known as bioaugmentation.

Basic Types of Bioremediation Techniques

Biostimulation provides nutrients and suitable physiological conditions for the growth of the indigenous microbial populations. This promotes increased metabolic activity, which then degrades the contaminants. Bioaugmentation means introduction of specific blends of laboratory cultivated microorganisms into a contaminated environment or into a bioreactor to initiate the bioremediation process. The process of developing bioremediation techniques may involve the following steps:

a. Isolating and characterizing naturally occurring microorganisms with bioremediation potential
b. Laboratory cultivation to develop viable populations
c. Studying the catabolic activity of these microorganisms in contaminated material through bench scale experiments
d. Monitoring and measuring the progress of bioremediation through chemical analysis and toxicity testing in chemically-contaminated media field applications of bioremediation techniques using either/both steps: (1) *in-situ* stimulation of microbial activity by the addition of microorganisms and nutrients and the optimization of environmental factors at the contaminated site itself (2) *Ex-situ* restoration of contaminated material in specifically designated areas by land farming and composting methods.

Mechanism of Heavy Metal Sorption/Decontamination

Since the early history, the association between heavy metals and the microbes was in vogue, this can be easily guessed by the range of divalent or transition metals at the active centres of many enzymes. Microbes have exploited the chemical properties of the metals for catalyzing key reactions and also for maintaining protein structure. These metals are therefore required by all forms of life even today in minute amounts for normal cell metabolism. The intake of the metals is subject to intricate homeostatic mechanisms that ensure that sufficient, but not excess quantities are acquired. While some metals are useful and selectively acquired by the microbes, many other metals that serve no biological role, or, instead, cause damage by their avidity for the sulfhydryl groups of proteins need to be avoided.

Therefore, from a physiological point of view, metals are divided into three categories: (i) essential and non toxic, (ii) essential but harmful at high concentration, and (iii) toxic even at low concentrations.

Thus, the living system needed mechanisms for two purposes simultaneously i.e., to acquire, and to reject metals selectively. This could only ensure the adaptation of microorganisms to the environment. Recently, the anthropogenic mobilization from the metal ores has put strong selective pressure for metal endurance on the microorganisms. Different organisms exhibit diverse responses to toxic ions. Eukaryotes in particular are more sensitive to metal toxicity than bacteria and they typically regulate intracellular metal ion concentrations by the expression of metallothioneins (MTs), which is a family of metal chelating proteins. Bacteria also have MTs that are functionally homologous to the MTs of eukaryotes. Prokaryotic MTs have been studied in detail only in the cyanobacterium *Synechococcus*. In this cyanobacteria they confer resistance to Zn^{2+} and Cd^{2+} (Blindauer *et al.*, 2001; Robinson *et al.*, 2001).

However, the use of these MTs only as the main mechanism of tolerance to metals (as in *Synechococcus*) is rarely seen in bacteria. Instead, a number of specific resistance mechanisms which include active efflux and sequestration or transformation to other chemical species are found to become effective above the homeostatic levels. *Ralstonia metallidurans* CH34, *a* β-proteobacterium (previously *Alcaligenes eutrophus* CH34) (Taghavi *et al.*, 1997), is an important example of heavy metal resistance. It has resistance determinants enabling it to grow at millimolar concentrations of nine different toxic metal ions (Collard *et al.*, 1994).

Sulfate – reducing bacteria (SRB) possess metal tolerance due to their unique metabolism. They immobilize toxic ions as metal sulfides, and along with this enzymatic metal reduction are also commonly seen in these bacteria (De Luca *et al.*, 2001).

Iron and sulfur oxidixing bacteria, which include the thiobacilli and thermophilic archaea are the part of the organisms' group that grow at highest metal concentrations. These are metal resistant due to their adaptation to very acidic environments, because under these conditions metal solubility is very high. As a conditional example, when the growth of *Thiobacillus ferrooxidans* depends on Fe (II), the bacterium becomes highly resistant to Al, Cu, Co, Ni, Mn and Zn (0.1-0.3 M; Hutchins *et al.*, 1986).

Of the variety of mechanisms that could be involved in bioremediation of toxic metals is use of living cells, non living biomass, or biopolymers as biosorbents. In this regard, specific pathways enabling bioprecipitation of heavy metals or their transformation to less toxic or easily recoverable forms have also been described (Gadd *et al.*, 1992). In microbes, metal binding by biomolecules or structural components or excreted polymers is fortuitous, and relative efficiencies are found to depend on attributes of the metal ion, as well as on the reactivity of the provided ligands. There are suggestions that the macromolecular composition of biosorbent should be manipulated by cultivation conditions (e.g., stress inducible fungal melanins) to improve its metal binding properties (Macaskie and Dean, 1990). The selectivity of biomolecules for the metal ions can be explained by semiempirical and qualitative theories; for example, HSAB (hard and soft acids and bases) principles and Irwing-Williams series of stability constants for divalent ions. Specific anchoring of the particular amino acid sequence to the biosorbent material, contributes to the selectivity for specific metal ions. In this way, biosorbents could be enriched with amino acids classified by HSAB principle to be stronger ligands of transition metals than those naturally present on the microbial surfaces (Macaskie and Dean, 1990). Extending the concept, surface exposure of metal binding peptides could improve metal binding properties of microorganisms, not only on biosorption but also on the metabolic activities located on the cell surface (Diels *et al.*, 1993; Macaskie *et al.*, 1987). In this reference, the metal binding ability of the *Escherichia coli* cell wall has been studied in detail previously (Hoyle and Beveridge, 1984; Hoyle and Beveridge, 1983). Toxic effect of heavy metals is probably exerted through free radical generation. When the levels of ROS formed exceed the ability of the antioxidant system to cope with them, damage to cellular components occurs. Increased activity of SOD was found in many organisms (Okamoto *et al.*, 2001; Rijstenbil *et al.*, 1994). It increased in *Scenedesmus bijugatus* exposed to different copper concentrations (Nagalakshmi and Prasad, 2001).

Making Use of Spirulina

Spirulina is a biomaterial whose role as an effective material to take up metal ions is being seriously examined (Chojnacka *et al.*, 2005; Hernandez and Oliguin, 2002). Many studies have been undertaken to analyze the toxic metal tolerance and sorption capacity of *S. platensis*.

Chojnacka *et al.*, (2005) studied the mechanism of biosorption of Cr^{3+}, $Cd2^{+}$, and Cu^{2+} in *S. platensis*. They found that chemisorptions rather than physical adsorption were the main mechanism for sorption of heavy metals. In their study the maximum contribution of physical adsorption in the overall biosorption process was 3.7%. In the cells of the cyanobacterium, functional groups on the cell surface contributed to the binding of metal ions by a biosorbent via equilibrium reaction. However, the cyanobacterial cells had to suffer damage when exposed to high concentration of heavy metal. Rangsayatorn *et al.*, (2002) found that cells were swollen and the filaments were fragmented at high concentration of Cd. Cd induced several alterations such as disintegration and disorganization of thylakoid membranes, presence of large intrathylakoidal space, increase of polyphosphate bodies and cell lysis. They also reported that the uptake of the heavy metal was not influenced by the temperature of the solution, but the pH of the solution was determinant of the sorption. Optimum pH for biosorption was 7. The maximum adsorption capacity for *S. platensis* was 98.04 mg Cd per g biomass. The rate of uptake of heavy metal was rapid, and upto 78% of the metal sorption was completed within first 5 minutes.

Chan and Pan (2005) studied biosorption of lead by *S. platensis*. They found that at low Pb^{2+} concentration (1.0 mg/L), only a slight inhibition of cell growth occurred. By contrast, the highest concentration (20 mg/L) caused a large number of cells to die at first (0–1 d), but most importantly, the cell growth recovered afterwards. Such responses of live and growing cells to high metal concentration are similar to the pure biological adsorption process using dead/treated biomass. They reported the EC_{50} at 72 h to be 11.46 mg/L.

Not only the living but the dried and re-hydrated biomass of *S. platensis* can also be used as a sorbent for heavy metal removal from water. (Solisio *et al.*, 2006) found that biomass re-hydrated for 24 h before use exhibited a shorter adsorption time as well as an increased percentage removal of copper when compared with simply dried biomass. The reason for this is that adsorption capacity could be due to the functional groups present on the cell surface, mainly protein $–COO^-$ groups and functional side chains of amino-acids, such as histidine, cysteine, aspartic acid and glutamic acid (Xue *et al*., 1988).

Kumar and Singh (1992) studied two strains of *platensis* by enriching with cobalt and iodine, the repeated sub-culturing resulted in increased resistance to these trace metals. The cobalt tolerant strain which grew at 55mg $CoCl_2.6H_2O$ 1^{-1} showed maximum uptake of 158.43 n mol Co ion mg^{-1} proteins which was 3.98 times higher than its parent. The iodine tolerant strain which grew at 7.0 g Kl 1^{-1} showed maximum uptake of 0.65 m mol $mg^{-1}$of protein which was 1.25 times higher than its parent.

Murali *et al.,* (2014) reported great accumulation and tolerance to heavy metals in *S. platensis* for two heavy metals viz., Co and Cr. The study was carried out by taking the heavy metals at different concentrations singly and in combination. The findings indicated that *S. platensis* was tolerant to moderate concentrations of heavy metals in either of the treatments.

CONCLUSION

Bioremediation is low cost than other technologies that are often used to clean up hazardous waste. There are a number of cost or efficiency advantages to bioremediation which can be employed in areas that are inaccessible without excavation. Bioremediation is carried out in the natural environment which contains diverse uncharacterized organisms and another difficulty is that no two environmental problems occur under completely identical conditions, for example, variations occur in the types and amounts of pollutants, climate conditions and hydro-geodynamics. These difficulties have caused the bioremediation field to lag behind knowledge-based technologies that are governed by common rationales (Watanabe, 2001) Bioremediation therefore, may make a significant contribution in situation such as accelerated habitat recovery by the removal of toxic hydrocarbon components to levels below the toxicity threshold. It is thus an important part of toolkits available for dealing with accidental and deliberate oil releases into the marine environment (Prince, 2002).

Even with the obstacles discussed above, there are tremendous market opportunities for bioremediation. With the next 10 years, soil clean-up costs alone are estimated to exceed 30 billion in Europe (Caplan, 1993). This greatly exceeds the amount of $1 billion spent thus far. If just 5% of this soil is cleaned using bioremediation, 1.5 billion dollars could be earned through bio-treatment methods.

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