International Journal of Microbiological Research 4 (3): 305-320, 2013
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2013.4.3.81228

Microbial Bioremediation of Chromium in Tannery Effluent: A Review

P. Saranraj and D. Sujitha

Department of Microbiology, Annamalai University, Annamalai Nagar,
Chidambaram- 608 002, Tamil Nadu, India

Abstract: Chromium, a priority pollutant is well known for its mutagenicity, carcinogenicity and teratogenicity in humans, experimental animals and plants. Extensive use of chromium in industries such as leather tanning, stainless-steel production, electroplating and wood preservatives have resulted in chromium contaminated soil and ground water at production sites which pose a serious threat to human health. Bioadsorption is the simple uptake of the constituents of the effluent by the organisms without any usage in its metabolism. Biodegradation means the breaking down of the constituents of the effluent into different fragments which will be either taken as a substrate by the organisms or simply remain in the system i.e., metabolism of the constituents by the organisms or simple cleavage by them into simpler fragments by the release of enzymes. Biodegradation is one of the biological processes facilitating the chemical changes of pollutants by microorganisms present in the polluted environment. Microorganisms are involved in the removal of toxic wastes, either in the environment or in controlled treatment systems.

Key words: Tannery Effluent · Chromium · Bioremediation · Bacteria and Fungi

INTRODUCTION

Elimination of heavy metals from industrial wastewater is important for preserving the quality of aquatic systems, streams and ground waters. Contaminated waters are generally cleaned by precipitation of a metallic oxyhydroxide sludge or by ion exchange with synthetic resins. Various types of nonliving biomass, bacteria, filamentous fungi, algae and higher plants can be profitably used in alternative metal removal processes because of their low cost and the high ion exchange capacity of cell walls. The hexavalent chromium compounds are comparatively more toxic than trivalent chromium compounds due to their higher solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids [1]. Accordingly, chromium and its compounds are placed on the priority list of toxic chemicals by US EPA [2]. A maximum acceptable concentration of 0.05 mg/l for hexavalent chromium in drinking water has been established on basis of health considerations. However, its high toxicity, mutagenicity and carcinogenicity renders it hazardous even at very low concentration.

Tanning industries worldwide generate approximately 40 million waste containing chromium (Cr) every year. With inadequate regulatory guidelines, wastes were largely disposed on land and water odies throughout the world. Studies showed that tannery waste disposals have led to severe contamination of productive agricultural land in Bangladesh, [3] in India [4] and in Australia [5]. Chromium is frequently one of the most toxic elements present in tannery waste. High chromium concentrations ranging from 1 to 50g/kg were reported in soils surrounding tannery waste disposal sites in India, with hexavalent chromium was present in groundwater at these sites [6].

Chromium level was found to increase with increase in the concentration of the tannery effluent. Chromium associated pollution is of increasing concern nowadays. Conventional methods for treatment of toxic chromate include chemical reduction followed by precipitation, ion exchange and adsorption on activated coal, alum, kaolinite and ash which require large amounts of chemicals and energy and therefore are unsuitable [7]. Several reports have indicated biological reduction of hexavalent chromium by microorganisms, both aerobes and anaerobes. Biological reduction of hexavalent chromium

Corresponding Author: P. Saranraj, Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram - 608 002, Tamil Nadu, India.

usually occurs at a neutral pH range and generates an insignificant quantity of chemical sludge as well as offers potential cost – effective remediation strategy. A number of chromium resistant microorganisms have been reported to detoxify hexavalent chromium, which include *Pseudomonas* sp., *Microbacterium* sp., *Desulfovibrio* sp., *Enterobacter* sp., *Escherichia coli, Shewanella alga* and *Bacillus* sp., where the method of detoxification is periplasmic biosorption, intracellular bioaccumulation and biotransformation through direct enzymatic reaction or indirectly with metabolites [8]. Some of the fungi that remove chromium from tannery wastes are *Aspergillus, Nostoc* and *Cyanobacteria* etc.

Biosorption by bacteria and fungi as an alternative treatment option for wastewater containing heavy metal has been reviewed by Kapoor and Viraghavan [9]. Any fungi can tolerate high concentration of potentially toxic metals and with other microbes; this may be correlated with decreased intracellular uptake or impermeability. A close relation between toxicity and intracellular uptake has been shown for Cu^{2+}, Cd^{2+}, Co^{2+} and Zn^{2+} in yeast *Saccharomyces cerevisiae*. Waste mycelia from industrial fermentation plants (*Aspergillus niger* and *Penicillium chrysogenum*) were used to as a biosorbent for removal of Zn ions from aqueous environments, both batch wise as well as in column mode. Under optimized conditions *Aspergillus niger* and *C. paspali* were found superior to *Penicillium chrysogenum*. Removal of lead ions from aqueous solution by non-living biomass of *Penicillium chrysogenum* was studied and observed that Pb^{2+} was strongly affected by the pH in the range of 4-5. Uptake of Pb^{2+} was 116 mg/g dry biomass, which was higher than that of activated carbon and some other microorganisms [10].

Chromium biosorption by non-living biomass of *Chlorella vulgaris, Clodophora crispate, Zoogloea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* was studied and observed that optimum initial pH (1.0-2.0) of the metal ion solution affects the metal uptake capacity of the biomass for all the microorganisms. Maximum adsorption rates of metal ions to microbial biomass were obtained at temperature in the range of 25-35°C. The adsorption rates increased with increasing the metal concentration of *Chlorella vulgaris, Clodophora crispate, Zoogloea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* up to 200, 200, 75, 125 and 100 mg/l respectively [11]. Dead cells of *Saccharomyces cerevisiae* removed 40% more uranium or zinc than the corresponding live cultures.

Biosorptive capacity of different biosorbents including dried mycelium of some species of fungi, baggase, rice rusk and fermented baggase by selected fungal species or natural micro flora was examined to remove cyanide from industrial effluent. The biomass of *Rhizopus sexualis* and the fermented baggase by *Rhizopus sexualis* or *Aspergillus terreus* showed higher sorption capacity than activated charcoal. The biomass of *Rhizopus sexualis* and *Mortierella ramanniana* exhibited higher CN sorptive capacity than ascomycetes e.g. *Aspergillus terreus* and *Penicillium capsulatum*. Maximal removal of Ni from electroplating industries occurred by 2.5 gm of biomass of *Saccharomyces cerevisiae* with in 5 hrs. Ni uptake capacity from aqueous solution was also studied in filamentous fungi such as *Rhizopus* sp., *Penicillium* sp. and *Aspergillus* sp. The metal uptake was highest by *Rhizopus* sp. [12].

Chromium: Chromium (Cr) is a transition metal present in group VI-B of the periodic table. Although it can exist in nine valence states, from -2 to +6 [13] only trivalent chromium Cr (III) and hexavalent chromium Cr (VI) are ecologically important because these are the most stable oxidation states in the natural environment. Chromium is a chemical element which has the symbol Cr and atomic number 24. It is a steely gray, lustrous, hard metal that takes a high polish and has a high melting point. It is odorless, tasteless and malleable metal. The name of the element is derived from the Greek word 'chroma' meaning color, because many of its compounds are intensely colored. Chromium is an important metal due to its high corrosion, resistance and hardness. It is used extensively in manufacturing of stainless steel. Chromium contamination of the environment is of concern because of the mobility and toxicity of Cr (VI).

Trivalent and hexavalent chromium differ widely in physicochemical properties and biological reactivity. While Cr (VI) species and dichromate's are extremely water-soluble and mobile in the environment, Cr (III) species are much less soluble and comparatively immobile. Moreover, Cr (VI) is recognized to be highly toxic, carcinogenic, mutagenic and teratogenic for mammals including humans, whereas Cr (III) is an essential trace element necessary for glucose, lipid and amino-acid metabolism as well as a popular dietary supplement [14].

Studies have revealed that Cr (VI) is approximately 100 times more toxic and 1000 times more mutagenic than Cr (III). Although, trivalent chromium (Cr (III) or Cr^{3+}) is required in trace amounts for sugar and lipid metabolism in humans, however its deficiency causes disease. Hexavalent chromium (Cr (VI) or Cr^{6+}) is a toxin and a carcinogen metal pollutant that tremendously affects the environment at abandoned chromium production sites. Hence its environmental cleanup is highly essential. Cr (VI) is highly toxic, carcinogenic and mutagenic. It causes severe diarrhea, ulcers, eye and skin irritations, kidney dysfunction and probably lung carcinoma. It is also associated with decrease in plant growth and changes in plant morphology. Chromium is present in the environment as either Chromium (III) or Chromium (VI). Chromate [Cr (VI)] is highly soluble in bacteria, it is transported rapidly across the cell membranes *via* the sulfate pathway and reduced in the cytoplasm to trivalent (III).Trivalent chromium, which interacts with proteins and nucleic acids, however, is far less soluble than hexavalent chromium and does not pass through biological membranes [15].

Bacterial reduction has been found in metallic minerals such as manganese, metal iron, mercury, selenite and tellurite. Some microbial transformations enable the bacteria to increase their tolerance toward toxic heavy metals. Chromium, a transition metal, is one of the major sources of environmental pollution. It is discharged into the environment through the disposal of wastes from industries like leather tanning, metallurgical and metal finishing, textiles and ceramics, pigment and wood preservatives, photographic sensitizer manufacturing etc. [16].

In the environment chromium occurs mainly in trivalent and hexavalent forms. The hexavalent chromium (Cr^{6+}) compounds are comparatively much more toxic than those of trivalent chromium (Cr^{3+}) [17]. There is only limited investigation for mining effluent treatment particularly Cr (VI) contaminants using microbial strains. Till date there is no literature cited on any molecularly identified and sequenced microbial species for chromium resistant and removal from Sukinda region. Thus our present study used potential indigenous microbial strains for treatment of industrial and mining effluent that may be suitable for biological treatment of Cr-contaminated waste of Sukinda mines. This study a remediation route for detoxification of Cr (VI) using an indigenous microorganism.

Bioremediation of Tannery Effluent by Using Microorganisms: Tannery effluents are a major source of aquatic pollution in India with chemical oxygen demand (COD), biological oxygen demand (BOD) and hexavalent chromium. Nearly 80% of the tanneries in India are engaged in the chrome tanning process. The tannery waste primarily consists of chromium and protein. Long term disposal of tannery wastes has resulted in extensive contamination of agricultural land and water sources in many parts of the India. There are more than 2500 tanneries in the country and nearly 80% of the tanneries are engaged in the chrome tanning process. In the process of tanning, chromium salts are used to convert hide to leather and the waste water generated is discharged into the environment which contains chromium salts in the excess of the maximum permissible limits. Tannery industry is one of the major industries in India. In tannery effluent, Cr (VI) is present as either dichromate ($Cr_2O_7^{2-}$) in acidic environment or as chromate (CrO_4^-) in alkaline environments [18].

Heavy metals exhibit toxic effects on soil biota and they can affect key microbial process and decrease the number and activity of soil microorganisms [19]. Metal contaminants are commonly found in soils, sediments and water. Metals pollutants can be produced through industrial processes such as mining, refining and electroplating. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. Metals can become toxic to many species. Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metals contamination. Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium and nickel have been identified and described in detail.

When looking at the microbial communities of metal-contaminated environments, it has been found that among the bacteria present, there is more potential for unique forms of respiration. Also, since the oxidation state of a metal ion may determine its solubility, many scientists have been trying to use microbes that are able to oxidize or reduce heavy metals in order to remediate metal-contaminated sites. Another implication of heavy metal tolerance in the environment is that it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure of the environment. Calomiris *et al.* [20] studied bacteria isolated from drinking water and found that a high percent of bacteria that are tolerant to metals are also antibiotic resistant.

Effluents from tannery, electroplating and electronic industries contain chromium which is highly toxic. This paper reported isolation of chromium tolerant microorganisms from solid waste as well as liquid effluent of an electroplating industry. Nine isolates were obtained that can tolerate chromium concentration up to 700 mg/L. They reached their stationary phase within 8-12 hours and can biosorbed 95% of initial 200 mg/L concentration of chromium within 4-10 hours. Fourier Transform Infra Red analysis of the biomass exposed to chromium indicated that amino and carboxylate groups in the biomass are involved in biosorption process. 16S RNA results of two most active organisms indicate that they are *Bacillus marisflavi* and *Arthrobacter* sp. and they show 98% and 96% homology similarity in the phylogenetic tree, respectively [21].

Bento *et al*. [22] observed that maximum chromium reduction occur at the optimum pH (7-9) and temperature (30°C) of growth by *Bacillus* sp. Ravibabu [23] studied the remediation of effluents using physical methods like activated charcoal, pH ranges and time periods and biological methods using the aquatic weed hyacinth as a biological pollutant removal.

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Microorganisms and microbial products can be highly efficient bioaccumulations of soluble and particulate forms of metals especially dilute external solutions. Microbes related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery. The present study deals with isolation, identification and characterization of heavy metal resistant bacteria was isolated from tannery effluent collected in and around Chennai, South India. Initially, a total of 50 isolates were screened from tannery effluent [24].

Chromium, a transition metal, is one of the major sources of environmental pollution. It is discharged into the environment through the disposal of wastes from industries like leather tanning, metallurgical and metal finishing, textiles and ceramics, pigment and wood preservatives, photographic sensitizer manufacturing etc. In the environment chromium occurs mainly in trivalent and hexavalent forms. The hexavalent chromium (Cr^{6+}) compounds are comparatively much more toxic than those of trivalent chromium (Cr^{3+}). The hexavalent chromium compounds are comparatively more toxic than trivalent chromium compounds due to their higher solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids [25]. Accordingly, chromium and its compounds are placed on the priority list of toxic chemicals by US EPA [26]. A maximum acceptable concentration of 0.05 mg/l for hexavalent chromium in drinking water has been established on basis of health considerations.

An *Arthrobacter* sp. and a *Bacillus* sp., isolated from a long-term tannery waste contaminated soil, were examined for their tolerance to hexavalent chromium [Cr(VI)] and their ability to reduce Cr (VI) to Cr (III), a detoxification process in cell suspensions and cell extracts. Both bacteria tolerated Cr (VI) at 100 mg/ml on a minimal salts agar medium supplemented with 0.5% glucose, but only *Arthrobacter* could grow in liquid medium at this concentration. *Arthrobacter sp*. could reduce Cr (VI) upto 50 µg/ml, while *Bacillus* sp. was not able to reduce Cr (VI) beyond 20 µg/ml. *Arthrobacter sp*. was distinctly superior to the *Bacillus* sp. in terms of their Cr (VI)-reducing ability and resistance to Cr (VI). Assays with permeabilized (treated with toluene or Triton X 100) cells and crude extracts demonstrated that the Cr (VI) reduction was mainly associated with the soluble protein fraction of the cell. *Arthrobacter* sp. has a great potential for bioremediation of Cr (VI) containing waste [27].

Cr (VI) (chromate) is a widespread environmental contaminant. Bacterial chromate reductases can convert soluble and toxic chromate to the insoluble and less toxic Cr (III). Bioremediation can therefore be effective in removing chromate from the environment, especially if the bacterial propensity for such removal is enhanced by genetic and biochemical engineering. Gram positive, chromium (Cr)-resistant bacterial strain (ATCC 700729) was isolated from effluent of tanneries. It was grown in media containing potassium dichromate concentration up to 80 mg ml^{-1} of the medium. The dichromate reducing capability of the bacterium was checked by estimating the amount of Cr (VI) in the medium before and after introduction of bacterial culture. The influence of factors like pH of the medium, concentration of Cr and the amount of the inoculum was studied to determine the ability of the bacterium to reduce Cr (VI) in the medium under various conditions. In a medium containing dichromate 20 mg ml^{-1} more than 87% reduction of dichromate ions was achieved within 72 hrs. The feasibility of the use of this bacterial strain for detoxification of dichromate in the industrial wastewater has been assessed. The isolated strain can be exploited for specific environmental clean-up operations [28].

Hexavalent chromium [Cr (VI)], is a toxic, water-soluble contaminant present in many soils and industrial effluents. Bacteria from various soils were examined for Cr (VI) resistance and reducing potential.

Microbes selected from both Cr (VI)-contaminated and non-contaminated soils and sediments were capable of catalyzing the reduction of Cr (VI) to Cr(III) a less toxic, less water-soluble form of Cr, demonstrating the utility of using a selection strategy for indigenous Cr(VI)-reducing bacteria in a bioprocess. As a result, indigenous Cr (VI)-reducing microbes from contaminated sites should provide the means for developing a bioprocess to reduce Cr (VI) to Cr (III) in non-sterile effluents such as those from soil washes. This approach also avoids the contamination problems associated with pure cultures of allochthonous microorganisms. In addition the apparent ubiquity of Cr (VI)-reducing bacteria in soil and sediments indicated the potential for in situ bioremediation of Cr (VI)-contaminated soils and ground water [29].

Viamajala *et al*. [30] studied the pollution effects of tannery water and reported that the salts of the tannery effluent percolates through the soil causing severe salinity to the land. Srinath *et al*. [31] found increased water soluble potassium, sulphur, iron, ammonium acetate, extractable iron and soil sulphur as well as traces of chromium in soils irrigated with tannery effluent. Sultan and Hasnain [32] observed a reduction in the micronutrient status with increasing concentrations of tannery effluent in soil (incubated for six months). The chromium level was also found to increase with increase in the concentration of the tannery effluent. Rajamani *et al*. [33] showed that chromates are absorbed in soils and low pH favoured their reduction in soils.

Megharaj *et al*. [34] isolated chromium resistant bacterial strain *Bacillus cereus* S-6 from effluents of tannery which was used for the reduction of toxic hexavalent chromium into less toxic trivalent chromium. At an initial hexavalent chromium concentration of 100 µg/ml, the cytosol and membrane preparation of the strain were able to reduce almost 67 and 43% of hexavalent chromium within 24 hrs incubation period while the heat killed cytosol and membrane preparation reduced 24 and 18% within the same time period. They reported that tannery effluent may change the characteristics of soil and interfere with the intake of water by plants. The presence of sulphide and chromium in tannery effluent affects plant life and soil productivity.

Many genera of microbes like *Bacillus, Enterobacter, Escherichia, Pseudomonas* and also some yeasts and fungi help in bioremediation of metals and chromium-contaminated soil and water by bioabsorption and bioaccumulation of chromium. The potential of bioremediation of metal toxicity and its impact on the environment was discussed [35]. Turick *et al*. [36] investigated several bacteria from various soils for hexavalent chromium resistance and reducing potential. Microbes selected from both hexavalent chromium-contaminated and non-contaminated soils and sediments were capable of catalyzing the reduction of hexavalent chromium to trivalent chromium a less toxic, less water-soluble form of chromium, demonstrating the utility of using a selection strategy for indigenous hexavalent chromium reducing bacteria in a bioprocess. As a result, indigenous hexavalent chromium reducing microbes from contaminated sites should provide the means for developing a bioprocess to reduce hexavalent chromium to trivalent chromium in non-sterile effluents such as those from soil washes.

The occurrence of metal tolerant and antibiotic resistant organisms was investigated in tannery effluent. Seventy-seven isolates comprising heterotrophs and coliforms which were tolerant to chromate level of >50µg/l were selected for detailed study. The majority of the coliforms were resistant to higher levels of chromate (200 µg/ml) whereas around 3% of the heterotrophs were resistant to Cr^{6+} at a level of >150 µg/l. All chromate tolerant heterotrophs were also tolerant to Cu^{2+} (100%) whereas only 58.53% coliforms were tolerant to Cu^{2+}. Except in the case of Cd^{2+} a higher number of heterotrophs were found tolerant to other heavy metals tested. Both groups of isolates were found sensitive to mercury. Resistance to cephaloridine was more abundant (P<0.001) in coliforms as compared to heterotrophs. On the other hand a significantly higher number (P<0.01) of heterotrophs showed resistance to streptomycin and carbencillin. All coliforms were sensitive to chloramphenicol. Around 80% and 31.70% of coliforms and heterotrophs exhibited a relationship to the combination of metals and antibiotics. Both heterotrophs and coliforms tolerant to Hg^{2+} were also resistant to polymixin-B [37].

Reduction of hexavalent chromium (chromate) to less-toxic trivalent chromium was studied by using cell suspensions and cell-free supernatant fluids from *Pseudomonas putida* PRS2000. Chromate reductase activity was associated with soluble protein and not with the membrane fraction. The crude enzyme activity was heat labile and showed a Km of 40, µM CrO_4^{2-} Neither sulfate nor nitrate affected chromate reduction either *in vitro* or with intact cells [38].

Bacterial strains (CrT-11, CrT-12, *Brevibacterium* sp. CrT-13, CrT-14) were isolated from the effluents of tanneries. All strains could resist very high concentration of K_2CrO_4 that is upto 40 mg ml^{-1} on nutrient agar and 25 mg ml^{-1} in nutrient broth. They have wide pH (5 to 9) and temperature (24 to 42°C) growth range. They exhibited multiple metals (Ni, Zn, Mn, Cu, Co and Pb) and antibiotics (streptomycin, ampicillin, tetracycline, kanamycin and chloramphenicol) resistances. All the strains were able to reduce Cr (VI) in to Cr (III) aerobically. *Brevibacterium* sp. CrT-13 accumulated and reduced more Cr (VI) at all the concentrations applied in comparison to the other strains. These bacterial strains also took up and reduced Cr (VI) present in industrial effluents and their reduction potential was not significantly affected in the presence of different metallic salts [39].

Several reports have indicated biological reduction of hexavalent chromium by microorganisms, both aerobes and anaerobes. Biological reduction of hexavalent chromium usually occurs at a neutral pH range and generates an insignificant quantity of chemical sludge as well as offers potential cost – effective remediation strategy [40]. Subsequent studies have shown that the capacity for hexavalent chromium reduction is widespread and is reported in organisms such as *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter, Eurydice, Micrococcus roseus and Escherichia coli* [41] as well as *Pseudomonas ambigua* [42], *Pseudomonas fluorescens* [43], *Enterobacter cloacae* [44], *Streptomyces* sp. [45], *Pseudomonas putida* [46], *D. desulfuricans* and *D. vulgaris* [47] and *Pseudomonas liquefaciens* [48].

Effect of Heavy Metals on the Growth of Bacteria: As chromium metal ion is present abundantly in the tannery effluents, the review in the present investigation focuses primarily on effect of chromium on the growth of bacteria. Germain and Patterson [49] inferred that wastewaters containing hexavalent chromium (chromate) are generated by many industries including the metal finishing industry, leather tanning, petroleum refining, textile manufacturing and pulp production. The chromium present in industrial wastes is primarily in the hexavalent form as chromate and dichromate. Petrilli and DeFlora [50] found that the hexavalent chromium is highly soluble in water while the reduced form of chromium, trivalent chromium, forms less soluble chromium hydroxides under non-acidic conditions. Hexavalent chromium is more toxic than trivalent chromium and has been shown to be mutagenic in a number of bacterial systems.

Flores and Perez [51] reported that about 800 acres of agricultural land in Dindigul have been seriously polluted by tannery effluents. All the fertile lands have become barren lands. Guha *et al*. [52] pointed out the characteristics of tannery effluent led into the land and said that the discharged effluents create severe land pollution. Jonnalagadda *et al*. [53] studied the pollution effects of tannery water and reported that the salts of the tannery effluent percolates through the soil causing severe salinity to the land. Komori *et al*. [54] assessed that effluents containing toxic chemicals have seeped into the arable land and agriculture has declined because of the effects of tannery effluent. With the declined with in productivity, the land value has also decreased. Lofroth and Ames [55] found increased water soluble potassium, sulphur, iron, ammonium acetate, extractable iron and soil sulphur as well as traces of chromium in soils irrigated with tannery effluent. Lovley and Phillips [56] showed that the experimental and epidemiological evidence exists for the carcinogenicity of some chromium compounds. Therefore chromium has been designated as a priority pollutant by US EPA.

Faisal and Hasnain [57] checked the multiple heavy metals (Ni, Zn, Mn, Cu, Co, Pb) and antibiotic resistances of *Brevibacterium* isolated from tannery effluents. Thacker and Parikh [58] identified that the *Brucella* sp. exhibit multiple heavy metals (Ni, Zn, Hg, Co, Pb) tolerance and resistance to various antibiotics. Middleton *et al*. [59] identified that *Ochrobactrum tritici* is resistant to chromium, nickel, cobalt, cadmium and zinc and able to grow in the presence of NaCl within the pH range of 4 -10.

Obbard [60] observed a reduction in the micronutrient status with increasing concentrations of tannery effluent in soil (incubated for six months). The chromium level was also found to increase with increase in the concentration of the tannery effluent. Park *et al*. [61] showed that chromates are absorbed in soils and low pH favoured their reduction in soils. Pedersen [62] reported that tannery effluent may change the characteristics of soil and interfere with the intake of water by plants. The presence of sulphide and chromium in tannery effluent affects plant life and soil productivity.

Chromium Adsorption by Bacteria: Srinath *et al.* [63] studied that the *Bacillus circulans* and *Bacillus megaterium* are able to bioaccumulate 34.5 and 32.0 mg chromium/g dry weight, respectively and brought the residual concentration of hexavalent chromium to the permissible limit in 24 hrs when the initial concentration was 50 mg hexavalent chromium/L. He stated that biosorption of hexavalent chromium was shown by *Bacillus megaterium* and another strain, *Bacillus* *coagulans*. Living and dead cells of *Bacillus coagulans* biosorbed 23.8 and 39.9 mg chromium/g dry weight, respectively, whereas, 15.7 and 30.7 mg chromium/g dry weight was biosorbed by living and dead cells of *Bacillus megaterium,* respectively.

Arvindhan and Madhan [64] assessed that the reduction of hexavalent chromium by intact cells and a cell-free extract of an actinomycete, *Arthrobacter crystallopoietes* isolated from soil contaminated with dichromate. Asatiani *et al*. [65] found that the *Arthrobacter* sp. could reduce hexavalent chromium upto 50 mg/ml, while *Bacillus* sp. was not able to reduce hexavalent chromium beyond 20 mg/ml. *Arthrobacter* sp. was distinctly superior to the *Bacillus* sp. in terms of their-hexavalent chromium reducing ability and resistance to hexavalent chromium. Assays with permeabilized (treated with toluene or Triton X 100) cells and crude extracts demonstrated that the reduction of hexavalent chromium is mainly associated with the soluble protein fraction of the cell.

Muhammed Faisal and Shahida Hasnain [66] demonstrated that the ability of *Brevibacterium* cells to accumulate toxic hexavalent chromium at different chromate concentrations (100, 500 and 1000 μg/ml) in different time intervals (15 min, 2 hours and 4 hours). They showed that the *Arthrobacter oxydans* does a complete uptake of hexavalent chromium concentration (35 mg/ml) in about 10 days.

Gonul Donmez and Nur Kocberber [67] isolated the microorganisms and their hexavalent chromium bioaccumulation capacities increased by enrichment procedure. At the end of the experiments, the highest specific chromium uptake was obtained at pH 8 as 109.45mgg^{-1} for 164.4mgl^{-1} initial hexavalent chromium concentration in the absence of NaCl. In the highest NaCl concentration the maximum specific chromium uptake was found at pH 9 (26.2mgg^{-1}) in samples with lower initial hexavalent chromium concentrations at pH 7 (as 87.5mgg^{-1}) for high initial hexavalent chromium concentrations.

Baldi *et al*. [68] studied the biosorption process for treatment of electroplating wastewater containing hexavalent chromium. They also explained that to treat chromium wastewaters is through the use of biomaterials as a low-cost adsorbent for chromium. Various biomaterials, such as dead biomass (of microalgae, seaweed, fungi and bacteria), agricultural waste biomass, industrial waste biomass and biomaterial-based containing activated carbons for the removal of chromium from aqueous solutions or wastewaters in a batch or column reactor system.

Rao *et al*. [69] investigated that the adsorption of hexavalent chromium ions from aqueous solutions on crude tamarind fruit shell, HCl treated and Oxalic acid treated shells at room temperatures The influence of different experimental parameters such as pH, effect of initial metal ion concentration and effect of dosage of adsorbent on biosorption were evaluated.

Chromium Adsorption by Fungi: Chromium and nickel are released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc. and the concentration levels of chromium and nickel in the environment widely varies. These two metals are of major concern because of their larger usages in developing countries and their non degradability nature. Hexavalent chromium is highly soluble in water and carcinogenic to human. Ni(II) is more toxic and carcinogenic metal when compared with Ni(IV). Due to their toxic effects on living systems stringent limits have been stipulated for the discharge of chromium and nickel into the environment. According to ISI: Bureau of Indian Standard (BIS) the industrial effluent permissible discharge level of Cr(VI) and Ni(II) into inland water is 0.1 and 3.0 mg L^{-1}, respectively. Gupta *et al*. [70] tested the tolerance and accumulation of hexavalent chromium by marine seaweed associated strains of *Aspergillus flavus* and *Aspergillus niger*. They revealed that both the isolates accumulated more than 25% of the chromium supplied. *Aspergillus flavus* invariably exhibited higher accumulation potential.

Igwe and Abia [71] found that the chromium was bioremoved from tannery industries effluent by *Aspergillus oryzae. Aspergillus oryzae* can grow in different concentration of chromium 120-1080 mg/L. They observed that maximum biomass growth and chromium removal rate at pH, 3.3, trivalent chromium concentration equal to 240 mg/L and inoculum size equal to 0.12% (dry weight) were 0.25 (dry weight) and 94.2%, respectively. They stated that bacterial strains were isolated and enriched from the contaminated site of Tamil Nadu Chromates and Chemicals Limited (TCCL) premises, Ranipet, Tamil Nadu, India. It was found that a bacterial concentration of 15+/-1.0 mg/g of soil (wet weight) 50 mg of molasses/g of soil as carbon source were required for the maximum hexavalent chromium reduction. The bioreactor operated at these conditions could reduce entire hexavalent chromium (5.6 mg /g of soil) in 20 days.

Srivastava and Thakur [72] explained the potency of *Aspergillus niger* evaluated in shake flask culture by absorption of chromium at pH 6, temperature 30°C. He inoculated *Aspergillus niger* in soil microcosm (40% moisture content) with different concentrations of chromate (250, 500, 1000, 1500 and 2000ppm), it removed more than 70% chromium in soil contaminated by 250 and 500ppm of chromate. However, chromium-contaminated soil (2000ppm of potassium chromate) mixed with compost (5% and 10%) significantly removed chromium in presence of fungus, *Aspergillus niger.*

Igwe and Abia [73] explained about the sorption behavior of some biosorbents with various heavy metals, their relative performance evaluated and a bioseperation process flow diagram for heavy metal removal from wastewater using biosorbents was proposed. Some biosorbents such as algae, fungi, bacteria have been investigated for their capacity towards heavy metals.

Chromium Reduction by Bacteria: Many industrial sites are contaminated with toxic trace metals which are then diverted into the environment, leading to pollution of surface and groundwater supplies. Heavy metals have a wide range of industrial applications such as electroplating, metal finishing or tanning and in mining industries. As a result, they are present in many industrial discharges. These heavy metals pose serious environmental implications as they remain mobilized in the food chain and are toxic to the biota [74]. Komori *et al*. [75] inferred that conventional methods for removing toxic chromium include chemical reduction followed by precipitation, ion exchange and adsorption on activated coal, alum, kaolinite and ash. However, most of these methods require high energy or large quantities of chemical reagents.

Jeyasingh and Philip [76] indicated that vast literature is available on chromate reduction by bacteria including physiological, biochemical and genetic aspects of chromate toxicity/resistance and reduction. Juliette Lamberta and Mohammed Rakib [77] explained that reduction of hexavalent chromium by the earthworm *Eisenia foetida*. He also suggested that *Eisenia foetida* play an important role during occasional hexavalent chromium pollution of soils. Kader *et al*. [78] explained that the technical process for removing trivalent chromium from tannery wastewater *via* precipitation.

Kamaludeen *et al*. [79] demonstrated that isolation of hexavalent chromium reducing anaerobes from hexavalent chromium contaminated and non contaminated environments. It provides the means for developing a bioprocess to reduce hexavalent chromium to trivalent chromium in non sterile effluents. This approach also avoids the contamination problems associated with pure cultures of allochthonous microorganisms. Kankal [80] added easily degradable organic substances of a very narrow C:N ratio and found marked hexavalent chromium reduction. Kapoor and Viraraghavan [81] investigated several bacteria from various soils for hexavalent chromium resistance and reducing potential. Microbes selected from both hexavalent chromium-contaminated and non contaminated soils and sediments were capable of catalyzing the reduction of hexavalent chromium to trivalent chromium a less toxic, less water-soluble form of chromium, demonstrating the utility of using a selection strategy for indigenous hexavalent chromium reducing bacteria in a bioprocess. As a result, indigenous hexavalent chromium reducing microbes from contaminated sites should provide the means for developing a bioprocess to reduce hexavalent chromium to trivalent chromium in non-sterile effluents such as those from soil washes.

Kapoor and Viraraghavan [82] reported that the reduction of hexavalent chromium to trivalent chromium decreases the toxicity and mobility of chromium contaminants in soils and water. In addition, the formation of a highly insoluble trivalent chromium product would decrease the likelihood of future trivalent chromium re-oxidation. Kapoor *et al*. [83] reported that chromium reduction by *Pseudomonas putida*. He explained the characterization of chromate reductase activities by a soluble protein fraction from *Pseudomonas putida.* Chromate reduction required either NADH or NADPH for maximum activity. Romanenko and Koren Ken [84] indicated that the early investigations demonstrate the facultative anaerobic bacteria such as *Pseudomonas dechromaticans, Pseudomonas chromatophila* and *Aeromonas dechromatica* remove hexavalent chromium from solution by the formation of a trivalent chromium precipitate.

Romanenko and Korenken [85] first reported that the *Aeromonas* sp. Capable of hexavalent chromium reduction >70% anaerobically. He also found that several facultative anaerobes tolerant to high levels of chromate (>400µg/ml) were isolated from tannery effluents. Laxman and More (2001) [86] found that chromate is a oxidizing agent that is reduced intracellularly to pentavalent chromium and reacts with nucleic acids and other cell components to produce mutagenic and carcinogenic effects on biological systems. LilianaMorales-Barrera and EliseoCristiani-Urbina [87] assessed that hexavalent chromium reduction to pentavalent chromium is responsible for chromate toxicity, further reduction to trivalent chromium leads to the formation of stable, less soluble and less toxic trivalent chromium. Reduction of hexavalent chromium to trivalent chromium is therefore a potentially useful process for remediation of hexavalent chromium affected environments.

Luef *et al*. [88] observed the microbial reduction of toxic hexavalent chromium has practical importance, because biological strategies provide green technology that is cost-effective. Mahvi *et al*. [89] inferred that the recovery of chromium III from tannery wastewater by using three aqueous oxidants, Hydrogen peroxide, Sodium Hypochlorite and Calcium Hypochlorite were independently oxidizing to soluble chromate under alkaline conditions. Megharaj and Naidu [90] highlighted that amino acid mixtures are the best electron donars for hexavalent chromium reduction. Infact, in one study, the number of viable cells decreased in the initial stages after hexavalent chromium was added and 50% of the added hexavalent chromium was reduced before viable cell numbers increased over what was present prior to hexavalent chromium addition. Modak *et al*. [91] stated that organic matter content and bioactivity were important factors in reducing almost 96% of the added hexavalent chromium under aerobic, field moist conditions. It suggests that organic amended soils can readily reduce hexavalent chromium and could promote excellent removal efficiency.

Rathinam Aravindhan *et al*. [92] demonstrated that the biological removal of carcinogenic hexavalent chromium using mixed *Pseudomonas* strains. Under optimal conditions, 100mg/L of hexavalent chromium was completely reduced within 180 min. Puranik and Paknikar [93] found that the hexavalent chromium was reduced by gram negative bacteria *Providencia* sp. It reduced chromate to 100% at a concentration ranging from 100-300 mg/L. It also exhibited multiple heavy metal tolerance. Quintelas and Fernandes [94] assessed that the low temperature reduction of hexavalent chromium by a *Arthrobacter aurescens*.

Urvashi Thacker and Rasesh Parikh [95] assessed that the reduction of chromate by cell-free extract of *Brucella* sp. Isolated from hexavalent chromium contaminated sites. High hexavalent chromium concentration resistance and high hexavalent chromium reducing ability of the strain make it a suitable candidate for bioremediation. Shaili Srivastava and Indu Shekhar Thakur [96] studied the relationship between the hexavalent chromium resistance of the culturable microbial community and the hexavalent chromium resistance and reducing ability strains of each population. Shaili Srivastava and Indu Shekhar Thakur [97] isolated chromium resistant bacterial strain *Bacillus cereus* S-6 from effluents of tannery was used for the reduction of toxic hexavalent chromium into less toxic trivalent chromium. At an initial hexavalent chromium concentration of 100 μg/mL, the cytosol and membrane preparation of the strain were able to reduce almost 67 and 43% of hexavalent chromium within 24 hrs incubation period while the heat killed cytosol and membrane preparation reduced 24 and 18% within the same time period.

Shikha Rastogi and Saxene [98] concluded that *Pseudomonas fluorescens* LB 300, reduces hexavalent chromium while growing aerobically glucose medium, also grows in an anaerobic chamber containing oxygen-free nitrogen on agar plates containing acetate as a potential electron donor and hexavalent chromium as the potential electron acceptor. However, no hexavalent chromium reduction occurs in anaerobic liquid cultures. Siegel *et al*. [99] demonstrated that *Enterobacter cloacae* strain HO1 reduces hexavalent chromium while growing under anaerobic conditions in a medium that contains acetate and amino acids as a potential electron donars. *Enterobacter cloacae* can grow anaerobically in the absence of added hexavalent chromium and no evidence for hexavalent chromium depended growth has been presented.

Srinath *et al*. [100] found that some organism reduce hexavalent chromium during anaerobic growth in media in which hexavalent chromium is provided as the sole electron acceptor, in no instance has hexavalent chromium reduction definitely been shown to yield energy to support anaerobic growth. For example, *Pseudomonas chromatophila* uses hexavalent chromium as an electron acceptor to support growth under anaerobic conditions with a variety of electron donars, including the non-fermentable substrate, acetate.

Saranraj *et al*. [101] isolated a bacterial strain from tannery effluent and identified it as *Enterococcus casseliflavus.* It showed a high level resistance of 800 μg/ml chromium. The minimal inhibitory concentration of chromium was found to be 512 μg/ml of potassium dichromate in Nutrient broth medium. The chromium adsorption was more significant by the live cells than killed cells at different time intervals. It was observed that, the inoculation of *Enterococcus casseliflavus* reduced the BOD and COD values of tannery effluent. The maximum adsorption of chromium was at a temperature of 35 to 45°C and at a pH of 7.0 to 7.5.

Subsequent studies have shown that the capacity for hexavalent chromium reduction is widespread and is reported in organisms such as *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter Eurydice, Micrococcus roseus* and *Escherichia coli* [102] as well as *Pseudomonas ambigua* [103], *Pseudomonas fluorescens* [104], *Enterobacter cloacae* [105], *Streptomyces* sp. [106], *Pseudomonas putida* [107], *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* [108] and *Pseudomonas liquefaciens* [109].

Chromium Reduction by Fungi: Filamentous fungi can be profitably used in processes for heavy metals removal from wastewater due to their low cost and to the high ion exchange capacity of their cell walls. This property arises from the large density of functional groups present in the cell wall (carboxyl, hydroxyl, amine, phosphoryl, sulfhydryl), creating a negatively charged surface [110]. The sorption properties of cell have been widely studied by solution chemistry [111], but the chemical nature of complexing groups is not known. The nature of Pb binding sites on the cell walls of the filamentous fungus *Penicillium chrysogenum* was investigated at the macroscopic level by sorption isotherm and at the molecular level by extended X-ray absorption free structure (EXAFS) spectroscopy by varying the metal concentration by two orders of magnitude, down to 4.8 10.3 m mol Ph/g.

ShailiSrivastava and InduShekharThakur [112] isolated the *Aspergillus niger* from soil and effluent of leather tanning mills had higher activity to remove chromium. He also indicated that removal of more than 75% chromium by *Aspergillus niger* determined by diphenylcarbazide colorimetric assay and atomic absorption spectrophotometry after 7 days.

LilianaMorales-Barrera and EliseoCristiani-Urbina [113] identified that hexavalent chromium was removed by a *Trichoderma inhamatum* fungal strain isolated from Tannery effluent. And the fungus exhibited a remarkable capacity to tolerate and completely reduced hexavalent chromium concentrations up to 2.43mM. He indicated that the *Trichoderma inhamatum* fungal strain may have potential applications in bioremediation of hexavalent chromium contaminated wastewaters.

Properties of the Tannery Effluents: Bioremediation has developed from the laboratory to a fully commercialized technology over the last 30 years in many industrialized countries. A successful bioremediation scheme relies on the management of soil microbial populations capable of catabolizing the contaminants. Heavy metals exhibit toxic effects on soil biota and they can affect key microbial processes and decrease the number and activity of soil microorganisms [114]. Microbial population has often been proposed to be an easy and sensitive indicator of anthropogenic effects on soil ecology. Cr (VI) has been reported to cause shifts in the composition of soil microbial populations and known to cause detrimental effects on microbial cell metabolism at high concentrations. Quite a few studies on soil contamination of heavy metal from industrial sites were reported [115].

Since, the discovery of the first microbe capable of reducing Cr^{6+} in the 1970s [116], the search for Cr^{6+}-reducing microorganisms (both aerobic and anaerobic) has been enthusiastically pursued, with numerous strains being isolated. Based on recent isolation and purification of Cr^{6+} reductases from aerobic bacteria and the fact that the process involved in Cr^{6+} reduction occurring under anaerobic conditions is starting to be understood, biological processes for treating chromium contaminated sites are becoming very promising. Some of the emerging technologies for the mitigation and remediation of Cr (VI) include microbial strategies for *in situ* and on-site bioremediation strategies and use in permeable reactive barriers.

Discovery of microorganisms capable of reducing Cr (VI) to Cr (III) have significant potential in development of *in situ* or on-site bioremediation strategies. In 1977, the first reported bacterial strains, *Pseudomonas,* were isolated from chromate (CrO_4^{2-} contaminated sewage sludge by Russian scientists N.A. Romanenko and V. Korenkov. Since 1977, several other CrO42- reducing strains have been reported, including other strains such as *B. cereus, B. subtilis, P. aeruginosa, P. ambigua, P. fluorescens, E. coli, Achromobacter eurydice, Micrococcus roseus, Enterobacter cloacae, Desulfovibrio desulfuricans* and *D. vulgaris* [117]. A number of bacteria in other genera, *viz*., *Bacillus s*pp., *E. coli* ATCC 33456, *Shewanella* alga BrY-MT and a few unidentified strains have also been shown to reduce Cr^{6+}[118]. Terry Beveridge [119] worked on isolation and characterization of a chromium reducing bacterium from a chromated copper arsenate contaminated site. Reports conclude a Gram-negative bacterium (CRB5) isolated from a chromium-contaminated site that was capable of reducing hexavalent chromium to an insoluble precipitate, thereby removing this toxic chromium species from solution.

Laxman and More [120] found that the effect of pH and temperature on maximum chromium reduction occurred in pH range of 6-7 and 37°C -50°C by *Streptomyces griseus*. Zainul Akmar Zakaria *et al*. [121] observed that maximum chromium reduction occur at the optimum pH (7 – 9) and temperature (30°C) of growth by *Bacillus* sp. Ravibabu [122] studied the remediation of effluents using physical methods like activated charcoal, pH ranges and time periods and biological methods using the aquatic weed hyacinth as a biological pollutant removal.

Yoshinobu Ishibashi and Simon Silver [123] explained about the various industrial discharges of toxic chemical pollutants requires biological treatment. He showed a permissible reduction of BOD (80%-90%) for both paper mill and dying industry effluents. Duangporn Kantachote *et al*. [124] identified the reduction of biological oxygen demand in tannery effluents from 4967 mg/L to 1010 mg/ml after inoculation of *Rhodopseudomonas blastica.* He also found the reduction of chemical oxygen demand from 7328 mg/L to 3371 mg/L in tannery effluents.

Young Hak Kwak and Han Bok Kim [125] demonstrated that the maximum amount of biomass growth and chromium removal rate occurred at pH 5 and were 0.35% (Dry weight) and 96.6%, respectively. And the enzymatic activities of *Aspergillus oryzae* at pH 5 was very suitable in which the living cell of fungi were able to grow significantly. He also observed that the maximum biomass growth and chromium removal rate was achieved at 30°C and were 0.29% (Dry weight) and 96.9%, respectively. While decreasing temperature below 24°C decreased fungal growth and enzymatic activity. Furthermore, increasing the temperature up to about 40°C, decreased the fungal growth and consequently the chromium removal extent.

Abubacker and Ramanathan [126] found that another approach was molecular techniques used to genetically engineer plants that could hyperaccumulate chromium and other heavy metals. In a recent study, it was found that there was a high correlation in chromium content in shoots of many plant species with the content of other heavy metals such as cadmium, copper, nickel and zinc. He stated that accumulations of these heavy metals in plant shoots were found to be associated. Using recent molecular approaches, it will be possible to evolve plants suitable for phytoremediation of soils and waters contaminated with chromium and other heavy metals.

Jonnalagadda Raghava Rao [127] reported biological removal of carcinogenic chromium (VI) using mixed *Pseudomonas* strains. In this study an aerobic reduction of Cr (VI) to Cr (III) by employing mixed *Pseudomonas* cultures isolated from a marshy land has been reported. The role of chromium concentration, temperature, pH and additives on the microbial reduction of Cr (VI) has been investigated. NADH was found to enhance the rate of reduction of Cr (VI). Complete reduction of Cr (VI) has been possible even at Cr (VI) concentrations of 300 ppm.

Sadeeshkumar *et al*. [128] studied that the removal of COD from tannery wastewater is attractive for the betterment of environment. Tanning wastewater containing high COD (3413 mg/ml) was oxidized with aqueous oxidants *i.e.* Hydrogen peroxide, Sodium Hypochlorite and Calcium Hypochlorite at different temperatures and reaction durations.

REFERENCES

1. Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Environment Contamination and Toxicology, 75: 699-706.
2. Cheung, K.H. and J.D. Gu, 2005. Reduction of chromate by an enrichment consortium and an isolate of marine sulphate-reducing bacteria. Chemosphere, 52: 1523-1529.
3. Thacker, U. and M. Datta, 2006. Reduction of toxic chromium and partial localization of chromium reductase activity in bacterial isolate DM1, World Journal of Microbiology and Biotechnology, 21: 891-899.
4. Park, D., Y.S. Yun, J.H. Jo and J.M. Park, 2000. Biosorption process for treatment of electroplating wastewater containing Cr (VI): Laboratory-scale feasibility test. Industrial Engineering and Chemical Research, 45(14): 5059-5065.
5. Megharaj, M., S. Avudainayagam and R. Naidu, 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology, 47: 51-54.
6. Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Environment Contamination and Toxicology, 75: 699-706.
7. Camargo, F.A.O., F.M. Bento, B.C. Okeke and W. T. Frankenberger, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate. Journal of Environmental Quality, 32: 1228-1233.

8. Camargo, F.A.O., F.M. Bento, B.C. Okeke and W. T. Frankenberger, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate. Journal of Environmental Quality, 32: 1228-1233.
9. Kapoor, A. and T. Viraraghavan, 1997. Heavy Metal Biosorption sites in Aspergillus niger. Bioresource Technology, 61: 221-227.
10. Niu H., X. Xu and J.H. Wang, 1993. Removal of lead from aqueous solutions by penicillin biomass. Biotechnology and Bioengineering, 42: 785-787.
11. Nourbakhsh, M., Y.S ag, D. Ozer, Z. Aksu, T. Katsal and A. Calgar, 1994. A comparative study of various Biosorbents for Removal of Chromium (VI) ions from Industrial wastewaters. Process Biochemistry, 29: 1-5.
12. Gill, R.K., V. Jindal, S.S. Gill and S.S. Marwaha, 1996. Studies on the biosorption of nickel from industrial effluent. Pollution Research, 15(3): 303-306.
13. Smith, W.A., W.A. Apel, J.N. Petersen and B.M. Peyton, 2002. Effect of carbon and energy source on bacterial chromate reduction. Bioremediation Journal, 6: 205-215.
14. Viamajala, S., B.M. Peyton, R.K. Sani, W.A. Apel and J.N. Petersen, 2004. Toxic effects of chromium (VI) on anaerobic and aerobic growth of Shewanella oneidensis MR-1. Biotechnology, 20: 87-95.
15. Costa, M., 2003. Potential hazards of hexavalent chromate in our drinking water. Toxicology and Applied Pharmacology, 188: 1-5.
16. Desh Deepak and Ajay Kumar Gupta, 1991. Cr VI removal from wastewater. Indian Journal of Environmental Health, 33(3): 297-305.
17. Schulin, R., 2007. Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria). Geoderma, 140: 52-61.
18. Srinath, T., P.W. Verma, P. Ramteke and S.K. Garg, 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Tannery Technology, 48 (4): 427-435.
19. Obbard, P., 2001. Ecotoxicological assessment of heavy metals in sewage sludge amended soils. Journal of Applied Geochemistry, 16: 1405-1411.
20. Calormiris, J., J.L. Armstrong and R.J. Seidler, 1984. Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. Applied Environmental Microbiology, 47(6): 1238-1242.
21. Park, D., Y.S. Yun, J.H. Jo and J.M. Park, 2000. Biosorption process for treatment of electroplating wastewater containing Cr (VI): Laboratory-scale feasibility test. Industrial Engineering and Chemical Research, 45(14): 5059-5065.
22. Bento, G. and K. Okeke, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate. Journal of Environment Quality, 32: 1228-1233.
23. Ravibabu, C.H., K. Nagaveni and N. Jamil, 2007. The toxic effects industrial influence on rats: Analysis and remediation methods. International Journal of Toxicology, 3(2): 45-47.
24. Flores, A. and J.M. Perez, 1999. Cytotoxicity, apoptosis and in vitro DNA damage induced by potassium chromate. Toxicology and Applied Pharmacology, 161: 75-81.
25. Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a Gram positive bacterium isolated from effluent of dying industry. Environment Contamination and Toxicology, 75: 699-706.
26. Cheung, K.H. and J.D. Gu, 2005. Reduction of chromate by an enrichment consortium and an isolate of marine sulphate-reducing bacteria. Chemosphere, 52: 1523-1529.
27. Megharaj, M., S. Avudainayagam and R. Naidu, 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology, 47: 51-54.
28. Thacker, U. and M. Datta, 2006. Reduction of toxic chromium and partial localization of chromium reductase activity in bacterial isolate DM1, World Journal of Microbiology and Biotechnology, 21: 891-899.
29. Park, D., Y.S. Yun, J.H. Jo and J.M. Park, 2000. Biosorption process for treatment of electroplating wastewater containing Cr(VI): Laboratory-scale feasibility test. Industrial Engineering and Chemical Research, 45(14): 5059-5065.
30. Viamajala, S., B.M. Peyton, R.K. Sani, W.A. Apel and J.N. Petersen, 2004. Toxic effects of chromium (VI) on anaerobic and aerobic growth of Shewanella oneidensis MR-1, Biotechnology, 20: 87-95.
31. Srinath, T., P.W. Verma, P. Ramteke and S.K. Garg, 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Tannery Technology, 48(4): 427-435.

32. Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Environmental Contamination and Toxicology, 75: 699-706.
33. Rajamani, S., T. Ramasami, J.S.A. Langerwerf and J.E. Schappman, 1995. Environment management in tanneries feasible chromium recovery and reuse system. In proceedings of 3rd international conference on appropriate waste management technologies for developing countries. Nagpur, 965-973.
34. Megharaj, M., S. Avudainayagam and R. Naidu, 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology, 47: 51-54.
35. Bento, H. and O. Okeke, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate. Journal of Environmental Quality, 32: 1228-1233.
36. Turick, M., F. Edwards and W. Carmiol, 1996. Isolation of Cr VI reducing anaerobes from Cr contaminated and non-contaminated areas. Applied Biotechnology and Microbiology, 44(5): 683-688.
37. Camargo, F.A.O., F.M. Bento, B.C. Okeke and W.T. Frankenberger, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate, Journal of Environmental Quality, 32: 1228-1233.
38. Cheung, K.H. and J.D. Gu, 2010. Reduction of chromate by an enrichment consortium and an isolate of marine sulphate-reducing bacteria. Chemosphere, 52: 1523-1529.
39. Costa, M., 2003. Potential hazards of hexavalent chromate in our drinking water. Toxicology and Applied Pharmacology, 188: 1-5.
40. Donati, E., C. Oliver and G. Curutchet, 2003. Reduction of chromium (VI) by the indirect action of Thiobacillus thioparus. Brazil Journal of Chemical Engineering, 20(1): 1999-2005.
41. Flores, A and J.M. Perez, 1999. Cytotoxicity, apoptosis and in vitro DNA damage induced by potassium chromate. Toxicology and Applied Pharmacology, 161: 75-81.
42. Guha, H., K. Jayachandran and F. Maurrasse, 2001. Kinetics of chromium (VI) reduction by a type strain Shewanella alga under different growth conditions. Environmental Pollution, 115: 209-218.
43. Jonnalagadda, R.R., A. Rathinam, J.S. Kalarical and U.N. Balachandran, 2007. Biological removal of carcinogenic chromium (VI) using mixed Pseudomonas strains. Journal of General and Applied Microbiology, 53(2): 71-91.
44. Komori, K.A., A. Rivas, K. Toda and H. Ohtake, 1990. A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of Enterobacter cloacae. Applied Microbiology and Biotechnology, 33: 117-119.
45. Romanenko, V.I. and V.N. Korenkov, 1977. A pure culture of bacterial cells assimilating chromates and bichromates as hydrogen acceptors when grown under anaerobic conditions. Mikrobiologiya, 46: 414-417.
46. Schulin, R., 2007. Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria). Geoderma, 140: 52-61.
47. Lovley, D.R. and E.J.P. Phillips, 1994. Reduction of chromate by Desulfovibrio vulgaris and its C3 cytochrome. Applied Environmental Microbiology, 60: 726-728.
48. Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a Gram positive bacterium isolated from effluent of dying industry. Environmental Contamination and Toxicology, 75: 699-706.
49. Turick, J. and R. Carmiol, 1996. Isolation of Cr VI reducing anaerobes from Cr contaminated and non contaminated areas. Applied Biotechnology and Microbiology, 44(5): 683-688.
50. Beszedits, S., 1988. Chromium removal from industrial wastewaters, In: J.O. Nriagu and E. Nieboer (Eds.), Chromium in the Natural and Human Environments, John Wiley, New York, pp: 232-263.
51. Flores, A. and J.M. Perez, 1999. Cytotoxicity, apoptosis and in vitro DNA damage induced by potassium chromate. Toxicology and Applied Pharmacology, 161: 75-81.
52. Guha, H., K. Jayachandran and F. Maurrasse, 2001. Kinetics of chromium (VI) reduction by a type strain Shewanella alga under different growth conditions. Environmental Pollution, 115: 209-218.
53. Jonnalagadda, R.R., A. Rathinam, J.S. Kalarical and U.N. Balachandran, 2007. Biological removal of carcinogenic chromium (VI) using mixed Pseudomonas strains. Journal of General and Applied Microbiology, 53(2): 71-91.

54. Komori, K.A., A. Rivas, K. Toda and H. Ohtake, 1990. A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of Enterobacter cloacae. Applied Microbiology and Biotechnology, 33: 117-119.
55. Lofroth, G. and B.N. Ames, 1997. Mutagenicity of inorganic compounds in Salmonella typhimurium: arsenic, chromium and selenium. Mutation Research, 53: 65-66.
56. Lovley, D.R and E.J.P. Phillips, 1994. Reduction of chromate by Desulfovibrio vulgaris and its C3 cytochrome. Applied Environmental Microbiology, 60: 726-728.
57. Muhammed Faisal and Shahida Hasnain. 2004. Microbial conversion of Cr VI to Cr III in industrial effluent. African Journal of Biotechnology, 3: 610-617.
58. Urvashi Thacker and Rasesh Parikh, 2006. Reduction of chromate by cell-free extract of Brucella sp. isolated from Cr VI contaminated sites. Bioresource Technology, 98: 1541-1547.
59. Middleton, S.S., R.B. Latmani, M.R. Mackey, M.H. Ellisman, B.M. Tebo and C.S. Criddle, 2003. Co-metabolism of Cr (VI) by Shewanella oneidensis MR-1 produces cell-associated reduced chromium and inhibits growth. Biotechnology and Bioengineering, 83: 627-637.
60. Obbard, P., 2001. Ecotoxicological assessment of heavy metals in sewage sludge amended soils. Journal of Applied Geochemistry, 16: 1405-1411.
61. Park, D., Y.S. Yun, J.H. Jo and J.M. Park, 2000. Biosorption process for treatment of electroplating wastewater containing Cr(VI): Laboratory-scale feasibility test. Industrial Engineering and Chemical Research, 45(14): 5059-5065.
62. Pedersen, N.B., 1982. The effects of chromium on the skin. In: S. Langards, editor. Biological and environmental aspects of chromium. Amsterdam: Elsevier Biomedical Press, pp: 249-75.
63. Srinath, T. Verma, P.W. Ramteke and S.K. Garg, 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Tannery Technology, 48(4): 427-435.
64. Arvindhan, S. and M. Madhan, 2004. Bioaccumulation of Cr from tannery wastewater. Journal of American Leather Chemist Association, 1: 197-203.
65. Asatiani, Z., N. Abuladze and H. Lejava, 2004. Effect of chromium VI action on Arthrobacter oxydans. Journal of Current Microbiology, 49(5): 321-326.
66. Muhammed Faisal and Shahida Hasnain, 2004. Microbial conversion of Cr VI to Cr III in industrial effluent. African Journal of Biotechnology, 3: 610-617.
67. Gonul Donmez and Nur Koçberber, 2005. Bioaccumulation of hexavalent chromium by enriched microbial cultures obtained from molasses and NaCl containing media. Journal of Process Biochemistry, 40(7): 2493-2498.
68. Franco Baldi, Ann M. Vaughan and Gregory J. Olson, 1990. Chromium(VI)-resistant yeast isolated from a sewage treatment plant receiving tannery wastes. Journal of Applied and Environmental Microbiology, 56(4): 913-918.
69. Srinivasa Rao, Popuri Ajithapriya and Jammala Krishnaiah Abburi, 2007. Biosorption of hexavalent chromium using tamarind (Tamarindus indica) fruit shell-a-comparative study. Electronic Journal of Biotechnology, 10(3): 69-75.
70. Gupta, R., P. Ahuja, S. Khan, R.K. Saxena and H. Mohapata, 2000. Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Current Science, 78(8): 967-973.
71. Igwe, J.C. and A.A. Abia, 2006. A bioseparation process for removing heavy metals from waste water using biosorbents. African Journal of Biotechnology, 5(12): 1167-1179.
72. Shaili Srivastava and Indu Shekhar Thakur, 2006. Evaluation of bioremediation and detoxification potentiality of Aspergillus niger for removal of hexavalent chromium in soil microcosm. Journal of Soil Biology and Biochemistry, 38(7): 1904-1911.
73. Igwe, J.C. and A.A. Abia, 2006. A bioseparation process for removing heavy metals from waste water using biosorbents. African Journal of Biotechnology, 5(12): 1167-1179.
74. Butter. T.J., L.M. Evison, I.C. Hancock, F.S. Holland and K.A. Matis, 1996. Removal and recovery of heavy metals from dilute aqueous streams by biosorption and electrolysis. Medical Genetics, 61(4b): 1863-1870.
75. Komori, K.A., A. Rivas, K. Toda and H. Ohtake, 1990. A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of Enterobacter cloacae. Applied Microbiology and Biotechnology, 33: 117-119.
76. Jeyasingh, J. and L. Philip, 2005. Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. Journal of Hazardous Materials, 118: 113-120.

77. Juliette Lamberta and Mohammed Rakib, 2006. Treatment of solutions containing trivalent chromium by electrodialysis. Journal of Ecobiotechnology, 34 (7): 100-106.
78. Kader, P., O. Sannasi, B.S. Othman, B. Ismail and B. Salmijah, 2007. Removal of Cr(VI) from Aqueous Solutions by Growing and Non-growing Populations of Environmental Bacterial Consortia. Global Journal of Environmental Research, 1(1): 12-17.
79. Kamaludeen, S.P., M. Megharaj, A.L. Juhasz, S. Sethunathan and N. Naidu, 2003. Chromium-microorganism interactions in soils: remediation implications. Journal of Environmental Toxicology, 178: 93-164.
80. Kankal, G., 1987. Dissolved oxygen and detention time as process parameters in sewage treatment by created lagoon. Recent Advances in Science and Technology, 14: 61-64.
81. Kapoor, A. and T. Viraraghavan, 1997. Heavy Metal Biosorption sites in Aspergillus niger. Bioresource Technology, 61: 221-227.
82. Kapoor, A. and T. Viraraghavan, 1998. Biosorption of heavy metals on Aspergillus niger: effect of pretreatment. Bioresource Technology, 63: 109-113.
83. Kapoor, A., T. Viraraghvan and D. Roy Cullimore, 1999. Removal of heavy metals using Aspergillus niger. Bioresource Technology, 70: 95-104.
84. Krantz-Rulcker, C., B.A. Uard and J.H. Ephraim, 1994. Environmental Science and Technology, 28: 1502-1505.
85. Romanenko, V.I. and V. N. Korenken, 1999. A pure culture of bacterial cells assimilating chromates and bichromates as hydrogen acceptors when grown under anaerobic conditions. Mikrobiologiya, 46: 414-417.
86. Laxman, S. and K. More, 2002. Reduction of hexvalent chromium by Streptomyces griseus. Journal of Environmental Toxicology, 27(4): 135-141.
87. Liliana Morales-Barrera and Eliseo Cristiani-Urbina, 2008. Hexavalent Chromium Removal by a Trichoderma inhamatum Fungal Strain Isolated from Tannery Effluent. Journal of Earth and Environmental Science, 187(4): 233-239.
88. Luef, E., T. Prey and C.P. Kubicek, 1991. Biosorption of Zinc by Fungal mycelial waste. Applied Microbiology and Biotechnology, 34: 688-692.
89. Mahvi, R. Nabizadeh, F. Gholami and A. Khairi, 2007. Adsorption of chromium from wastewater by Platanus orientalis leaves. Iranian Journal of Environmental Health, Science and Engineering, 4(3): 191-196.
90. Megharaj, L. and M. Naidu, 2003. Toxicity of Cr VI and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiology, 47(1): 51-54.
91. Modak, J.M., K.A. Natarajan and B. Saha, 1996. Biosorption of copper and zinc using waste Aspergillus niger biomass. Minerals and Metallurgy Process, 13(2): 52 -57.
92. Rathinam Aravindhan and Balachandran Unni Nair, 2006. Biological removal of carcinogenic Cr VI using mixed Pseudomonas strains. Bioresource Technology, 11(3): 11-17.
93. Puranik, P.R. and K.M. Paknikar, 1999. Biosorption of lead, cadmium and zinc by Citrobacte strain MCM B-181: characterization studies. Biotechnology Progress, 15: 228-237.
94. Quintelas, J. and M. Fernandes, 2012. Biosorption of Cr (VI) by an Escherichia coli Biofilm Supported on GAC. Journal of Applied Sciences, 34(6): 223-231.
95. Urvashi Thacker and Rasesh Parikh, 2007. Reduction of chromate by cell-free extract of Brucella sp. isolated from Cr VI contaminated sites. Bioresource Technology, 98: 1541-1547.
96. Shaili Srivastava and Indu Shekhar Thakur, 2006. Biosorption potency of Aspergillus niger for removal of chromium (VI). Journal of Biomedical and Life Sciences, 53(3): 232-237.
97. Shaili Srivastava and Indu Shekhar Thakur, 2007. Evaluation of biosorption potency of Acinetobacter sp. for removal of hexavalent chromium from tannery effluent. Journal of Earth and Environmental Science, 18(5): 637-646.
98. Shikha Rastogi and Saxene, 2003. BOD analysis of industrial effluents: 5 days to 5 min. Journal of Current Applied Physics, 2(3): 191-194.
99. Siegel, S.M., M. Balun and B.Z. Siegel, 1996. Water, Air and Soil Pollution, 53: 335-344.
100. Srinath Verma, P.W. Ramteke and S. K. Garg, 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Tannery Technology, 48(4): 427-435.
101. Saranraj, P., D. Stella, D. Reetha and K. Mythili, 2010. Bioadsorption of chromium resistant Enterococcus casseliflavus isolated from tannery effluent. Journal of Ecobiotechnology, 2(7): 17-22.
102. Sudha Nayar and P. Ramasami, 1985. Bacterial accumulation of chromium. Journal of Leather Science, 32: 88-90.
103. Sumit Yadav, O.P. Shukla and U.N. Rai, 2005. Chromium Pollution and Bioremediation. Journal of Environmental Pollution, 11(1): 534-538.

104. Thiruneelakantan Srinath and Shilpi Khare, 2001. Isolation of hexavalent chromim-reducing Cr-tolerant facultative anaerobes from tannery effluent. Journal of General Applied Microbiology, 47: 307-312.
105. Turick, M. and C. Carmiol, 1996. Isolation of Cr VI reducing anaerobes from Cr contaminated and non contaminated areas. Applied Biotechnology and Microbiology, 44(5): 683-688.
106. Urvashi Thacker and Rasesh Parikh, 2007. Reduction of chromate by cell-free extract of Brucella sp. isolated from Cr VI contaminated sites. Bioresource Technology, 98: 1541-1547.
107. Vidal Nieto, K. Cooman, M. Gajardo and C. Bornhardt, 2004. Unhairing effluents treated by an activated sludge system. Journal of Hazardous Materials, 112: 143-149.
108. Lovley, D.R. and E.J.P. Phillips, 1994. Reduction of chromate by Desulfovibrio vulgaris and its C3 cytochrome. Applied Environmental Microbiology, 60: 726-728.
109. Krantz-Rulcker, C., B.A. Uard and J.H. Ephraim, 1994. Environmental Science and Technol., 28: 1502-1505.
110. Schulin, R., 2007. Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria). Geoderma, 140: 52-61.
111. Wase, J. and C. Forster, 1997. Biosorbents for metal ions. London: Taylor & Francis.
112. Shaili Srivastava and Indu Shekhar Thakur, 2006. Biosorption potency of Aspergillus niger for removal of chromium (VI). Journal of Biomedical and Life Sciences, 53(3): 232-237.
113. Liliana Morales-Barrera and Eliseo Cristiani-Urbina, 2008. Hexavalent Chromium Removal by a Trichoderma inhamatum Fungal Strain Isolated from Tannery Effluent. Journal of Earth and Environmental Science, 187(4): 233-239.
114. Obbard, P., 2001. Ecotoxicological assessment of heavy metals in sewage sludge amended soils. Journal of Applied Geochemistry, 16: 1405-1411.
115. Schulin, R., 2007. Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria). Geoderma, 140: 52-61.
116. Romanenko, V.I. and V.N. Korenkov, 1977. A pure culture of bacterial cells assimilating chromates and bichromates as hydrogen acceptors when grown under anaerobic conditions. Mikrobiologiya, 46: 414-417.
117. Wase, J. and C. Forster, 1997. Biosorbents for metal ions. London: Taylor & Francis.
118. Camargo, F.A.O., B F.M. Ento, B.C. Okeke and W.T. Frankenberger, 2003. Chromate reduction by chromium resistant bacteria isolated from soils contaminated with dichromate, Journal of Environmental Quality, 32: 1228-1233.
119. Terry, J., Beveridge, S.M. Jeff and P. David, 2008. Isolation and characterization of a chromium-reducing bacterium from a chromated copper arsenate contaminated site. Environmental Microbiology, 2(6): 611-619.
120. Laxman, S. and K. More, 2002. Reduction of hexvalent chromium by Streptomyces griseus. Journal of Environmental Toxicology, 27(4): 135-141.
121. Zainul Akmar Zakaria, Zainoha Zakaria, Salmijah Surif and Wan Azlina Ahmad, 2007. Hexavalent chromium reduction by Acinetobacter haemolyticus isolated from heavy-metal contaminated wastewater. Journal of Hazardous Materials, 146: 30-38.
122. Ravibabu, C., H. Nagaveni and K. Jamil, 2004. The toxic effects industrial influence on rats: Analysis and Remediation methods. Internet Journal of Toxicology, 3(2): 45-47.
123. Yoshinobu Ishibashi and Simon Silver, 1990. Chromium reduction in Pseudomonas putida. Applied and Environmental Microbiology, pp: 2268-2270.
124. Duangporn Kantachote, Salwa Torpee and Kamontam Umsakul, 2005. The potential use an oxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electronic Journal of Biotechnology, 8(3): 138-143.
125. Young Hak Kwak and Han Bok Kim, 2003. Vibrio harveyi is also a chromate reductase. Applied Environmental and Microbiology, 69: 4390-4395.
126. Abubacker, G. and H. Ramanathan, 2003. Bioremediation of tannery effluent pollutants. Bioresource Technology, 19 (3): 237-242.
127. Jonnalagadda, R.R., A. Rathinam, J.S. Kalarical and U.N. Balachandran, 2007. Biological removal of carcinogenic chromium (VI) using mixed Pseudomonas strains, Journal of General and Applied Microbiology, 53(2): 71-91.
128. Sadeeshkumar, R., P. Saranraj and D. Annadurai, 2012. Bioadsorption of the toxic heavy metal Chromium by using Pseudomonas putida. International Journal of Research in Pure and Applied Microbiology, 2(4): 32-36.