Isolation and Characterization of Chromium Resistant *Enterobacter aerogenes* from Marine Soil

Aproject submitted

by

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This work is committed to my family for their adoration andunswerving support.

Certification Statement

This is to certify that this project titled “Isolation and Characterization of Chromium Resistant *Enterobacter aerogenes* from Marine Soil” is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Mohammad Samiul Alam Rajib, Senior Lecturer, Department of Pharmacy, BRAC University and this project is the result of the author’s original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project itself.

Signed

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Countersigned by the supervisor

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Abstract

Hexavalent Chromium is carcinogenic, highly toxicand mobile in nature. It is considered as an ecological contamination which is utilized as a part of the majority of the commercial industries and is discharged without treatment. Hexavalent form of Chromium can be reduced into trivalent form of Chromium which is water insoluble. The trivalent form is less toxic due to a decrease in bioavailability and they can be used in bioremediation process. Samples were gathered from the Sitakunda Ship Breaking Yard, seaside locales of Bangladesh, for isolation, characterization and identification of the bacteria which have the potential to diminish the cancer-causingChromium (VI) to Chromium (III). Nutrient agar medium was used which was supplemented with Chromium (VI) as potassium chromate. By purification on nutrient agar plates, containing different concentrations of Chromium, the isolate was obtained. Further the sample was studied. The isolated strain was examined for Chromium (VI) diminishment capacity in development subordinate way. It was found that the isolate was resistant to Cr (VI) as well as has the reducing activity.

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LIST OF ABBREVITIONS

Cr	Chromium
DPCZ	Diphenyl Carbazide
ETP	Effluent treatment plant
Kg	Kilogram
MCL	Maximum contaminant level
mg	Milligram
mL	Milliliter
mM	Millimolar
NADH	Nicotinamide adenine dinucleotide
NB	Nutrient broth
nm	Nanometer
O.D.	Optical density
OSHA	Occupation safety and health administration
PEL	Permissible exposure limit
ppm	Parts per million
RPM	Rotation per minute
rRNA	Ribosomal ribonucleic acid
µM	Micro molar

Chapter 1: Introduction

1.1 Background

The hexavalent form of Chromium is a cancer-causing agent and a potential mutagen which is introduced frequently into the environment by variety of activities, including leather tanning, pigment manufacturing and electroplating (McGrath & Smith, 1990). Hexavalent Chromium is mutagenic and toxic to human being and other organisms (Thacker & Madamwar, 2005). Distribution of Chromium is very wide in the nature. It holds the 21st position in the file of most usually occurring components in the crust of Earth (Chandra & Kulshreshtha, 2016). It is widely present in plants, animals, rocks and soils. The unregulated transfer of Chromium containing effluents causes contamination of aquatic sediments, soil, and ground and surface water. By these pathways, these lethal heavy metals enter in our daily life food habit and cause different complexities like cancer.

Abiotic process is utilizing for the treatment of most contaminated sites over the world. Dig-and-treat or pump-and-treat methods are used for the implementation of the abiotic process and it requires regular follow-up of immobilization steps or precipitation. For the treatment of wastes containing hexavalent Chromium, biological reduction method is the most useful method. Recently, fixed-film and continuous-flow bioreactors have been utilized for biological reduction of Cr(VI). In these reactors, carbon sources as electron contributors are supplied remotely to the wastewater relying on the necessity (Elangovan, Philip, & Chandraraj, 2010).

An alternative option for chemical and physical treatment technology is the bioremediation technology.Ion exchange, electrolytic reduction, liquid–liquid extraction, electro-coagulation and adsorption, reverse osmosis, and membrane filtration can be utilized for removal of Cr(VI). These techniques have a few disadvantages, for the most part high cost, low productivity at lower fixations, and the production of poisonous slime or different squanders that needs to be disposed. Thus bioremediation technology has the most advantage above all these methods as it is inexpensive and environmentally friendly (Nandi, Laskar, & Saha, 2016).

The current study gives us the chance to assess the obscure Chromium resistive bacterial strains that can be utilized as the main remedy for Chromium intoxication. It can be done either by reducing the hexavalent Chromium before interacting with the human body or it can serve as

apotential source forChromium reductase enzyme which is developing as a likely-looking chemotherapeutical operator (Nandi et al., 2016).

1.2 Methodology

Biochemical tests were utilized to characterize the isolated culture. Later during the investigation microscopic morphological imaging was also conducted.

The critical part of the present study was to discover a reasonable microbial strain that will be resistive as well as have the capability to reduce Chromium (VI) that is available in the medium in amomentous manner. The techniques we used to assess the reduction profile were led controllably with the goal that we can distinguish the particular bacterial strain. The laboratory analyses were led in Chromium supplemented nutrient broth media. Before assessing the Chromium reducing capability of the isolated strain, an intensive isolation procedure was done repeatedly unless the unadulterated colonies of bacteria were distinguished morphologically.

1.3 Objectives

The main target of the investigation was to assess the possibility of Chromium reducing bacteria as a specialist for bioremediations of carcinogenic Chromium and additionally the foreseeable source of Chromium reductase enzyme to keep away from other concoction. Taking after undertakings were performed in order to accomplish the objectives:

- Assessment of the execution of the confined bacteria in Chromium defiled environment.
- Investigation of Minimum Inhibitory Concentration of Chromium to evaluate the resilience of the isolated bacteria.

Chapter 2: Review of Literature

2.1 Introduction

Chromium is used for the manufacture of steel and different alloys (Rifkin, Gwinn, & Bouwer, 2004).Chromium is a transition metal and its distribution is very wide in the nature. It occupies the 21st position in the index of most commonly occurring elements in the Earth's crust(Chandra & Kulshreshtha, 2016). Chromium shows several oxidation states which ranges from $Cr2^{+}$ to $Cr6^{+}$(Chandra & Kulshreshtha, 2016). The trivalent and the hexavalent form of Chromium is most commonly found in the environment (Chandra & Kulshreshtha, 2016).Chromium is used to tan leather, preserve food and electroplate metals. In refractory bricks Chromium is used as pigments, high temperature furnaces, drilling muds, dyes, inhibitors of rust and corrosion, toner for photocopies and textiles (Rifkin et al., 2004).Chromium contamination is occurring in the biosphere due to release of untreated industrial effluents containing Chromium compounds into the natural resource like water, sediment, soil and air. It is occurring both in underdeveloped and developed countries (Thacker & Madamwar, 2005). Usually Chromium exists primarily in the nature as trivalent and hexavalent forms (Rifkin et al., 2004). For nucleic acid stabilization, proper glucose metabolism and stimulation of enzyme system Chromium is considered as an essential micronutrient but it is toxic at elevated level (Thacker & Madamwar, 2005).Hexavalent Chromium is mutagenic and toxic to human being and other organisms (Thacker & Madamwar, 2005). In aquatic systems mobility of Cr (VI) is greater than Cr (III) (Rifkin et al., 2004). Once it enters into the body it crosses the cell membrane readily and oxidize the intracellular compounds. It is carcinogenic to human when inhaled. Dermatitis can occur due to dermal contact also numerous adverse systemic effect can occur due to ingestion (Thacker & Madamwar, 2005). In contrast, the trivalent Chromium form is mostly found in undissolved form and it hardly pass through the cell membrane. Its oxidative potential is also different from hexavalent form of Chromium(Thacker & Madamwar, 2005).

2.2 Chemistry

Chromium is symbolized as Cr and has an atomic number of 24. Chromium is silvery grey in color. It is brittle, hard and a lustrous metal which can be highly polished.It has a melting point of 1907°C and boiling point of 2672°C ("Chemical properties of Chromium - Health effects of Chromium - Environmental effects of Chromium," 1998-2016). Chromium is tasteless and odorless ("Agency for Toxic Substances and Disease Registry," 2011). The name Chromium has derived from the Greek word “chroma” which means “color”, as it has various colored compounds (Horn, 2013). The hardness and resistance to corrosion and rust makes Chromium a very useful metal. Thus it is used to manufacture stainless steel and other alloys (Rifkin et al., 2004).

In spite of the fact that Chromium is a crucial micronutrient for legitimate glucose digestion system, incitement of chemical framework and adjustment of nucleic acid, hoisted levels of Chromium is dangerous, however disease may occur due to its deficiency. Hexavalent Chromium is lethal and mutagenic to most living beings and is known not aggravation, consumption of the skin and respiratory tract; it likewise causes lung carcinoma in people (Thacker & Madamwar, 2005). This toxin hugely influences the earth at relinquished Chromium generation locales. Henceforth its natural cleanup is exceedingly crucial. Complete information on the concoction component Chromium is given in Table 2.1.

Table 2.1: Comprehensive data of element Chromium("American Elements," 2016; "Chromium," 2013)

Name	Chromium
Symbol	Cr
Atomic number	24
Element category	transition metal
Group, period, block	6, 4, d
Appearance	silvery metallic/ silver-grey
Atomic weight	51.996
Electron configuration	[Ar] $3d^5$ $4s^1$
Crystal structure	body-centered cubic
Oxidation state	-2, -1, 1, 2, 3, 4, 5, 6
Atomic radius	128pm
Thermal conductivity	93.9 $W.m^{-1}.K^{-1}$

2.3 Occurrence and Sources of Chromium compounds

Chromium is normally happening element found in rocks, animals, plants, and soil. Chromium exists in numerous oxidation states, of which the hexavalent (Chromium VI) and trivalent (Chromium III) states are most common naturally. Chromium is known not different compound and organic responses in characteristic frameworks. Both oxidation of Chromium (III) and diminishment of Chromium (VI) can happen in geologic and sea-going situations.In the environment Chromium VI may respond with dust particles or different substances and might be changed over to Chromium (III) (Chandra & Kulshreshtha, 2016).

Chromium (VI) happens in rare minerals and might be actually happening in groundwater, be that as it may, Chromium (VI) in the earth is completely introduced from human exercises (Chandra & Kulshreshtha, 2016). An essential source is the generation and utilization of

Chromium mixes (for the most part ammonium dichromate,Chromium trioxide, sodium dichromate,sodium chromate and potassium dichromate) and in addition the release of industrial wastes containing Chromium mixes.

As condensed by the United States Environmental Protection Agency (US EPA), basic Chromium is found in air, water, soil and biota with groupings of 1.0–2,000 mg/kg soil (normal of 40 mg/kg soil), 0.1−6.0 µg/L crisp water and 0.2−50 µg/L ocean water (EPA, 2010). In contaminated areas, Chromium fixations might be higher, e.g. up to 30 µg/L in new water (ATSDR, 2000a).

The most mined mineral is ferric Chromite, $FeCr_2O_4$. Fundamentally found in South Africa. The chromite metal store in South Africa speaks to around 72% of the earths recognized sources. Different nations with exploitable mineral stores incorporate Zimbabwe,Russia, Finland, Kazakhstan,India the Philippines and Brazil (figure: 2.1)

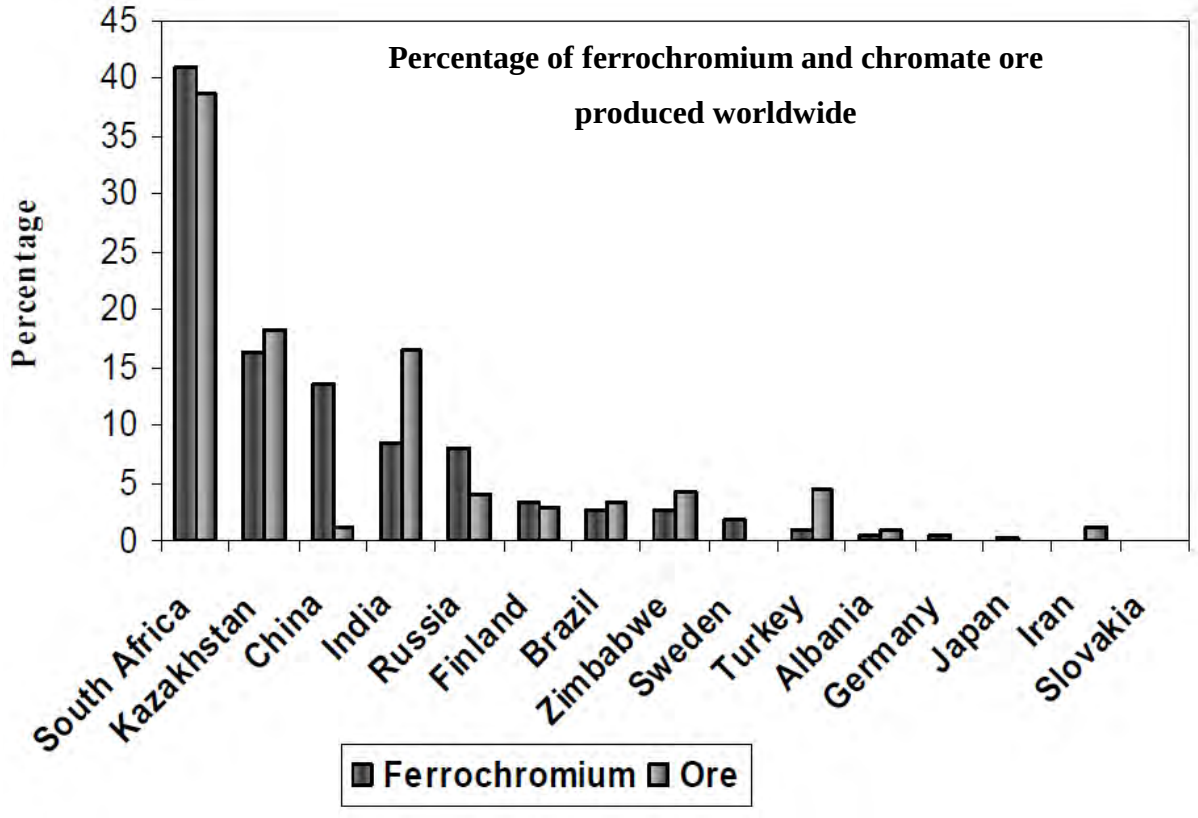

Figure2.1: Percentage of Ferrochromium and Chromite ore produced worldwide (Papp, 2006)

2.4 Uses of Chromium

Some core employments of Chromium(VI)compounds incorporate electroplating, dyes and pigment manufacturing,leather tanning, chemical synthesis, preservatives for wood, refractory production and corrosion inhibitors (EPA, 2010). Chromium (VI) compounds are also found in numerous buyer items, for example, treatment of wood using copper dichromate or tanning of leather using chromic sulfate.

2.4.1 Applications

I. In metallurgy, to give erosion resistance and a gleaming completion:
   - as an amalgam constituent, for example, in stainless steel in cutlery
   - in chrome plating,
   - In anodized aluminum, truly transforming the surface of aluminum into ruby.

II. As paints and dyes:
   - Chromium (III) oxide is a metal shine known as green rouge.
   - Chromium salts shading glass an emerald green.
   - Synthetic rubies are produced as Chromium is the compound that causes the red color of ruby.
   - Makes a splendid yellow for painting.

III. As a catalyst.

IV. Chromite is utilized to make molds for the terminating of blocks.

V. For the tanning of leathers Chromium salts are utilized.

VI. Laboratory glassware are cleaned using potassium dichromate, also used as an agent for titration. For fabric dyes it is used as a mordant.

VII. Chromium (IV) oxide (CrO_2) is utilized for the manufacture of magnetic tape, where it's higher coercively than iron oxide tapes give better execution.

VIII. Anti-corrosive agent in well drilling muds.

IX. In pharmaceutical, as a dietary supplement or thinning help, for the most part as Chromium (III) chloride or Chromium (III) picolinate.

X. Chromium hexacarbonyl ($Cr(CO)_6$) is utilized as a fuel (gasoline) added substance.

XI. Chromium boride (CrB) is utilized as a high-temperature electrical conveyor.

XII. Chromium (III) sulfate ($Cr_2(SO_4)_3$) is utilized in ceramics, as a green pigment present in paints, inks and varnishes and in addition in chrome plating.

XIII. Chromium (VI) is utilized as a part of the post Ballard readiness of Gravure (rotogravure) printing Forme Cylinders. By electroplating the metal onto the second layer of copper (after the Ballard skin), the life span of the printing barrel is expanded.

XIV. Micronutrient present in "health" aware beverages, which is known to increase the quantity of energy that we get from food.

2.5 Regulation regarding level of Chromium:

Variety of adverse effect occurs in plants, animals and human being due to presence of Chromium (VI) in water bodies greater than standard limit. Consequently, stringent controls have been forced by different associations. As indicated by the World Health Organization (WHO) drinking water rules, the greatest reasonable point of confinement is 0.05 and 2mg/L for hexavalent Chromium and total Chromium [including Cr (III), Cr (VI) and other forms], individually (Gupta and Rastogi 2009). The Maximum Contaminant Level (MCL) of Chromiumis 0.1 mg/L (absolute Chromium) as indicated by Safe Drinking Water Act. Most extreme reasonableChromiumconcentration is 0.1 mg/L in filtered water. In color additives, Chromiummust not be more than 50 ppm. Chromium might be utilized as a part of hydrolyzed leather feast utilized as a part of food for animals and must not contain Chromium at levels above 2.75% of the aggregate by weight. The Permissible Exposure Limit (PEL) for Cr (VI) was declared by Occupational Safety and Health Administration (OSHA) to be 0.1 mg/m^3 (taking into account chromic acid and chromates posting). National Institute for Occupational Safety and Health (NIOSH) demonstrates Immediately Dangerous to Life and Health (IDLH) limit as 15 mg/m^3as Chromium (VI) (For chromates and chromic acid posting). Suggested Limit of Exposure is confined to be 0.001 mg/m^3 (forchromyl chloride, chromatesand chromic acid listings).

2.6 Chromium Toxicity and Health Effects

2.6.1 Toxicity of Chromium

People exposed to Cr (VI) can be affected by ulceration,sensitivities, aggravations, dermatitis, skin and nasal disturbances, respiratory track issue,puncturing of eardrum and lung carcinoma(Poopal & Laxman, 2009). Also, Cr(VI) confirmations the ability to gather in the placenta, harming fetal improvement (Poopal & Laxman, 2009). Cr(VI) contamination in nature changes the soil microbial groupstructures and diminishing microbial development and corresponding enzymatic reactions, with an ensuing perseverance of soils organic matter and amassing of Cr(VI) (Shi, Becker, Bischoff, Turco, & Konokpa, 2002). The harmful activity of Cr(VI) is because of its capacity to effortlessly infiltrate cell layers, and cell film harms brought on by oxidative stress incited by Cr(VI) which has been broadly reported, both in prokaryotic and in eukaryotic cells, with impacts, for example, decrease in membrane unification or hindrance of electron transport chain (Codd, Rillon, Levina, & Lay, 2001; Francisco, Moreno, & Vasconcelos, 2010). Also, Cr(VI) enters into the cells by utilizing the sulfate transport arrangement of the membrane present in the cells of living beings that can utilize sulfate (Ohtake, Cerventes, & Silver, 1987; Otha, Glasworthy, & Pardee, 1971). The cell layer is almost impermeable to Cr(III), therefore, Cr(III) has in this manner just around one thousandth of the poisonous quality of Cr(VI) (Polti, Amoroso, & Abate, 2010a). Checking these contemplations, it is conceivable to reason that, contingent upon its oxidation state, Chromium might have distinctive biologicalimpacts, with Cr(VI) which is very lethal to most life forms, and Cr (III) which is generally harmless (Katz & Salem, 1993; Wong & Trevors, 1988).

2.6.2 Health effects

The SCHER construct its appraisal in light of data gathered in late audits and evaluations, for example, compounds of inorganic Chromium VI (WHO/IPCS, 2013), Toxicological Profile for Chromium (ATSDR, 2012), Establishing a reference dosage reaction relationship for cancer-

causing nature of hexavalent Chromium (RAC, 2013), Chromium in drinking water (WHO, 2003), Chemicals in toys (RIVM, 2008),compounds of Chromium VI (IARC, 2012), Scientific Opinion on the dangers to general wellbeing identified with the nearness of Chromium in sustenance and drinking water (EFSA, 2014) and Public Health Goal for Hexavalent Chromium in Drinking Water, California Environmental Protection Agency (OEHHA, 2011). Late writing (up to October 2014) was counseled with particular spotlight on new accessible proof on the potential cancer-causing impacts of Chromium VI. The SCHER concentrated fundamentally on data on wellbeing impacts taking after oral presentation and saw that different Chromium salts were utilized to regulate Chromium VI in animal studies and also in in vitro examines. These incorporate strontium, zinc, dipotassium, calcium, disodium, and diammonium salts which have distinctive degrees of solvency. In epidemiological studies, co-presentation to Chromium III and Chromium VI happened.

2.6.2.1 Kinetics

The model was extended for people orally presented to Chromium (VI). Consequently, data from studies and from the published literature with respect to the toxicokinetics for aggregate Chromium in people was utilized. Based on a model of blended second-order, pH-subordinate process and gave a decent portrayal of Chromium toxicokinetics consistent with current information on humans exposed in Chromium. Gastric lumen pH, gastric lumen travel time, gastric lumen volumes, and gastric juice creation were distinguished as critical hotspots for human variability for which information is deficient so as to further create key suppositions made in the PBPK models and to permit enhanced wellbeing hazard appraisal (Kirman et al., 2013). Chromium VI is very receptive in biological systems and can quickly be diminished to Chromium III which is less promptly retained and far less harmful than Chromium VI. In humans there is an expansive between and intra-singular variability in regards to retention of Chromium VI since the transformation relies on upon the centralizations of both Chromium VI and the local diminishing specialists and in addition on the gastric substance and on pH. Particularly acidic situations with high natural substance advance the diminishment of Chromium VI to Chromium III. Small thiols and ascorbate are the chief organic reducer of Chromium VI, representing more than 80% of its digestion system (Zhitkovich, 2011).

Chromium VI as chromate basically takes after sulfate and phosphate and is promptly taken up by every cells and organs all through the body by means of sulfate transporters (Costa, 1997). This varies from Chromium III which is not taken up by cells similarly. The last uptake is dictated by rivalry between extracellular decrease of Chromium VI and its quick intracellular ingestion. Chromium levels were just unassumingly hoisted after exposure to Chromium VI (Costa, 1997; Thomann et al., 1994; Witmer et al., 1989; Collins et al., 2011; Witt et al., 2013).

Physiologically-based kinetic (PBK) models were created for rats and mice orally presented to Chromium (Kirman et al., 2012; Schlosser, 2014). Presentation – tissue fixations were direct or supra straight showing that the exposures did not immerse gastric diminishing limit (Collins et al., 2010).

2.6.2.2 Mode of action

The heaviness of proof backings the credibility that Chromium (VI) may act through a mutagenic and genotoxic mode of action (MOA). Likewise, Chromium VI has been appeared to deregulate cell development (IARC, 2012). Chromium VI promptly crosses cell membrane through sulfate transporters. Inside the cell, exceptionally receptive Chromium VI is lessened in this manner creating oxidative harm to proteins, lipids and DNA. Hereditary sores incorporate DNA adducts, Cr-DNA adducts, DNA-Cr-DNA adducts, protein-Cr-DNA adducts,oxidized bases, abasicsites,DNA-strand breaks, DNA-protein crosslinks and DNA between and intrastrand crosslinks (Wise, Holmes, & Wise, 2008). In the cell, Chromium (III) can tie to DNA and produce DNA adducts prompting mutation and genomic instability as is seen by in vitro ponders in bacterial and human cells (Quievryn et al., 2003). Moreover, Chromium (VI)reduction can bring about DNA harm from ROS (reactive oxygen species). The mutagenic and clastogenic actions of Chromium are very complex. Cr (III) enters into the cell very poorly and is less harmful for the body. However, if it is formed inside the cell by the reduction of Cr(VI) which can enter into the cell readily, the Cr(III) may cause arrest of the DNA polymerase by producing cross-links with DNA. When Cr(VI) is being reduced to Cr(III), ROS is being generated. By the presence of H_2O_2 the Cr(III) itself causes the level of ROS to rise within the cells that causes the production of 8-hydroxydeoxyguanosine in DNA *in vitro*Cr(III) can also bind outside the cell

membrane to generate ROS that penetrates into the cell causing harm to the cell.(Hadjiliadis, 2012).

In vitro, low Chromium VI fixations cause relentless initiation of the mitogen-actuated protein kinases ERK-1, ERK-2, JNK and p38 (Chuang and Yang, 2001; Kim and Yurkow, 1996) and the phosphorylation of the mitogenic interpretation components NFκB, ATF-2 and c-Jun (Samet et al., 1998; Ye et al., 1995). As these protein kinases and interpretation components constitute essential mediator in inflammatory procedures and tumor development, impacts on cell signal transduction that deregulate cell development are additionally not out of the ordinary on account of Chromium VI, notwithstanding the direct genotoxic systems included (Hartwig, 2007, 2010).

In vivo, Chromium VI has been appeared to be genotoxic by all courses of organization in rodents treated with high measurements of Chromium VI (ATSDR, 2000b; ATSDR, 2008; OEHHA, 2011). In a 90-day animal study intended to expand upon and extend the NTP's study, inflammation was seen in the small digestion tracts of mice that were orally exposed at cancer-causing measurements of Chromium (VI). These are liable to be the outcome of oxidative damage and are recommended that they may go before regenerative hyperplasia and tumor arrangement (Thompson et al., 2013). Entire genome microarray examination of duodenal epithelial specimens recognized changes in genes required in oxidative stress reaction, cell cycle control, or lipid digestion system (Kopec et al., 2012). Inhalation due to occupational exposure has been appeared to bring about DNA damage in lymphocytes (IARC, 2012).

2.6.2.3 Effects in Animals

Distinctive studies tended to the intense lethality of Chromium (VI). In outline, acute oral lethal dose (LD_{50} value) in rats presented to Chromium (VI)compounds shifted somewhere around 13 and 29 mg/kg b.w. relying upon the compound managed and the sex of the rodent (Gad et al., 1986). The principle impacts saw in rodents after medium-term oral introduction to Chromium mixes were declines in body weight pick up and changes in immunity and hematological parameters. The rats and mice were uncovered for a long time to sodium dichromate regulated in drinking water was utilized to infer endured every day consumption levels for non-cancer-causing impacts.

Introduction of rats through inward breath brought about aspiratory irritation and neutrophil relocation (Cohn et al., 1998).

Different studies demonstrated that Chromium compounds instigated diseases in trial creatures taking after assorted introduction pathways including oral route, intrapleural,intratracheal, inhalation, intraperitoneal, intra muscular, subcutaneous andintravenous infusions (ATSDR, 2008). Carcinogenesis happened for the most part at the site of organization. Inhalation increased the chance of lung cancer in rats (Glaser et al., 1986; Glaser et al., 1988) and mice (Nettesheim et al., 1971). By repository infusion a few Chromium compounds (lead chromate,calcium chromate, strontium chromate, zinc chromate) brought about local sarcomas. The potassium chromate which was given orally upgraded UV-incited skin cancer, showing tumor systemic impacts (Davidson et al., 2004).

2.6.2.4. Effects in humans

In people most information on impacts are gotten from reported instances of coincidental presentation to high measurements and by inhalation from occupational exposure. It is for the most part laborers in chromate generation, Chromium electroplatingandchromate pigment formation who are presented to compounds containing Chromium.

Skin contact with Chromium (VI) containingcompounds causes rashes and ulcers. Dermal presentation to Chromium (VI) compound has additionally been connected to unfavorably susceptible contact dermatitis. Utilizing a patch test, 2 μg was required to bring out a positive skin response in hypertensive subjects. The predominance of Chromium affectability in the all-inclusive community has been evaluated to be somewhere around 0.5% and 1.7% in research in a few European nations (Peltonen and Fräki, 1983; Hartwig, 2007; Hartwig, 2010). Be that as it may, sharpening properties of Chromium VI are not tended to by the SCHER in this conclusion. Inward breath in occupationally uncovered specialists prompted impacts in the aviation routes, for example, septal aperture and nasal mucosal ulceration. Changes in lung capacity parameters were additionally watched. Exposure was evaluated taking into account the introduction time frame (characterized as theinterval in-between when a laborer was enlisted and the time manifestations were initially distinguished) and also on mean and middle yearly Chromium VI

fixations prone to be knowledgeable about the occupation position held when the side effects initially happened (Finley, Proctor, & Paustenbach, 1992; Lindberg & Hedenstierna, 1983).

Chromium VI has appeared to bring about DNA harm (DNA–protein crosslinks, micronuclei, DNA strand breaks, chromosomal abnormalities or sister chromatid trades) within the lymphocytes of specialists (welders,electroplaters or ferrochromium composite foundry laborers who were principally uncovered during inhalation, as surveyed in WHO/IPCS, 2013). Not all human studies indicated reliable results. They were constrained in a few viewpoints: for the most part, the levels of presentation to Chromium VI were not known and uncovered and non-uncovered gatherings were analyzed frequently taking into account set of working responsibilities. A portion of the studies utilized groups that were too little to have the measurable power to dependably evaluate the cytogenetic changes in laborer.

With respect to impact of Chromium VI on nasal sinusand nasal diseases, the epidemiological confirmation stays suggestive yet uncertain (IARC, 2012).

A relationship between theabdominal tract growth and introduction of hexavalent Chromiumin pure drinkable water is been accounted for at a defiled area in China (Zhang and Li, 1997). Be that as it may, there are significant instabilities with respect to the study result, particularly in the estimation of exposure (Brandt-Rauf, 2006; Beaumont et al., 2008 and follow-up creator correspondence; Smith, 2008). A backgroundstudy did not uncover any expansion in growths in the gastro-intestinal tract in laborers who were exposed for the most part by breathing (Gatto et al., 2010), however the individual investigations were little and translation was retarded by absence of sufficient exposure estimations and absence of data on main confounders, for example, smoking, liquor utilization, dietary variables, and financial status.

2.7 Carcinogenesis induced by Chromium

Hexavalent Chromium mixes have been considered as powerful human cancer-causing agents and have been appeared to bring about changed sorts of DNA damage including DNA-protein cross-linking in different cells and tissues. Strangely, Cr (VI) does not tie to DNA or proteins in cell-free frameworks (Fornace et al, 1981; Koster, 1985). Be that as it may, Cr (VI) exists as an oxyanion at physiological pH, is promptly transported into the cell through the cell's sulfate

anion transport framework (Jenette, 1981; Arslan, 1987). Cr (VI) is acceptedinside the cell to be decreased by the cellular redox framework to its naturally most stable structure, Chromium (III) (Conett, 1983; Nieboer, 1988). Cr (III) ties to DNA and also proteins in cell-free frameworks (Tsapakos, 1983) and has high fondness for some other organic ligands (Earley 1965). Cr (III), notwithstanding, is inadequately taken up into the cell and is thought to be noncarcinogenic (deFlora, 1985). Amid the intracellular lessening of Cr(VI) to Cr(III), responsive species, for example, intermediate valance conditions of Chromium and dynamic oxygen species are created (Conett, 1983; Mattagajasingh, 1995; 1997), which may, thusly, start the cancer-causing process by changing the structure of DNA (Kawanishi, 1986). Hydroxyl radicals (·OH), which are created amid the cell diminishment of chromate (Shi, 1990) are additionally equipped for bringing on DNA-protein cross-linkage (Margolis et al., 1988; Gajewski, 1990) and are considered as "ultimate agents" in chromate carcinogenesis (Shi, 1990). Cr(III) and the receptive intermediate conditions of Chromium may likewise be considered as cancer-causing in light of the fact that -OH radicals are appeared to be produced by redox cycling of Cr(III) (Sugden, 1992), and DNA damage has been appeared to be brought on by moderate valence conditions of Chromium, for example, Cr(V) (Kortenkamp et al., 1989). In spite of the fact that chromate-actuated DNA-protein edifices are embroiled in chromate cancer-causing nature, the mechanism of their development, synthesis, and organic importance are not surely knowing. It has been proposed that cross-linkage of proteins to DNA could disturb chromatin structure and the ordinary direction of gene expression (Bedinger et al., 1983). This, thusly, could assume a part in carcinogenesis in that erasure of DNA bases may come about when segments of recreating DNA are covered under DNA-protein edifices (Briggs, 1988). Such cancellations to "tumor suppressor genes" (Bouck and Benjamin, 1989) may offer ascent to misfortune or inactivation of the gene, prompting carcinogenesis. Moreover, amid typical control of gene expression, proteins, either alone or in collaboration with different proteins, reversibly cooperate with particular DNA successions (Stein, 1979). Cross-linkage of DNA with unseemly proteins could upset the ordinary direction of DNA-protein cooperation, bringing about genuine hereditary outcomes, incorporating interruption in or adjustment of gene expression. In this way, it is important to distinguish of the proteins that take an interest in chromate-instigated DNA-protein edifices and the way of their collaboration with DNA. Distinguishing proof of proteins cross-linked to DNA

may likewise help with our comprehension of chromatin structure and protein collaborations, including the three-dimensional introduction of proteins around DNA.

2.8 Conventional methods for remediation of Chromium toxicity

Distinctive common systems to decrease Cr (VI) from waste water stream consolidates physical and mixture techniques, for instance, molecule exchange, precipitation, filtration, electrochemical treatment, adsorption, chemical lessening, film developments and vanishing recovery (Ahluwalia & Goyal, 2007; Al-Sou'od, 2012)

2.8.1 Electro Chemical Precipitation

This methodology utilizes an electrostatic potential to increase the expulsion of generous metal from polluted waste water over the customary manufactured precipitation procedure (Kurniawana, Chana, Loa, & Babelb, 2006). This technique is the best widely recognized technique for evacuating harmful overwhelming the level of metals from water in parts per million (ppm). Polprasert and Kongsricharoern in 1995 explored the Cr (VI) expulsion utilizing the ECP procedure from an electroplating wastewater. Using this system Cr (VI) obsession could be removed from 3,860 mg/L to 0.2 mg/L.

Regardless of the way that the technique is smart its adequacy is impacted by low pH level and the proximity of various salts (particles). The system requires development of various chemicals, which finally prompts the period of an abnormal state water content slime and the exchange is cost genuine. Precipitation with particle trade, lime or disulphide does not have the specificity. At lowfixation it is inadequate in evacuation ofthe metal particles.

2.8.2 Ion Exchange

Among the physicochemical methodologies made for Chromium ejection from wastewater, the ion exchange strategy is transforming into a notable system that has become much thought starting late. Ion exchange is a unit system in which the particles of a specific species variety sorts are unstuck from the insoluble exchange material by the particles of a substitute species in course of action. The courses of action containing Chromium enters through one side of the segment underweight, then passes through the resin bed, lastly Chromium is ousted. Right when the limit of resin is drained, the portion is released to clear the accumulated solids and after that recouped. Routinely, the used cross sections are synthetic natural ion exchange resins which are utilized for ion exchange.

Manufactured Dowex 2-X4 ion exchange resin was used to examine the intake of Cr (VI) from authentic plating wastewater (Sapari, Idris, & Hamid, 1996). An unequivocally major anion sap in hydroxide structure was used as a part of the sections as an anionic exchanger. Around 100% clearing of Cr (VI) was refined in the examination. Another designed ion exchange resin and Ambersep 132 was moreover researched to recover chromic acid from manufactured plating course of action in a four-phase ion exchange method (Lin and Kiang 2003).

An impediment of particle trade system for Chromium clearing is that the particle trade saps are to a great degree particular.The chosen resin must be capable of specifically expelling the metal contaminant of concern. Further, insufficient removal of Chromium may occur and the ion exchange equipment can be costly. Moreover, it can't manage concentrated metal arrangements as the system gets easily fouled by organics and diverse solids in the wastewater. Likewise, ion exchange is disregardful and it is particularly fragile to pH.

2.8.3 Biosorption

Chromium biosorption from watery courses of action is modestly another methodology that has shown outstandingly reassuring in the ejection of contaminations from liquid effluents. The adsorbent materials acquired from ease farming wastes can be used for the effective ejection and recuperation of Chromiumfrom wastewater streams. Biosorption or metal is a to some degree complex system affected by a couple of variables. System required in the biosorption method fuse incorporate complexation,chemisorption, adsorption–complexation on pores and

surface,micro precipitation, ion exchange, significant metal hydroxide development onto the surface adsorption and bio surface (Gardea-Torresdey, Rosa, & Peralta-Videa, 2004). This procedure encounters less power of biosorption and low adsorption limit.

2.8.4 Adsorption using activated carbon

It is observed that the activated carbon got from various crude materials, like nutshells, sawdust, coconut shells etc. are more capable of adsorbing Chromium(Mohan & Pittman, 2006). (Demirbasa, Kobyab, Senturkb, & Ozkana, 2004) studied on the clearing of Cr (VI) using GAC sort Filtrasorb Water Air Soil Pollut 400 from aqueous solution. It was found that decline in adsorbents particle size manufactures its surface area for the adsorption of metal, and it brings about higher decrease profitability on Cr^{6+}. Additionally, it showed that the Cr^{6+} adsorption was more positive at higher temperature. From *Terminalia arjuna* nuts, few enacted carbons were arranged and were activated chemically utilizing zinc which demonstrated most compelling removal of Chromium at pH 1.0 (Mohanty et al. 2005). (Natale et al. 2007) used activated carbon conveyed by Sutcliffe Carbon starting from a bituminous coal to adsorb Cr (VI). The adsorption was found to be limited for the enacted carbon firmly depends on upon pH of the solution and saltiness. The crucial deficiency of the method lies in its consistent recovery and desorption and is depends on the adsorbent'slife.

2.8.5 Membrane filtration

Membrane filtration procedure has gotten a noteworthy consideration for the wastewater treatment. It considers the use of water hydraulic pressure for the desired separation through the semipermeable membrane. Different sorts of membranes, for example, inorganic, polymeric, and fluid films can be utilized for Cr (VI) evacuation. (Pugazhenthi et al. 2005) arranged upheld non-interpenetrating altered ultrafiltration carbon layer by gas stage nitration utilizing NOx and amination utilizing hydrazine hydrate. The film was utilized for the division of Cr (VI) from the

fluid arrangement. Allotment tests the chromic acid course of action was done using unmodified (96% release), aminated (88% release) and nitrated (84% release) carbon film. Expulsion of Chromium VI was examined by (Muthukrishnan and Guha 2008) for changing pH and concentration of the membrane feed solution by using different nanofiltration composite polyamide layers. For this examination two films were used, the first one is a high rejection membrane (NFI) and the other one is a low rejection membrane(NFII). The release rate of Chromium was found to increase with the extension of feed solution pH.

The genuine inconvenience of this system isolated from being fiscally immoderate have obstacles like high vitality,deficient metal ejection, reagent consumption and generation of destructive ooze or waste materials that require exchange.

The drawback of this technique for the treatment of Cr (VI) contaminated soil and groundwater is that, high amount of energy is used continuously, use of reductants which are poisonous and costly.

These techniques are costly and in some cases the secondary wastes require proper treatment.Bioremediation is the best alternative for the treatment of Chromium contamination as it is cost effective and harmless to the environment (Kamaludeen, Arunkumar, Avudainayagam, & Ramasamy, 2003).

In Situ bioremediation advancement can be associated with evade the limitations of chemical and physical techniques. Some researchers have recorded direct metabolic decrease of Cr (VI) by bacteria organisms. Cr (VI) bioreduction emits an impression of being inescapable since, Cr (VI) diminishing consortia were isolated from Cr (VI) contaminated areas and furthermore uncontaminated goals. After microbial reduction, it is ordinarily acknowledged that Cr (VI) forms are changed into stationary insolubleand Chromium hydroxide. From now on, this development can be associated at field regions for the immobilization of Cr below the earth surface.

2.9 Metals and Microorganisms

2.9.1 The mechanism of Metal resistance in Bacteria

The inescapable method for metals in nature has achieved the limitless appearance of the resistance of metals in microorganisms. The metal resistanceof microbes is heterogeneous in both their innate and biochemical bases and may be plasmid, transposonor chromosomally encoded along with other qualities. The five segments that are all around proposed considerable metal resistant in bacteria (Rouch et at., 1995) are given as follows;

1. Elimination of metals by porousness hindrance.
2. Elimination of metal from cell by active export.
3. Intracellular physical segregation of metal by restricting proteins to keep it from harming metal-delicate cell material.
4. Extracellular segregation.
5. Detoxification of metal where the metal is artificially adjusted to render less dynamic.

2.9.2 Metal sensitive cellular components

Metal ions is capable to diminish or build catalyst activity, or adjust compound specificity by actuating conformational change in enzyme or by the locking the enzymes in particular compliances; or by shaping creating bonds with the active and other vital locales in chemicals and transport systems, for this reason their function is prevented.

Metal ion can specifically harm DNA structure, for instance by deliveringcross link strandsor they may impact the data substance of DNA in a roundabout way by reducing the devotion of DNA synthesis(Beyersman, 1994).

In spite of the fact that an extensive variety of cell parts are potential focuses for the metal-induced damages, a subject of these segments are important for crucial cell capacity, for example, DNA used for replication. Cell passing will come about because of inactivation due to metal-inductionby metal sensitivities so that as the centralization of a specific metal ascents, if the concentration reaches to the critical level then their function is inactivated. Along these lines, contingent on the convergence of the metal, the cell must have a few methods for assurance for one or more target sites for their survival. The more noteworthy the grouping of metal the more noteworthy the quantity of fundamental parts that requires protection. For instance, E. coli,

generation of the main proteins can be avoided by transformation in a single gene bringing about expanded metal resistance (Lutkenhaus, 1977).

2.9.3 Metal uptake system and resistance

For the cells which have very metal-sensitive fundamental cell segments are situated within the cytoplasm, the resistance mechanism is decided by the quantity of uptake system for the passage of metal into the cell. The lipid part of the cell membrane is exceptionally impermeable to hydrophilicions, like, the metal cations (Anderson, 1978). Metal, hence, for the most part goes through the membrane where resistant sites are less. For instance, if there should arise an occurrence of E. coli, Cobaltand Arsenate enters through the Pit or phosphate transport framework and the mgt or Magnesium Transport framework. (Silver et al., 1975).

2.9.4 Metal as a biological requirement

Various substantial metals are important to keep up fundamental metabolic exercises of bacterial cells. Copper, iron and nickel are required by the greater part of the bacterial species, and Molybdenum, tungstenand cobalt in a few species. When all is said in done, these metals will be less harmful to the cell when contrasted with metal with no positive metabolic activities, as cell will have proper systems that will adapt up to the little functions in local concentrations.

2.9.5 Gene cassette versus chromosome-mutation-determined resistance

The hereditary premise of metal resistance in safe microscopic organisms will be dictated by the accessibility in the nearby population of a performed gene cassette that determines a committed component of resistance. These are suggested to have been embraced to give resistancesuccessful by evolutionary selection. Furthermore, can be borne on chromosome, transposon orplasmid. The last two competitors can advance exchange of the connected resistance cassette between the bacterium. Cassette intervened resistance may give more elevated amounts of resistance than the accessible chromosomal mutation may permit. The bigger a

populace, the more prominent is the likelihood of a significant cassette bearing hereditary component being available.

2.10 Mechanism of Chromium resistance in Bacteria

The chromosomal resistance in bacteria makes utilization of techniques like particular or unspecific Cr(VI) reduction, free radical detoxifying exercises, repair of DNA damage (Morais et al., 2011) and forms connected with sulfur or iron homeostasis (Ramirez-Diaz et al., 2008). Numerous microorganisms can possibly survive dangerous metal-dirtied situations by creating systems to stay away from toxicity of metals like, adsorption uptake, DNA methylation, metal efflux, and metal biotransformation either specifically by enzymatic decrease to less portable and harmful structures or by implication through making complexes with metabolites, (for example, H2S) (Camargo et al., 2005; Pei et al., 2009; Soni et al., 2012). Microbial lessening of Cr(VI) to Cr(III) is especially critical from bioremediation perspective which can be considered as an extra chromate resistance mechanism (Cervantes et al., 2001). An assortment of Cr- resistant microorganisms with high Cr(VI)- diminishing potential have been accounted for including *Pseudomonas, Deinococcus,Bacillus, Enterobacter,Escherichia,Shewanella, Agrobacterium, Thermus* and different species (Ohtake et al., 1987). It has been accounted for that both chromate resistant and in addition non-resistant strains can reduce chromate however the development of later are altogether hindered at higher chromate concentrations (Bopp and Ehrlich, 1988). In this manner, the bacterial property, which is especially helpful for a successful bioremediation methodology, is one that joins high resilience/resistance with the capacity to reduce Cr(VI) to Cr(III) (Dhal et al., 2013). A few microorganisms displaying Cr (VI) diminishing activities and resistance have been disengaged and distinguished from chromatecontaminated environment andalso in uncontaminated biological systems (Schmieman et al., 1998; Turick et al., 1996; Wang and Shen, 1995). Microorganisms that can diminish Cr(VI) are generally called as Chromium reducing bacteria (CRB). Among CRB, the Gram-positive bacteria are appeared to have huge resistance to Cr(VI) toxicities at moderately high concentrations, while Gram-negative bacteriahavecomparatively less tolerance to Cr(VI) (Coleman, 1988). Microorganisms found in metal tainted environment are actually safe for such metals. An examination completed by Das et al. (2013) uncovered that the bacteria collected from chromite mine soils are safe towards Cr(VI)

alongside other substantial metals. It is notable that chromate resistance and reduction are not as a matter of course interrelated, and not all Cr (VI) resistive bacteria can reduce Cr(VI) to Cr(III). In this way, both Chromium resistance and reducing are observed to be autonomous properties of bacteria (Bopp and Ehrlich, 1988; Silver, 1997). Bacterial Cr(VI) decrease can either happen "directly" by the utilization of enzymes or "indirectly", where Cr(VI) diminishment is catalyzed by the metabolic deciding items, for example, Fe(II) and HS- of iron and sulfate-reducingbacteria (Hwang et al., 2002). Bacteria utilize distinctive resistance components to overcome the Cr(VI) harmfulness in the earth which incorporate the reduced uptake of Cr(VI), extracellular Cr(VI)reduction, reactive oxygen species (ROS) detoxifying intracellular Cr(VI) reduction, DNA repair enzymes, efflux of Cr(VI) from cell and ROS rummaging are portrayed in Fig. 2.2 (AeF).

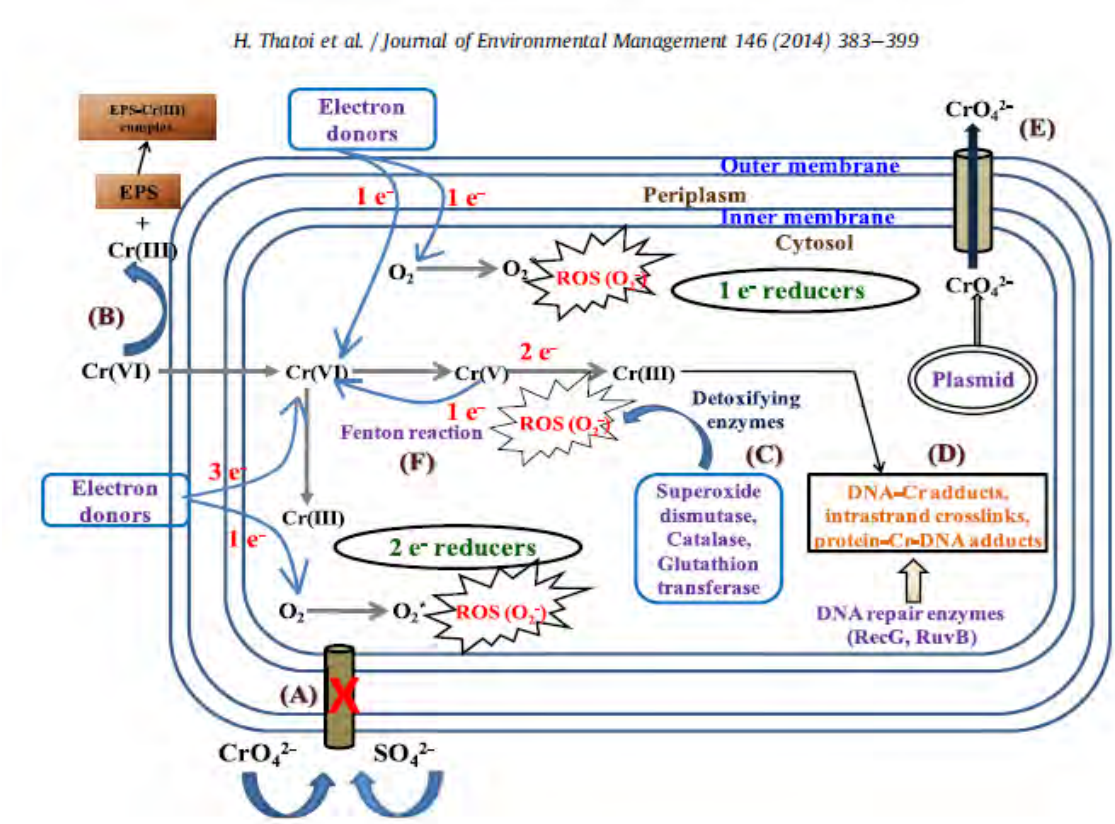

Figure 2.2: Mechanisms of chromate resistance in bacterial cells. (A) Mutation in chromosome-encoded sulfate uptake transporters (B) Extracellular reduction of Cr(VI) to Cr(III) (C) Intracellular reduction of Cr(VI) to Cr(III) by chromate reductase (D) Function of SOS repair

system in reducing oxidative stress (E) Efflux of chromate from the cytoplasm. (F) Action of ROS scavenging enzyme in reduction of oxidative stress.

(A) Reduced uptake of Cr (VI)

One of the proficient defensive systems against the deadly impacts of Cr(VI) is likely connected with reduced uptake of Cr(VI) like sulfate uptake pathway and with sulfur or iron homeostasis. Since chromate ions (CrO_4^{2-}) has basic takes after with tetrahedral sulfate ions (SO_4^{2-}) (Fig. 2.2), it can without much of a stretch go through cell membranes by means of SO_4^{2-}transport pathway, with the assistance of non-particular anionic (SO_4^{2-}, PO_4^{3-}) bearers (Wenbo et al., 2000). The movement of chromate is decreased if the chromosome encoded sulfate uptake pathway in bacteria is mutated (Ramirez-Diaz et al., 2008). The microorganisms present in metal tainted environment experience speedy transformation to create Cr(VI) resistance that prompts diminished Cr(VI)uptake by sulfate transport pathway. Defenseless organisms can get to be inhumane by change or by consolidation of the hereditary data which encodes the resistance (Kümmerer, 2004).

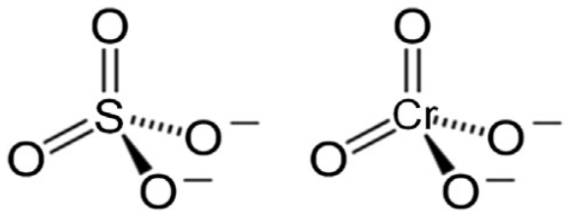

Figure 2.3: Structural similarity of chromate and sulfate ions.

(B) Extracellular Cr (VI) reduction

Extracellular reduction of Cr(VI) to Cr(III) trailed by its binding with the functional group on bacterial cell surface is another resistance system (Ngwenya and Chirwa, 2011). Binding of reduced Cr(III) to bacterial cell surface helps its simple expulsion from the debased environment. Peptidoglycan segments present in the cell dividers of these organisms observed to be intense binder of Cr(III) (Hoyle and Beveridge, 1983). It has exhibited that a few types of bacteria have adsorptive properties which encourage the expulsion of metal species from aquatic solution. These properties are generally subject to the circulation of reactive functional groups like

carboxyl, hydroxyl, amine, sulfhydryl and phosphate group on the cell divider surface of the bacteria (Parmar et al., 2000). Along these lines there is no entry of Cr(VI) in the cell when its reduction happens extracellularly.

(C) ROS detoxifying enzymes/Intracellular Cr (VI) reduction

Amid Cr(VI) diminishment to Cr(III) a fleeting, very reactive intermediate Cr(V) radical is produced which redox cycles. In this manner, Cr(V) is oxidized back to Cr(VI), giving its electron to dioxygen and creating reactive oxygen species, alluded as ROS. Generations of ROS results in oxidative stress in the organisms. In this procedure, the bacterial proteins are likewise impelled by chromate in the protection against oxidative stress prompting an extra system of chromate resistance (Ramirez-Diaz et al., 2008). Be that as it may, the oxidative stress emerging because of the ROS are invalidated to a vast degree by detoxifying catalysts like superoxide dismutase (SOD), glutathionetransferase,catalase, etc. (Ackerley et al., 2004b).

(D) DNA repair enzymes

Security of bacterial cells by DNA repair enzymes of damaged DNA created by Cr(VI) is another protection shield. Cr(VI) enters the bacterial cell which is promptly diminished to Cr(III) by the activity of different enzymatic or non-enzymatic exercises that produces the generation of ROS, which thus applies injurious impacts on protein and DNA in the cell. The ROS produced causes damage to DNA, similar to base change, single-strand breaks, double strand breaks. Such harms brought about to DNA can be repaired by exceptional DNA repair mechanism like the SOS response enzymes (RecA, RecG, RuvB) (Hu et al., 2005). For instance, in Escherichia coli, Cr(VI) has for quite some time been known not the E. coli SOS repair framework that shields DNA from oxidative damage (Llagostera et al., 1986). Also DNA helicases like RecG and RuvB, segments of the recombination DNA repair systems, have appeared to take an interest in the reaction to DNA damage brought on by chromate in Pseudomonas aeruginosa (Miranda et al., 2005). Cell Cr(VI) reduction is the enactment procedure, creating redox-dynamic intermediates Cr(V/IV) and stable Cr(III) framing Cr-DNA adducts which is the most plenteous type of DNA damage that causes changes and chromosomal breaks (Zhitkovich, 2011).

(E) Efflux of Cr (VI) from cell

Chromate ion efflux from the cell cytoplasm, intervened by transporters encoded by particular plasmid-borne genes, is additionally a resistance instrument found in bacteria. Efflux of chromate is by all accounts an effective and widespread mechanism of resistance, which prevents the collection of toxicions inside the bacterial cells (Ramirez-Diaz et al., 2008). The best comprehended chromate resistance framework is that given by *P. aeruginosa*. ChrA protein has a place with the chromate ion transporter CHR superfamily. ChrA, is a hydrophobic membrane protein, encoded by plasmids pUM505 of *P. aeruginosa* and pMOL28 from *Cupriavidusmetallidurans* (in the past *Alcaligeneseutrophus* and *Ralstoniametallidurans*) (Cervantes et al., 1990; Nies et al., 1990) that have been shown as included in chromate resistance by chromate efflux component (Ramirez-Diaz et al., 2008). ChrA protein performs as a chemiosmotic pump that fares chromate from cytoplasm or periplasm to outside driven by the proton thought process power (Alvarez et al., 1999). CHR proteins from some bacteria have been exhibited as included in chromate resistance by chromate efflux system (Ramirez-Diaz et al., 2008).

(F) ROS scavenging

Cr(VI) subsequent to entering the cell can be diminished to Cr(V). Electron contributors like NAD(P)H or some other natural mixes (like glucose) give electrons to Cr(VI), prompting the development of relative temperamental poisonous intermediate Cr(V). In spite of the fact that the chromate reinstructs diminish Cr(V) further to Cr (III) by a two-electron exchange (through "semi-tight" mechanisms), sometimes this response is not exceptionally quick. Therefore a segment of Cr (V) intermediate is immediately reoxidized to Cr (VI) along these lines producing ROS by a Fenton-like response. Amid this procedure hydroxyl radical (-OH) are shaped in the microbial cells (Shi and Dalal, 1994) as delineated in the condition beneath:

$$Cr\ (V) + H_2O_2 \longrightarrow Cr\ (VI) + -OH + OH$$

Amid the diminishment procedure, atomic oxygen is lessened to O_2 -radicals, which create H_2O_2 by means of dismutation. Hexavalent Chromium responds with H_2O_2 to create -OH radicals through a Fanton like response. This step is like the oxidation of Fe(II) with H_2O_2 in the Fenton response as the creation of -OH from Fe(II) through Fenton response is encouraged enormously by the arrangement of Fe(II) complex that have empty locales for H_2O_2 coordination.

Table 2.2 summarizes the bacterial strategies and have related to chromate tolerance.

Table 2.2: Bacterial Mechanisms of Chromate resistance

Enzyme/ system	**Species**	**Function**	**Reference**
Transport			
ChrA transporter	*Pseudomonas Aeruginosa*	Efflux of cytoplasmic Chromate	Alvarez et al. 1999
Cys operon products	*Shewanella Oneidensis*	Sulfate transport	Brown et al. 2006
TonB receptor, hemin Transporter	*S.oneidensis*	Iron transport	Brown et al. 2006
Reduction			
Chromate reductases	Diverse species	Reduction of Cr(VI) to Cr(III)	Cervantes et al. 2001
SOD, catalase	*Escherichia coli*	Combat of oxidative Stress	Ackerley et al. 2004

Outer membrane Proteins	*Caulobacter Crescentus*	General stress Response	Hu et al. 2005
DNA repair			
RecG and RuvB DNA Helicases	*Pseudomonas Aeruginosa*	Repair of DNA Damage	Miranda et al. 2005
SO0368, UvrD, and HrpA helicases	*Shewanella Oneidensis*	Repair of DNA Damage	Chourey et al. 2006
Other Mechanisms			
Cys operon products	*S. oneidensis*	Sulfur metabolism	Brown et al. 2006
Enzyme/ system	**Species**	**Function**	**Reference**
Adenylyl sulfate Kinase	*S. oneidensis*	Sulfur metabolism	Brown et al. 2006
Ferritin	*S. oneidensis*	Iron binding	Brown et al. 2006

2.11 Microbial reduction of hexavalent Chromium

Bioremediation has created from the research facility to a completely popularized innovation in the course of the most recent 30 years in numerous industrialized nations. A fruitful bioremediation plan depends on the administration of soil microbial populaces fit for catabolizing the contaminants. Heavy metals show dangerous impacts on soil biota, and they can influence key microbial procedures and abatement the number and movement of soil microorganisms (Obbard et al., 2001). Microbial populace has regularly been proposed to be a simple and touchy marker of anthropogenic consequences for soil ecology. Cr (VI) has been accounted for to bring about movements in the arrangement of soil microbial populaces, and causes inconvenient consequences for microbial cell digestion system at high concentration. Many studies on soil defilement of heavy metal from mechanical destinations were accounted for

("Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria)," 2007). Since the revelation of the primary organism fit for decreasing Cr^{6+} in the 1970s (Zhu et al., 2008), the quest for Cr^{6+} reducing microorganisms (both high-impact and anaerobic) has been energetically sought after, with various strains being secluded.

2.11.1 Bioremediation of Chromium

Customary techniques for expelling metals from tainted destinations incorporate compound precipitation, oxidation/diminishment, ion exchange, filtration, membrane utilization, evaporation and adsorption on actuated coal, alum, kaolinite, and cinder (Barceloux & Barceloux, 1999; Otha, Galsworthy, & Pardee, 1971). Notwithstanding, the majority of these strategies require high vitality or substantial amounts of chemical reagents, with conceivable creation of secondary pollution (Jeyasingh & Ligy, 2005; Komori, Rivas, Toda, & Ohtake, 1990). Concerning expulsion of Cr(VI), traditional methodologies incorporate compound decrease took after by precipitation, adsorption and ion exchange on enacted carbon, alum, kaolinite and of slag, and the vast majority of these techniques require a high energy and a lot of chemical reagents (A. Ganguli & A. Tripathi, 2002). Additionally, expensive safe transfer of harmful muck, fragmented reduction of Cr(VI) and high cost for Cr(VI) reduction, particularly for the expulsion of moderately low convergences of Cr(VI) are non-advantageous from the efficient perspective (Kratochvil, Pimentel, & Volesky, 1998; Patterson, 1985).

An inventive innovation is spoken to by bioremediation, which utilizes the metabolic capability of microorganisms to expel harmful metals, with a specific end goal to purify the dirtied territories. Bioremediation systems can be named in situ or ex situ depending, separately, on whether the mediation is completed with reasonable bacteria specifically on the contaminated site, or on segments of natural frameworks, for example, water, dregs or soil, subsequent to being expelled and transported in appropriate facilities for treatment (Pattanapipitpaisal & Reakyai, 2013). Cr(VI)- resistant microorganisms speak to a vital chance to have sheltered, temperate and ecologically neighborly techniques for reducing Cr(VI) to Cr(III), for conceivable bioremediation applications (Raspor et al., 2000). The diminishment of Cr(VI) to Cr(III) is then a potential helpful procedure for the recuperation of destinations debased by Cr(VI) (Polti, Amoroso, & Abate, 2010b). Cr(VI)- evacuation in light of microorganisms is presently thought

to be a powerful option strategy to the routine procedures, and is accepting awesome consideration for potential application in bioremediation(Dey & Paul, 2013), (A Ganguli & AK Tripathi, 2002). Checking that the insolubility of Cr(III) encourages its precipitation and expulsion, the biotransformation of Cr(VI) to Cr(III) has been considered as an option procedure for treating Cr(VI)- tainted wastes (Cervantes et al., 2001; Ohtake, Cervantes, & Silver, 1987). Among biotechnological approaches, microbial reduction of Cr(VI) is financially savvy and eco-accommodating and can offer a practical option (Ge, Zhou, Dong, Lu, & Ge, 2013). Microbial reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Chromiumresistivemicroorganisms are capable of the natural reduction of Cr(VI) into the less portable Cr(III), and its resulting precipitation, could speak to a viable technique for detoxification of Cr(VI) polluted locales and have a potential use in bioremediation (Jain, Amatullah, Alam, & Mahmud, 2012).

2.11.1.1 Phycoremediation

Incorporated into the bioremediation advances, phycoremediation is the utilization of photosynthetic microorganisms as microalgae, macro algae and cyanobacteria for the evacuation of pollutants as metals. Moreover, it is vital to comprehend the conveyance of the metal adsorbed onto the surface in connection to the metal accumulated inside the cell, keeping in mind the end goal to comprehend the overwhelming evacuation mechanisms and to settle on choices of the feasibility of the recuperation of the adsorbed metals (Olguin & Sanchez-Galvan, 2012).

2.11.1.2 Biosorption and bioaccumulation

Biosorption and bioaccumulation of Chromium for bioremediation purposes have been illustrated. Yeasts and molds have been most generally explored from this angle, and the mechanisms of Chromium resistance of chosen microorganisms are of specific significance in bioremediation technologies. The systems of Chromium lethality and detoxification have been concentrated widely in yeasts and fungi, and some encouraging results have developed around there (Poljsak, Pocsi, Raspor, & Pesti, 2010). The capacity existing in various natural microorganisms, known for their ability to bind with metals, can be confirm in human gastrointestinal bacteria. Bacterial species having a place with the class Lactobacillus, occupant

in various regions as the human body and in matured nourishments, can bind with metals, including Cr(VI), and to detoxify them from various areas(Monachese, Burton, & Reid, 2012).

2.11.1.3 Bio augmentation-assisted phytoextraction

A technique for bioremediation of contaminated sites by metals, including Chromium, is spoken to by bio augmentation assisted phytoextraction, in which fungi and bacteria, connected with plants ready to accumulate metals were broke down on the premise of a proposed as bioprocess for a bioremediation approach. The execution of bio augmentation to support the microbial survival was recommended keeping in mind the end goal to upgrade the microbial-plant affiliation and the proficiency of the procedure (Lebeau, Braud, & Jezequel, 2008).

2.11.1.4 Biomineralization

Biomineralization is a procedure by which microorganisms change aqueous metal ions, including Chromium, into indistinct or crystalline precipitate. Biomineralization is viewed as a promising and financially savvy procedure for remediating Chromium contamination. A case of arsenic precipitation was considered as a conceivable system for arsenic bioremediation of sediments debased by arsenic (Focardi et al., 2010). Naturally mediated change, immobilization, and mineralization of poisonous metals may speak to an essential point of view for bioremediation(Cheng, Holman, & Lin, 2012).

Chapter 3: Materials and Methods

3.1 Introduction

This section portrays materials utilized and diagrams the exploratory configuration for biodegradation of hexavalent Chromium through batchprocess. It likewise gives an overview of the disengagement and portrayal of bacterial strains from Marine sample. Qualities of soil and water samples were acquired from Sitakunda Ship Breaking Yard were all around archived. Enhancement of culture and procedure parameters for the cell development and Cr (VI) debasement kinetics was likewise concentrated on.

3.2 Chemicals

Immaculate and logical evaluation chemicals were utilized as a part of all trials including media arrangement for development. Nutrient agar (Merck, India) and nutrient broth(Merck India), Potassium chromate (Merck, India) and DPCZ (Merck, India) were utilized as a part of this study.

3.3 Glassware and Apparatus

The instruments and apparatus utilized all through the investigation are recorded underneath in

Table 3.1

Table 3.1: List of Instruments used during the whole experiment and function

Instruments	**Functions**
Vertical Autoclave	Sterilization
Analytical Balance	Weight Measurement
Laminar airflow	Aseptic Environment
pH	Measurement of pH

BOD Incubator	Incubation of cultures
Water system	Preparation of the stock solution, throughout the experiment etc.
Spectrophotometer(UV/Vis)	Estimation of cell growth and Cr (VI) degradation
Centrifuge	Collection of pellet and Cr (VI) estimation
Microscope	Microbial morphology Observation
Shaker	For incubation of Bacterial culture at room temperature.
Micro Pipette	To draw trace amount of media and chemicals.

3.4 Sample Collection

Ship breaking industry is a standout amongst the most monetarily critical and developing ventures in the seaside areas of Bangladesh that has a considerable measure of ecological disadvantages. This industry is arranged in the Fauzdarhat shoreline, Sitakunda (Bhatiary to Barwalia) (Fig-3.1) which covers 16 km range close to the coastline of the Bay of Bengal, around 20km to southwest of Chittagong (DNV, 2001). Beach front environment is very dynamic and naturally assorted going with basic earthly and oceanic territories, for example, mangrove timberlands, regularly and for all time immersed wetlands and salt marshes. Ship breaking is a procedure of disassembling out-of-administration ships keeping in mind the end goal to reuse the scrap metals. Bangladesh is the pioneer in separating of extensive ships (Ahmed et al., 2013). It breaks principally expansive lethal ships likecargo ships, tankers and compartment ships (Frey, 2013). Almost all the ships took back to Bangladesh are poisonous, obsolete ships age around 20-30 years (Sarraf et al., 2010). By and large, a boat comprises of roughly 95 percent of steel and 5 percent of unsafe materials (Khan et al., 2011). The ocean is a dynamic biological community since ship disassembling happens in shoreline zone, along these lines tidal zone, sub-tidal zone, remote ocean slowly gets to be host to the diverse sort of squanders. These squanders contain bacterial contaminants, petroleum hydrocarbons andsubstantial metals (Reddy et al., 2005).

Heavy metals can be amassed by the marine spineless creatures, partner with particulates and adsorb by the silt. All these could be suspended or make dissolvable complex to

Chromiumdeposition (Reddy et al., 2005). By and large, a ship, with mass in-between 5000 to 40000 ton, is covered with paint of 10 to 100 ton which contains heavy metal (Khan et al., 2011).

The water and soil tests gathered (0–15 cm depth) from various areas of the Chromium testimony and sullied site at Ship Breaking Yard of Sitakunda.

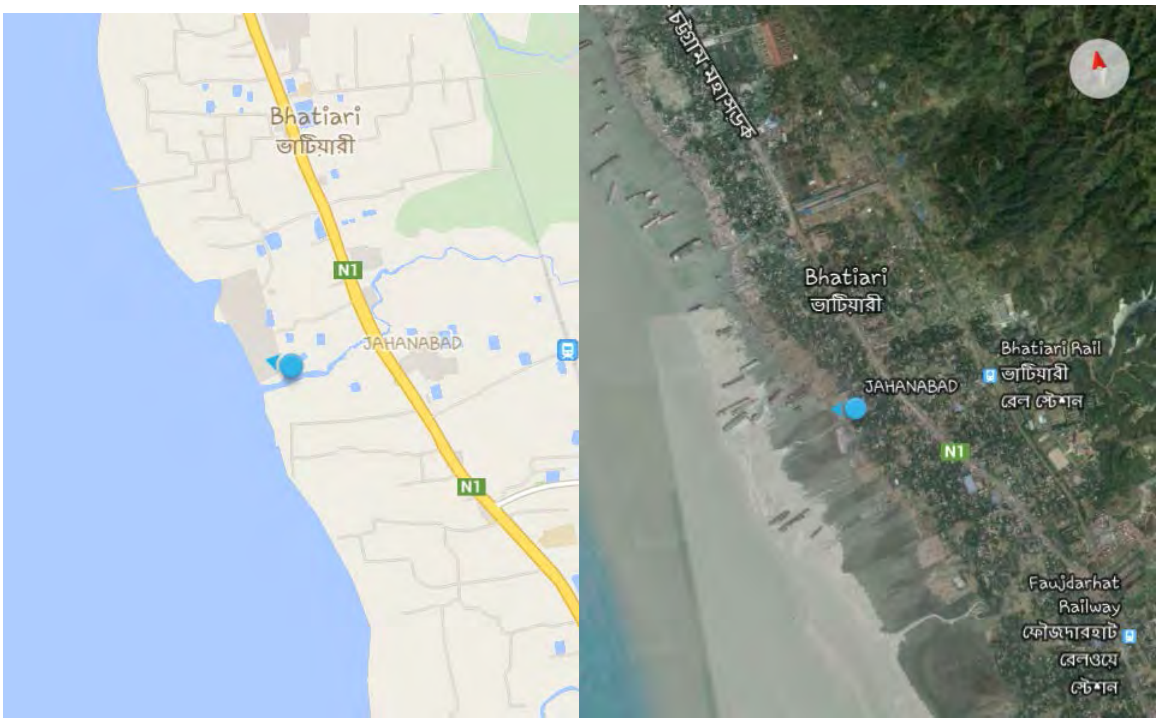

Figure 3.1: Area of Ship Breaking Industry, Bangladesh (Google Map, 2014)

3.5 Isolation and Culture Condition

Seclusion of bacteria from the soil specimens gathered was done according to standard systems. For disengagement of Chromiumresistant bacteria, we immunized 100 µL of waste water sample and 100 µL of soil sample (Fresh specimen) (1gm of soil was taken in a tube containing 9 ml of saline water to break down the dirt) by spreading system in nutrient agar plate containing 2mM of Cr^{6+} supplemented as K_2CrO_4 to the media and incubated. The colonies were seen following 24 hours of incubation at 37^oC.

Nutrient agar media was set up by dissolving 2.8g of nutrient agar powder to 100 mL water. The medium was autoclaved at 121^{o}C and maintaining a pressure of 15 Lb. for 45 min. At that point K_2CrO_4 was added to the media lastly the media was filled the plate to set up the nutrient agar plate. Isolated colonies were gotten with sanitized tooth pick and streaked on nutrient agar medium plate containing 2mM, 3mM, 4mM, and 5mM Cr^{6+}.It was again incubated at 37^{o}C for 24 hours. This procedure was rehashed with progressively higher concentrations (6mM, 7mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM) of Cr^{6+} until the minimum inhibitory concentration (MIC) of bacterial confine was acquired. Huge development and quick Cr (VI) degradation kinetics of the particular bacterial species within the sight of 40mM Chromium (VI) amid twenty-four hours of incubation at 37°C, were considered as Cr (VI) resistant. A solitary strain fit for developing at this condition was chosen for further investigation. We isolated 8 single colonies from various Nutrient Agar plates containing distinctive Chromium fixations toward the end of the confinement procedure and gave the name relating to their unique Chromium focus, for example, 2mM soil (3) and 2mM Soil (4).

3.6 Chromium reduction profile of Chromium resistant bacteria

Diphenyl carbazide examine for estimation of Cr^{6+} was adjusted from Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992) and also the strategies recorded in Turick et al., 1996. A standard curve was set up to institutionalize the diminishment profile of the Chromium resistant bacteria.

3.6.1 Preparation of Chemicals

3.6.1.1 Preparation of 10ml 3M H_2SO_4

Firstly, in a falcon tube 8mL distilled water was taken. Then concentrated sulphuric acid of 1670 µL was added drop by drop to the falcon tube containing 8ml of distilled water. After that, the volume of the solution was made up to 10mL by adding 330 µL distilled water.

3.6.1.2 Preparation of Diphenyl Carbazide

In a falcon tube 0.025g of DPCZ powder was taken. After that 9.67 mLof acetone followed by 330 µL 3M $H_2SO_4$was introduced in the falcon tube containing DPCZ powder. The falcon tube was shaken well for the preparation of homogenous Diphenyl Carbazide solution.

3.6.1.3 Preparation MOPS buffer

Firstly, for the preparation of 50mL 1N NaOH, 0.1g NaOH was added in 50ml of water. After that 334.88mg of MOPS was added with 80ml of distilled water for the preparation of 20mM MOPS buffer.By adding sufficient amount 1N NaOH in the buffer solution the pH of MOPS buffer was adjusted to 7.

3.6.1.4 Preparation of 5mM 10ml K_2CrO_4

Firstly, 19.4g of K_2CrO_4 was dissolved in 10mL distilled water to prepare 1M K_2CrO_4 solution. After that, the solution was filtered using membrane filter having pore size0.45 micron. Finally, the solution was diluted to 5mM concentration and was stocked for further use.

3.6.2 Experimental procedures

3.6.2.1 Preparation of Standard Curve

3.6.2.1.1 Preparation of sample for reaction

The following sample solutions of different strength were prepared. The final volume of each sample was 1ml.

Table 3.2: Preparation of sample for Standard Curve

Concentration Final	**Quantity of 5mM K_2CrO_4solution**	**Amount of NB added**	**Final Volume to solution**
50 µM	10 µL	990 µL	1 ml
100 µM	20 µL	980 µL	1 ml
150 µM	30 µL	970 µL	1 ml

200 µM	40 µL	960 µL	1 ml
300 µM	60 µL	940 µL	1 ml
400 µM	80 µL	920 µL	1 ml
500 µM	100 µL	900 µL	1 ml
600 µM	120 µL	880 µL	1 ml
700 µM	140 µL	860 µL	1 ml
800 µM	160 µL	840 µL	1 ml
900 µM	180 µL	820 µL	1 ml
1000 µM or 1mM	200 µL	800 µL	1 ml

3.6.2.1.2 Reaction protocol for standard curve

At first, in a falcon tube 600 µL sample was taken. After that 1.2 ml 20mM MOPS buffer, 99 µL 3M H_2SO_4, 981 µL distilled water and 120 µL DPCZ were added gradually to the sample. Finally, a homogenous mixture was prepared. After the reaction, the solution changed its color. Thenat 540nm the absorbance of the reaction solution was measured using a UV- Visible spectrophotometer.

3.6.2.2 Evaluation of reduction profile of selected isolates at room temperature

3.6.2.2.1 Procedure

- **Day 0:**

In two separate conical flasks (10 mL each)nutrient broth was prepared. After that, sample was taken from stock culture and inoculated in the nutrient broth media. The uninoculated nutrient broth media was considered as control. Then both flasks were incubated overnight on an incubator.

❖ **Day 1:**

The next day, in two separate conical flasks (25 mL each) nutrient broth was prepared. Into the nutrient broth 25 μL K_2CrO_4 was added for the preparationof 1mM K_2CrO_4. Next, from the culture flasks 3 mL of culture solution was withdrawn which was incubated at room temperature at the previous day. Then the O.D. of the culture solution (both and sample and negative control) was measured using UV-Visible Spectrophotometer at 600nm wavelength for the observation of cell growth. After that, to obtain O.D. of 0.2 in the experiment culture the amount required for the overnight culture was calculated. Then, a sterile falcon tube was used to withdrawn the required amount of overnight culture (both sample and negative control). Then the samples were centrifuged for 5 minutes at the speed 4000 rpm. Subsequent to centrifuging the required measure of overnight culture pellets were taken and moved into the recently arranged 25ml nutrient broth supplemented with 1mM Cr (VI) and was vortexed well to acquire 0.2 optical density of the culture. Then at room temperature (25^oC) the freshly prepared culture was incubated. After 1.5hours, 2 ml of freshly prepared experiment cultures (from both specimen and control) were taken from the conical and O.D. were measured at 600 nm wavelength to observe the growth of the cell. At that point, these 2ml cultures were centrifuged for 5 minutes at 4000 RPM and the supernatants were taken. After that the same response strategy as expressed in segment 3.6.2.1.2 was led and the O.D. were recorded at 540 nm wavelength. The procedure was rehashed after 3^{rd}, 4.5^{th}, 6^{th} hour and the last reading was taken after overnight growth i.e. after 24hours. This informationwas placed in OriginPro v8.0 programming to acquire the reduction profile.

Chapter 4: Results

4.1 Isolation data of Chromium resistant microorganism

Two single colonies were isolated from various Nutrient Agar plates with different Chromium concentrations. The names were given according to their unique Chromium concentration like S_2 and S_4.

4.2 Chromium reduction profile of Chromium resistant microorganism

4.2.1 Standard Curve

For obtaining a standard curve, the procedure mentioned in 3.6.2.1.2 was repeated twice. Then using the mean absorbance value, a graph was plotted utilizing Microsoft Excel 2016 software. The results found are as follows:

Table 4.1: Standard curve data of Chromium (VI)

Concentration (µM)	Absorbance (R1)	Absorbance (R2)	Mean Absorbance
50	0.12	0.08	0.1
100	0.460	0.468	0.464
150	0.908	0.912	0.91
200	1.11	1.05	1.08
300	1.592	1.586	1.589
400	2.070	2.080	2.075
500	2.447	2.445	2.446

600	2.801	2.821	2.811
700	2.865	2.837	2.851
800	3.012	3.028	3.02
900	3.127	3.101	3.114
1000	3.120	3.136	3.128

From the above data, the following Standard Curve was obtained:

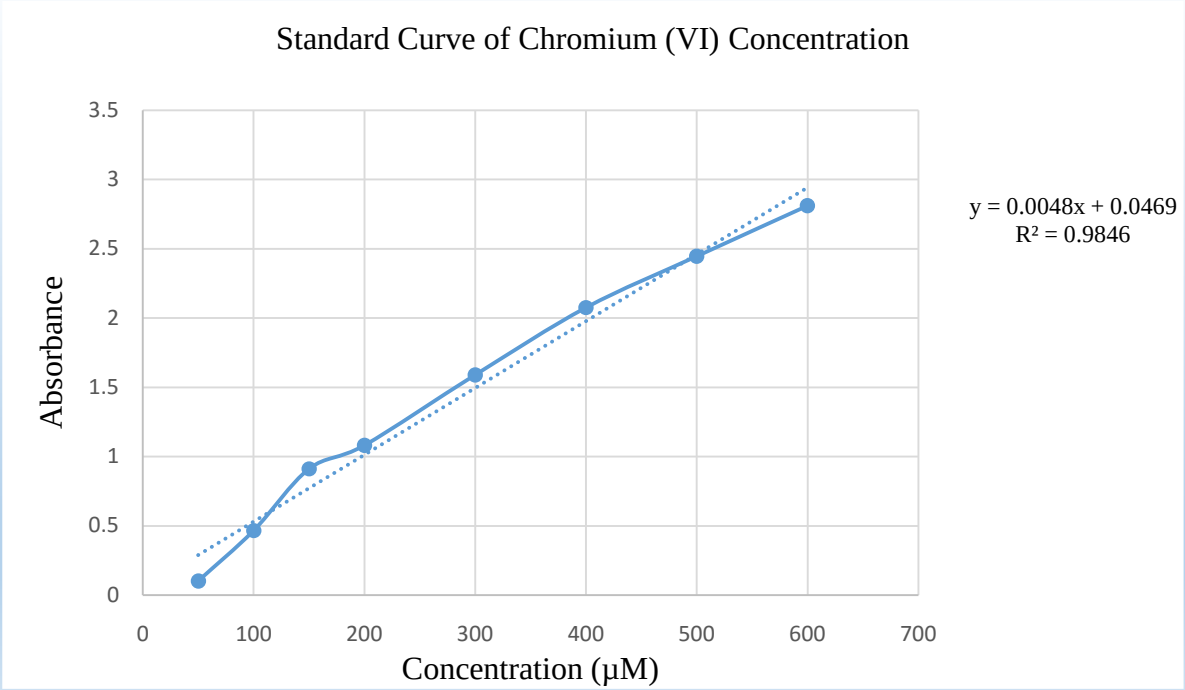

Figure4.1: Standard curve of Chromium (VI)

4.2.2 Reduction profile of Isolate: S_4

The experiment was performed at different parameters for example, changing the temperature and pH. The results obtained are summarized below:

Table 4.2: Isolate- S_4: Chromium reduction profile Vs. Cell Growth at 25^{o}C, pH 7.0

Sample		
Time (Hours)	**Concentration of Bacteria at 600nm(Cell density)**	**Concentration of Chromium at 540nm(Chromium density)**
0	0.224	587.125
1.5	0.297	569.375
3.0	0.442	559.5
4.5	0.457	529
6.0	0.503	506.25
24	1.61	180.625

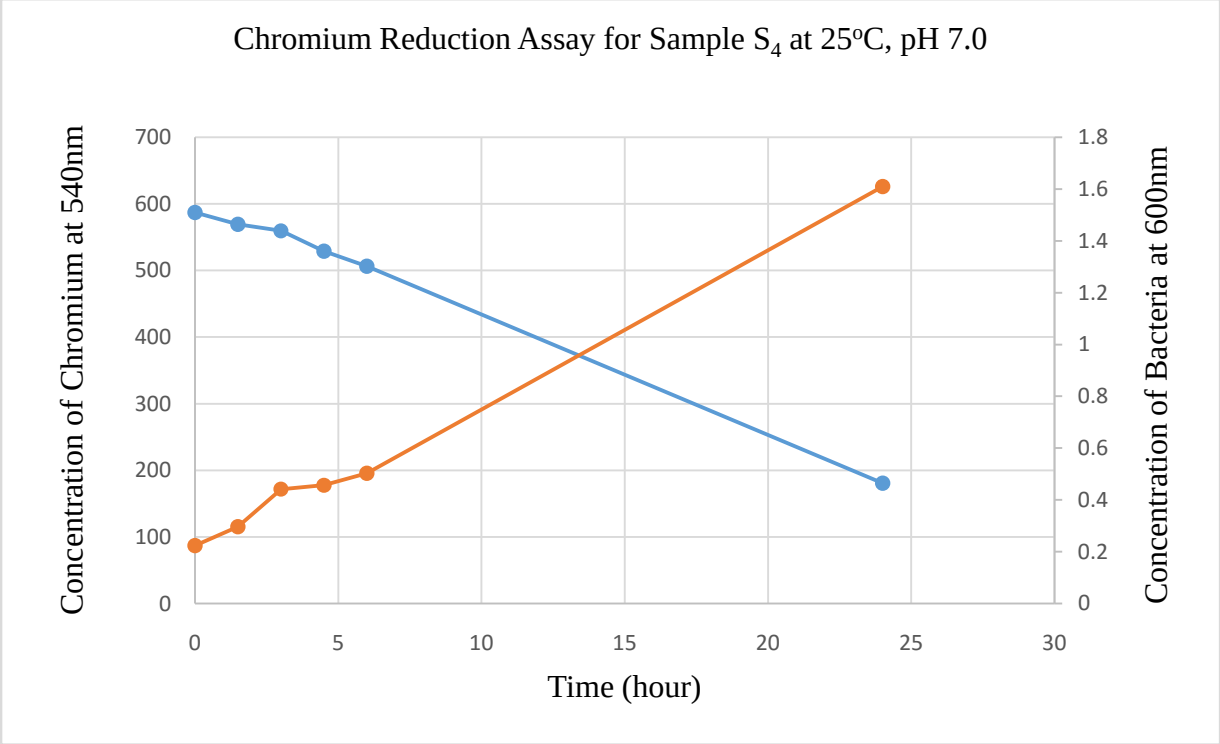

Figure4.2: Chromium reduction Vs. Cell Growth in S_4 isolate at 25^{o}C, pH 7.0

From the above Figure 4.2.1 it can be clearly seen that there is a significant reduction of Chromium concentration at 540nm that is from 587.125 to 180.625. A significant growth of bacterial concentration at 600nm was also observed. Thus it can be concluded that the isolated strain S_4 is not only resistant to Chromium but has also the property to reduce the Chromium.

Table 4.3: Isolate- S_4: Chromium reduction profile Vs. Cell Growth at 37^oC, pH 5.5

Sample		
Time (Hours)	**Concentration of Bacteria at 600nm(Cell density)**	**Concentration of Chromium at 540nm(Chromium density)**
0	0.332	634.25
1.5	0.426	560.75
3.0	0.564	541.125
4.5	0.686	511.5
6.0	0.734	490.625
24	1.495	310.5

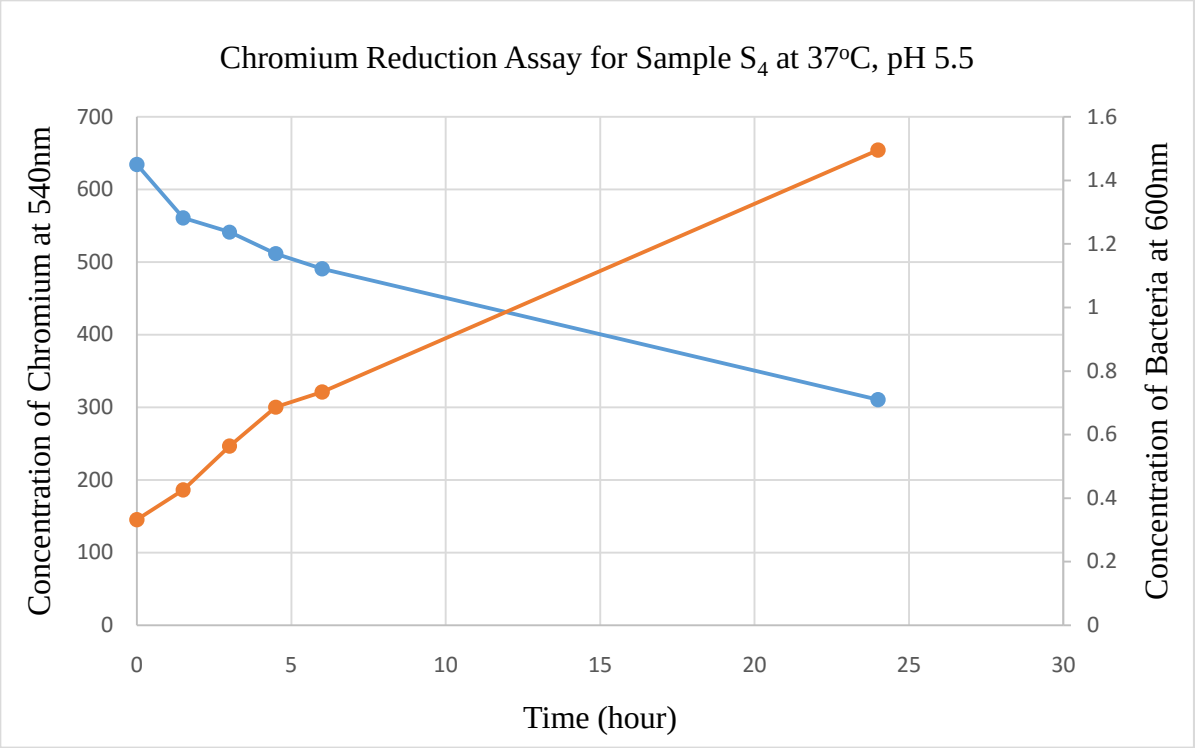

Figure 4.3: Chromium reduction Vs. Cell Growth in S_4 isolateat 37^{o}C, pH 5.5

From theFigure 4.2.2 it can be seen that there is a significant reduction of Chromium concentration at 540nm that is from 634.25 to 310.5. A significant growth of bacterial concentration at 600nm was also observed. Thus it can be concluded that the isolated strain S_4 is not only resistant to Chromium but is also capable in reducing the Chromium.

Table 4.4: Isolate- S_4: Chromium reduction profile Vs. Cell Growth at 37^{o}C, pH 7.0

Sample		
Time (Hours)	**Concentration of Bacteria at 600nm(Cell density)**	**Concentration of Chromium at 540nm(Chromium density)**
0	0.143	485.125
1.5	0.211	459.5
3.0	0.293	447.5
4.5	0.511	397.875
6.0	0.593	360.25
24	0.901	244.5

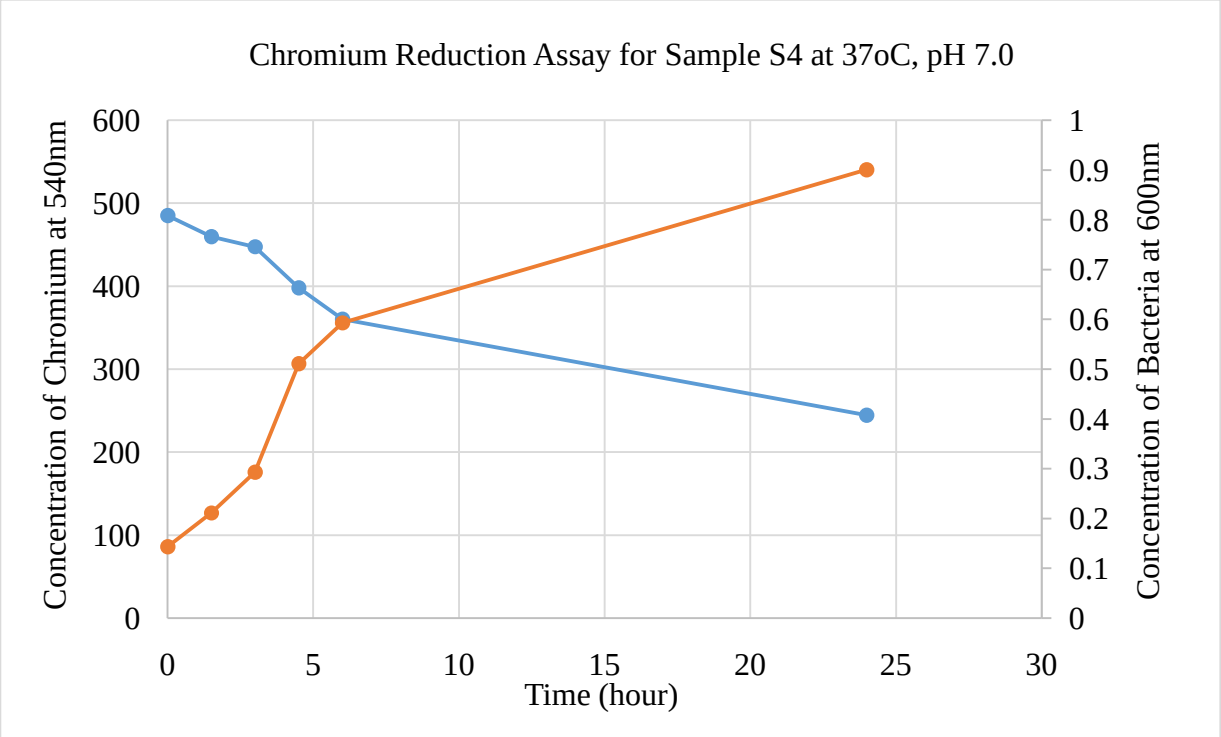

Figure 4.4: Chromium reduction Vs. Cell Growth in S_4 isolateat 37^{o}C, pH 7.0

In the Figure 4.2.3 it can be seen that there is a reduction of Chromium concentration at 540nm which is from 485.125 to 244.5 also there is a significant growth of bacterial concentration at 600nm. Thus it can be said that the isolated strain S_4 is resistant to Chromium as well as capable in reducing the Chromium.

Table 4.5: Isolate- S_4: Chromium reduction profile Vs. Cell Growth at 37^oC, pH 8.5

Sample		
Time (Hours)	**Concentration of Bacteria at 600nm(Cell density)**	**Concentration of Chromium at 540nm(Chromium density)**
0	0.217	849.25
1.5	0.376	844
3.0	0.537	833
4.5	0.7	830.125
6.0	0.884	790.25
24	1.83	356.25

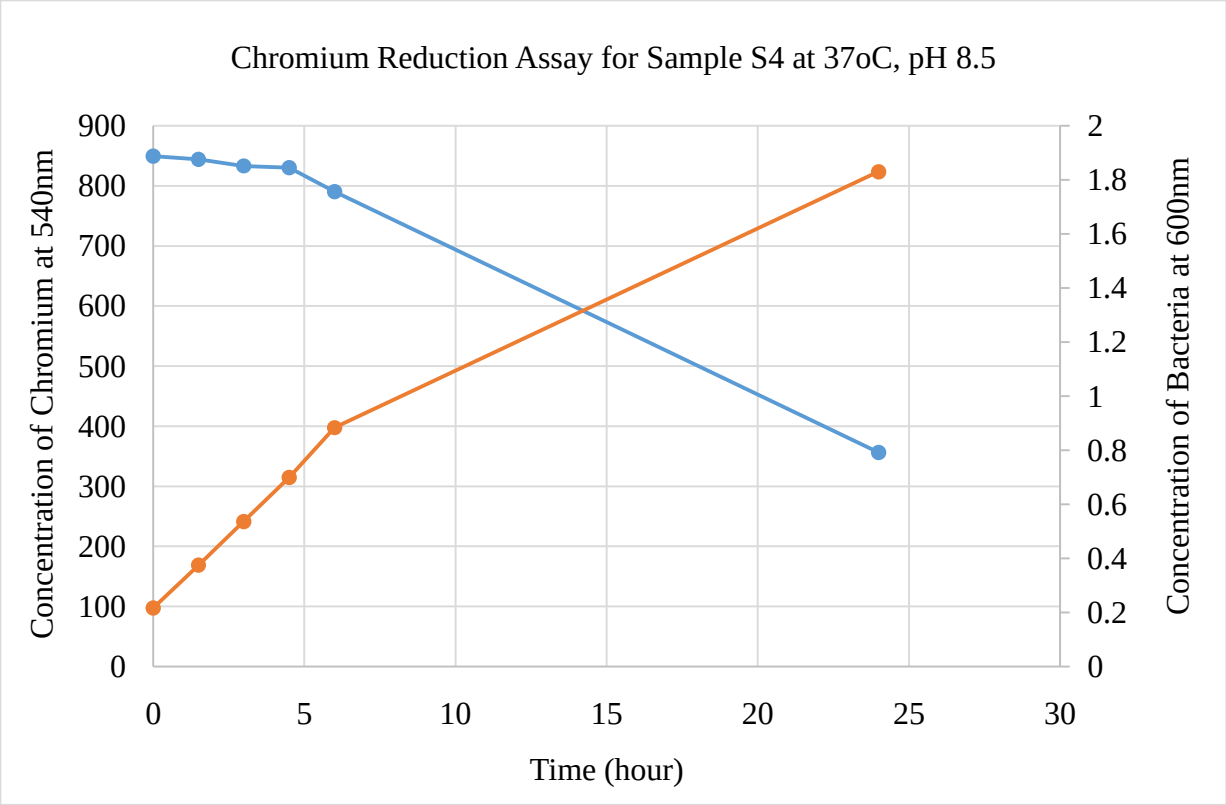

Figure 4.5: Chromium reduction Vs. Cell Growth in S_4 isolateat 37^{o}C, pH 8.5

Figure 4.2.3 shows that there is a reduction of Chromium concentration at 540nm which is from 849.25to 356.25 and also there is asignificant growth of bacterial concentration at 600nm. Thus it can be said that the isolated strain S_4 is resistant to Chromium and is also capable of reducing the Chromium.

Table 4.6: Isolate- S_4: Chromium reduction profile Vs. Cell Growth at 42^{o}C, pH 7.0

Sample		
Time (Hours)	**Concentration of Bacteria at 600nm(Cell density)**	**Concentration of Chromium at 540nm(Chromium density)**
0	0.282	598.875
1.5	0.394	588.375
3.0	0.599	544.5
4.5	0.664	538.5
6.0	0.736	524.625
24	1.303	63.375

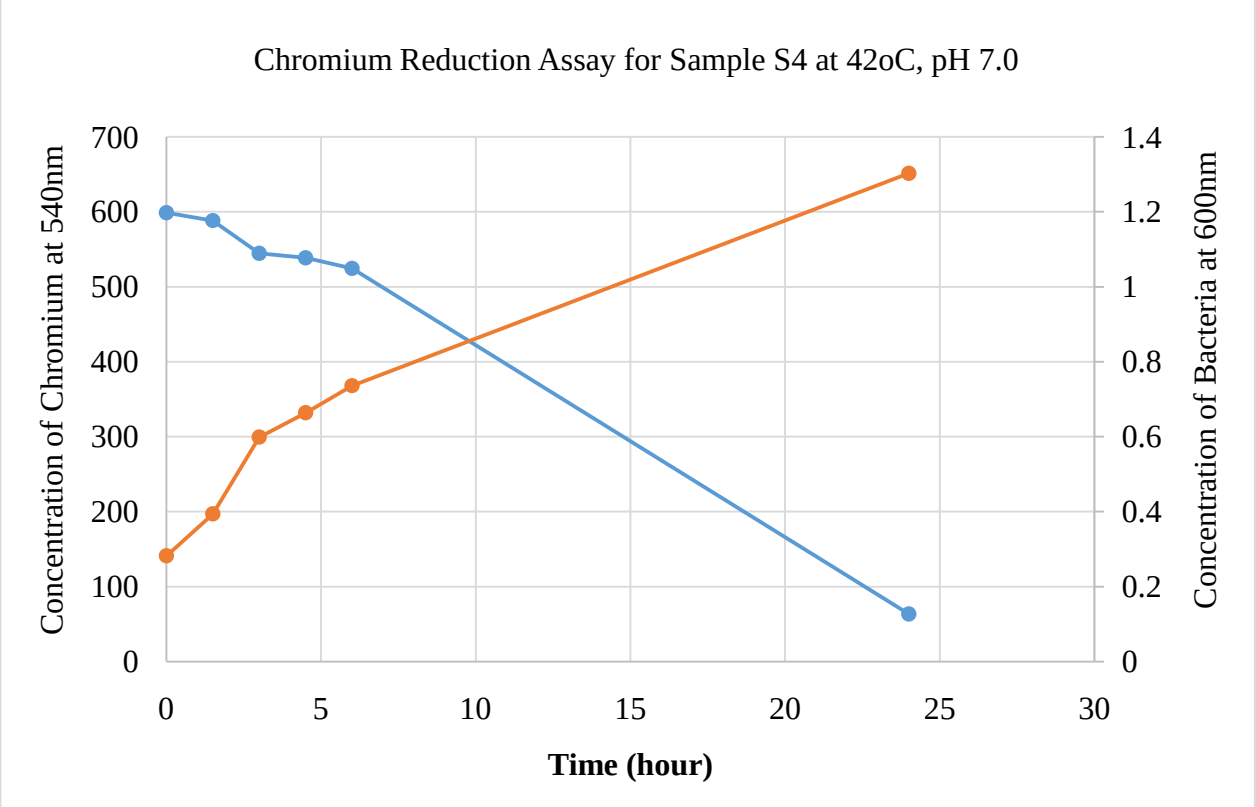

Figure 4.6: Chromium reduction Vs. Cell Growth in S_4 isolateat 42^{o}C, pH 7.0

From the above Figure 4.2.5 it is clearly seen that there is a significant reduction of Chromium concentration at 540nm that is from 598.875 to 63.375. It is also observed that there is asignificant growth of bacterial concentration at 600nm. Thus it can be concluded that the isolated strain S_4 is not only resistant to Chromium but has also the property to reduce the Chromium.

4.2.3 Discussion

Vast amounts of Chromiumare being released in the nature due to prevalent use of Chromium in the industries like textile, leather tanning, pulp production and dyeing manufacturing etc. Hexavalent form of Chromium is extremely poisonous and demonstrates carcinogenic and mutagenic effect on biological system because of its strong oxidizing nature. Microorganisms have developed various resistance and reduction capacity to adapt to chromate toxicity.

In this study, Chromium resistance microorganism was isolated and was found to have the capability to reduce the carcinogenic Chromium very successfully.

The experiment was performed with the isolate S_4 at different parameters. Using the results, graphs were plotted. From the data it is clearly seen that the isolate S_4 is resistive and at the same time is capable to reduce the Chromium. The outcomes of this investigation has characterized and recognized a new Chromium resistive and Chromium reducing strain. So it can be established that Chromium resistant bacteria are pervasive in Chromium concentrated areas and have level with capability of reducing chromate in aerobic condition, a procedure of ecological and biotechnological centrality. Finally, we conducted biochemical test for the identification of the isolated bacterial strain.

4.2.4Future Direction

The experiment can be further continued by investigating the antibiotic resistance of the bacterial strain. Investigating the antibiotic resistance will give a further scope to correlate the antibiotic resistance and heavy metal reducing capacity. The 16s rRNA sequencing can also be done to specifically identify the bacterial strain.

Chapter 5: Conclusion

Conclusion

The consequences of this study have affirmed that the isolated bacteria are a standout amongst the most encouraging bacterial isolate that could endure and reduce Chromium (VI). The isolated bacteria have an incredible potential for Chromium bioreduction. The reduction rate is proportionally related with the incubation time. The isolate could provide as a potential source of Chromium reductase enzyme which could be used as a chemotherapeutic agent after further development. The data collected through this study uncovered that among different sorts of Chromium resistant bacteria strain the isolated strain can be utilized with other microorganisms found by different researchers to mitigate the harmfulness impact of Chromium in the natural specimens.Finally, biochemical test was carried out and the isolated bacteria indicated likenesses with the species *Enterobacter aerogenes*.

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