14 Microbial Bioremediation of Industrial Effluents

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14.1 Introduction

The contamination of air, water, and soil with toxic chemicals causes high risks for the ecosystem both directly and indirectly. Rapid industrialization and explosive development of chemical and mining industries vis-a-vis population explosion have resulted in global deterioration of environmental quality. The environment is sensitive to heavy metals due to their longevity and toxicity (Aravindhan et al., 2007). Extensive utilization of minerals for human need finds application in various industries. Therefore, mining activities along with rapid industrialization are generally considered indices of progress in any country. India is endowed with various types of minerals. Because of commercial importance of minerals, policy planners have emphasized on rapid mining process to overcome the need of the time by industrialization. However, as a fallout of extensive mining and industrial activities, heavy metal contaminated land and water has become a serious environmental health issue in India. In this regard, industrial wastes are the major source of contamination of toxic metals like Hg, Zn, Cr and Al.

Chromium is one of the toxic chemicals considered to be a more hazardous pollutant even at low concentration. Chromium compounds are widely used in leather tanning, steel production, and alloy formation, as metal corrosion inhibitors, and in paints as pigment and various other applications. Chromium generally occurs in two oxidation states, Cr^{3+} and predominantly Cr^{6+}, in air, water, and soil (Cheung and Gu, 2006; Daulton et al., 2007). Hexavalent chromium is 100 times more poisonous and 1000 times mutagenic than Cr^{3+}; hence, it has been listed as a priority pollutant and a human carcinogen by the United States Environmental Protection Agency (USEPA) (Cheung and Gu, 2006). Hexavalent chromium is highly soluble in water and mobile through the ecosystem, while Cr^{3+} is insoluble and forms a precipitate with organics in nature (Bajgai et al., 2012).

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Many of today's environmental problems, as well as their potential solutions, are intimately interwoven with the microbial component of the global ecosystem. Increasingly, scientists have recognized that microorganisms occupy a key position in the orderly flow of materials and energy through the global ecosystem by virtue of their metabolic activities to transform organic and inorganic matter. The bioactive potentiality of microorganisms in nature is a ready answer for degradation and recycling of the hazardous compounds being added to our environment because of the industrial and chemical boom. They can reduce toxicity of various pollutants and wastes that are detrimental to the valuable gift of nature. Many microorganisms such as bacteria, fungi, and algae have been recognized for their ability to resist either the toxic effect of hexavalent chromium or the biotransformation of Cr^{6+}; thus, it becomes less toxic or nontoxic to them via sequestration mechanisms such as reduction, complexation, alkylation, and precipitation (Iihan et al., 2004; Ertugrul et al., 2009). Through these mechanisms, microorganisms are also able to bioabsorb and bioaccumulate hexavalent chromium in their cells with the help of numerous binding sites present on their cell wall.

Ironically, some of these mechanisms make an environment susceptible to heavy metal toxicity. For example, reduction of toxic heavy metal ions to relatively less toxic (Cr^{6+} to Cr^{3+}) makes the heavy metal ions mobile through the water in the soil; therefore, chances of its presence in nonpolluted sites and the probability of its getting into runoff water increase. Toxic metals classified as environmental pollutants cannot be degraded, but their oxidation state can be changed to another less toxic state by microorganisms. Most of the microorganisms are antipolluters that metabolize toxic chemical substances and convert recacitrant compounds to its simpler form present in the pollutant. The virtually omnipresent microorganisms are powerful tools for bioremediation. It is an important and unique biological process, which is globally recognized as a cost-effective and eco-friendly technology. Thus, bioremediation of hexavalent chromium aims at extracting the metals to make them unavailable to flow into the ecosystem, or extract to mobilizing them for reuse or safe disposal (Crawford and Crawford, 1995). The various properties of microorganisms like reduction, adsorption, and bioaccumulation of heavy metals give the potential for a cheap alternative method of heavy metal removal from soil and industrial wastewaters. Both living and dead biomaterials are capable of removing heavy metal ions from the heavy metal−contaminated sites through diverse mechanisms (Vindhan, 2004).

Conventional treatment of Cr^{6+} waste involves a two-stage process such as chemical reduction of Cr^{6+} to Cr^{3+} followed by precipitation of Cr^{3+} by using lime, caustic soda, or sodium bicarbonate (Cushnie, 1985). Even though the process is quite effective, the large volume of sludge generated and the release of dangerous gases and cost of the chemical-reducing agents make it imperative to look into safe and cheaper alternatives. The biological system seems to be a more suitable approach. Therefore, bioremediation has become as a cost-effective, efficient, and environmentally friendly alternative for removing heavy metals from industrial effluents. The advantage of bioremediation is that this process does not require using aggressive and concentrated chemicals, and metal ions bound biomass

could be reused after elution (Chojnacka, 2007). Bioremediation is a suitable alternative to conventional methods, but the presence of co-contaminants of chromium such as Cu, Fe, Hg, Ni and Co may limit its application (Singh and Tripathi, 2007). Microbial populations in heavy metal−polluted environments harbour microorganisms that have adapted to the toxic heavy metals and become "metal resistant" (Kasan and Baecker, 1989). Bioreduction of Cr^{6+} occurs directly due to microbial metabolism or indirectly by bacterial metabolites (Losi et al., 1994).

Many scientists have investigated and demonstrated the feasibility of using biological processes for the treatment of Cr^{6+}-contaminated sites and industrial effluents by either pure culture or a consortium of Cr^{6+}-reducing bacteria (Romanenko et al., 1976; Bopp and Ehrlich, 1988; Lupton et al., 1992; Turick and Apel, 1997; Camargo et al., 2003).

14.2 Chromium Production

Chromium, the 24th element in the periodic table, was first discovered in Siberian red lead ore by French Chemist Nicholas Louis Vanquelin in 1978. It was named "Chrom" from the Greek word "$\chi\rho\omega\mu\alpha$" because of its brilliant hues. This first-row transition metal finds a variety of uses in industries exploiting its color, strength, hardness, corrosion resistance, and oxidizing capabilities (Darrim, 1956). Chromium is extracted from chromite ore, which has large deposits in South Africa, the Philippines, Southern Zimbabwe, and Turkey (Mathews and Morning, 1980). South Africa is the world's largest producer of ferrochrome. The country holds about 70% of the world's total chrome reserves, most of it derived from the Bushveld Igneous Complex (BIC) ores. South Africa produces an estimated 7,417,329 tons of chromium. India is the third leading chromite ore producer globally with an output of about 3.5−4 megatons (MT) per year. Chromite ore is mainly produced in the state of Odisha, with a large portion of the chromite produced consumed by local ferrochrome-producing companies (Ferrochrome Facts, 2007). Odisha is rich with various types of minerals, chromite being chief among them. The Sukinda mining area of Jajpur in Odisha has 97% of India's chromite deposits and has been declared one of the most polluted places in the world (Blacksmith Institute Report, 2007) due to chromium pollution. The total production of Odisha in 2004−05 was 3,123,386 MT, of which Jajpur's deposits alone contribute 3,035,201 MT (ENVIS Newsletter, 2006).

14.3 Chromium Toxicity

Chromium plays a key role in the biological system, but beyond a certain level, it is toxic (Balamurugan et al., 2004), mutagenic (Gili et al., 2002), carcinogenic (Codd et al., 2003) and teratogenic (Asmatullah et al., 1998). Moreover, the metal contamination imparts many adverse effects on human beings such as brain

damage, reproductive failure, nervous system failure, and tumor formation. Chromium and its compounds are widely used in different industries and then enter into the ecosystem (Figure 14.1) through effluent.

Generally chromium is present in the environment in two different oxidation states like Cr^{3+} and Cr^{6+} (Devi et al., 2012). The naturally occurring trivalent chromium is less toxic and nonbioleachable. It is an essential micronutrient in animal physiology, playing a role in glucose and lipid metabolism (Anderson, 1989; Mertz, 1993). It is involved in peripheral action of insulin, normal glucose utilization, and stimulation of enzyme systems (Mertz, 1969), and possibly in the stabilization of nucleic acids (Huff et al., 1964). The Cr^{6+} is water soluble, toxic, and bioleachable, as well as mutagenic, carcinogenic, and teratogenic; it is a powerful epithelial irritant as many researchers have reported (Petrilli and Flora, 1977; Gale, 1978; Gruber and Jennette, 1978; Langand, 1983; IARC, 1990; Daulton et al., 2007). Hexavalent chromium is present in the effluents from electroplating, paint, pigment, cement, mining, dyeing, fertilizer, and photography industries. At high concentrations, all compounds of chromium are toxic. Ingestion of chromium may cause epigastric pain, nausea, vomiting, and severe diarrhea. According to USEPA, the tolerance limit of Cr^{6+} in drinking water is 0.05 mg/L. Hexavalent chromium is carcinogenic in nature (Devi et al., 2012). Hence, it is highly imperative to treat the industrial effluent containing Cr^{6+} before its discharge.

Hexavalent chromium is a strong oxidant, and the ion can pass through cell membranes (Figure 14.2) many times faster than the trivalent form, which is a

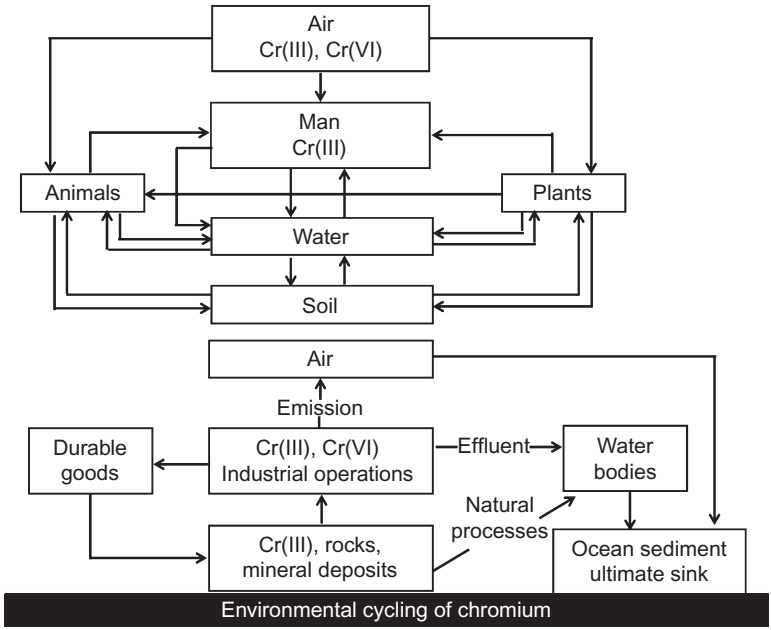

Figure 14.1 Chromium cycle in the environment.

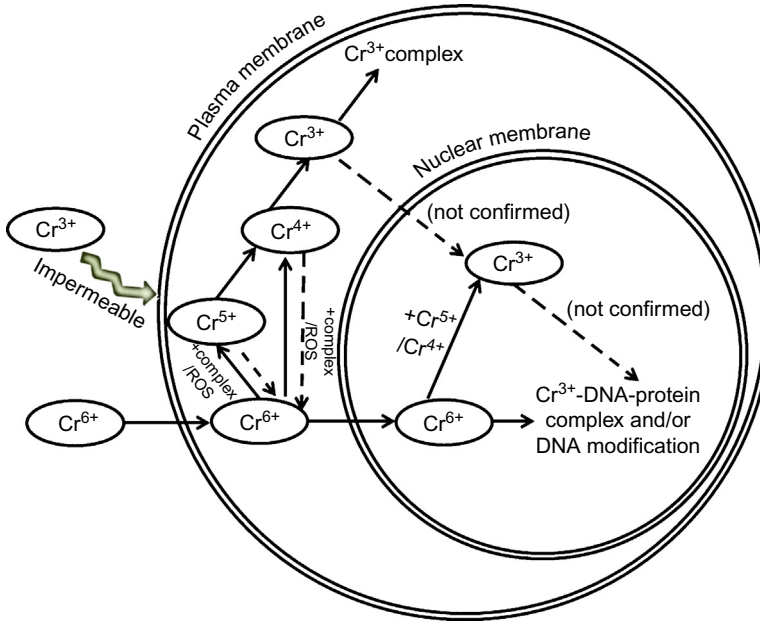

Figure 14.2 Vincent schematic diagram of hexavalent chromium toxicity.

major reason for its carcinogenicity. Intracellularly, it is then reduced to the trivalent form by various reducing agents like ascorbic acid, sodium sulfite, glutathione, Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), and Nicotinamide Adenine Dinucleotide Hydrogen (NADH) (Petrilli and Flora, 1978). The trivalent form binds and reacts with nucleic acid and other cell components by producing free radicals (Medeiros et al., 2003). A low concentration of chromium has been reported to stimulate plant growth; however, chromium concentration of 5−60 mg/kg of soil can damage plant roots (Pratt, 1966). Hexavalent chromium has been shown to affect growth, photosynthesis, morphology, and enzyme activities in algae and is toxic in concentrations ranging from 20 to 10,000 ppb as suggested by Schroll (1978), Silverberg et al. (1977), and Towill et al. (1978).

Microorganisms require a very low concentration of chromium for their growth and development, but a high concentration is toxic for them. Many microorganisms can accumulate chromium (Dursun et al., 2003; Pas et al., 2004), but the negative effects in bacterial cells such as cell elongation, cell enlargement, and reduction in cell division lead to cell growth inhibition as reported by Paran (1983) and Theodotou et al. (1976). Hexavalent chromium in the range of 0.05−5 mg/L of medium is generally toxic to microorganisms, though an internal concentration of chromium is species dependent (Babich et al., 1982). When Cr^{6+} concentration increases from 0.1 to 0.4 mg/L in aqueous systems, diatoms have been found to be replaced by algae and cyanobacteria. In *Escherichia coli* strain (NR 9064), high

concentrations of chromium led to formation of DNA−DNA cross-links and decreased polymerase activity (Snow, 1994). However, in fungi, chromium toxicity leads to reduced growth of mycelia (Babich et al., 1982). It is also toxic even in low concentration (1 mg/kg of soil), which reduced soil microbial transformations like nitrification (Ross et al., 1981). Cr^{6+} has been shown to be mutagenic to *E. coli*, *Bacillus subtilis*, and *Salmonella typhimurium* as reported by Nishoka (1975), Petrilli and Flora (1977) and Venitt and Levy (1974), respectively, generally causing breaks in DNA strands. The genotoxic effects of hexavalent chromium on bacterial cells include frame shift mutation and base pair substitution (Petrilli and Flora, 1978).

14.4 Bioremediation of Chromium Toxicity: The Green Chemistry

Conventional techniques of Cr^{6+} effluent treatment is quiet effective, but not economical and eco-friendly. Thus application of microorganisms is highly advantageous in this regard. Aerobic microorganisms such as *Pseudomonas auroginosa*, *Alcaligenes eutrophus*, *Waustersia eutropha*, *Pseudomonas fluorescens*, *Pseudomonas synxantha*, *E. coli*, *Bacillus megaterium*, *Bacillus* sp., and *Pseudomonas maltophila*, and anerobes like *Pseudomonas dechromaticans*, *Pseudomonas chromatophila*, *Aeromonas dechromatica*, *B. subtilis*, *Bacillus cereus*, *Pseudomonas auroginosa*, *Pseudomonas ambigua*, *Micrococcus roseus*, *Enterobacter cloacae*, and *Desulfovibrio desulfuricans* are involved (Cheung and Gu, 2006) in detoxification of hexavalent chromium. However, biomonitoring of hexavalent chromium is also possible using phenotypic responses of a unique blue color pigment producing bacterium *Vogesella indigofera*, following exposure to Cr^{+6} (Cheung and Gu, 2002).

Microorganisms develop different resistance mechanisms to chromium for survival in Cr-contaminated sites. The microbial response depends on the nature of the toxic elements. The resistance mechanism can be exclusion by permeability barriers, exclusion by active transport, intracellular sequestration by binding proteins of the cell, extracellular sequestration, and detoxification by chemical modification of the metal from toxic to nontoxic forms. The reduction in metal sensitivity to cellular targets can be by mutation to decrease metal sensitivity, increased production of damaged cell components, increased efficiency of repair of damaged cell components, plasmid encoded mechanism, etc.

Chromium resistance in bacteria is either chromosomal or plasmid mediated (Peitzsch et al., 1998; Juhnke et al., 2002). Plasmid-associated resistance has been observed in *Streptococcus lactis* (Efstathiou and Mckay, 1977), *Pseudomonas* sp. (Summers and Jacoby, 1978), *Alcaligenes eutrophus* (Nies and Silver, 1989; Cervantes and Silver, 1992; Peitzsch et al., 1998) etc. Some chromium-reducing bacteria can grow by reducing hexavalent chromium and simultaneously detoxifying the environment. Most hexavalent chromium-reducing bacteria reported so far are gram negative (Baldi et al., 1990; Francis et al., 2000). Bacterial chromium reduction can be direct, enzymatic, or indirect by bacterial metabolites. Enzymatic

reduction by some bacteria is mainly by soluble or membrane-bound enzyme systems (Figure 14.3). Membrane-associated chromate reductase activity was first reported by Wang et al. (1989) in *E. cloacae* (H01), which reduces hexavalent chromium to trivalent form by precipitation. *Shewanella putrefaciens* (MR-1) also shows cytoplasmic membrane-associated chromate reductase activity in anaerobic conditions using NADH and formate as electron donors for the enzyme (Myers et al., 2000). Rahman et al. (2007) reported that *Pseudomonas* sp. (C-171) showed resistance to 2000 ppm of Cr^{6+} in the form of $K_2Cr_2O_7$. In this bacterium, the growth rate and reduction of chromium was found to be inversely proportional to the Cr^{6+} supplementation whereas, slight elongation of bacterial cell due to accumulation of chromium hydroxide has been observed.

Chromium resistance in bacteria is mediated through two different mechanisms such as efflux mediated and reduction of Cr^{6+} to Cr^{3+}. Chromate efflux by *chrA* transporter has been established in *Pseudomonas aeruginosa* and *Cupriavidus metallidurans*, consisting of an energy-dependent process driven by the membrane potential. Most characterized enzymes for chromate reduction belong to the NADPH-dependent flavoprotein family of reductase. Expression of components of the machinery for repair of DNA damage and systems related to the homeostasis of iron and sulfur are also mechanisms of bacterial resistance to chromate (Ramirez et al., 2008). A study on reduction of Cr^{6+} by using *Pseudomonas putida* (PRS-2000) reveals that chromate reductase activity is associated with soluble protein and not with the membrane fraction. Crude enzyme activity is heat labile, and sulfate or nitrate does not affect reduction (Ishibashi et al., 1990). Evidence reveals that Cr^{6+} reduction is dependent upon pH, temperature, inoculum concentration, and Cr^{6+} concentration (Camargo et al., 2003). An alkaline pH and 30°C is the optimum

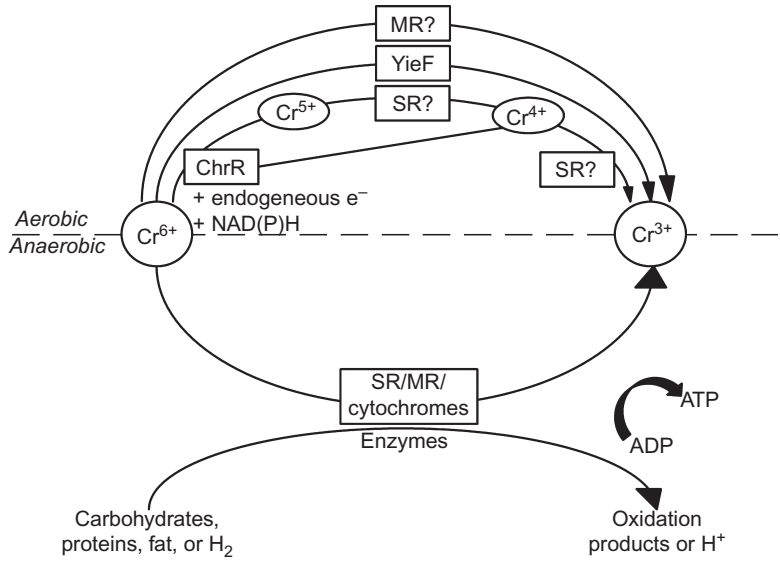

Figure 14.3 Mechanism of enzymatic hexavalent chromium reduction.

bioparameter for growth of chromium-resistant or -reducing bacteria such as *B. cereus*, *Bacillus thuringensis*, and *Arthrobacter crystallopoites*. As Cr^{6+} reduction is enzyme mediated, changes in pH affect the degree of ionization of enzymes, changing the protein conformation and affecting enzyme activity (Farrell and Ranallo, 2000). Generally, most chromium-resistant bacteria can carry out Cr^{6+} reduction at an optimum temperature range of 30−37°C (Losi et al., 1994).

However, soluble chromium reductase activity has been observed in *E. coli* (Shen and Wang, 1993), *Pseudomonas* sp. (CRB5) (McLean and Beveridge, 2001), and *Bacillus coagulans* (Philip et al., 1998). Indirect chromium reduction is due to changes in pH, redox potential during growth, and production of metabolites.

Indirect reduction of Cr^{6+} by bacterial isolates in the medium resulted in the production of off-white residues, which were the sign of chromate reduction. Bacterial conversion of Cr^{6+} to Cr^{3+} is due to production of metabolite (Smillie et al., 1981; Fude et al., 1994; Rahman et al., 2007; Mishra et al., 2010) like H_2S in the medium. The H_2S produced by the bacteria reduces Cr^{6+} to Cr^{3+}, and the trivalent chromium reacts with H_2S to form chromium sulfide, which is not stable at aqueous solution; it is deposited in the form of chromium hydroxide precipitate (off-white) in the medium. Hexavalent chromium reduction by bacteria is also due to production of acidic metabolic by-products from aerobic and anaerobic respiration. These metabolites decrease pH and redox potential (Beveridge, 1989; McLean and Beveridge, 2001), which favors conversion of Cr^{6+} to Cr^{3+} and production of chromium oxides and hydroxide. Chemical thermodynamics predict that low redox potential results in precipitation of these chromium oxides and hydroxide.

14.5 Case Study

A similar study was also undertaken to understand and elucidate the complex microbial activity in the Sukinda Valley, because of the alarmingly high level of chromium pollution in that particular area. In our experiment, an attempt was made to detoxify hexavalent chromium by chromium-resistant bacterial isolates from this area. Random soil, sediment, and water samples were collected aseptically from four different sites: Kalarangi, South Kaliapani, Kamardha, and the Dumsala canal of the Sukinda mining area of Jajpur district of Odisha. Physiochemical parameters like temperature, pH, moisture content, total chromium, and hexavalent chromium content of the samples were estimated. The average pH of soil, sediment, and water was 8.59, 6.99, and 7.84, respectively. The total chromium content was 4.24 g/kg, 5.7 g/kg, and 1.12 mg/L in soil, sediment, and water samples, respectively. The hexavalent chromium content of soil, sediment, and water was (Mishra et al., 2010) 39 mg/kg, 46.74 mg/kg, and 0.689 mg/L, respectively. Four bacterial isolates, namely, *Micrococcus luteus*, *P. putida*, *Serratia marcescens*, and *Acinetobacter calcoaceticus*, tolerated hexavalent chromium beyond 500 ppm were selected for reduction at different pH, temperatures, times of incubation, and concentrations of hexavalent chromium.

For screening of hexavalent chromium-reducing bacteria, the 24-h Cr^{6+} reduction test was conducted in a nutritive media (LB) at pH 7.0 (optimum for bacteria)

and 30°C (optimum for bacteria isolated from environment). The result reveals that out of all four isolates, *A. calcoaceticus* reduced 67.14% Cr^{6+}, which was the highest of all. The percentages of hexavalent chromium reduction under similar conditions by other isolates like *S. marcescens*, *M. luteus*, *P. putida* were 65.02%, 53.14%, and 50.72%, respectively. However, no reduction of hexavalent chromium was observed in a control set without bacteria, which indicates the reduction of Cr^{6+} due to the presence of bacteria. The reduction of Cr^{6+} by these bacterial isolates in the medium resulted in the production of off-white residues, which were the sign of chromate reduction.

However, in a nonnutritive (Samantaray and Mishra, 2012) medium, *A. calcoaceticus* reduced Cr^{6+} by 38.1% at 30°C/24 h/pH 7.0 and all other bacterial isolates like *S. marcescens*, *M. luteus*, *P. putida* reduced 37.05%, 31.02%, and 26.05%, respectively, but no reduction was observed in the control. This difference in trend of reduction in a nonnutritive medium in comparison to a nutritive medium may be due to a decrease in physiological and metabolic activities of the isolates (Losi et al., 1994; Camargo et al., 2003) and viability after some time and possible inhibition of biomass activity by prolonged chromate toxicity in a nonnutritive medium. Comparative analysis of Cr^{6+} reduction in nutritive and non-nutritive medium indicated that *A. calcoaceticus* possesses the higher potential among the isolates and selected for further studies.

Effect of hexavalent chromium concentration on the growth of viable cell numbers of *A. calcoaceticus* indicates that the viable cell count was higher, i.e., 9.6×10^7 CFU/mL at 100 ppm of Cr^{6+}, and then the trend was decreased (Samantaray and Mishra, 2011) up to 800 ppm in comparison to the control. However, the viable cell count was 8.4×10^7 CFU/mL at 50 ppm of Cr^{6+}, which is higher than the control, i.e., 8.1×10^7 CFU/mL, which indicates requirement of hexavalent chromium as a substrate for their optimal growth and development. Pei et al. (2009) and Zakaria et al. (2007) found that growth of *Acinetobacter haemolyticus* was higher at 90 ppm and reduced to 48% at 110 ppm in LB medium, which is due to apparent hexavalent chromium toxicity. Thus, 100 ppm of hexavalent chromium was selected and supplemented in the LB medium during the period of experimentation.

Effect of inoculum size on Cr^{6+} reduction was also studied and it was found that, increase in the rate of Cr^{6+} reduction with increase in inoculum size up to a limit. The optimum reduction of Cr^{6+} (74.62%) was observed with 5% inoculum volume at an increasing trend from 1−5% and decreased (Samantaray and Mishra, 2011) further above 5%. Thus, 5% inoculum was supplemented during the period of study. A similar result was observed by Rahman et al. (2007), who found that maximum reduction was recorded at 30% (v/v) inoculums among 10% and 20% (Wang et al., 1989; Rahman et al., 2000; Pei et al., 2009). They reported that the higher the cell density, the greater the percentage of reduction. This may be due to the fact that, increased bacterial cells in terms of inoculum size increases the rate of H_2S production thus fasten the rate of Cr^{6+} reduction.

The highest hexavalent chromium-reducing bacterial isolate, *A. calcoaceticus*, was selected for parametric studies. Hourly Cr^{6+} reduction results reveal that

A. calcoaceticus could reduce 85% of hexavalent chromium optimally at pH 8.0, in LB within 24 h. For most of the isolates, the optimum pH for growth correlates with the highest rate of hexavalent chromium reduction. The trend increases with increase in time, i.e., up to 24 h. Thus, pH 8.0 was kept constant for Cr^{6+} reduction. For most of the isolates, the optimum pH for growth correlates with the highest rate of hexavalent chromium reduction (Camargo et al., 2003). The relationship between pH and Cr^{6+} reduction was not surprising because chromate is the dominant chromium species in aqueous environments at pH 6.5−9.0 (McLean and Beveridge, 2001). The optimum pH for growth of Cr^{6+}-resistant bacteria was reported at 7−7.8 (Losi et al., 1994), but hexavalent chromium forms are soluble over a wide pH range and generally mobile in soil−water systems (Losi et al., 1994). Similar results were also obtained by Wang et al. (1990), reporting that Cr^{6+} reduction in *E. cloacae* occurred at pH 6.5−8.5 and was inhibited at pH 5−9. As Cr^{6+} reduction is enzyme mediated, changes in pH will affect the degree of ionization of the enzyme, changing the protein conformation and affecting the enzyme activity.

The hexavalent chromium reduction profile monitored at different temperatures ranging from 20°C to 37°C in LB for 24 h at 8.0 hexavalent chromium reduction by *A. calcoaceticus* in LB indicates that the percentage of reduction (85%) is higher at an optimum temperature of 30°C. The decreasing trend of Cr^{6+} reduction was also observed with an increase in temperature for which it was kept constant at 30°C in order to study the effect of metal on Cr^{6+} reduction. As observed, the highest reduction was reported at an optimum temperature of 30°C and the percentage of reduction decreased with an increase in temperature. This is possibly because of decreased enzyme activity with increase in temperature. This could be due to loss of viability or metabolic activity of the cells on prolonged incubation at higher temperature (Aravindhan et al., 2007). Similar results were also obtained by Camargo et al. (2003). Losi et al. (1994) reported an optimum temperature of 30−37°C for chromate reduction. However, Wang et al. (1990) reported that no chromate reduction was observed at 4°C and 60°C. Temperature is an important selection factor for bacterial growth and affects enzymatic reactions necessary for chromate reduction.

Consortium study was undertaken to know effectiveness of the synchronized use of the two isolates for Cr^{6+} reduction. However, *S. marcescens* was found to inhibit the growth of *A. calcoaceticus* in LA medium. Thus, consortia hexavalent reduction was not possible by these two desired bacterial isolates. This might be due to the production of red coloured water soluble pigment prodigiosin by *S. marcescens.* Khanafari et al. (2006) reported that the red prodigiosin pigment produced by *S. marcescens* has antimicrobial, immunosuppressive, and anti-proliferate activity. In our study, *S. marcescens* was capable of producing red water-soluble pigment in Nutrient Agar (NA), Nutrient Broth (NB), Luria-Bertani Agar (LA), and Luria-Bertani Broth (LB), respectively. However, pigment production was also observed in a wide range of pH 4−14, Cr^{6+} concentration up to 1000 ppm, and in a temperature range of 20−37°C.

Natural habitats are generally characterized by the coexistence of a large number of toxic and nontoxic substances for which it is imperative to study multiple metal effects on the physiology and biochemistry of microorganisms (Verma and Singh,

1995). *A. calcoaceticus* showing the highest hexavalent chromium reduction was tolerant to a broad range of heavy metals like Fe^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, and Co^{2+} up to concentrations of 1000, 900, 1000, 100, and 300 ppm, respectively. Among all these metals tested, the highest tolerance was observed toward Fe^{2+} and Ni^{2+}. These observations assume great significance because effluents from any metal related to industry have several metal ions or contaminants. Tolerance to other metals has an added advantage of withstanding the presence of other metal ions while performing the desired activity. There are reports of the use of Cr^{6+} reducing microorganisms for treatment of other waste materials (Lovely, 1995). Thus, these locally isolated strains possess huge credentials for detoxification of hexavalent chromium from industrial effluent and chromium contaminated sites.

Effects of metal on hexavalent chromium reduction by *A. calcoaceticus* indicate that, in 1 ppm of copper, 89.39% Cr^{6+} reduction was observed (Samantaray and Mishra, 2012) at 30°C/24 h/pH 8. In the presence of iron, 68.44% hexavalent chromium was reduced, and the rate of reduction decreased in the presence of nickel as compared to control. Although the organism showed more tolerance to iron, no change in chromium reduction was observed. The increase in reduction in the presence of copper may be due to enhanced enzyme activity of chromate reductase (Pal and Paul, 2004; Faisal and Hasnain, 2004; Elangovan et al., 2006) as it also acts as a micronutrient for optimal growth of the bacteria.

14.6 Conclusion

This case study revealed that 89.39% Cr^{6+} reduction was observed by *A. calcoaceticus* at 30°C/24 h/pH 8 and in the presence of 1 ppm copper in a nutritive (LB) medium. Thus, it is concluded that *A. calcoaceticus* may be used in the bioremediation of hexavalent chromium toxicity. Understanding the potential of microorganisms in recycling of metals may lead to improved processes for bioremediation of metal-contaminated areas. Hexavalent chromium toxicity is a major concern, thus there is a high level of interest in developing methods aimed at detoxifying chromium-contaminated areas at minimal costs with fewer side effects. The process, which is in its nascent laboratory stage, is now moving on to the developmental stage. Slowly, microorganisms are proving to be the right tools for environmental pollution control. Our current state of knowledge about the state of affairs in chromium-contaminated areas leaves us with many queries, answers to which can help us to find a solution to control chromium pollution.

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