Bioremediation of Wastewater Chromium through Microalgae: A Review

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***Abstract* - With growing industrialization and urbanization, the waste discharged to the river streams continuously pose health hazards. The wastewater discharged contains high amount of organic matter as well as heavy metals like zinc, chromium, lead, cobalt, nickel etc. Conventional methods are available to clean wastewater but they might generate large amount of sludge, leads to improper handling, disposal problem of sludge and high capital cost Therefore, there is a need of novel and sustainable technology for wastewater treatment to resolve the issue of limitations of physicochemical treatments. The use of microbial biomass (bioremediation) is considered to be a viable alternative to conventional methods.**

Keywords— Pollution, Bioremediation, algae, chromium

INTRODUCTION

Environmental pollution is a very serious threat in present scenario. Rapid industrialization and urbanization are the main reason for water pollution as they are continuously discharging waste into the river. Many industries like electroplating, tanning, paper, textile etc are key component of discharging effluents causing heavy metal pollution. Heavy metal pollutants like Mercury (Hg), Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni) and Zinc (Zn) causes poisoning which can occur through drinking water or intake via food chain. These heavy metals accumulate in the food chain of aquatic and terrestrial ecosystem posing health hazards [1]. Many conventional methods are available to sequester toxic heavy metals like reverse osmosis, chemical precipitation, electro dialysis, ion-exchange and ultrafiltration. These methods and techniques are functional for removal of heavy metals with high concentration but ineffective for low concentration (1-100 ppm level) and not cost-effective. Therefore, a novel technology is needed to overcome the limitation of conventional methods which is effective and inexpensive yet providing heavy metal concentration to at least environmentally acceptable standards. Many studies have been reported investigating the potential of yeast, fungi, algae, bacteria, agricultural waste and some aquatic plants to sequester metal concentration from dilute aqueous solutions [2-4]. Out of all the heavy metals, Chromium is found to be highly toxic and carcinogenic. This paper reviews about the occurrence, sources and properties of chromium along with its immediate human health effects. The present study also gives viewpoint about how algae can be used as biosorption of chromium.

I. CHROMIUM AS HEAVY METAL

Chromium is the 21st most abundant element in the earth's crust. It is the 24th element in the periodic table with atomic weight 52, atomic no. 24, atomic weight 51.996 g/mol and melting point 1903^{o}C. Chromate was first discovered by L.N. Vauquelin in 1797 at Siberian red lead ore. The word chromate comes from Greek word *chroma,* meaning *"color"*. Chromium is a silver grey in color, lustrous, brittle, hard metal, when heated it burns and forms the green chromic oxide. Chromium exists in oxidation state -2 to +6 but abundantly in trivalent form. Divalent chromium (+2) is unstable in most compounds as it forms trivalent compound when oxidized by air. It persists in environment in two oxidation states Cr (III) and Cr (VI) [5-6]. Hexavalent Chromium is more toxic than trivalent chromium and often present in wastewater as chromate and dichromate. Chromium is corrosion resistant. Chromium compounds have a common origin: chromite ore (Cr_2O_3, FeO). Chromium and its compounds are used in industrial sector including electroplating, chromate manufacturing, leather tanning etc [7]. In India as mentioned below in table 1, Chromate reserves are mainly (93 %) found in Odisha, mostly in the Sukinda valley in Cuttack and Jajpur districts and other reserves are located at Manipur, Nagaland, Karnataka, Jharkhand, Maharashtra, Tamil Nadu and Andhra Pradesh.

TABLE 1

Chromium compounds are found to be carcinogenic and mutagenic. Health effects of chromium compounds can vary with route of exposure. Chromium enters our body due to inhalation, ingestion or dermal contact. During occupational exposure, the absorption of inhaled Cr (VI) depends on the oxidation state, particle size, and solubility of the compound. In humans, it poses health problems like DNA damage, nausea, vomiting, nasal irritation and ulceration, skin irritation, eardrum perforation and lung carcinoma [9]. Therefore, it is very necessary to combat harmful effect of chromium which is being disposed off in water bodies through industries. In this context, Bioremediation plays an important role in sequestering chromium from wastewater. It is an innovative technology which uses the metabolic potential of microorganisms to remove toxic metals, in order to decontaminate the polluted areas [10,11]. The major advantages of bioremediation over conventional treatment methods include low cost; high efficiency; minimisation of chemical and/ or biological sludge; no additional nutrient requirement; regeneration of biosorbent; and possibility of metal [12].

II. BIOREMEDIATION OF CHROMIUM THROUGH ALGAE

Many Studies have been carried out on application of algae like biofuel production, biomass production and assessment of water contamination with heavy metals and pesticides. Of all the microbes, algae are able to take up, accumulate and concentrate heavy metals in significant amounts from the aqueous solution. Algae have also been considered to be potential biosorbents because of their easy handling, cheap availability, relatively high surface area and high binding affinity [13,14,1]. Microalgae remove heavy metals directly from polluted water by two major mechanisms; the first is a metabolism dependent uptake into their cells at low concentrations, the second is biosorption which is a non-active adsorption process [15,16,17]. Phycoremediation is defined as a use of microalgae or macroalgae to detoxify heavy metals [18,11]. Table 2 describes that algae have many unique characteristics which make it suitable for removal of selected metals from wastewater

TABLE 2

Algae have high metal binding capacities, since polysaccharides, proteins or lipids on the surface of their cell walls have some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals The presence of functional groups with binding abilities do not always gurantee biosorption due to steric or conformational hindering or other barriers. [19,20,21,22, 23]. Algae also has the the ability to grow both autotropically *and* heterotrophically *[24*].

It is clear from the table above that work has been done on Chromium removal by algae e.g immobilized algal biomass was characterized for removal of chromium which resulted in maximum metal uptake of 11.494 mg/g [27]. Chlorella Pyrenoidosa was tested for its Chromium (VI) removal capacity from synthetic wastewater using immobilization (calcium alginate and carrageenan). Maximum metal uptake by the algae immobilized with calcium alginates beads was observed at pH 3 and concentration of 75mg/l [28]. Chromium removal capacity was estimated for dried green alga *Ulva lactuca* and activated carbon. The maximum efficiency was found to be 92% with absorption capacity of 10.61 mg/g [29]. Metal uptake studies have been conducted mainly employing laboratory grown algal species, using single metal ions. There are limited studies which employed algal species, naturally growing in polluted water to remove multimetal ions from solution. Also, there are not many studies done comparing the capacity of freshwater algae and brackish water algae to sequester heavy metal. Since industrial effluents may contain more than one metal ion, and the algae growing in metal polluted water may have higher biosorption potential.

III. HEAVY METAL ACCUMULATION AND ITS MECHANISM

Heavy metals enter micro algal cells through micronutrient transporters. As they enter the cell, binding to specific intracellular compounds and/or transport of the metals to specific cellular compartments takes place which helps in heavy metal detoxification. Another method is chelation in which it converts heavy metals into other forms to reduce their toxicity. The detoxification mechanisms of heavy metals by algae include systems such as Metallothioneins (MTs) and Phytochelatins, Cell wall components (Alginates and guluronic acid, sulfated polysaccharides and alginates [29, 30]. The biosorption process takes place in two steps: first rapid physical adsorption (between metal ions and cell surface) and then slow chemical adsorption [31,32, 17]. The principle mechanism of removal of metallic ions involves formation of complexes between metal ions and functional groups (hydroxyl, phosphoryl, amino, carboxyl, sulphydryl, amine, imidazole, sulphate, phosphate, carbohydrate) which are present on surface of algal cell wall [33,12]. The algae and metal ion interaction depends on various factors such as physiological condition of algal cells, form of metal (chemical speciation), influence of other ions (Na^{2+}, Ca^{2+}), influence of trace metals, inherent tolerance of algal cells. Metal removal by biosorbents from wastewater is strongly influenced by number of abiotic and biotic factors such as pH, Chelating agents, redox potential, temperature, light, cellular activity, algal biomass concentration and extracellular products [34,35].

IV. ROLE OF MOLECULAR STUDY IN BIOREMEDIATION

Rapid Identification of specific microorganism with high affinity to absorb/ adsorb the heavy metals as well as its modification for the specific role like bioremediation is required for its commercial use. Degradation of the contaminants from the environment become much easier by using molecular ecological techniques (Fig. 1) such as direct DNA isolation from environmental samples, denaturing gradient gel electrophoresis, PCR methods and nucleic acid hybridization. These techniques have proved to have advantage over conventional methods of isolation and identification of microorganisms (taxonomical and morphological). In algal research, molecular biology acted revolutionary in studying the DNA regions by amplifying it and using Polymerase Chain Reaction (PCR). The PCR has a property of amplifying a target sequence from crude DNA model. In the laboratory, DNA fragments are synthesized and product contains many copies of fragment which can be used for identification [36,37]. Due to the limitations of morphological identification of microalgae, molecular markers such as rbcL [38] and 18s rDNA [39] has proved to be very useful. Recently, colony PCR for microalgae has been reported using Chelex-100 for DNA extraction and amplification using the cells from the liquid cultures (Wan et al. 2011) [41].

Wu et al. 2013 [43] isolated 4 strains of Chlorella and analyzed nuclear and chloroplast encoded rDNA sequences using PCR technique. Another study was done by Ponnusawamy et al. 2013 [44] for identification of microalgae using genomic DNA, and 16S rRNA gene amplification and resultant showed Chlorella vulgaris. The 16S rRNA gene sequencing is mainly used to determine the phylogenetic position of the unknown microbe among known microorganisms. This is achieved by submitting the unknown microbe sequence to the GenBank 16S rRNA sequences database in National Center for Biotechnology Information [NCBI]. The pair of sequences from different organisms are aligned and differences in their nucleotide sequence is counted. The similarity between a pair 16S rRNA sequence is done by a comparative tool named BLAST [45,46,47]. PCR method is mainly used for tracking genetically modified microorganisms, monitoring indicator pollutants in water, soils and sediments, cloning genes, measuring gene expression by viable m/o as well as detecting specific population based on gene sequence [48,49,50].The 18S rRNA gene sequence was studied for identification of microalgae and amplified gene sequence found to have >99% identity with Coelastrum sp. strains in the NCBI database [51].

V. CONCLUSION

Various contaminants like metals and heavy metals are constantly released into the environment by anthropogenic activities. From this exhaustive assessment of literature, it is concluded that heavy metal pollution is one of the major threat to water bodies with special reference to chromium, which is been discharged into the river streams industries. Bioremediation of heavy metals is considered to be economically promising alternative to conventional methods of remediation. There is a tremendous capacity of different species of algae in removal of chromium from wastewater which should be exploited. Molecular tools are among some steps towards phycoremediation

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TABLE 1: Production of Chromite , 2008-09 to 2010-11 (By States) (Qty in tonnes; value in Rs)

STATES	2008-09		2009-10		2010-11	
	Qty	Value	Qty	Value	Qty	Value
India	**4073479**	**22633627**	**345580**	**10453620**	**4262207**	**22955675**
Karnataka	4115	36475	6483	30856	8491	36851
Maharashtra	-	-	66	489	-	-
Odisha	4069364	2257152	3419031	10422275	4253716	22918824

Source: Indian Minerals yearbook (2011) [8]

TABLE 2: Uptake and Accumulation of Chromium by algal Species.

Biosorbent	Adsorption Capacity(mg/g)	References
Padina tetrastromatica	5.5	[52]
Sargassum weghtii (brown)	65.96	[53]
Pithophora sp.	4.9	[54]
Scenedesmus quadricanda	1.98-81.98	[55]
Spirulina sp	9.62	[56]
Chlorella vulgaris	23.6	[57]
Cladophora crispata	30.4	[58]
Chlorella vulagris	23.0	[59]
Scenedesmus obliquus	15.6	[59]
Synechocystis sp.	19.2	[59]
Padina boergesenli	49	[60]
Pithophora	434.10	[61]
Acid-treated Pithophora	666.21	[61]
Nizamuddina zanadinii	32.72	[22]
Stoechospermum marginatum	32.63	[22]
Cystoseira indica	43.38	[22]
Padina australis	37.82	[22]
Sargassum glauceseers	47.62	[22]
Spirogyra sp.	265	[26]
Desmococcus olivaceus	16	[11]
Scenedesmus quadricauda	12	[23]
Ceramium virgatum	26.5	[25]

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FIGURE 1: Schematic showing molecular technique for environmental samples (soil, sediment and water) [42].