Isolation and Characterization of Chromium Resistant Bacteria from Sea Bed Soil

A project submitted
by
Kazi Nuruddin Al Masud
ID: 12346012
Session: Summer 2012
to
The Department of Pharmacy
in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

August 2017

This work is dedicated to my parents to whom I owe my achievements.

Certification Statement

This is to certify that this project titled “Isolation and Characterization of Chromium Resistant Bacteria isolated from Sea Bed 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 SamiulAlamRajib, 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

_____

Countersigned by the supervisor

_____

Acknowledgement

First of all, I am grateful to the Almighty for giving me the strength to complete this project. Without his blessings, I would never be able to do all the works regarding my project.

After that I would like to thank my supervisor Mohammad SamiulAlamRajib, Senior Lecturer, Department of Pharmacy, BRAC University. I am extremely thankful and indebted to him for his guidance, support, understanding and encouragement, throughout my project work. Without his unbelievable patience and wisdom, my project work would be really frustrating.

I submit my heartiest gratitude to Professor Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, BRAC University, for her sincere guidance and encouragement which was a great help throughout my research work.

Further thanks to all the Lab Officers and Lab Assistants for their help and concerns.

Finally, I would like to thank the faculty members of Department of Pharmacy at BRAC University, my parents and my fellow mates. Without their continuous support and help, I would not be able to complete this project.

Abstract

Chromium (VI) is carcinogenic, highly toxic and mobile in nature. It is considered as an ecological contaminant 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 water insolublechromium. The trivalent form is less toxic due to a decrease in bioavailability and they can be used in bioremediation process. Water and soil 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 convert the cancer-causing chromium (VI) to chromium (III). Nutrient agar medium was used which was supplemented with chromium (VI) as potassium chromate(K_2CrO_4). 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) reduction capacity in development subordinate way. It was found that the isolate was resistant to Cr (VI) as well as has the reducing activity. The isolated strain was also examined for different antibiotic resistance profile and the result was, the isolate showed mild to moderate resistant to antibiotic. Furthermore, Minimum Inhibitory Concentration was also determined for the isolated strain. Finally the identification of isolate was done by 16s rDNA sequencing method.

CONTENTS

LIST OF TABLES

Page

LIST OF ACRONYMS

BLAST	Basic Local Alignment Search Tool
Cr	Chromium
DPCZ	Diphenyl Carbazide
EPA	Environmental Protection Agency
ETP	Effluent treatment plant
IDLH	Immediately Dangerous to Life and Health
Kg	Kilogram
MCL	Maximum contaminant level
mg	Milligram
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
mL	Milliliter
mM	Millimolar
NADH	Nicotinamide adenine dinucleotide
NB	Nutrient broth
NIOSH	National Institute for Occupational Safety and Health
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
SCHER	Scientific Committee on Health and Environmental Risks
WHO	World Health Organization
ZOI	Zone of Inhibition
µM	Micro molar

Chapter 1: Introduction

1.1 Background

Chromium is widely distributed in the nature**.** It can be found in the list of naturally most occurring metals where it acquire the twenty first position. (Chandra & Kulshreshtha, 2016). Chromium has two chemical forms. One is Trivalent whereas other is Hexavalent. This hexavalent Chromium is cytotoxic which means carcinogenic. It also contain the potency of possible mutation(Thacker & Madamwar, 2005). Often hexavalent form of Chromium is used in many activities. For instance tannery industry, production of coloring material and plating on different metallic products through the process called electrolysis (McGrath & Smith, 1990). The metal is extensively seen in plants, animals, rocks and soils. Aquatic sediments, soil, ground and surface water are being contaminated by the unregulated transfer of Chromium containing effluents. This lethal heavy metal makes their way into our food habits of everyday and result in different complications such as cancer through these pathways.

Abiotic systemic process is used to treat most of the debased locales around earth where we live in. Digging and treating or pumping and treating strategies that are utilized in the purpose of execution of the biological procedure and which recommends normal immobilization steps or precipitation that should be followed up. To treat the squanders that contain hexavalent Chromium, organic diminishment strategy is the most helpful technique. As of late, settled film and nonstop stream biological reactors that have been widely [used] in the purpose of natural decrease of Hexavalent Chromium. In these biologically worked reactors, sources that contain carbon as electron patrons those are provided promptly into the effluent water depending upon the need (Elangovan, Philip, and Chandra raj, 2010).

The option that can be used instead for substance and physical reduction innovation is the technology of bioremediation. Exchange of ions, decrease in electrolytes, extraction through liquid and liquid, coagulation through electrolytic process and adsorption on any physical product, turn around osmosis process, and film filtration process can also be used in the

purpose of expulsion of chromium(VI). These processes have a couple disservices, generally costly, low profitability at lower obsessions, and the generation of harmful ooze or diverse wastes that should be arranged. Therefore bioremediation innovation has the most preferred standpoint over every one of these strategies as it is cheap and earth well disposed (Nandi, Laskar, and Saha, 2016).

The present review allows us to survey the dark chromium resistant bacterial isolates which could be used as one of the principle solution for the Cr (VI) inebriation. That would be possible by lessening chromium(VI) before communicating with the living body neither which can fill in like a viable chromium(VI) reductase protein producing source that is creating like a conceivable looking cancer causing prevention administrator (Nandi et al., 2016).

1.2 Methodology

Isolated culture was characterized through the use of biochemical tests. Afterward, to know the physical structure of isolated strains SEM (Scanning Electron Microscope) was done. 16s DNA sequencing was done for the identification later on.

The crucial stage in the analytical process was to isolate the feasible microbiological strain which will be resistant to Cr and in the meantime, it will have the potential to convert Chromium (VI) which are readily found in the nature with a high level. The methods that we are utilized to determine the profiles of reduction were done where our goal was to determine a particular strain to distinguish as a possible Chromium reduction enzyme producer. Chromium supplemented nutrient broth media was used for laboratory analyses. Before assessing the reduction capacity of the isolated strain of Chromium, to isolate a visible purified single bacteria an intensive agar medium culture was done where all the distinguished colonies are isolated as purified bacterial strain.

1.3 Aims and Objectives

The purpose of this analytical investigation was to study the possibility of Chromium (VI) reducing bacterial strain which can be used as a pathway for bioremediation of cancer causing Cr 6^+ and moreover, search for a source of Cr 6^+ reduction enzyme. Having al the proper steps and that were executed in the purpose of attaining the objectives:

- In Cr 6^+ rich environment to examine the reducing capacity of a particular bacteria.
- Examination of MIC of Cr to assess the resistant level or the Cr tolerance limit of the isolated bacteria.
- Evaluation of anti-microbial resistance profile.

Chapter 2: Literature review

2.1 Introduction

In the modern time of industrialization using metals is must. Every day we using up a huge amount of metals for different purposes. Use of heavy metals in today's world is increasing day by day. Mostly used metals are Chromium, Nickel, Lead, Arsenic Cupper, Iron, Zinc etc. As the use of heavy metals is increasing the amount of industrial wastage is also increasing constantly of which all of them containing trace of heavy metals. Chromium is one of the heavy metals that are used in industries. For the production of steel and many alloys Chromium is used in a huge amount (Rifkin, Gwinn, & Bouwer, 2004). In the production of alloys Chromium and Nickel is used in the same proportion (McGrath & Smith, 1990). Chromium, in the periodic table we can get it between group two and group three, which we called the transitional metals. This metal is distributed widely in the nature as a result it obtain the 21st position in the index of mostly occurring metals (Chandra &Kulshreshtha, 2016). Like different transitional metals Chromium also shows different oxidation number. $Cr2^{+}$ to$Cr6^{+}$ is the range of Chromium oxidative state(Chandra &Kulshreshtha, 2016). Though it is true that there is different oxidation state of Chromium but $Cr3^{+}$ and $Cr6^{+}$ is the most abounded form of Chromium that are found in the nature (Chandra &Kulshreshtha, 2016).In this industrialized world Chromium is used to tan animal skin in tanning industry, to produce rusting proof metallic product, in the production of color, in ship as rust proof metalloid. The utilization of Chromium is also huge in pigmentation, preservation of food materials. Furnaces containing high temperature, mudsa drilling and photocopy toner also contain the Chromium (Rifkin et al., 2004). Cr (IV) sullying is occurring in the biosphere for the arrival of untreated cutting-edge effluents that contains Chromium mixes into the not unusual asset like water, dregs, soil and air. Its miles going on each in immature and created international locations (Thacker and Madamwar, 2005). In t6e light of data from EPA (Environmental Protection Agency) Chromium is taking its pace in the list of top 4 heavy metal air pollutants (Swift, Howell & Tedder, 2006). Every Cr found upon in the crackpot as 3^{+} and 6^{+} forms (Rifkin et al., 2004). Due to nucleic pungent satisfaction, fair to middling metabolism of glucose and incitement of biologically working protein practices Chromium

is cool as an vacant micronutrient but it is tedious at on cloud nine consider (Thacker &Madamwar, 2005). Hexavalent Chromium contain the potency of possible mutation and precise to mortal uncultured and rotation organisms (Thacker &Madamwar, 2005). For oceanic frameworks versatility from claiming Cr (VI) may be more terrific over Cr (III) (Rifkin et al., 2004). When it enters under those body it passes those cell layer promptly and oxidize those intracellular mixes. It is causing cancer when mankind breathe in. Dermatitis camwood happen because of dermal contact also various unfriendly systemic impact camwood happen because of ingestion (Thacker &Madamwar, 2005). In set side by side, the trivalent Chromium arrival is habitually inferior in undissolved manner and it verging on gorge flick through the apartment glaze. Its oxidative capability is including possibility exotic hexavalent aspect of Chromium (Thacker &Madamwar, 2005). Though Chromium is harmful for living organisms, it has some nutritious value as well (Beveridge et al., 1994). The most furious thing is that hexavalent form of Cr can accumulate in plants and vegetables (Ahmed &Goni, 2009). The most possible way of exposing human to heavy metals is to contaminate the food chain (Khan et al., 2008).

2.2 Chemistry

Chromium invented within 1797 by way of Louis-Nicholas Vauquelin and extracted one year later. Cr is determined within the middle of the periodic table, a chart that shows how chemical elements are associated with each different. Elements in Group three through 12 are known as the transition metals. These elements all have similar bodily and chemical properties. They have got a bright, vibrant surface and high melting factors.Chromium is the 24th detail within the periodic desk and its miles discovered in approximately 0.0122% of the Earth's crust.Cr 3^{+} oxide (a famous inexperienced color) is the 9th maximum plentiful chemical compound in the earth's crust. It will be named following those Greek expression "chroma," intending shade. This element make many beautifully colored compounds, in addition to a numerous array of colored solutions.Cr is likewise a totally necessary commercial metallic.Approximately three-quarters of chromium produced these days is utilized in alloys, which include chrome steel. A metal is made by using melting and grinding two or greater metals. The combination has various homes than the character metals. Chromium is also used to cowl the surface of other metals. This approach protects

the bottom metal and gives the surface a bright, brilliant look at a low fee. (http://www.chemistryexplained.com/elements/A-C/Chromium.html#ixzz4k3pTsh3U).

- 3rdC BC: As stated by renowned archaeologists, Qin Dynasty and the Terracotta armed forces weapons were covered for chromium.
- 1761: Johann Gottlob Lehmann observed an orange colored-crimson steel inside the Ural Mountains that became later determined as Lead chromate.
- 1770: Peter Simon Pallas reached to an end in which Lehmann found the steel and Pallas observed the metallic to be beneficial in paint.
- 1797: Louis Nicolas Vauquelin observed that he could isolate steel Cr with the aid of heating the oxide in a charcoal oven. This result in the invention of chromium metal.
- 1800s: Cr was normally utilized in colors and tanning salts.
- Today: Cr is now normally used as metallic mixtures and in business factories.

Cr, additionally loosely referred to as Chrome, is the twenty-first element in relative abundance with recognize to the earth's crust, but is the seventh most considerable detail due to the fact Cr is focused within the earth's core and mantle. It has atomic wide variety 24 and belongs to the institution 6(VI B) of the Periodic table. On the tonnage basis, Cr ranks forth a few of the metals and 13th of most mineral household things in business manufacturing. The primary conclusive proof demonstrating a metabolic position for chromium changed into obtained through Mertz and Schwartz' in a chain of investigations the primary of which seemed in 1955.(N.V. MANDICH,1998)

Cr has a ground state electron configuration **2, 2, 6, 2, 6, 5, 1**. As to increase the stability factor of the atom the half-filled **3d** shell with **1** electron in each of the orbitals, the distribution is favored over **3d** and **4s** orbital where we can find **4** and **2** electrons respectively. Because of the exchange of large amount of energy the half-filled shell leads to **S** state (L=O) that are commonly stable. Ions that interact with the chemical environment their **d** orbital is closer to the surface. (N.V. MANDICH,1998).

A typical transition element, Cr which is responsible for forming many compounds which are painted and magnetic. Cr has oxidation states which are: **Negative 2, 1, 0 and Positive 1, 2, 3,4,5,6**. The maximum oxidation number, **6**, relates to the accummulation of the numbers of **3D** and **4s** electrons. The lowest **Negative 2, 1, 0** and **Positive 1** is the formal oxidation

states displayed through chromium in compounds consisting of carbonyls, nitrosyls and organo metallic complexes (N.V. MANDICH,1998).

It is fragile, difficult and a luminous steel that may be notably polished. Melting factor of 1907°C moreover boiling factor of 2672°C ("Chemical properties of Chromium - fitness effects of Chromium -Environmental results of Chromium," 1998-2016) observed. Chromium has no taste and odor ("organization for toxic substances and disease Registry," 2011).The rigidness and resistance to corrosion and preventiveness to rusting makes Cr a completely beneficial metal. As a consequence it is used to produce the product stainless-steel and different alloys (Rifkin et al., 2004).

Despite the reality that chromium may be a vital micronutrient to real glucose assimilation system, prompting about compound skeleton. Also modification from claiming nucleic acid, hoisted levels about chromium is dangerous, however illness might happen because of its lack. Hexavalent chromium will be disappointment and mutagenic on the majority living creatures. Furthermore it may be referred to not aggravation, utilization of the skin What's more respiratory tract; it similarly makes lung carcinoma for skin (Thacker &Madamwar, 2005). This poison immensely impacts those earth at surrendered Chromium producing localities.Henceforth its natural cleanup is rather crucial.

In metal form chromium, it shows paramagnetic houses. Lately, which is already been observed that chromium can show distinctive attracting houses relying on its heating and cooling, which impacts spinning alignments of electron. Compounds of Cr, which includes chromium dioxide, are taken into consideration to be ferromagnetic. That ferromagnetic characteristic of these compounds permits them to be used in records tape, a manner to preserve data. Chromium may be known to other compounds whilst preserving its magnetic houses, and this varies on the number of other factors within the compound. Likewise, a few stainless-steel products are magnetic dependent on the quantity of Cr they include.

Cr will make many kinds of oxides that show off trendy acid-base behavior in addition to showing a different number of various colors.chromium(II) oxide, CrO, is primary. It is located within the shape of an insoluble powder which is black in color.Chromium 3^+ oxide,

Cr_2O_3 is the principle oxide of Cr. It’s miles amphoteric and while it's not soluble in water, it'll soluble in acid. Its miles observed in environment inside the form of an extraordinary required metal, escalate. Its miles used as a pigment, generating a deep green color.Chromium dioxide or chromium(VI) oxide, CrO_2, in its environmental state looks like crystals which are black in color. It is well-known for showing ferromagnetic properties and changed into as soon as extensively used as a artificial magnet in magnetic information used as collection source in audio tapes for cassette. It changed into considered to be one of the most ideal magnetic pigments for recording tapes because of its thin, long, glass rod like crystals. This amorphous strong can be formed thru the thermal decomposition of dichromate complexes.Chromium trioxide or Cr 3^+ oxide, CrO_3 is acidic oxide, or acidic anhydride of chromic acid. It react with water to shape chromic acid and could react with a base to shape a chromium salt. In stable shape, it's far a dark red-orange granular complex. It’s miles used in chrome-plating as a sturdy oxidizer, however, it's far extraordinarily toxic.Chromate, is a salt of chromic acid. This salt is related to a yellow coloration in primary situations, as an instance $KCrO_4$. Dichromate, is a salt of dichromic acid. This salt is related to a strong orange coloration in acidic situations, for instance potassium dichromate. But, compounds of chromate or dichromate with heavy metals normally display a crimson color. Dichromate is a robust oxidizing agent but it's far a terrible sedimenting agent. Chromate then again is used as a sedimenting agent but its miles a terrible agent for oxidizing.

Table 2.1: Chromium's widespread data ("American Elements," 2016; "Chromium," 2013)

Emblem	Cr
Term	Chromium
Class	Transition metal
Group, period, block	6, 4, d
Atomic number	24
Atomic weight	51.996
Atomic radius	128pm
Appearance	Silver-grey
Electronic configuration	[Ar] $3d^5$ $4s^1$
Thermal conductivity	93.9 $W.m^{-1}. K^{-1}$
Oxidation state	-2, -1, 1, 2, 3, 4, 5, 6
Crystal structure	Body-centered cubic

2.3 Occurrence and Sources of Chromium compounds

Cr is usually occurring element observed in hard rocks, animals, flowers, and soil. Cr present in many oxidation states, of which the hexavalent (Chromium VI) and trivalent (Chromium III) states are maximum not unusual certainly. Chromium is thought not various compound and natural responses in feature frameworks. Both oxidation of Chromium (III) and diminishment of Cr 6^+ can occur in geologic and sea-going conditions. Inside the surroundings Cr 6^+ may also reply with dirt debris or many kinds of substances and is probably changed over to Chromium (III) (Chandra &Kulshreshtha, 2016).

Chromium (VI) occurs in uncommon minerals and is probably certainly taking place in groundwater, be that as it could, Chromium (VI) within the world is absolutely delivered from human exercises (Chandra &Kulshreshtha, 2016). An important sector is the technology and proper utilization of Chromium mixes (for the most part ammonium

dichromate, Chromium trioxide, sodium dichromate, sodium chromate and potassium dichromate) and moreover the discharge of industrial wastes containing Chromium mixes.

As condensed by means of the USA Environmental protection business enterprise (US EPA), simple Cr is determined in air, soil, water, 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 adulterated regions, Chromium fixations have to be better, e.g. up to 30 µg/L in new water (ATSDR, 2000a).

The most found mineral is ferric Chromite, $FeCr_2O_4$. Basically got in South Africa. The chromite steel keep in South Africa talks to around 72% of the earths identified assets.

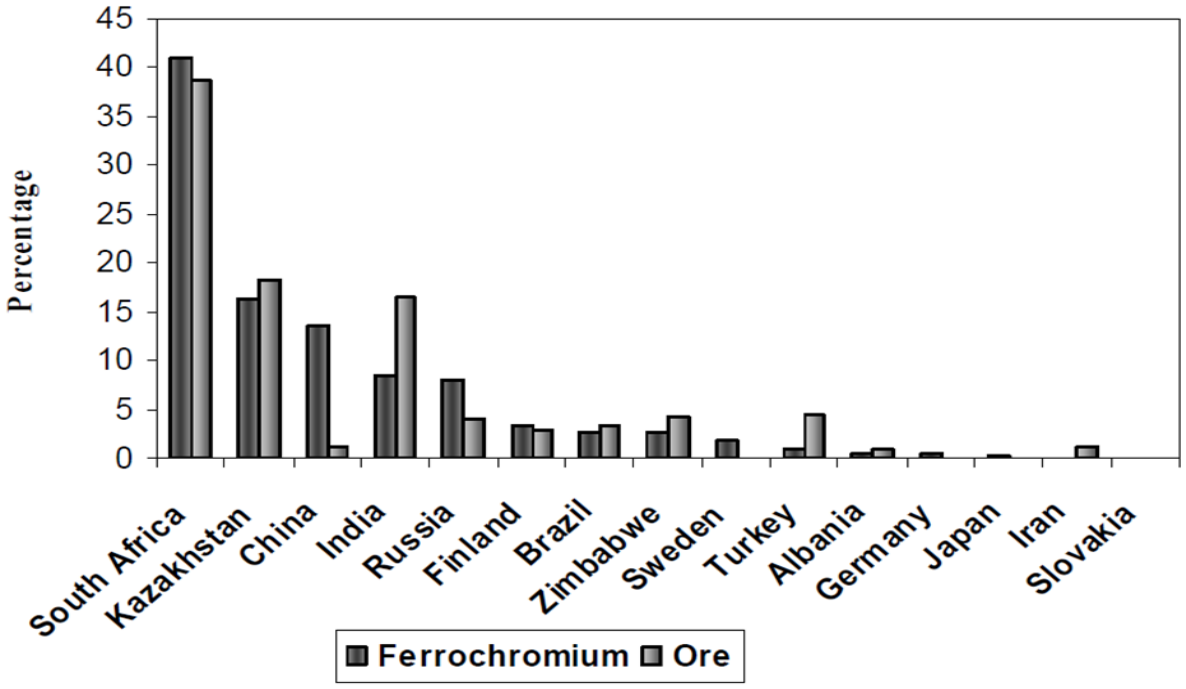


Figure 2.1: Ore of chromite and Ferrochromiumfractioncreated globally (Papp, 2006).

2.4 Chromium utilization

Chromium is essential in the manufacturing of stainless steel. Maximum chrome steel incorporates approximately 18 percent chromium; it's far what hard and tough metallic and would raise its resistance to decay, especially at excessive heat. The cause that the fact stainless steel does not rust and is effortlessly sterilized, it is a part of many gadgets we use in our each day lives. a number of the maximum recognizable of those objects consist of

kitchen appliances, food processing system, and clinical and dental gear. Among the decorations on motors, including embellishes, trim, and hubcaps, are chromium plated. Chromium in super metals (high performance alloys) allows jet engines to run in an excessive-temperature, highly stress, chemically oxidizing surroundings. On U.S. roadways, chromium pigments are used to make the yellow lines that suggest visitor's lanes. Chromium-containing pigments discover their manner into a variety of beauty products. Chromite is conducted in excessive-temperature packages, inclusive of blast furnaces and molds for burning bricks, because it holds power at high temperature. Chromium is likewise crucial for proper health. Inadequate amounts result in glucose intolerance in human beings. Organ mushrooms, meats, broccoli and wheat germ are all appropriate dietary assets of chromium.Some highly assigned Chromium 6^{+} compounds comprise electroplating, pigment and dyes, leather-based tanning, synthesis of chemical, and preservatives for wooden, refractory production and erosion inhibitors (EPA, 2010). Chromium^{6+} compounds are also determined in many buyer objects, for example, treatment of timber using Cu dichromate or leather based tanning the use of chromic sulfate.

2.5 Regulation regarding level of Chromium

Form of different detrimental worse effect takes place in flora, living animals and person because of the attendance of Cr 6^{+} in watery places greater at more than a well-known level. Consequently, different controlling work has been done by several associations. World health organization has given a level which can be affordable by a human being is 0.05mg and 2mg/L for Cr $^{(VI)}$ and trivalent Cr as well,(Gupta and Rastogi 2009). According to MCL Cr concentration in water is 0.1mg/L which is absolute Chromium and that was declared by safe water ingesting act. In purified water 0.1mg/L is the maximum permissible level. 50 ppm is the maximum level up which a color can contain Cr. Cr is used to tan lather, in food for animal and the level should be not more than 2.75%of its weight. OSHA has already declared a PEL for Cr which is zero.1 mg/m^3.

2.6 Toxicity of Chromium and the effect of it towards health

2.6.1 Chromium toxicity

Ulcer, sensitiveness, aggravates, dermis infection, skin infection and nasal infection, respiratory tract infection, eardrum puncturing and lung carcinogenic infection can affect the people who are exposed mostly to Cr^{6+}. Cr^{6+} also can be found into the placenta which is dangerous for fetal improvement. Cr (VI) contamination in nature causes change in the structure of microbial structures, lowers the development of microbes and related enzymatic reactions which is delaying the development of organic soil matter and increasing the amount of Cr (VI). Cr (VI) causes harm by infiltrating the cell layers and by oxidative stress. It causes harm to cell films in both prokaryotic and eukaryotic cells for instance decreased in unification of membrane or barriers in electron transport system which has been broadly reported. Cr (VI) utilizes the sulfate from the sulfate transport arrangement in the cells of living beings. On the other hand, Cr (III) has less permeability with the probability of thousand in one that of Cr (VI). To sum up we can say that Chromium upon oxidation becomes more lethal Cr (VI) which has adverse biological impacts on most forms of life.

2.6.2 Effects on health

SCHER assesses depending on the data retrieved from later search for instance, inorganic Cr 6^{+} compounds (WHO, 2013), Cr Toxicology Profile (ASTDR, 2012), taking preparation for a reaction for dosage referencing in relation to carcinogenic effect of Cr^{6+} (RAC, 2013), Drinking water Cr (WHO, 2003), Chemical compounds which are used to prepare baby items (RIVM, 2008), Chromium VI components (IARC, 2012). Later literature enlightened on newly available evidences regarding carcinogenic effect of Cr^{6+}. The SCHER mainly focused on good impact after orally given doses also revealed the salts of Cr used up in *In Vivo* and *In Vitro* studies. This includes strontium, zinc, dipotassium, calcium, disodium and diammonium salts having specific solvency degree.

2.6.2.1 Kinetics

There was an extension of the model for the people those have oral disposal of Chromium. Subsequently data from various studies and literature on aggregation of Chromium (VI) were used with respect to toxicokinetics. Blended second-order and pH-subordinate process portrays the consistency of toxicokinetics with respect to human who are exposed to Chromium. pH of gastric tract, gastric tract food remaining time, volumetric area of gastric tract, and production of gastric enzyme specified for parameters that calculate human being variability to make key suppositions in PBPK models if there is deficiency in any of the mentioned parameters for better assessment on wellbeing hazard. Cr (VI) can easily surpass the biological systems and reduced to Cr (III) which is less harmful. Acidic condition with other natural substances increase the reduction rate from Cr^{6+} to Cr^{3+}. Mainly small thiols moreover ascorbate works as reducing agents from $Chromium^{6+}$ to $Chromium^{3+}$. Cr^{6+} is taken up earlier than Chromium(III) and this happens because of competitive ingestion intracellularly and lowering the level of Cr^{6+}.

2.6.2.2 Mode of action

Previously discussed issues show the credibility of Chromium working with an ability to possible mutation and toxicity to genes. Similarly, Cr^{6+} has appeared with the capability of hampering the cell regulation process (IARC, 2012). $Chromium^{6+}$ passes easily throughout the cell membranes and oxidation of DNA, protein and lipids. Genomic sources combines with Cr^{6+}& forms DNA adducts, Chromium-DNA complex, DNA-Chromium-DNA complex, proteins-Chromium-DNA combinations, nitrogen bases those are oxidized, abasicsites, DNA-double helix broken apart, crosslinking of DNA- protein and intra strand cross linkage(Wise, Holmes, & Wise, 2008). Usually Cr (VI) ties with DNA and produces DNA adducts which leads to genomic mutation as seen in in vitro study of human and bacteria. Cr (VI) reduction causes harm in DNA by producing reactive oxygen species (ROS). Cr (III) is less mobile and less harmful to the cells. Again, if this is produced within the cell causes ready movement of Cr (VI) and it arrests the DNA polymerase by crosslinking with DNA. This causes the rise in the ROS level. ROS is formed when $Cr^{(VI)}$ is converted into Cr^{3+}. Presence of high level of hydrogen peroxide, influence Cr^{3+} to raise the

level of ROS in the cell which produce 8-hydroxydeoxyguanosine within DNA afterward which also come out of the cell membrane that hinders the cell cycle(Hadjiliadis, 2012).

2.6.2.3 Effects in Animals

The intense lethality of Chromium (VI) has been experimented through the conduction of several studies. Depending upon the compound managed and the sex of the rodent, Chromium (VI) was administered to rats in oral lethal dose varying from 13 and 29mg/kg b.w. Through this oral administration a few major changes were observed which includes loss in body weight and immunity and hematological parameter change. To understand the everyday consumption levels for non-cancer causing impacts, the mice and guinea pigs used were to sodium dichromate regulated in the taking water for a long time. The rats were introduced through inward breathing which lead to aspiratory irritation and neutrophil relocation. Several studies report thatChromium compounds initiates disease through the administration via oral route, intra muscular, and intra thecal, intra peritoneal administration. Majority of cases shows Carcinogenesis in the site of origin and lung cancer occurs in mice and rats because of increased inhalation. Local sarcomas were also observed through repository infusion of a limited number of Chromium compounds (lead chromate, calcium chromate, strontium chromate, zinc chromate). Orally administered Potassium chromate raised UV-incited skin cancer, showing tumor systemic impacts.

2.6.2.4 Human effects

Reports for the instances of coincidental presentation to high measurements and occupational inhalation serve as the source of information on impacts in most of the people. Workers who work in chromate generation, Chromium electroplating and chromate pigment formation are at great risk because of exposure to the compounds containing chromatin.

Rashes, ulcers and susceptible contact dermatitis occurs when skin come in contact with Chromium (VI) contained materials. In patch test, to bring out a positive skin response in hypertensive subjects 2 μg chromatin was required. Chromatin affectability should be around 0.5% though European nations suggest 1.7% in a research (Peltonen and Fräki, 1983; Hartwig, 2007; Hartwig, 2010). In this case, Sharpe properties of $Chromium^{6+}$ are not tended to by the SCHER pungent properties of Cr^{6+} are not recommended. Occupationally

uncovered breathing may lead to aperture in the septal area and ulcer in the mucosal area in the nasal rout. Volume of the lung and exposure was evaluated in a particular time frame (Finley, Proctor, & Paustenbach, 1992; Lindberg & Hedenstierna, 1983).

Cr^{6+} damage DNA of body defense cells (welders, electroplaters or ferrochromium composite foundry laborers who were principally uncovered during inhalation, as surveyed in WHO/IPCS, 2013). Though many of the those results are not trust-worthy as Chromatin VI presentation level was not known, subjects were analyzed taking set of responsibilities and a portion of the subjects were too little to observe the cytogenic changes.

Impacts of Cr^{6+} on nose sinus and nose infections, where the diseases occurrence information is not known (IARC, 2012).

Incorporation of hexavalent Chromium in pure drinkable water has a co-relation with abdominal tract growth and considered as polluted in China (Zhang and Li, 1997). There is various irregularity in the study results especially in calculating of exposures (Brandt-Rauf, 2006; Beaumont et al., 2008 and follow-up creator correspondence; Smith, 2008). Unfortunately, the study could not reveal the abdominal tract growth of the laborers who were exposed because of breathing (Gatto et al., 2010).

2.7 Carcinogenesis induced by Chromium

Cr (VI) is defined as the dangerous cancer-causing agent and brought about changes in human DNA. It is not like Cr (III) which binds with DNA polymerase rather it stays as oxyanion and survives through sulfate anion framework. Level of acceptation of Cr (VI) within cell decreased because redox framework to its most stable structure Cr (III) which binds with DNA and proteins with in cellular framework having high amount of affinity for organic ligands. Amongst the intracellular decreasing of Cr^{6+} to Cr^{3+}, temperamental species, for instances, intermediate valance of Cr and dynamic oxygen species are made (Conett, 1983; Mattagajasingh, 1995; 1997), that may commence the cancer-causing activity by altering the structure of DNA (Kawanishi, 1986). Hydroxyl that is generated because of Cr reduction causes cross linkage with DNA-protein and expressed as agents for chromate carcinogenesis. Medium valance of Cr causes DNA damage as well. Furthermore, among

regular control of gene flourishing, proteins, either alone or in combination with various proteins, oppositely contribute with particular DNA successions (Stein, 1979). Cross linking of DNA molecules with other proteins causes upset in the regular DNA-protein incorporation, brings heredity outcomes and causes interruption in gene expression. The specified proteins and other things need to be detected to be sure what the main reason behind Cr carcinogenesis is.

2.8 Traditional methods for reduction of chromium toxicity

Particular processes to reduce Cr^{6+} from contaminated water flow includes many physical and mixture techniques like exchange of molecule, sedimentation, filtering, electrochemical treatment, development of flim and vanishing recovery (Ahluwalia & Goyal, 2007; Al-Sou'od,2012)

2.8.1 Electro chemical precipitation

This system uses an electronic potential to enhance the removal of various metal from the used water over traditional manufactured process (Kurniawana, Chana, Loa, & Babelb,2006). This method is broadly professed method for the assessment of detrimental metal's level from water parts per million (ppm). Polprasert and Kongsricharoern in 1995 discovered the Cr^{6+} expulsion exerting the ECP procedure from an electricity guided plating contaminated water. Cr (VI) can be reduced from 3,860mg/L to 0.2mg/L by this method. Irrespective of the smartness of the method, the efficiency of this method is based on low P^H level and presence of various. This method requires generation of different types of chemical which increase the unusual state of water content slime's time and the interchange has an authentic cost. Precipitation with particle trade, lime or disulphide does not have any specific rule. It becomes hard to release the metals at low fixation.

2.8.2 Exchanging of Ion

Though ion exchange method has delayed to be started, it is becoming now a significant method among all methods established to reduce Chromium from waste water. Particles of any distinctive species are removed from insoluble exchange material by the particles of substitute species during the course of action in ion exchange method. Chromium enters through the one side of the segment underweight, passes through the resin bed and finally

Chromium is released. Accumulated solids are cleared when the amount of resin is decreased. Cross section natural resin is used in ion exchange method.

Manufactured Dowex 2-X4 resins are utilized to evaluate the intake of Cr (VI) from genuine plating waste water (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% removal of Cr (VI) was purified in the treatment. Ambersep 132 which is another designed resin is further analyzed to chromic acid from manufacturing plating course of action in a four-phase ion exchange method (Lin and Kiang).

An obstacle of particle trade system for chromium removal is that particle trade fluid is to a great extent specific. The selected resin must be capable of removing the metal contaminant of concern. In addition to that, inadequate clearing of Chromium may happen and the ion exchange equipment can be expensive. Moreover, it can't handle concentrated metal arrangements. As the systems gets easily fouled by organics and diverse solids in waste water. Likewise, ion exchange is disregardful and it is particularly weak to P^H.

2.8.3 Biosorption

It is a new invention for the recovery of cancer causing Chromium from the water. Low cost agricultural wastage material can be used in this regard (Basso et al. 2002; Park et al. 2006). There are several variables on which the metal sorption is dependent and the process is also very complex. Many mechanisms like sorption of chemicals, formation of complex, adsorption after complexation on pores, exchange of ions, micro form of precipitation and adsorption on the surface (Gardea-Torresdey et al. 2004).

2.8.4 Utilizing activated carbon for adsorption

It is observed that Cr can be adsorbed very well on carbon material that has been found in like Coconuts. At high temperature Cr adsorption take place very well (Mohan & Pittman, 2006). On the p^H and salt concentration of a solution adsorption is totally dependent of Chromium.

2.8.5 Filtration through Membrane

Membrane filtration procedure is so tough and considered as so costly. *In Situ* bioremediation is handled on this process.

2.9 Metals and Microorganisms

2.9.1 Metal resistance mechanism into Bacteria

There is unbound aspect of the resistance of metals in microorganisms gained by this inescapable method for metals present in nature. Opposition of microbes towards metal is heterogeneous in innate base and also in biochemical base. Along with that, they can may be plasmid, transport of chromosome encrypted with other proficiency. The five segments of metal resistant in bacteria (Rouch et at., 1995) are given below:

1. Exclusion of metals by porousness hindrance.
2. Dismissal of metal by active export from cell.
3. Intracellular physical dissociation of metal by preventing proteins to keep it from harming subtle metal cell material.
4. Extracellular separation.

Detoxification of metal where the metal is spuriously set to term less dynamic

2.9.2 Metal sensitive cellular components

Metals have the ability to decrease or assemble activity of catalyst, modify specificity of the compound by causative following change in enzyme or by sealing the enzymes in discrete assent. Metal ion can do harm specifically in DNA structure, like delivering cross link strands or they can influence the data of DNA in an indirect process by decreasing the constancy of DNA synthesis(Beyersman, 1994).

Massive diversity of cell are major focal points for the metal-introduced destructions, an issue of these portions are major for critical cell proportions, for instances, DNA that is used for replication. Progression of cell will occur due to discharge of metal-induction by metal reactivity so that as the concentration of a specific metal conquest, if the concentration comes to a crucial level then it is considered that their progress is inactivated. With the facts, chance of confluence of the metal, the cell have to have some techniques for undertaken conditions for one or many target sites to survive. Significant of metal for grouping the more

unusual the number of basic parts that needs protection. For instances, E. coli, production of the major proteins may be ignored by transformation in a single gene bringing about extended resistance of metal (Lutkenhaus, 1977).

2.9.3 Uptake system of metal and resistance

Metal ions have ability to minimize the catalyst movement, change of elements specificity by causative change in enzyme or by sealing the enzymes in detached assent. Metal ion can do harm specifically in DNA structure, like delivering cross link strands or they can influence the substance of data of DNA in an indirect method by minimizing the firmness of DNA synthesis (Beyersman, 1994).

Due to the reason that an innumerous alteration parts of the cell are chiefly focal points for the metal-initiated deterioration, an issue of these portions are major for critical cell proportions, for example, DNA used for replication. Cell advancement will take place because of dismissal of due to metal-introduction by metal reactivity so that as concentration of a specific metal is seen, if the concentration reaches to a crucial level then their function is inactivated. With these facts, chance of confluence of the metal, the cell must have some techniques for undertaken conditions for one or more target sites to survive. The importance of the grouping of metal needs protection. For example, E. coli, origination of the main desired proteins can be ignored by transformation in a single gene bringing about extended metal resistance (Lutkenhaus, 1977).

2.9.4 Metal as a biological requirement

To continue principle metabolic contemplation of bacterial cells different necessary metals are very crucial. The larger part of bacterial species needs copper, nickel and iron, in some other species Molybdenum, Tungsten and cobalt are required. Less detrimental effect to the cell differing with metals and found no activities of positive metabolism. In local concentration cell will work less in appropriate system.

2.9.5 Gene cassette versus chromosome-mutation-determined resistance

The gene adjuration in safe microscopic organisms of metal resistance would be imposed by the availability in population of accomplished gene cassette that apprehends assigned ingredients of counteraction. It is exposed that these have already been adopted to prevent resistance through evolutionary volition. Moreover, it may be pulled on chromosome. Last two antagonists can forward the cassette exchange resistance between bacteria. The strength intervened in the cassette can give great amounts of resistance with respect to the possible accessible chromosomal mutation. As one of the foremost, the most prominent population is likely to have an important hereditary component in the cassettes.

2.10 Mechanism of Chromosome resistance in Bacteria

In a wide range of bacterial species it is found that, chromate ion passes the cell cytoplasm by sulfate uptake pathway and it reflects the chemical similarities of the two oxyanions (Cervantes and Campos- Garcıa 2007). As trivalent Chromium forms obscure compounds, it slowly passes through the cell membrane (Cary 1982). Numerous enzymatic or non-enzymatic activities cause reduction of hexavalent Chromium to trivalent Chromium in the cell and the acquired trivalent Chromium by this way may show toxic effects in the cell's cytoplasm (Cervantes et al. 2001). The procedures of resistance to chromate-resistant bacterial isolates has been found and this is done by plasmids or chromosomal genes (Cervantes and Campos-Garcıa 2007). The initiation of discharge of chromate ions from the cytoplasm of cell occurs by the genes found in plasmids which conceals membrane transporters. However, different methods like, reduction of hexavalent Chromium, removal of toxic substance from free-radical, replacement of damaged DNA, and techniques of sulfur or iron homeostasis are correlated to the resistance system found in the chromosomes of bacteria. Researches on Cr (VI) reductases focusses on enzymes with higher reductive activity.

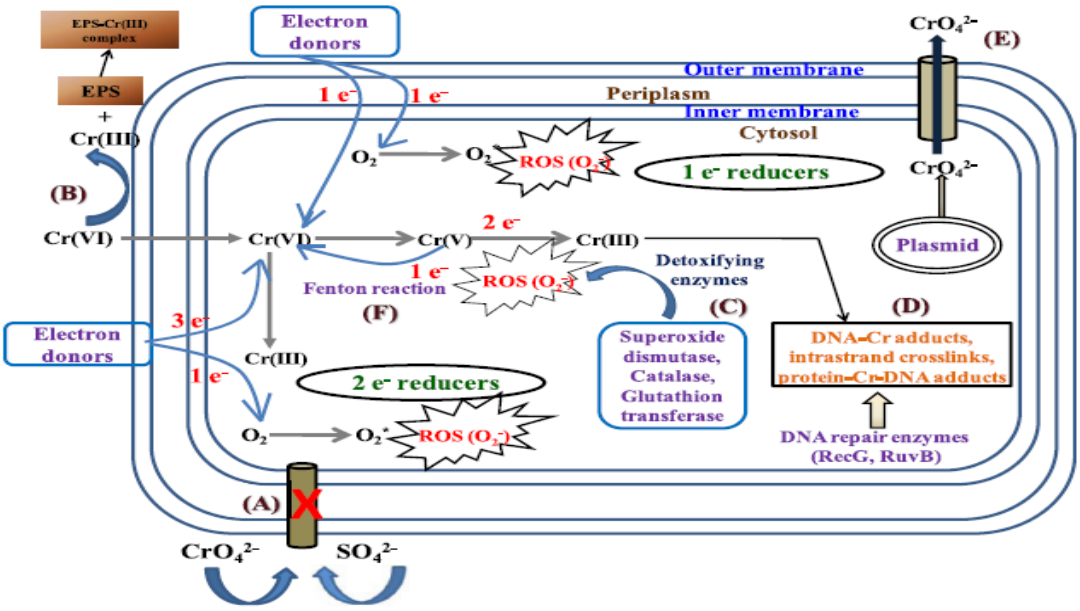


Fig 2.2 Resistance of chromate mechanisms into the cells of bacteria. A: Transformation in the chromosome-encrypted take-up transporter of sulfate, B: Lessening of hexavalent Chromium extracellularly into trivalent Chromium, C: Lessening of hexavalent Chromium intracellularly to trivalent Chromium through chromate reductase, D: Utility of the healing method of SOS in diminishing oxidative pressure, E: Chromate efflux from cytoplasm, F: ROS foraging enzyme activity to diminish oxidative pressure (Thatoi et al., 2014).

(A) Reduced uptake of Cr (VI)

Decreased intake of Cr $^{6+}$ as sulfate additionally homeostasis of iron or sulfur is also related with the protecting systems against the effects Cr $^{6+}$. Tetra dimensional sulfate molecule that is ionized is related with the uptake of Chromate CrO_4^{2-} which is also ionized (Fig. 2.2), for this reason Chromate can go through the cell membrane by the sulfate ion pathway, where SO_4^{2-} and PO_4^{3-} anions are used as bearers(Wenbo et al., 2000). When mutation occurs in the pathway of SO_4^{2-} uptake, Cr uptake in the cell also decreased where the pathway is encoded with chromosome (Ramirez-Diaz et al., 2008). Microorganisms those are residing in metal enriched atmosphere can demonstrated a high speed transporting capacity to generate Cr $^{6+}$ resistance which will cause a low uptake of Cr $^{6+}$ through the pathway of

sulfate transportation. Genomic information that encodes resistance or changing characteristics a microorganism can change its resistance to environment (Kümmerer, 2004).

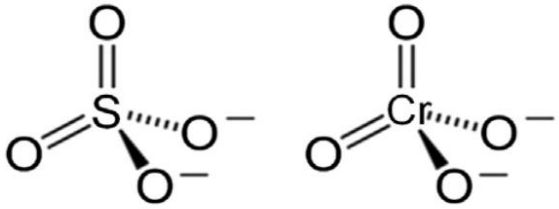


Fig 2.3: Resemblance among structures of the ions of sulfate and chromate

(B) : Detoxifying enzymes of ROS or Cr (VI) reduction intracellularly:

Forming of Cr^{3+} from $Cr^{(VI)}$ is redox by the process by highly reactive intermediate Cr^{5+}. Term ROS is defined as the process that is followed by Cr^{5+} to convert itself into Cr^{6+} through giving its electron to dioxygen. Organisms face oxidative stress due to the formation of ROS. In this manner, chromate impels the bacterial proteins which gives protection against oxidative stress prompting an extra system of chromate resistance (Ramirez-Diaz et al., 2008).

(C) DNA repair enzymes

By DNA repair enzymes of damaged DNA Cr (VI) creates indemnity of bacterial cells which works as another protection shield. Entrance of the bacterial cell by Cr (VI) which is quickly shortened to Cr (III) by the action of various enzymatic or non-enzymatic practices that creates the propagation of ROS, which thus imbeds detrimental influence on protein and DNA in cell. Some damages are happened to DNA due to the ROS which is analogous to base change, single-strand breaks, double strand breaks. These types of injury that occurs to DNA can be mended by anomalous DNA reparation mechanism like the SOS repercussion enzymes (RecA, RecG, RuvB) (Hu et al., 2005). For example, for quite some time in Escherichia coli, Cr^{6+} has been familiar not the E. coli SOS reparation framework which shields DNA from oxidative depreciation (Llagostera et al., 1986). Segments of the recombination DNA refit systems corresponding to DNA helicases like RecG and RuvB, apparently stimulant reaction to DNA depreciation fetched by chromate in Pseudomonas

aeruginosa (Miranda et al., 2005). Reduction of Cr (VI) cell creates redox-progressive interim Cr (V/IV) and static Cr (III) framing Cr-DNA adducts which is the most abundant type of DNA embezzlement which causes changes and chromosomal breaks (Zhitkovich, 2011).

(D) Scavenging of ROS

Cr^{6+} following to accessing the cell can be shortened to Cr (V). Electron helper deliver electrons to Cr (VI) causing improvement of toxic Cr (V). Although, the chromate rebuild minimized Cr (V) further to Cr (III) by a two-electron interchange (through "semi-tight" mechanisms), sometimes this repercussion is not anomalously fast. So, a portion of Cr^{5+} mediate is instantly reoxidized to Cr^{6+} with these lines generating ROS by a Fenton-like repercussion. Among this process hydroxyl radical (-OH) are formed in the microbial cells (Shi and Dalal, 1994) as de drawn in the situation below:

$$Cr\ (V) + H_2O_2 \rightarrow Cr\ (VI) + \text{-}OH + OH$$

While the process goes on, atomic oxygen is diminished to O_2 -radicals producing H_2O_2 dismutation. Hexavalent Chromium conforms H_2O_2 to produce -OH radicals by a Fanton like response which is like the oxidation of Fe (II) with H_2O_2 in the Fenton repercussion. The production of -OH from Fe (II) by Fenton is exhorted extremely by the disposition of Fe^{2+} complex which have void locales for H_2O_2 adjustment.

Table 2.2: Methods of bacteria for the imperviousness of chromate

Enzyme/System	Species	Function	Reference
Transport			
ChrA transporter	*Pseudomonas Aeruginosa*	Efflux of cytoplasmic chromate	Alvarez et al. 1999
Cys operon products	*ShewanellaOneidensis*	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	*Eschericia coli*	Combat of oxidative stress	Ackerley et al. 2004
Outer membrane proteins	*CaulobacterCrescentus*	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	*ShewanellaOneidensis*	Repair of DNA damage	Chourey et al. 2006
Other Mechanisms			
Cys operon products	*S. oneidensis*	Sulfur metabolism	Brown et al. 2006
Adenylyl sulfate	*S.oneidensis*	Sulfur metabolism	Brown et al.

Kinase			2006
Ferritin	*S.oneidensis*	Iron binding	Brown et al. 2006

2.11 Microbial reduction of hexavalent Chromium

In recent years most of the industrialized countries adopt bioremediation as a popular innovation which was previously confined to research laboratory only. A fruitful bioremediation plan depends on the administration of soil microbial populaces fit for catabolizing the contaminants. Heavy metals can be hazardous to soil biota, and for this reason can play a vital role in key microbial procedures and abatement the number and movement of soil microorganisms (Obbard et al., 2001). Microbial are considered to be a simple and touchy marker of anthropogenic consequences for soil ecology. Movements of soil microbial populaces are caused by Cr (VI) and in high concentration it causes inconvenient consequences for microbial cell digestion system. Since the revelation of the primary organism fit for decreasing $Cr^{(VI)}$ in the 1970s (Zhu et al., 2008), the quest for $Cr^{(VI)}$reducing microorganisms (both high-impact and anaerobic) has been energetically sought after, with various strains being secluded.

2.11.1 Bioremediation of Chromium

There are different types of techniques available 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). Major drawback is, most of these strategies need high vitality or significant amounts of chemical reagents with acceptable rate of creation of secondary pollution (Jeyasingh & Ligy, 2005; Komori, Rivas, Toda, & Ohtake, 1990). Keeping in mind the 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). Moreover , from the perspective of efficiency, 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 not beneficial (Kratochvil, Pimentel, & Volesky, 1998; Patterson, 1985). Bioremediation opens a new pathway where in order to purify the dirtied territories, the metabolic capability of microorganisms is utilized to expel harmful metals. Bioremediation systems can be named as in situ or ex situ. It depends 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, which needs to being expelled and transported in appropriate facilities for treatment later on (Pattanapipitpaisal & Reakyai, 2013). Cr(VI)- resistant microorganisms can influence the procedure of reduction of Cr(VI) to Cr(III), for the purpose of beneficial bioremediation applications (Raspor et al., 2000). Regression of Cr(VI) to Cr(III) is a potential helpful procedure for the recuperation of destinations debased by Cr(VI) (Polti, Amoroso, & Abate, 2010b). In today's concept of application of bioremediation, Cr(VI)-evacuation in light of microorganisms is considered as a prevailing strategy (Dey & Paul, 2013), (A Ganguli & AK Tripathi, 2002). Considering the fact that, the insolubility of Cr(III) encourages its precipitation and expulsion, the biotransformation of Cr(VI) to Cr(III) has been chosen as an option procedure for treating Cr(VI)- tainted wastes (Cervantes et al., 2001; Ohtake, Cervantes, & Silver, 1987). Microbial reduction of Cr(VI) is financially savvy and eco-accommodating and can offer a practical option comparing to all other biotechnological approaches (Ge, Zhou, Dong, Lu, & Ge, 2013). Microbial reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Chromium resistive microorganisms are capable of the natural reduction of Cr (VI) into the less portable Cr (III). This is a feasible 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

Phycoremediation is the utilization of photosynthetic microorganisms as microalgae, macro algae and cyanobacteria for the evacuation of pollutants as metals. It is very important here to comprehend the transference of the metal adsorbed onto the surface related to the metal

accumulated inside the cell. The end goal which is to comprehend the overwhelming evacuation mechanisms and to settle on choices of the feasibility of the recuperation of the adsorbed metals should also be considered during the procedure (Olguin & Sanchez-Galvan, 2012).

2.11.1.2 Biosorption and bioaccumulation

Yeasts and molds have been most widely explored in case of Bio sorption and bioaccumulation of Chromium for bioremediation. Mechanisms of Chromium resistance of chosen microorganisms are of specific significance in bioremediation technologies. Some satisfying results have been developed while the systems of Chromium lethality and detoxification have been concentrated widely in yeasts and fungi, (Poljsak, Pocsi, Raspor, & Pesti, 2010). The ability of natural microorganisms to bind with metals can be confirmed 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 phyto-extraction

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

2.11.1.4 Bio-mineralization

Bio mineralization is a procedure of changing aqueous metal ions, including Chromium, into indistinct or crystalline precipitate by using microorganisms. The process is thought to be a promising and financially savvy procedure for remediating Chromium contamination. For example, a case of arsenic precipitation was considered as a possible system for arsenic

bioremediation of sediments debased by arsenic (Focardi et al., 2010). Some essential perspectives for bioremediation are, naturally mediated change, immobilization, and mineralization of poisonous metals (Cheng, Holman, & Lin, 2012).

Chapter 3: Materials and Methods

3.1 Introduction

This segment contains materials applied and diagrams outlining the configuration required for the explanation of biodegradation of hexavalent Chromium thru batch process. It likewise offers an overview of the disengagement and portrayal of bacterial traces from Sea Bed pattern. Features of soil and water samples were acquired from Sitakunda ship breaking dockyard have been all round archived. Enhancement of tradition and process parameters for the cell development and Cr (VI) debasement kinetics changed into likewise concentrated on.

3.2 Chemicals

Chemicals which are unadulterated and analytical mark were applied into every investigations comprising organization of media for advancement. Following chemicals have been applied into this investigation:

1) Extract of beef
2) Extract of yeast
3) Agar of nutrient
4) Broth of nutrient
5) Potassium chromate (K_2CrO_4)
6) Diphenyl Carbazide powder &
7) MOPS buffer
8) Mueller Hinton Agar (MHA)

3.3 Glassware and Apparatus

The devices and tools applied during the research are itemized beneath into Table 3.1

Table 3.1: Total number of toolsutilized throughout the research and their role

Instruments	**Functions**
Vertical Autoclave	Sterilization
Analytical Balance	Measurement of weight
Laminar airflow	Aseptic atmosphere
pH meter	pH measurement
BOD incubator	Culture incubation
Water system	Stock solution preparation
UV-vis spectrophotometer	Growth of the cell measurement and degradation of hexavalent Chromium measurement
Centrifuge	Pellet collection and measurement of hexavalent Chromium
Water bath	Solubilization of media
Microscope	Observation of bacterial morphology
Shaker	To incubate the culture of bacteria at the temperature of room
Micro pipette	For withdrawing chemical and media's trace quantity

3.4 Collection of sample

"Ship breaking industry" is one of the emerging sectors & creating wanders of Bangladesh's ocean side regions which shows an impressive measure of biological weaknesses. This commerce is orchestrated inside the oceanfront of Fauzdarhat, Sitakunda from

BhatiaryuptoBarwalia (Figure: 3.1). It concealments sixteen kilometer extend near the Bay of Bengal's seashore, about twenty kilometer of Chittagong's southwest (DNV, 2001). Forward- facing atmosphere of seashore is extremely powerful and normally grouped running with essential natural and maritime domains, like, woodland of mangrove, consistently and forever submerged marshlands and swamps of salt. Breaking of ship is one method for dismantling unobtainable management ship remembering the true objective for reutilizing piece elements. Bangladesh has been the forerunner into widespread boat's splitting (Ahmed et al., 2013). Mainly costly deadly ships such as ships that are freight and also have partition, tankers are halted by it (Frey, 2013). All the ships reclaimed towards Bangladesh are toxic and outdated ships have age about the years of twenty to thirty (Sarraf et al.,2010). All things considered, a watercraft includes around the steel's ninety-five percent & risky ingredient's five percent (Khan et al., 2011). Sea is known as one energetic natural group from the time from when dismantling of ship occurs into seashore region, thusly the region of tidal and sub-tidal, isolated sea gradually acquires the chance for hosting the differing kind of wastes. Toxins of microbe, hydrocarbon of petroleum and generous elements are encompassed by the wastes generated through them (Reddy et al., 2005). Substantial elements are combines through oceanic boneless living being, join forces within particulates & adsorption occurs through residue. Everything might be deferred or soluble compound could be created for the storage of Cr (Reddy et el., 2005). All around, one ship which have somewhere five thousand to forty thousand ton of quantity is covered with the dye of ten to one hundred ton and it holds substantial element (Khan et al., 2011).Assessments of liquid and dirt with the deepness of zero to fifteen centimeter accumulated from different regions where the evidence has been found on the presence of Cr and destroyed spot of Sitakunda's Ship breakage Yard.

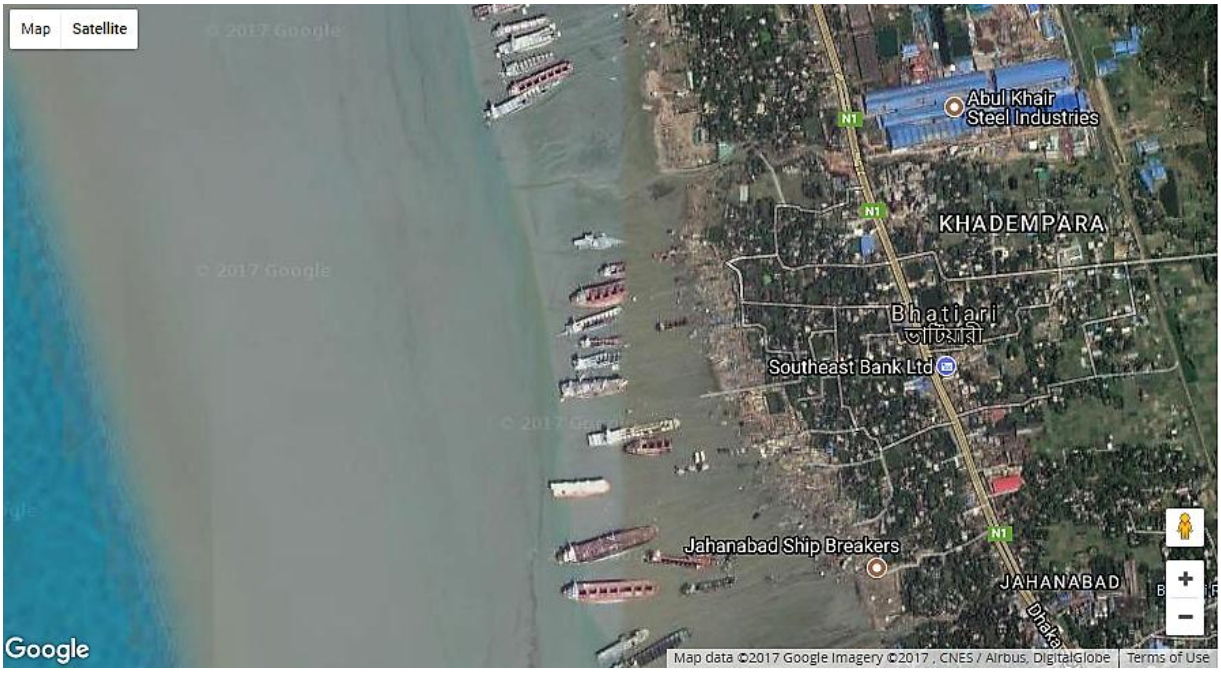


Fig 3.1: Ship breakage industry zone of Chittagong, Bangladesh (Google map, 2017).

3.5 Isolation and condition of the culture

Segregation of microorganisms from samples of the soilassembled had been finished by standard framework. For the withdrawal of microorganism that show imperviousness to Chromium, inoculation was done for one hundred micro-liter specimen of effluent liquid & one hundred micro-liter specimen of soil obtained from unspoiled sample (Into a tube, one gram of dirt had been occupied comprising nine milliliter of the liquid of saline for crushing the soil) through scattering framework into agar dish of nutrient towards media comprising two millimolar of hexavalent chromium complemented as potassium chromate (K_2CrO_4) and then incubation was done. Some colony of bacteria had been observed taking after incubation for twenty-four hour at thirty-seven degree Celsius(37^0C) temperature. Establishment of the media of nutrient agar was accomplished through melting 2.8gram powder of nutrient agar into one hundred milliliter of H_2O. Sterilization was done for media at 121^0 C for forty-five minuteswith keeping fifteen Lb pressure. By then potassium chromate(K_2CrO_4) was incorporated into media and finally that media had been occupied throughout the dish for setting the arrangement of agar dish of nutrient. Bacterial colony that

has been segregated contracted with toothpick which were sterilized and speckled upon the agar dish of nutrient on medium comprising two, three, four and five millimolar hexavalent Chromium. Once more it's incubation was done for twenty-four hours at thirty-seven degree Celsius. This system has been repeated with continuously greater focuses of six, seven,ten, fifteen, twenty, twenty-five, thirty, thirty-five, forty millimolar of hexavalent Chromium till MIC (minimum inhibitory concentration) of the restrain of bacteria has been procured. Enormous improvement &rapid debasement energy of hexavalent Chromiumof specific species of bacteria inside the forty millimolar hexavalent Cr in the midst of the incubation of 24 hours at thirty-seven degree Celsius has been reflected as impervious to hexavalent Chromium. An introverted strain appropriate for emerging at such circumstance has been decided to do the advance examination. Segregation was done for eight solitary colonies obtained through different agar dishes of nutrient comprising characteristic Cr obsessions near the quarantine technique's termination and provided the label linking with their special center of Cr, such as, two millimolar of dirt for (3) and (4).

3.6 Chromium reduction profile of Chromium resistance bacteria

To investigate H_2O and effluent H_2O, test of Diphenyl Carbazide complex formation assay was done to estimate hexavalent Chromium was attuned through standard procedures (Greenberg et al., 1992) and likewise these approaches were documented into Turick et al., 1996. Arrangement was done for one curve that is standard for institutionalization of depletion chart of the microorganism that show imperviousness to Chromium.

3.6.1 Chemical preparation

3.6.1.1 10ml 3M H_2SO_4 preparation

At first, 8mL purified H_2O was poured into one falcon tube. After that, into falcon tube, 1670 µL conc. H_2SO_4has been putted on droplet through droplet comprising 8mL purified H_2O. Next, solution's capacity was prepared to the equal of 10mL though putting on 330µL purified H_2O.

3.6.1.2 Diphenyl Carbazide preparation

0.025 diphenyl carbazide powder was added into one falcon tube. Then into falcon tube, 9.67mL acetone and afterward 3M sulphuric acidof 330µL were taken comprising diphenylcarbazaide powder. Mixing was finely done of that falcon tube to prepare uniform solution of DPCZ.

3.6.1.3 MOPS buffer preparation

At first, into 50mL H_2O, 0.1g sodium hydroxide has been taken to prepare 50mL 1N sodium hydroxide. Then, to prepare 20mM buffer of MOPS, MOPS powder of 334.88mg has been putted on with purified H_2O of 80mL. MOPA buffer's pH was attuned into 7 through the addition of adequate quantity of 1N sodium hydroxide inside the solution of buffer.

3.6.1.4 5mM 10mL K_2CrO_4 preparation

At first, for the solution preparation of 1M potassium chromate, 19.4g potassium chromate has been melted into 10mL purified H_2O. Then, filtration was done for this solution utilizing membrane sieve containing 0.45micron size of the pore. At last, dilution was done for this solution upto 5mM and it was preserved so that it can be utilized in future.

3.6.2 Processes of experiment

3.6.2.1 Standard curve preparation

3.6.2.1.1 Sample preparation for reaction

Subsequent solutions of specimen of various strength have been arranged. Individual specimen's absolute volume was one milliliter.

Table 3.2: Sample preparation for standard curve

Concentration Final	**Quantity of 5mM K_2CrO_4 solution**	**Amount of NB added**	**Final volume to solution**
50µM	10µL	990µL	1ml
100µM	20µL	980µL	1ml
150µM	30µL	970µL	1ml
200µM	40µL	960µL	1ml
300µM	60µL	940µL	1ml
400µM	80µL	920µL	1ml
500µM	100µL	900µL	1ml
600µM	120µL	880µL	1ml

3.6.2.1.2 Reaction protocol for standard curve

Firstly, sample of 600 µL was added into one falcon tube. Then into the sample, 1.2ml 20mM buffer of MOPS, 99 µL 3M sulfuric acid, 981 µL purified H_2O and diphenyl carbazide of 120 µL were taken progressively and thus an uniform blend has been made. When the response occurs into the sample and solution forms, then the solution alters its shade into purple. Finally, the response solution's absorbance has been measured through UV-Visible spectrophotometer at 540nm.

3.6.2.2 Evaluation of reduction profile of selected isolates at room temperature

3.6.2.2.1 Procedure

- **Day 0:**

Broth of nutrient was made into two distinct conical flasks. Volume of each flask is 10mL. From that point forward, from stored culture, specimen was added and then into the broth medium of nutrient, inoculation was done. Control reflected that broth medium of nutrient where inoculation was not done. Finally, upon the rotating incubator, both of the 10mL conical flasks were given for incubation for 24hours.

- **Day 1:**

On following day culture containing 25mL nutrient broth put into two distinct conical flasks. To prepare 600µL potassium chromate (K_2CrO_4), 15µL K_2CrO_4 was putted on nutrient broth. Then 2mL solution of the culture was removed from that flask containing culture and at former day, it was in the incubator at room temperature. At next, for sample. positive control (E.coli) and negative control (blank), the solution of the culture's OD (optical density) has been recorded through UV-visible spectrophotometer at the wavelength of 600nm to observe the development of cell. Furthermore, calculation was done for the quantity needed from the 24hours culture for obtaining 0.2 optical density into test culture. After that, for withdrawing the needed quantity of 24hrs culture, utilization was done for one sterilized falcon tube for the sample, blank and E. coli. Then these were vortexed and after that for five minute, centrifugation was done for samples, blank and E. coli at the rate of 4000 rotation per minute. After the centrifugation of the needed quantity of the overnight pellets of culture was done, then these have been carried and putted in 25ml nutrient broth that were freshly organized and it were complemented with 600µL potassium chromate which contain hexavalent Chromium and moreover, it has been vortexed finely for obtaining culture's OD of 0.2. Afterward, at the temperature of room, culture that has been newly made, was given for incubation. Subsequently to 1.5hours, from 25ml conical flask of sample, positive and

negative control,2ml test cultures were withdrawn and optical density has been recorder at the wavelength of 600nm for observing the cell development. At next, these cultures of 2ml were vortexed and then for five minutes, centrifugation was done at the rpm of 4000 and thus bacteria were precipitated at the bottom of falcon tube and supernatant were obtained at the top of falcon tube and 600µL was withdrawn from the upper supernatant. Then the indistinguishable reaction procedure as stated into 3.6.2.1.2 section was driven and optical density was measured at the wavelength of 540nm. This methodology was reiterated following 3rd hour, 4.5th hour, 6th hour and after the growth of bacteria for 24hours (overnight), the final measurement was recorded.Microsoft office excel 2016 were used for obtaining the reduction profile.

3.7 Antibiotic resistance among Chromium resistant isolates:

3.7.1 Strain Culture preparation in Nutrient broth (NB)

For the sensitivity investigation of antibiotic, broth of nutrient was made for culturing strains. Into one conical flask, nutrient broth of 20ml was added and inoculation was done for the subsequent strains in the individual conical flasks comprising 20ml nutrient broth and incubation was done for overnight at 37 degree Celsius. Labeling was done into the conical flasks.

3.7.2 Inoculation of test plates

For the preparation of test plates, Mueller Hinton Agar (MHA) was utilized. Incubated strains of culture were withdrawn after overnight for their inoculation in the plates of MHA. One cotton swab which were sterilized was immersed in conical flask comprising the preparation of culture strain. Inoculation was done on MHA plate's dry exterior through moving the cotton swab upon whole sterile surface of the MHA. This method has been reiterated through moving 2 more periods, for each period, circling that plate about 60º for ensuring inoculum's uniform spread. Finally, the agar's rim was mopped.

For three to five minutes, the cover was opened, however, it could not left to be open after exceeding fifteen minutes so that no extra moisture of the surface can be absorb before giving antibiotic disks.

3.7.3 Application of Antibiotic discs

Antibiotic disc's fixed battery was distributed on MHA plate's surface which has been gone through inoculation. Individual discs were pushed down for ensuring of the thorough contact within the surface of agar. Following antibiotic discs were utilized in this test:

- ✓ Chloramphenicol (C:30 mg)
- ✓ Ciprofloxacin (Cip:5 mg)
- ✓ Gentamicin (CN: 10 mg)
- ✓ Ofloxacin (OF: 5 mg)
- ✓ Vancomycin (VA: 30mg)
- ✓ Sulphametronazol / Trimethoprim (SXT: 25 mg)
- ✓ Azithromycin (AZM:15mg)
- ✓ Neomycin (N: 30 mg)
- ✓ Ceftriaxone (CRO: 30 mg)
- ✓ Cefuroxime Sodium (CXM: 30 mg)
- ✓ Penicillin-G (P: 10 mg)

3.7.4 Incubation

Into the incubator, within fifteen minutes, the test plates were positioned at 37ºC for 24hours after discs were placed.

3.8 Determination of Minimum Inhibitory Concentration (MIC):

Into microbiology, MIC is an anti-bacterial minimum concentration which will prevent one bacteria's observable development after the incubation of 24hours. Chromate's minimum inhibitory concentration for individual isolate was expressed through counting technique of colony. Inoculation was done of the plates comprising agar media of nutrient complemented with various concentrations of potassium chromate (K_2CrO_4) from 2mM – 30mM with 50µL culture of 24hours old developed into nutrient broth media. Then all of these plates was given for incubation at the temperature 37ºC for forty-eight hours. Finally, the bacterial development was measured through counting of the colony using digital colony counter.

3.9 Identification of the isolate S_4:

After obtaining sequence data file of S_4 isolate by 16s rDNA sequencing,the sequence chromatogram was observed finch TV tool and sequence data was purified and saved the file format as FASTA.BLAST (Basic Local Alignment Search Tool) was performed of the query sequence with existing database from NCBI (National Center for Biotechnology Information).Possible bacterial strains was identified on the basis of maximum similarity score.

Chapter 4: Results

4.1 Isolation data of Chromium resistance bacteria

Isolation was done for the individual colonies of two from the Agar dishes of nutrient with various concentrations of Cr

4.2 Chromium reduction profile of Chromium resistant bacteria

4.2.1 Standard Curve

Method stated into 3.6.2.1.2 was done to obtain the standard curve. After that, utilizing the value of absorbance, plotting was done of one graph using Microsoft Excel 2016. Results which have been obtained are given below:

Table 4.1: Data of standard curve of hexavalent Chromium:

Concentration (µM)	Absorbance
50	0.294
100	0.624
150	0.907
200	1.214
300	1.675
400	2.117
500	2.587
600	2.875

From these data, one standard curve was found and that is given below:

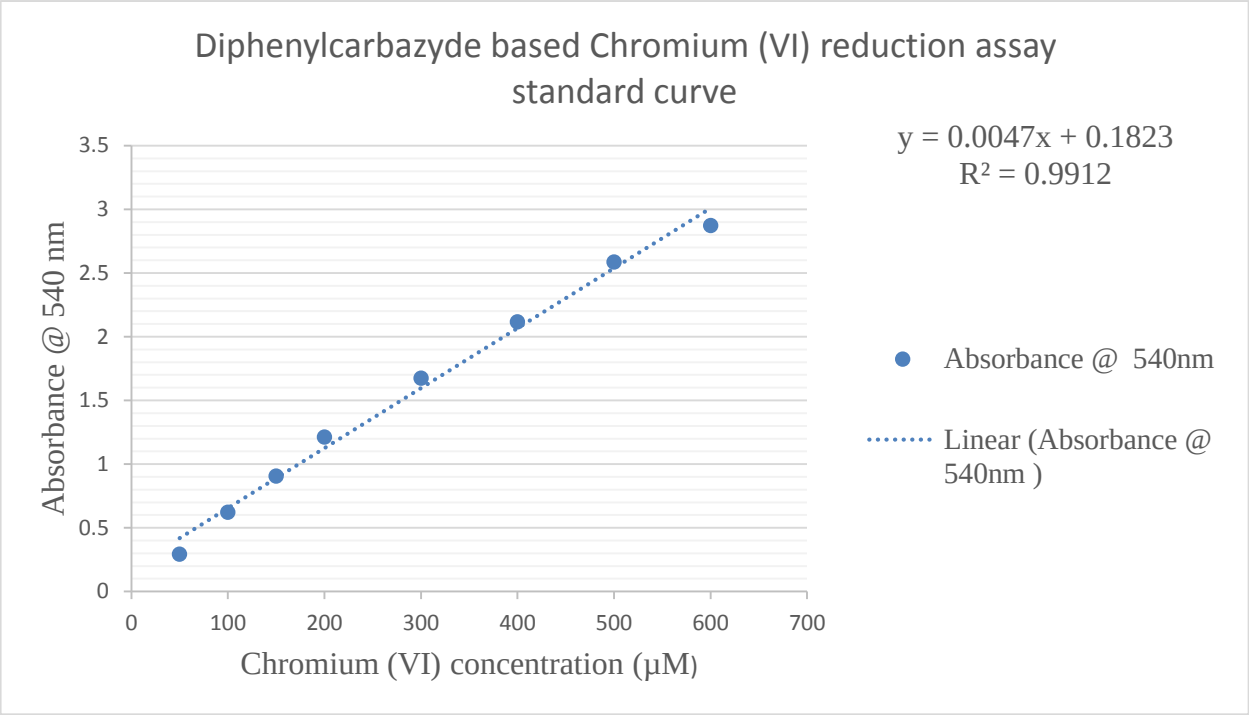


Figure 4.1: Standard curve of hexavalent Chromium absorbance taken at 450nm wavelength

4.2.2 Reduction profile of Isolate: S_4

At various constraints, this test was done such as, altering the pH and temperature and the outcomes which was attained precise underneath:

Table 4.2: Isolate S_4: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 7

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	279.5106383	0.881	432.2765957	0.098
1.5	274.4751773	1.106	366.9574468	0.108
3.0	244.8297872	1.694	385.4680851	0.109
4.5	230.929078	2.083	344.1914894	0.099
6.0	214.1205674	2.227	385.4680851	0.098
24	103.3404255	2.716	386.106383	0.102

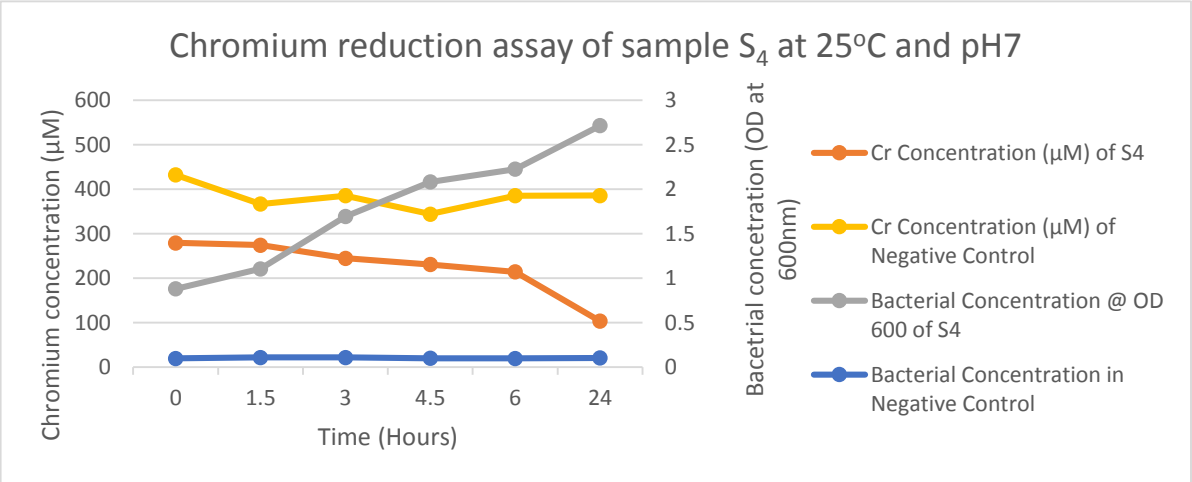


Figure 4.2: Chromium reduction Vs. Cell Growth in S_4 isolate at 25^0 C, pH 7

From the above **Figure 4.2** it was clear that in sample S_4 at 0hour, Chromium concentration was around 377.67 at 540nm. Then, a dramatic fall of the concentration of Chromiumwas seen after 1.5hour, which was 282.06 at 540nm. Chromium concentration continued to decrease in the following hours and lastly, a marked reduction of Chromium has been observed after 24hours, which was 86.177. Therefore, 77% reduction of Chromium in 24 hours was obtained at 540nm. Moreover, in sample S_4, a sharp growth of bacterial concentration was observed at 600nm from 0.872 to 2.771 in 24 hours. But no significant reduction of chromium concentration or bacterial growth was obtained in the negative control. Hence, it is observable that, sample $S_4$is not only resistant towards Chromium, but also proficient of lessening the number of Chromium at 25ºC, pH 7.

Table 4.3: Isolate – S_4: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 5.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	362.2056738	0.853	432.2765957	0.098
1.5	310.7163121	0.902	366.9574468	0.108
3.0	304.5460993	0.989	385.4680851	0.109
4.5	276.5319149	0.966	344.1914894	0.099
6.0	256.8865248	0.102	385.4680851	0.098
24	137.9503546	0.079	386.106383	0.102

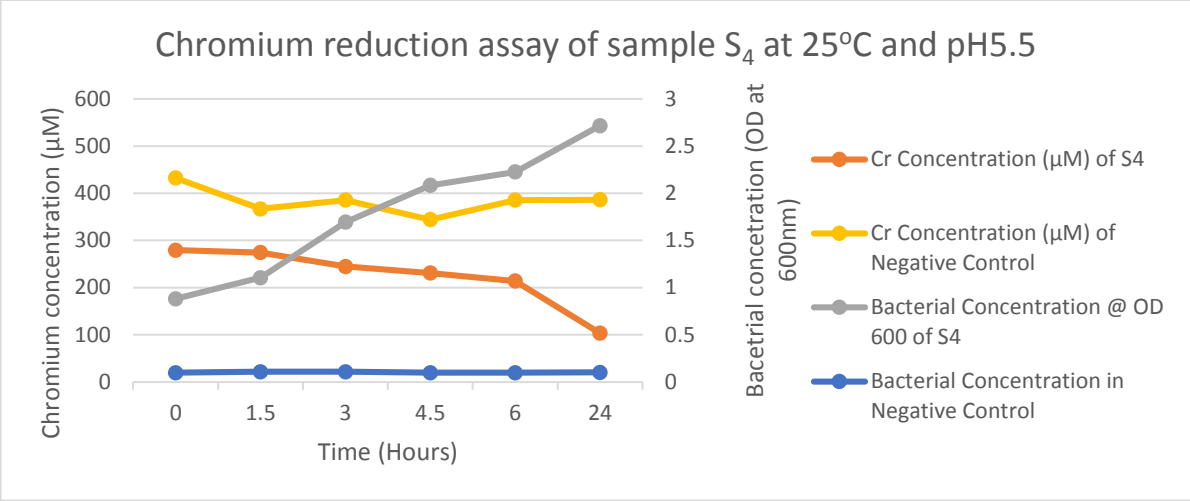


Figure 4.3: Chromium reduction Vs. Cell Growth in S_4 isolate at 25ºC, pH 5.5

From the above **Figure 4.3** it was clear that, in sample S_4 at 0hour, Chromium concentration was around 219.44 at 540nm. Then, gradually, the decrease in Chromium concentration was seen in the following hours at 540nm. Finally, a marked reduction of the concentration of Chromium was found after 24 hours which was 22.63. Therefore, in 24 hours, 89% reduction of Chromium was obtained at 540nm. Moreover, minimal growth of bacterial concentration was observed in sample S_4 from 0.599 to 0.707 in 24 hours at 600nm. But no significant bacterial growth or Chromium reduction was obtained in the negative control. Therefore, it is observable that, sample S_4 can reduce the number of Chromium and also shows minimal resistant to Chromium at 25ºC, pH 5.5.

Table 4.4: Isolate-S_4: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 8.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	332.0638298	0.811	432.2765957	0.098
1.5	245.964539	1.006	366.9574468	0.108
3.0	242.1347518	1.498	385.4680851	0.109
4.5	237.1702128	1.968	344.1914894	0.099
6.0	202.4184397	2.019	385.4680851	0.098
24	149.6524823	2.675	386.106383	0.102

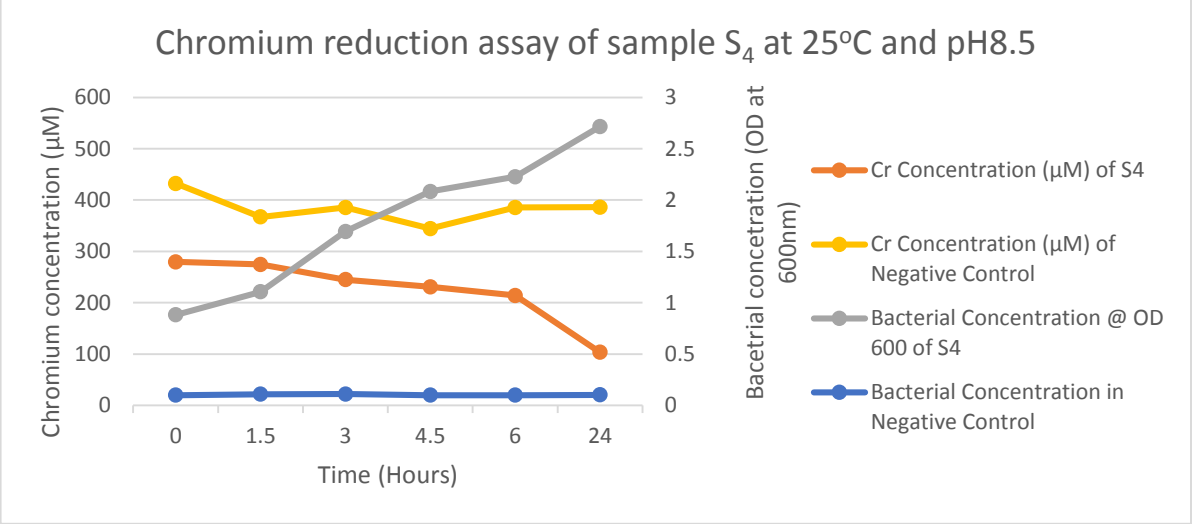


Figure 4.4: Chromium reduction Vs. Cell Growth in S_4 isolate at 25ºC, pH 8.5

From the above **Figure 4.4** is has been observed that, at 0hour, concentration of Chromium was approximately 223.76 at 540nm in sample S_4. After that, concentration of Chromium was rapidly declining and after 6hour, Chromium concentration in that sample was 98.09 at

540nm. At last, significant drop of Chromium concentration was found after 24 hours which was 31.709. Hence, 86% reduction of Chromium was seen for sample S_4at 540nm. Meanwhile, a sharp growth of bacterial concentration of S_4 was recorded at 600nm from 0.735 to 2.665. Yet, no noteworthy reduction of the concentration of Chromium or growth of bacteria was obtained in negative control. As a result, it can be said that, sample S_4 is both resistant towards Chromium and capable of reducing Chromium concentration at 25ºC, pH 8.5.

Table 4.5: Isolate-S_4: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 7

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	328.0212766	0.983	286.5319149	0.138
1.5	290.1489362	1.027	286.106383	0.149
3.0	267.3829787	1.621	344.8297872	0.102
4.5	252.7730496	2.059	322.4893617	0.1
6.0	171.212766	2.283	345.0425532	0.098
24	1.85106383	2.278	314.1914894	0.131

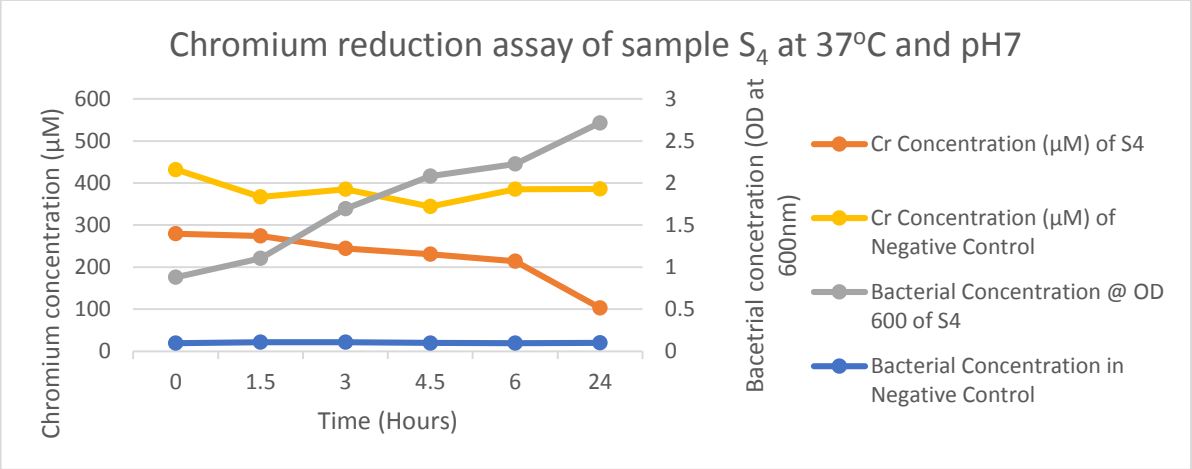


Figure 4.5: Chromium reduction Vs. Cell Growth in S_4 isolate at 37ºC, pH 7

From the above **Figure 4.5** it was clear that, at 0hour, Chromium concentration was around 376.18 at 540nm in the sample of S_4. Concentration of Chromium was gradually decreasing until 6hour, which was 244.55. But a dramatic fall down of Chromium concentration was observed after 24 hours which was approximately 47.95. Thus 87% reduction of Chromium was obtained between 24 hours at 540nm. In addition, a rapid growth of bacterial concentration of S_4 was seen at 600nm from 0.871 to 2.728. But no significant reduction of Chromium concentration or bacterial growth was obtained in the negative control. Therefore, it can be said that, sample S_4 shows remarkable resistance to Chromium and it can also decrease Chromium number.

Table 4.6: Isolate-S_4: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 5.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	358.8723404	0.113	286.5319149	0.138
1.5	298.8014184	0.118	286.106383	0.149
3.0	274.1205674	0.184	344.8297872	0.102
4.5	263.2695035	0.195	322.4893617	0.1
6.0	224.4042553	0.339	345.0425532	0.098
24	17.5248227	2.69	314.1914894	0.131

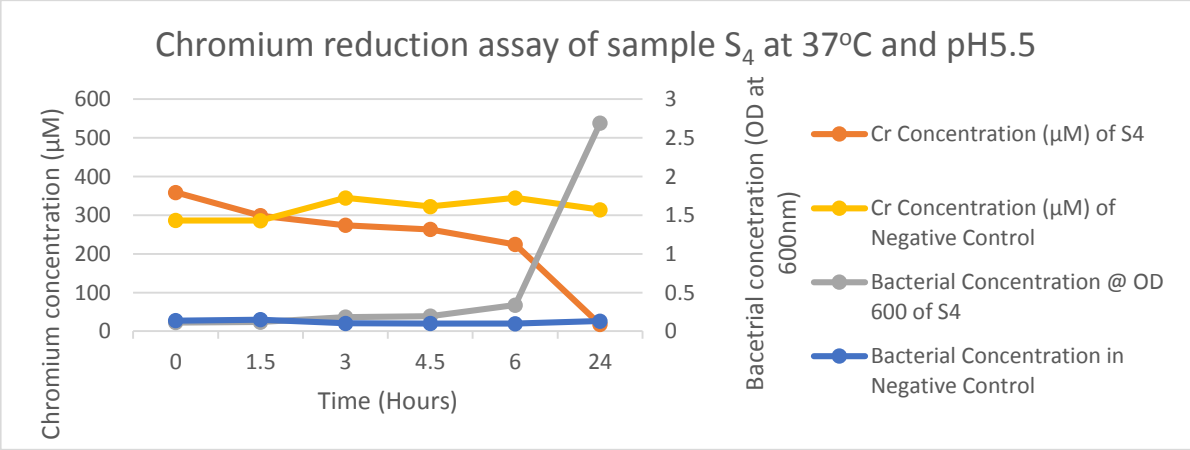


Figure 4.6: Chromium reduction Vs. Cell Growth in S_4 isolate at 37ºC, pH 5.5

From the about **Figure 4.6** it can be seen that, at 0hour in sample S_4, concentration of Chromium was around 358.87 at 540nm. Then, a sharp decrease of Chromium concentration was seen after 1.5hour, which was 298.81 of that sample. After 24 hours, Chromium concentration was dramatically reduced into 17.53. Thus, 95% reduction of Chromium concentration was done by S_4 isolate at 540nm. Meanwhile, bacterial growth was observed gradually from 0.113 to 2.69 in sample S_4 at 600nm. However, no significant bacterial growth or reduction of Chromium was seen in the negative control. Therefore, it has been clear that, sample S_4has the capability to show noteworthy resistance to Chromium and to reduce the concentration of Chromium at 37ºC, pH 5.5.

Table 4.7: Isolate-S_4: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 8.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	303.9078014	0.853	286.5319149	0.138
1.5	273.1276596	0.867	286.106383	0.149
3.0	228.8014184	1.319	344.8297872	0.102
4.5	219.5815603	1.889	322.4893617	0.1
6.0	170.3617021	2.213	345.0425532	0.098
24	15.68085106	2.806	314.1914894	0.131

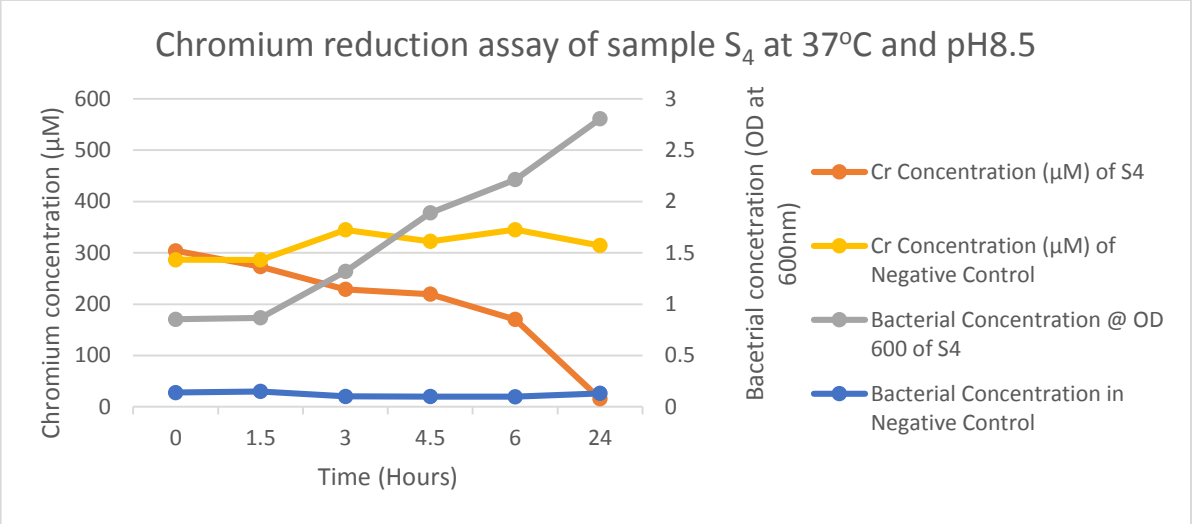


Figure 4.7: Chromium reduction Vs. Cell Growth in S_4 isolate at 37ºC, pH 8.5

From the above **Figure 4.7** it wasclear that, in sample S_4 at 0hour, Chromium concentration was 291.92 at 540nm. The concentration of Chromiumof that sample was gradually decreasing until 6hour, which was 173.63 and then, a marked reduction of Chromium concentration was observed after 24 hours, which was 65.18. Hence, 77% reduction of Chromium concentration was seen at 540nm. Moreover, a sharp growth of bacteria was observed in sample S_4 from 1.188 to 2.987 at 600nm. But in the negative control, no significant reduction of Chromium concentration or bacterial growth was obtained. Therefore, it can be said that, sample $S_4$is not only resistant towards Chromium, but also proficient of lowering the number of Chromium at 37ºC, pH 8.5.

Table 4.8: Isolate-S_4: Chromium reduction profile Vs. Cell Growth at 42ºC, pH 7

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	311.141844	0.621	350.787234	0.084
1.5	218.5886525	0.958	350.1489362	0.098
3.0	110.7163121	1.458	346.3191489	0.091
4.5	104.7588652	1.936	326.3191489	0.098
6.0	60.14893617	2.343	325.893617	0.086
24	0.929078014	2.431	338.0212766	0.079

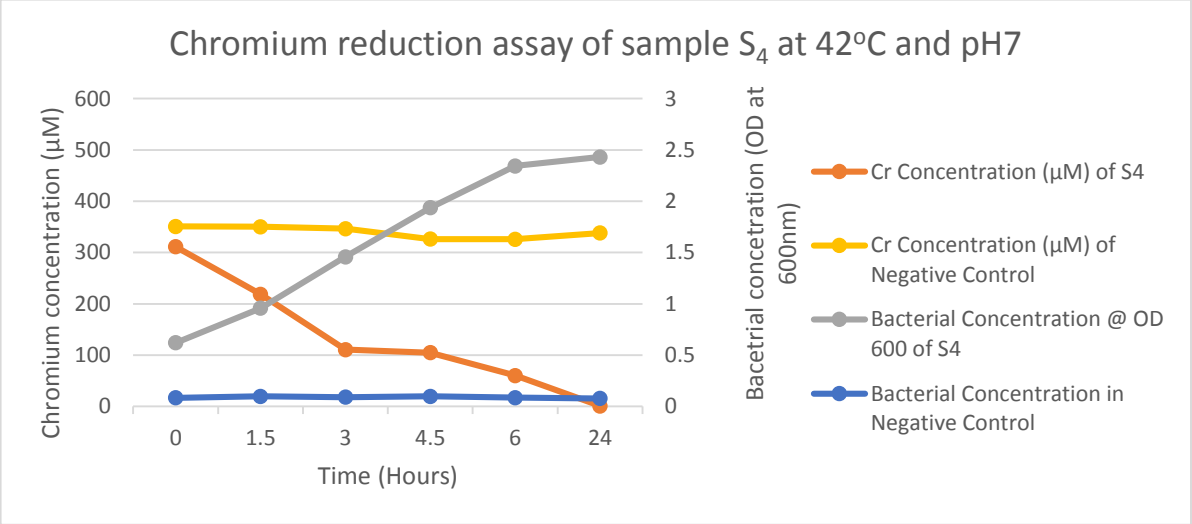


Figure 4.8: Chromium reduction Vs. Cell Growth in S_4 isolate at 42ºC, pH 7

From the above **Figure 4.8** it can be said that, at 0hour in sample S_4, Chromium concentration was around 335.61 at 540nm. After passing 3hour, a dramatic decrease of Chromium concentration was observed which was 158.73 in that sample. Finally, after 24

hours Chromium concentration was rapidly reduced into 31.57. Thus, 90% reduction of the concentration of Chromium was obtained in 24 hours at 540nm. Moreover, in sample S_4, a sharp growth of bacterial concentration was seen at 600nm from 0.413 to 2.404. However, in the negative control, no growth of bacteria or no noticeable reduction of Chromium was observed. Therefore, it has been clear that, sample S_4 is resistant to Chromium and can also decrease the number of Chromium at 42ºC, pH 7.

Table 4.9: Isolate-S_4: Chromium reduction profile Vs. Cell growth at 42ºC, pH 5.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	257.1702128	0.412	350.787234	0.084
1.5	215.0425532	0.727	350.1489362	0.098
3.0	144.9007092	0.902	346.3191489	0.091
4.5	117.0992908	1.303	326.3191489	0.098
6.0	38.87234043	1.377	325.893617	0.086
24	0.219858156	1.071	338.0212766	0.079

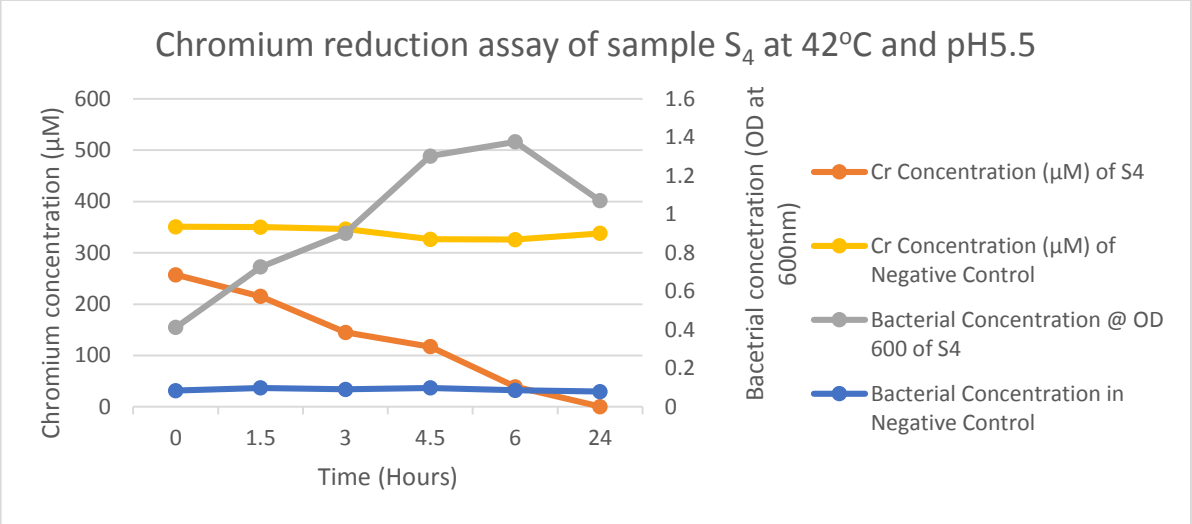


Figure 4.9: Chromium reduction Vs. Cell Growth at 42ºC, pH 5.5

From the above **Figure 4.9** it was clear that, in sample S_4 at 0hour, Chromium concentration was 335.61 at 540nm. Then, a dramatic fall of the concentration of Chromium was seen after 3hour, which was 132.98 at 540nm. Chromium concentration continued to decrease in the following hours and lastly, a marked reduction of Chromium concentration was observed after 24 hours, which was 15.54. Hence, 96% reduction of Chromium concentration was obtained between 24 hours at 540nm. Moreover, in sample S_4, a sharp growth of bacterial concentration was observed from 0.316 to 1.41 at 600nm in 24 hours. But no reduction of Chromium or bacterial growth was seen in the negative control. Therefore, it can be said that, sample S_4 is not only resistant towards Chromium, but also proficient of lessening the Chromium at 42ºC, pH 5.5.

Table 5.0: Isolate-S_4: Chromium reduction profile Vs. Cell growth at 42ºC, pH 8.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	224.3333333	0.382	350.787234	0.084
1.5	177.2411348	0.737	350.1489362	0.098
3.0	120.7163121	1.082	346.3191489	0.091
4.5	100.929078	2.009	326.3191489	0.098
6.0	101.070922	2.29	325.893617	0.086
24	53.55319149	2.435	338.0212766	0.079

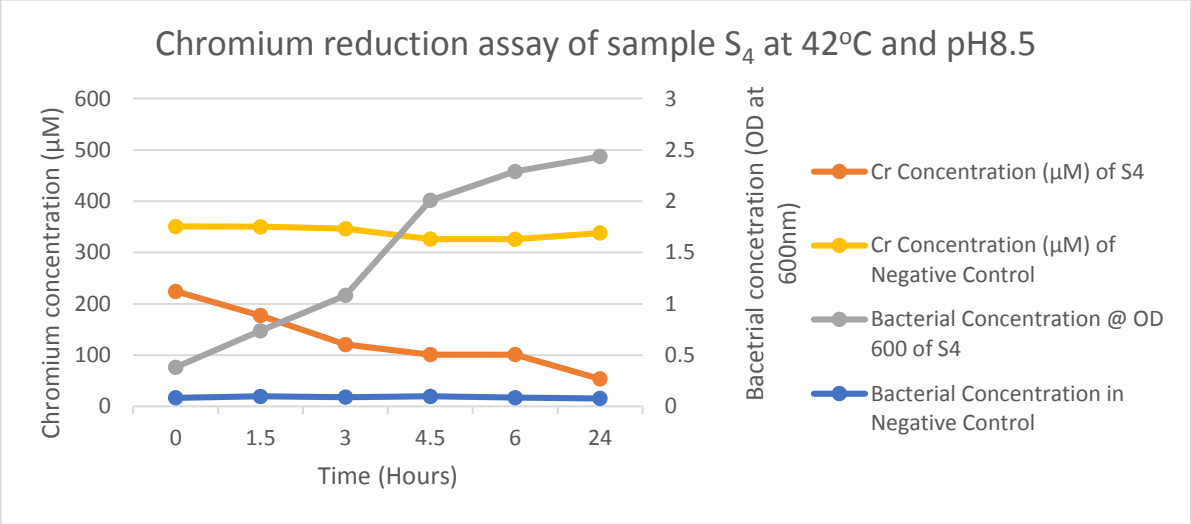


Figure 5.0: Chromium reduction Vs. Cell Growth in S_4 isolate at 42ºC, pH 8.5

From the above **Figure 5.0**it can be observed that, concentration of Chromium was approximately 298.45 at 540nm in sample S_4 at 0hour. After that, Chromium concentration was decreasing rapidly and at 6hour, the concentration was 121.43 at 540nm. Finally, after

24 hours, the concentration of Chromium was dramatically reduced and became 14.05 of sample S_4. Thus, between 24 hours, 95% reduction of the concentration of Chromium occurred at 540nm. Meanwhile, a sharp growth of bacterial concentration was obtained at 600nm from 0.457 to 2.007 in the sample of S_4. However, no bacterial growth or reduction of Chromium was happened in the negative control. Therefore, it can be said that, sample S_4 can decline the Chromium concentration and it is also capable to show resistance to Chromium at 42ºC, pH 8.5.

4.3 Antibiotic resistance among Chromium resistant isolate S_4:

This test was done by distributing and fixing 11 discs of Antibiotic on the surface of MHA plates which have been gone through inoculation and then incubation was done for 24 hours. After incubation, the activities of antibiotic discs against S_4 isolate were determined by measuring the diameter of zone of inhibition in millimeter with the help of a transparent scale. The outcomes which has been attained are given below:

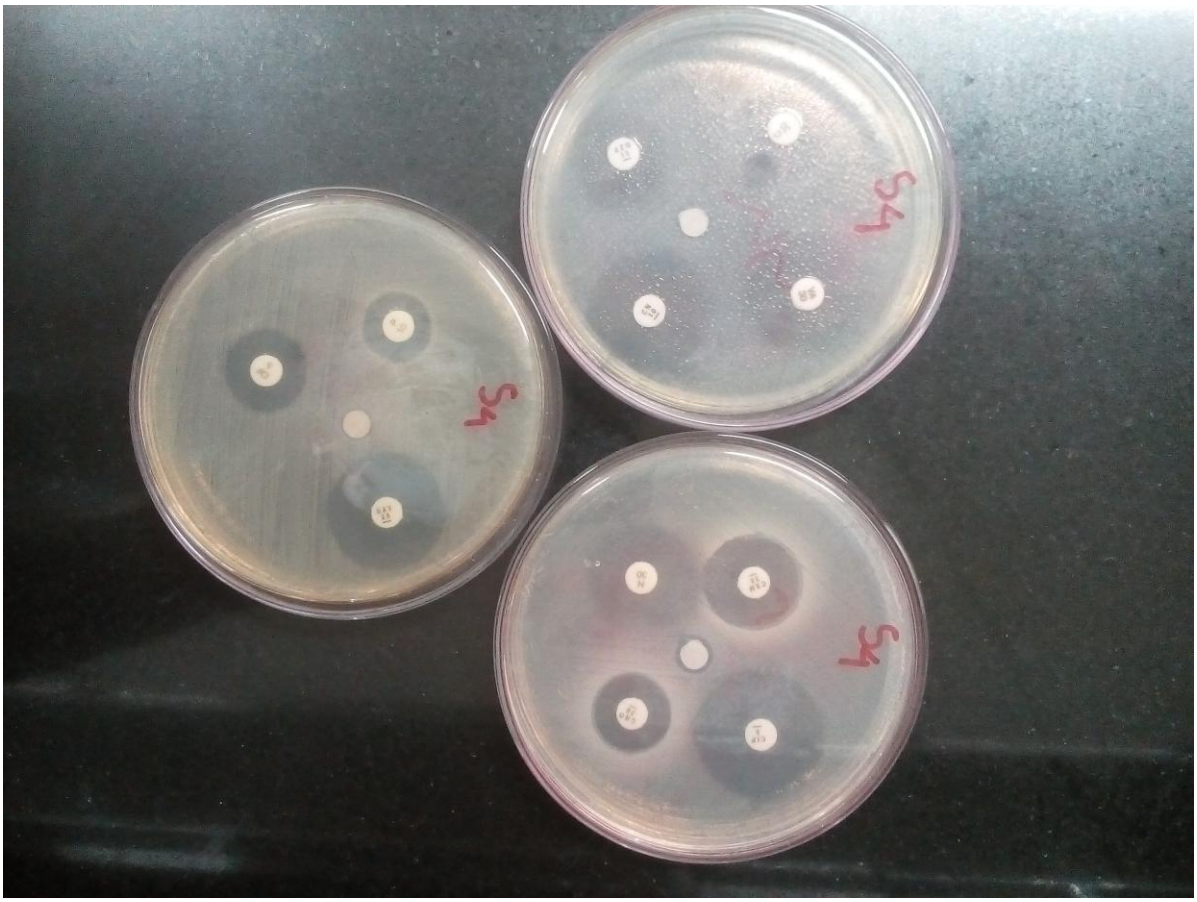


Figure 5.1: Zone of Inhibition of Antibiotic discs in S_4 isolate

Table 5.1: Isolate-S_4: Antibiotic resistance profile

Name of Antibiotic disc	Diameter of Zone of Inhibition (ZI) of S_4 on (mm)
1. Chloramphenicol (C:30mg)	22
2. Ciprofloxacin (Cip:5mg)	25
3. Gentamicin (CN:10mg)	24
4. Ofloxacin (OF:5mg)	16
5. Vancomycin (VA:30mg)	20
6. Sulphametronazol/Trimethoprim (SXT:25mg)	23
7. Azithromycin (AZM:15mg)	19
8. Neomycin (N:30mg)	17
9. Ceftriaxone (CRO:30mg)	14
10. Cefuroxime Sodium (CXM:30mg)	18
11. Penicillin-G (P:10mg)	15

From the **Table 5.1** it can be said that, for bacterial isolate S_4, the highest zone of inhibition was recorded for Ciprofloxacin which was 25 mm. Therefore, the performance of Ciprofloxacin was more potent than all other antibiotics against the bacterial isolate of S_4

because this antibiotic disc was capable for killing the most bacterial cells of S_4 strain surrounding the disc. S_4 could not show that much resistance against this antibiotic or could not resist the effects of this antibiotic and S_4 was mostly susceptible against Ciprofloxacin. In addition, the lowest zone of inhibition was recorded for Ceftriaxone which was 14 mm. Hence, function of Ceftriaxone was very poor than all other antibiotics against S_4 isolate because Ceftriaxone could not inhibit the bacterial growth of S_4 strain significantly. S_4 isolate was mostly resistant against Ceftriaxone and less susceptible against this antibiotic. Overall, isolate S_4 was more or less susceptible against the remaining antibiotic discs. Ciprofloxacin, Chloramphenicol, Gentamicin, Vancomycin exhibited intensive antibiotic activity from 26mm-22mm respectively and Ofloxacin, Neomycin, Ceftriaxone, Azithromycin, Cefuroxime Sodium showed mild to moderate antibiotic activity from 20mm-16mm accordingly.

4.4 Minimum Inhibitory Concentration of Chromium to inhibit the growth of Chromium resistance bacteria:

4.4.1 MIC of isolate: S_4

In different concentrations of Chromium, different number of colonies have been found and they are tabulated below:

Table 5.2: MIC of isolate S_4

Concentration	**Number of Colonies**
2mM	29
4mM	31
6mM	27
8mM	22
10mM	19

12mM	16
14mM	17
16mM	17
18mM	14
20mM	9
22mM	7
23mM	7
24mM	3
25mM	0
27mM	0
28mM	0
29mM	0
30mM	0

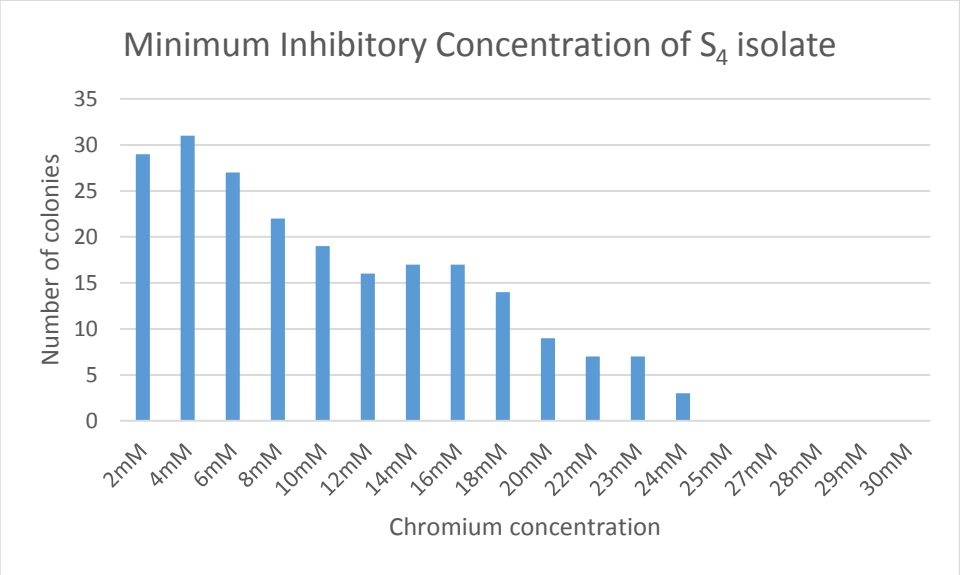


Figure 5.2: MIC of isolate: S_4

From the **Table 5.2** it was seen that, the isolate S_4 tolerates the Chromium concentration up to 24mM, which means, S_4 was able to show resistance till the Chromium concentration of 24mM. But isolate S_4 was totally susceptible from the Chromium concentration of 25mM because no colony was found in 25mM Chromium plate and also in more higher concentration Chromium plate. Thus, 25mM was the Minimum Inhibitory Concentration for S_4 isolate.

4.5 Identification of isolate S_4:

16s rDNA sequencing was retrieved using "sanger sequencing method". The sequence is given in Appendix A. For the identification of the bacterial isolate, BLAST was performed with the purified sequence data. The result is tabulated in Appendix B.

Table 5.3: BLAST result summary of S_4

Sl.	Description	Max Score	Total Score	Query cover	E value	Identity	Accession number
1	Bacillus anthracis strain ATCC 14578	1989	1989	100%	0.0	99%	NR 041248.1
2	Bacillus cereus strain ATCC 14579	1988	1988	100%	0.0	99%	NR 074540.1
3	Bacillus cereus strain JCM 2152	1988	1988	100%	0.0	99%	NR 113266.1
4	Bacillus cereus strain CCM 2010	1988	1988	100%	0.0	99%	NR 115714.1
5	Bacillus cereus strain NBRC 15305	1988	1988	100%	0.0	99%	NR 112630.1
6	Bacillus cereus strain ATCC 14579	1988	1988	100%	0.0	99%	NR 112630.1
7	Bacillus cereus strain IAM 12605	1988	1988	100%	0.0	99%	NR 112630.1
8	Bacillus toyonensis strain BCT7112	1971	1971	100%	0.0	99%	NR 112630.1
9	Bacillus thuringiensis strain ATCC 10792	1971	1971	100%	0.0	99%	NR 112630.1
10	Bacillus thuringiensis strain IAM 15305	1971	1971	100%	0.0	99%	NR 112630.1
11	Bacillus thuringiensis strain NBRC 12077	1967	1967	100%	0.0	99%	NR 112630.1
12	Bacillus pseudomycoides strain NBRC 101232	1965	1965	100%	0.0	99%	NR 112630.1

4.5 Discussion

People are discharging huge measures of Chromium into atmosphere because of pervasive utilization of Chromium into industries such as, textile, tannery of leather, generation of pulp & production of dyes and so forth. Cr (VI) is to a great degree toxic and exhibits mutagenic cancer-causing impact on organic framework in light of the solid nature of oxidization of it. Different diminishment and resistance ability have been created by bacteria for adjusting to the poisonousness of chromate.

Into this research, isolation was done for those bacteria which show resistance to Chromium and the capacity for reducing the cancer-causing Chromium effectively was discovered in those bacteria.

This analysis was done with S_4 isolate at various parameters. Plotting of graphs were done through utilizing the outcomes. By investigating all the data, it was obviously observed that, the S_4 isolate showed resistance and meanwhile, was capable for reducing Chromium concentration at different temperature and pH. Isolate S_4 showed significant reduction of Chromium when they were incubated at 37ºC and pH 5.5, then at 42ºC in the pH of 7, 5.5, 8.5. Among all parameters, optimum temperature and pH for the reduction of Chromium concentration was 42ºC, pH 5.5 because 96% reduction was done in this parameter. This examination's result has portrayed and perceived one fresh strain which has resistive and diminishing power against Chromium.

In the test of antibiotic resistance profile, it was clear that, isolate S_4 showed resistance against Ofloxacin, Neomycin, Ceftriaxone, Azithromycin and Cefuroxime Sodium. It showed the greatest resistance against Penicillin-G. Here, the isolate exhibited the greatest susceptibility towards the Sulphametronazol/Trimethoprim. It also exhibited moderate susceptibility towards Ciprofloxacin, Chloramphenicol, Gentamicin and Vancomycin.

In the test of Minimum Inhibitory Concentration (MIC) of the Chromium resistant bacteria, it has been found that, the isolate S_4 tolerated the Chromium concentration up to 23mM. But no colony was found in 24mM Chromium plate. Therefore, the Minimum Inhibitory Concentration for this isolate is about 24mM.

Therefore, it can be said that, microorganisms which show resistance towards Chromium are persistent in the Chromium contaminated regions and they have the ability to reduce chromate into the aerobic situation, a methodology that carries biotechnological and environmental significance.

At last, 16s rDNA sequencing was done to identify the isolated strains of bacteria.

Chapter 5: Conclusion

5.1 Conclusion

The results of this investigation have asserted that, secluded microorganisms are the standout among maximum promising isolate of bacteria that have been able to persist and lessen hexavalent Chromium. The secluded microorganisms carry an amazing potential to biologically reduce Chromium. Rate of decrease of Chromium concentration is relatively associated with the time of incubation. The isolated bacteria is capable to perform as a significant foundation of the Chromium reductase enzyme that can be utilized as one of the source of chemotherapy if future advancement can be done. The information gathered through this examination revealed that, amongst various types of microbial strain that show resistance towards Chromium, the secluded strain is ready to use with different microbes discovered through distinct scientists for relieving toxic effect of the Chromium into normal samples.

5.2 Future Direction

Investigation can be done the correlation between antibiotic resistance profile and Chromium reduction assay. Further studies like cell free extract will help to elucidate the mechanisms responsible for the reduction of Chromium. Isolate S_4 can be a potential source for Chromium reductase enzyme. Therefore, further investigation of the synthesis of Chromium reductase enzyme can be done and then, to identify the exo-enzyme or endo-enzyme, examination can be carried out.

Reference

Ackerley, D. F., Gonzalez, C. F., Park, C. H., Blake, R., Keyhan, M., & Matin, A. (2004). Chromate-Reducing Properties of Soluble Flavoproteins from Pseudomonas putida and Escherichia coli. Applied and Environmental Microbiology, 70(2), 873-882. doi:10.1128/aem.70.2.873-882.2004

Ahluwalia, S. S., & Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour Technol, 98*(12), 2243-2257. doi:10.1016/j.biortech.2005.12.006

Al-Sou'od, K. (2012). Kinetics of the adsorption of hexavalent Chromium from aqueous solutions on low cost material.

Barceloux, D., & Barceloux, D. (1999). Chromium. *Clinical Toxicology*, 37, 173-197.

Briggs, J. (1988). Characterization of chromium effects on a rat liver epithelial cell line and their relevance to in vitro transformation. 48(22):6484-90

Cervantes, C., Campos-Garcia, J., Devars, S., Gutierrez-Corona, F., Loza-Tavera, H., Torres-Guzman, J., & Moreno-Sanchez, R. (2001). Interactions of Chromium with microorganismsand plants. *FEMS Microbiology Reviews, 25*, 335-347.

Chandra, P., & Kulshreshtha, K. (2016). Chromium Accumulation and Toxicity in Aquatic Vascular Plants *70*, 313-327

Chemical properties of Chromium - Health effects of Chromium - Environmental effects of Chromium. (1998-2016).

Cheng, Y., Holman, H.-Y., & Lin, Z. (2012). Minerals, microbes, and remediation: Remediation of Chromium and uranium contamination by microbial activity. *Elements, 8*, 107-112.

Chromium. (2013). In R. H. Kretsinger, V. N. Uversky, & E. A. Permyakov (Eds.), *Encyclopedia of Metalloproteins* (pp. 595-595). New York, NY: Springer New York.

Codd, R., Rillon, C., Levina, A., & Lay, P. (2001). Studies on the genotoxicity of Chromium: from the test tube to the cell. *Coordination Chemistry Review*, 537-582.

Coleman, R. (1988). Chromium toxicity: effects on microorganisms with special reference to the soil matrix

Das, S., Ram, S. S., Sahu, H. K., Rao, D. S., Chakraborty, A., Sudarshan, M., & Thatoi, H. N. (2012). A study on soil physico-chemical, microbial and metal content in Sukinda chromite mine of Odisha, India. Environmental Earth Sciences, 69(8), 2487-2497. doi:10.1007/s12665-012-2074-4

Demirbasa, E., Kobyab, M., Senturkb, E., & Ozkana, T. (2004). Adsorption kinetics for the removal of Chromium (VI) from aqueous solutions on the activated carbons prepared from agricultural wastes.

Dey, S., & Paul, A. K. (2013). Hexavalent Chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden *Brazilian Journal of Microbiology, 44*.

Dhal, B., Thatoi, H. N., Das, N. N., & Pandey, B. D. (2013). ChemInform Abstract: Chemical and Microbial Remediation of Hexavalent Chromium from Contaminated Soil and Mining/Metallurgical Solid Waste: A Review. ChemInform, 44(30). doi:10.1002/chin.201330273

Elangovan, R., Philip, L., & Chandraraj, K. (2010). Hexavalent Chromium Reduction by Free and Immobilized Cell-free Extract of Arthrobacter rhombi-RE.

Finley, B. L., Proctor, D. M., & Paustenbach, D. J. (1992). An alternative to the USEPA's proposed inhalation reference concentrations for hexavalent and trivalent Chromium. *Regul Toxicol Pharmacol, 16*(2), 161-176.

Focardi, S., Pepi, M., Ruta, M., Marvasi, M., Bernardini, E., Gasperini, S., & Focardi, S. (2010). Arsenic precipitation by an anaerobic arsenic-respiring bacterial strain isolated from polluted sediments of the Orbetello Lagoon, Italy. *Letters in Applied Microbiology, 51*, 578-585.

Francisco, R., Moreno, A., & Vasconcelos, M. P. (2010). Different physiological responses to chromate and dichromate in the Chromium resistant and reducing strain Ochrobactrum tritici 5bvl1. *BioMetals, 23*, 713-725.

Ganguli, A., & Tripathi, A. (2002). Bioremediation of toxic Chromium from electroplating effluent by chromate-reducing Pseudomonas aeruginosa A2Chr in two bioreactors. *58*(3), 416-420.

Ganguli, A., & Tripathi, A. (2002). Bioremediation of toxic Chromium from electroplating effluentby chromate-reducing Pseudomonas aeruginosa A2Chr in two bioreactors. *AppliedMicrobiology and Biotechnology*, *58*, 416-420.

Gardea-Torresdey, J. L., Rosa, G. d. l., & Peralta-Videa, J. R. (2004). Use of phytofiltration technologies in the removal of heavy metals. *76*.

Gatto, N. M., Kelsh, M. A., Mai, D. H., Suh, M., & Proctor, D. M. (2010). Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: A meta-analysis. Cancer Epidemiology, 34(4), 388-399. doi:10.1016/j.canep.2010.03.013

Ge, S., Zhou, M., Dong, X., Lu, Y., & Ge, S. (2013). Distinct and effective biotransformation of hexavalent Chromium by a novel isolate under aerobic growth followed by facultative anaerobic incubation. *97*(5), 2131–2137.

Gupta, V., & Rastogi, A. (2009). Biosorption of hexavalent chromium by raw and acid-treated green alga Oedogonium hatei from aqueous solutions. Journal of Hazardous Materials, 163(1), 396-402. doi:10.1016/j.jhazmat.2008.06.104

Hadjiliadis, N. (2012). Cytotoxic, Mutagenic and Carcinogenic Potential of Heavy Metals Related to Human Environment.

Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria). (2007). *140*(1–2), 52–61.

Horn, J. D. V. (2013). Chromium, Physical and Chemical Properties. 666-669.

Hu, P., Brodie, E. L., Suzuki, Y., Mcadams, H. H., & Andersen, G. L. (2005). Whole-Genome Transcriptional Analysis of Heavy Metal Stresses in Caulobacter crescentus. Journal of Bacteriology, 187(24), 8437-8449. doi:10.1128/jb.187.24.8437-8449.2005

Hwang, I., Batchelor, B., Schlautman, M. A., & Wang, R. (2002). Effects of ferrous iron and molecular oxygen on chromium(VI) redox kinetics in the presence of aquifer solids. Journal of Hazardous Materials, 92(2), 143-159. doi:10.1016/s0304-3894(02)00006-7

Jain, P., Amatullah, A., Alam, R. S., & Mahmud, R. H. (2012). Antibiotic resistance and Chromiumreduction pattern among Actinomycetes. *American Journal of Biochemistryand Biotechnology*, *8*, 111-117.

Jeyasingh, J., & Ligy, P. (2005). Bioremediation of Chromium contaminated soil: optimization ofoperating parameters under laboratory conditions. *Journal of Hazardous Materials*, 118,113-120.

Kamaludeen, S. P. B., Arunkumar, K. R., Avudainayagam, S., & Ramasamy, K. (2003). Bioremediation of Chromium contaminated environments.

Katz, S., & Salem, H. (1993). The toxicology of Chromium with respect to its chemical speciation: a review. *Journal of Applied Toxicology*, *13*, 217-224.

Kirman, C., Aylward, L., Suh, M., Harris, M., Thompson, C., Haws, L., . . . Hays, S. (2013). Physiologically based pharmacokinetic model for humans orally exposed to chromium. Chemico-Biological Interactions, 204(1), 13-27. doi:10.1016/j.cbi.2013.04.003

Komori, K., Rivas, A., Toda, K., & Ohtake, H. (1990). A method for removal of toxic Chromium usingdialysis-sac cultures of a chromate-reducing strain of Enterobacter cloacae. *AppliedMicrobiology and Biotechnology*, 33, 91-121.

Kopec A, Kim S, Forgacs A, et al. 2012a. Genome-wide gene expression effects in B6C3F1 mouse intestinal epithelia following 7 and 90 days of exposure to hexavalent chromium in drinking water. Toxicol Appl Pharmacol 259, 13-26.

Kopec A, Thompson C, Kim S, et al. 2012b. Comparative toxicogenomic analysis of oral Cr(VI) exposure effects in rat and mouse small intestinal epithelium. Toxicol Appl Pharmacol 262, 124-138.

Kratochvil, D., Pimentel, P., & Volesky, B. (1998). Removal of trivalent and hexavalent Chromiumby seaweed biosorbent. *Environmental Science and Technology*, 32, 2693-2698.

Kümmerer, K. (2011). Antibiotics in the Aquatic Environment. Antimicrobial Resistance in the Environment, 325-335. doi:10.1002/9781118156247.ch18

Kurniawana, T. A., Chana, G. Y. S., Loa, W.-H., & Babelb, S. (2006). Physico–chemical treatment techniques for wastewater laden with heavy metals. *118*.

Lebeau, T., Braud, A., & Jezequel, K. (2008). Performance of bioaugmentation-assisted phytoextractionapplied to metal contaminated soils: A review. *Environmental Pollution, 153*, 497-522.

Lindberg, E., & Hedenstierna, G. (1983). Chrome plating: symptoms, findings in the upper airways, and effects on lung function. *Arch Environ Health, 38*(6), 367-374.

Lin, S., & Kiang, C. (2003). Chromic acid recovery from waste acid solution by an ion exchange process: equilibrium and column ion exchange modeling. Chemical Engineering Journal, 92(1-3), 193-199. doi:10.1016/s1385-8947(02)00140-7

McGrath, S., & Smith, S. (1990). Chromium and nickel. In: Alloway B.J. (ed.) Heavy Metals inSoils. New York: Wiley. 125-150.

Mohan, D., & Pittman, C. U. (2006). Activated Carbons and Low Cost Adsorbents for Remediation of Tri- and Hexavalent Chromium from Water.

Mohanty, K., Jha, M., Meikap, B. C., & Biswas, M. N. (2005).Removal of chromium(VI) from dilute aqueous solutionsby activated carbon developed from Terminalia arjuna nutsactivated with zinc chloride. Chemical Engineering Sci-ence, 60(11), 3049–3059

Monachese, M., Burton, J., & Reid, G. (2012). Bioremediation and tolerance of humans to heavymetals through microbial processes: a potential role for probiotics? *Applied and EnvironmentalMicrobiology*, *78*, 6397-6404.

Muthukrishnan, M., & Guha, B. (2008). Effect of pH on rejection of hexavalent chromium by nanofiltration. Desalination, 219(1-3), 171-178. doi:10.1016/j.desal.2007.04.054

Nandi, R., Laskar, S., & Saha, B. (2016). Surfactant-promoted enhancement in bioremediation of hexavalent Chromium to trivalent Chromium by naturally occurring wall algae.

Natale, F. D., Lancia, A., Molino, A., Musmarra, D. (2007).Removal of chromium ions from aqueous solutions byadsorption on activat ed carbon and char. Journal ofHazardous Materials, 145(3), 381–390

Ngwenya, N., & Chirwa, E. M. (2011). Biological removal of cationic fission products from nuclear wastewater. Water Science & Technology, 63(1), 124. doi:10.2166/wst.2011.021

Obbard. P, (2001). Ecotoxicological assessment of heavy metals in sewage sludge amended soils J. Appl Geochem, 16: 1405-1411.

OEHHA. (2011). Public Health Goals for Chemicals in Drinking Water: Hexavalen Cr.

Ohtake, H., Cervantes, C., & Silver, S. (1987). Decreased chromate uptake in Pseudomonas fluorescenscarrying a chromate resistance plasmid. *Journal of Bacteriology, 169*, 3853-3856.

Ohtake, H., Cerventes, C., & Silver, S. (1987). Decreased chromate uptake in Pseudomonas fluorescens carrying a chromate resistance plasmid. *Journal of Bacteriology, 169*, 3853-3856.

Olguin, E., & Sanchez-Galvan, G. (2012). Heavy metal removal in phytofiltration and phycoremediation:the need to differentiate between bioadsorption and bioaccumulation. *New Biotechnology, 30 Number 1*.

Otha, N., Galsworthy, P., & Pardee, A. (1971). Genetics of sulfate transport by Salmonella typhimurium. *Journal of Bacteriology*, 105, 1053-1062.

Otha, N., Glasworthy, P., & Pardee, A. (1971). Genetics of sulfate transport by Salmonella typhimurium. *Journal of Bacteriology, 105*(1053-1062).

Pattanapipitpaisal, P., & Reakyai, T. (2013). Cr(VI) reduction by cell-free extract of thermophillic Bacillus fusiformis NTR9. 407-414.

Patterson, J. (1985). Industrial wastewater treatment technology.

Pugazhenthi, G., Sachan, S., Kishore, N., & Kumar, A. (2005). Separation of chromium (VI) using modified ultrafiltration charged carbon membrane and its mathematical modeling. Journal of Membrane Science, 254(1-2), 229-239. doi:10.1016/j.memsci.2005.01.011

Kongsricharoern, N. and Polprasert, C. (1995) Electrochemical Precipitation of Chromium (Cr6+) from an Electroplating Wastewater. Water Science and Technology, 31, 109-117. http://dx.doi.org/10.1016/0273-1223(95)00412-G

Poljsak, B., Pocsi, I., Raspor, P., & Pesti, M. (2010). Interference of Chromium with biological systemsin yeasts and fungi: a review. *Journal of Basic Microbiology, 50*, 21-36.

Polti, M., Amoroso, M., & Abate, C. (2010a). Chromate reductase activity in Streptomyces sp. MC1. *Journal of General and Applied Microbiology, 56*.

Polti, M., Amoroso, M., & Abate, C. (2010b). Chromate reductase activity in Streptomyces sp. MC1. *Journal of General and Applied Microbiology*, *56*, 11.

Poopal, A., & Laxman, R. (2009). Chromate reduction by PVA-alginate immobilized Streptomyces griseus in a bioreactor. *Biotechnology Letters, 31*, 71-76.

Wenbo, Q., Reiter, R. J., Tan, D., Garcia, J. J., Manchester, L. C., Karbownik, M., & Calvo, J. R. (2000). Chromium(III)-Induced 8-Hydroxydeoxyguanosine in DNA and Its Reduction by Antioxidants: Comparative Effects of Melatonin, Ascorbate, and Vitamin E. Environmental Health Perspectives, 108(5), 399. doi:10.2307/3454379

Quievryn G, Peterson E, Messer J, and Zhitkovich A (2003) Genotoxicity and mutagenicity of chromium(VI)/ascorbategenerated DNA adducts in human and bacterial cells. Biochemistry 42, 1062-1070.

Raspor, P., Batic, M., Jamnik, P., Josic, D., Milacic, R., Pas, M., . . . Skrt, M. (2000). The influence of Chromium compounds on yeast physiology. *Acta MicrobiologyImmunology Hung*, 47, 143-173.

Rifkin, E., Gwinn, P., & Bouwer, E. (2004). Chromium and SedimentToxicity.

Sapari, N., Idris, A., & Hamid, N. H. A. (1996). Total removal of heavy metal from mixed plating rinse wastewater.

Shi, W., Becker, J., Bischoff, M., Turco, R., & Konokpa, A. (2002). Association of microbial community composition and activity with lead, Chromium, and hydrocarbon contamination. *Applied and Environmental Microbiology, 68*, 3859-3866.

Thacker, U., & Madamwar, D. (2005). Reduction of Toxic Chromium and Partial Localization of Chromium Reductase Activity in Bacterial Isolate DM1. *21*(Issue 6), 891–899.

Thatoi, H., Das, S., Mishra, J., Rath, B. P., & Das, N. (2014). Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: A review. Journal of Environmental Management, 146, 383-399. doi:10.1016/j.jenvman.2014.07.014

Thompson C, Proctor D, Suh M, et al. (2013a). Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent and relevance to humans. Crit Rev Toxicol 43, 244-274.

Wise, S. S., Holmes, A. L., & Wise, J. P., Sr. (2008). Hexavalent Chromium-induced DNA damage and repair mechanisms. *Rev Environ Health, 23*(1), 39-57.

Wong, P., & Trevors, J. (1988). Chromium toxicity to algae and bacteria. In: Nriagu JO., Nieboer E. (eds.), Chromium in the Natural and Human Environments., 305-315.

Zhang, J., & Li, S. (1997). Cancer Mortality in a Chinese Population Exposed to Hexavalent Chromium in Water. Journal of Occupational & Environmental Medicine, 39(4), 315-319. doi:10.1097/00043764-199704000-00008

Zhitkovich, A. (2011). Chromium in Drinking Water: Sources, Metabolism, and Cancer Risks. Chemical Research in Toxicology, 24(10), 1617-1629. doi:10.1021/tx200251t

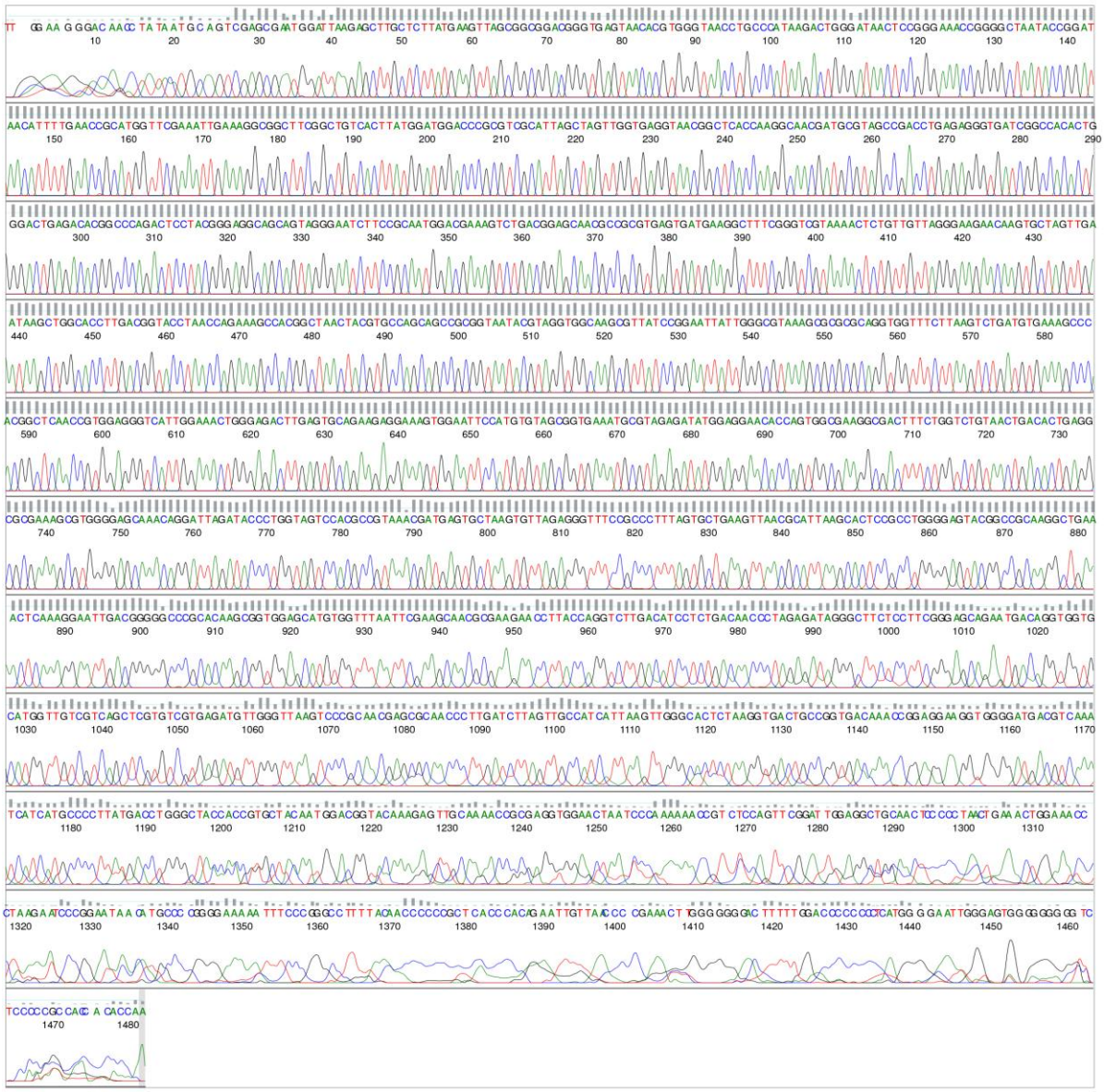
Zhu, W., Chai, L., Ma, Z., Wang, Y., Xiao, H., & Zhao, K. (2008). Anaerobic reduction of hexavalent Chromium by bacterial cells of Achromobacter sp. Strain Ch1. *163*(6), 616–623.

Chapter 7: Appendix

File: 1st_BASE_2808070_S4_27_F.AB1

Geospiza
www.geospiza.com

Sample Name:	2808070_S2_27_F	Signal Strengths:	A = 1685, C = 1290, G = 1195, T = 1240
Mobility:	KB_3730_POP7_BDTv3.mob	Lane/Cap#:	63
Spacing:	14.8022	Matrix:	n/a
Comment:	n/a	Direction:	Native



Printed: Aug 15, 2017 11:14PM FinchTV v.1.4.0 Page 1 of 1