REMOVAL OF ARSENIC (III) AND CHROMIUM (VI) FROM THE WATER USING PHYTOREMEDIATION AND BIOREMEDIATION TECHNIQUES

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Under the guidance of

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CERTIFICATE

This is to certify that the dissertation entitled " Removal of arsenic (III) and chromium (VI) from the water using phytoremediation and bioremediation techniques" being submitted by Sri Anil Kumar Giri for the award of Ph.D. degree is a record of bonafied research work carried out by him under my supervision. In my opinion, the work fulfills the requirements for which it is being submitted.

The work incorporated in this thesis has not been submitted elsewhere earlier, in part or in full, for the award of any other degree or diploma of this or any other Institution or University.

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ABSTRACT

Advancement in science and technologies parallel to industrial revolution has opened new vistas to exploit the inherent traits of natural resources including green plants and microorganisms to overcome the damage to the environment by pollutants.

The present work was aimed to develop the phytoremediation potential of the aquatic plant *Eichhornia crassipes* for arsenic (III) and chromium (VI) from water. The accumulation, relative growth and bio-concentration factor of plant on treatment with different concentrations of arsenic(III) and chromium(VI) solution significantly increased ($P<0.05$) with the passage of time. Plants treated with 0.100 mg/L arsenic (III) accumulated the highest concentration of arsenite in roots (7.20 mg kg^{-1}, dry weight) and shoots (32.1 mg kg^{-1}, dry weight); while those treated with 4.0 mg/L of chromium (VI) accumulated the highest concentration of hexavalent chromium in roots (1320 mg/kg, dry weight) and shoots (260 mg/kg, dry weight) after 15 days. The plant biomass was characterized by SEM, EDX, FTIR and XRD techniques. Microwave-assisted extraction efficiency is investigated for extraction of arsenic from plant materials by comparison of the results by three extractant solutions: (i) 10% (v/v) tetramethylammonium hydroxide (TMAH) (ii) Deionized water and (iii) Modified protein extracting solution at different temperature and times. Extraction of chromium ions was carried by same procedure from plant materials using three extractant solutions: (i) 0.02 M ethylenediaminetetraacetic acid (EDTA), (ii) Deionized water and (iii) HCl solution at different temperature and times. Chromatograms are obtained for arsenic and chromium species in plant shoot biomass by using HPLC-ICP-MS.

The biosorption of arsenic (III) and chromium (VI) from water is studied by living cells of *Bacillus cereus* biomass as bioremediation. *Bacillus cereus* biomass is characterized, using SEM-EDX, AFM and FTIR. Dependence of biosorption was studied with variation of various parameters to achieve the optimum condition. The maximum biosorption capacity of living cells of *Bacillus cereus* for arsenic (III) and chromium (VI) was found to be 32.42 mg/g and 39.06 mg/g at pH 7.5, at optimum conditions of contact time of 30 min, biomass dosage of 6 g/L, and temperature of 30 ± 2°C. Biosorption data of arsenic (III) chromium (VI) are fitted to linearly transformed Langmuir isotherm and pseudo-second order model with R^2 (correlation

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coefficient) > 0.99. Thermodynamic parameters reveal the endothermic, spontaneous, and feasible nature of sorption process of arsenic (III) chromium (VI) onto *Bacillus cereus* biomass. The arsenic (III) and chromium (VI) ions are desorbed from *Bacillus cereus* using both 1M HCl and 1M HNO_3.

The biosorption data of both arsenic (III) and chromium (VI) ions collected from laboratory scale experimental set up is used to train a back propagation (BP) learning algorithm having 4-7-1 architecture. The model uses tangent sigmoid transfer function at input to hidden layer whereas a linear transfer function is used at output layer.

The removal of chromium (VI) from aqueous solutions by activated carbon prepared from the *Eichhornia crassipes* root biomass. The maximum removal capacity of activated carbon was found to be 36.34 mg/g for chromium (VI), at pH 4.5, contact time of 30 min, biomass dosage of 7 g/L, and temperature of 25 ± 2 °C. The adsorption mechanisms of chromium (VI) ions onto activated carbon prepared from the *Eichhornia crassipes* root biomass are also evaluated in terms of thermodynamics, equilibrium isotherm and kinetics studies. Column studies are also performed to know the breakthrough point with an initial concentration of 10 mg/L.

Key words- *Eichhornia crassipes* ; Phytoremediation ; Arsenic (III); Chromium(VI); Microwave assisted extraction; Bio-concentration factor; *Bacillus cereus*; Biosorption isotherm, Biosorption kinetics; Thermodynamic parameters; Regeneration and reuse; Atomic force microscopy; HPLC-ICP-MS; SEM-EDX; XRD, FTIR; HG-AAS; ANN; Activated carbon; Column studies.

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Abbreviations

AFM	Atomic force microscopy
ANN	Artificial neural network
ANOVA	Analysis of variance
ArsC	Arsenate reductase
As_2O_3	Arsenite
ATPase	Adenine tri phopatase
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	Bio-concentration factor
BET	Brunauer-Emmett-Teller surface area analyzer
BPNN	Back propagation neural network
DO	Dissolved oxygen
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy dispersive X-ray
FAAS	Flame atomic absorption spectroscopy
FTIR	Fourier transfer infrared spectroscopy
GSH	Glutathione
HCl	Hydrochloric acid
HG-AAS	Hydride generation atomic absorption spectroscopy
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma-mass spectroscopy
$K_2Cr_2O_7$	Anhydrous potassium dichromate
Mg/g	Milligram per gram
Mg/Kg	Milligram per kilogram
Mg/L	Milligram per liter
$NaBH_4$	Sodium tetrahydroborate
PCs	Phytochelatins

SEM	Scanning electron microscopy
SOD	Superoxide dismutase
TDS	Total dissolved solids
TMAH	Tetra methylammonium hydroxide
USEPA	United States Environmental Protection Agency
WHO	World health organization
XRD	X-ray diffract meter
γ - EC	γ-glutamylcysteine
γ - ECS	γ-glutamylcysteine synthetase

Chapter-1

1. **Introduction**

The quality of life on Earth is linked inextricably to the overall quality of the environment. In early times, it was believed that land, water and other natural resources have unlimited abundance but today, it is not true; in greater or lesser degree the human society has shown the carelessness and negligence in using them. The problems associated with contaminated sites now assume increasing prominence in many countries throughout the globe. The living conditions today have surely improved to a great extent at the cost of environmental degradation. Global development, however, raises new challenges, especially in the field of environmental protection and conservation (Duruibe *et al*., 2007). Consequent to globalization, industrialization and urbanization creates the problem of pollution including heavy metal pollution .The pollution due to heavy metals and metalloids is a widespread problem and causes a major environmental degradation in each segment of environment. It is indeed a matter of concern to everybody as it has direct effect on human and environmental health (Hogan, 2012). The development is dominated by two prime factors. Firstly, each developed or developing countries are facing unprecedented problems in the environmental areas like issues of population, extremes of affluences, drinking water, global warming, stratospheric ozone depletion, ground water contamination, management of wastes and other pollution problems. Secondly, these problems invoke opportunities of parallel scope to solve the above problems. The air, water and soil are getting filled up with hazardous wastes. The very nature of environmental problems has changed (Agarwal, 2009) in the last few decades. Natural resources may be renewable or non-renewable are made by nature, not by human beings. A resource is anything which the environment provides to meet our needs and desires, which have dependability through time (University of Texas, 2003).

Among the various environmental resources water is one of the most important commodities which require special attention. Water is a vital natural resource which forms the basis of all life. About 97% of the earth's surface is covered by water and most of the animals and plants have 60-65% water in their body. Every year 111,000 km^3 of water falls on land and 70, 000 km^3 returns to the atmosphere by evapotranspiration from wet surfaces and plants. The remaining 41, 000 km^3, is the runoff, which eventually reaches the oceans. If the runoff were divided evenly, it could provide each person with 6,760 m^3 a year of freshwater (2000 population). The major amount of water available for human consumption is also polluted. Any human activity that impairs the use of water as a resource may be called water pollution. The burgeoning population is exhausting the available fresh water resources as a result of which, the world is heading towards water crises (Saeijs and Van, 1995; Rosegrant and Cai, 2001). It is predicted that in future most of the social conflicts are going to be water based as predicated by the great scientist of present world Albert Einstein. At present, one liter of packaged water costs rupees 12-15 whereas the milk is costing rupees 10-16 in villages and in future, pure water will be a heavily priced commodity.

In 2012, the world population reaches 7 billion marks. The UN estimates that, by 2050 there will be an additional 2.1 billion people (UN News Centre, 2012) with most of the growth in developing countries that already are under water stress. Former Secretary-General of the United Nations, Kofi Annan, who in 2001 said, "Fierce competition for fresh water may well become a source of conflict and wars in the future." In appreciation of this situation, several steps being taken to control water pollution which seems to be insufficient. More research inputs are required to avoid conflicts in future. Water in the earth's biosphere is used and reused again and again. This is called water cycle or continuous movement of water between the earth and the atmosphere. Water can change states among liquid, vapor, and solid at various places in the water cycle. Although the balance of water on Earth remains fairly constant over time, individual water molecules can come and go, in and out of the atmosphere.

Heavy metals/metalloids can be found in industrial wastewater/effluents and are deemed undesirable proclaimed by many researchers/environmentalists. Prolonged exposure to heavy metal ions has been found to cause the degradation of flourishing ecosystems which harm the inhabitants. Heavy metals/ metalloids are the main group of inorganic contaminants. A

considerable large area of land is contaminated with them due to the use of sludge or municipal compost, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, residues from metalliferous mines, and smelting industries (Jackson *et al*., 2012). Although metals are present naturally in the Earth's crust at various levels but many metals are essential for cells (e.g. copper, iron, manganese, nickel, zinc). All metals are toxic at higher concentrations. Specifically, any metal (or metalloid) species may be considered as ''contaminant'' if it occurs where it is unwanted, or in a form or concentration that causes a detrimental human or environmental effect (Matschullat, 2002). Metal concentrations in soil typically range from less than one to as high as 100 000 $mgkg^{-1}$.

Among the various heavy metals, Arsenic (III) and Chromium (VI) are well-known toxic metal that is considered as priority pollutant. The adverse effects of these two metals are well documented. Arsenic contamination in natural water possesses a great threat to millions of people in many regions of the world such as China, Bangladesh, Nepal, Myanmar, Cambodia and Thailand. Ground water contamination by arsenic has been a major problem in the northeastern parts of India like West Bengal, Assam and in few regions of Odisha. Arsenic occurs naturally in soil and water, and it also enters the environment due to anthropogenic activities. Common chemical forms of arsenic in the environment include arsenate, arsenite, dimethyl arsenic acid, and mono methyl arsenic acid. Epidemiological studies demonstrated that there is close link between the chronic exposure to arsenic in drinking water and some medical disorders and cancers. Chronic arsenic poisoning can cause a lot of human health problems through either contaminated drinking water or agriculture products irrigated by contaminated water (Zhao *et al*., 2012; Wang *et al*., 2007; Guha Mazumder, 2007).

Chromium is another heavy metals found in effluents discharged from industries involved in electronics, electroplating, metallurgical, leather tanning and wood preservatives. The speciation of chromium in contaminated environments becomes critical for understanding its fate and exposure. At pH value less than 6.5 and at high chromium concentration; $Cr_2O_7^{2-}$ predominates, whereas CrO_4^{2-} predominates at pH value greater than 6.5. Strong exposure to Cr (VI) may cause epigastria pain, nausea, vomiting, severe diarrhea and cancer in the digestive tract and lungs (Saçmac *et al*., 2012; Megharaj *et al*., 2003). Many methods have been documented for removal of excessive heavy metals/metalloids from water such as coagulation, ion exchange, precipitation, electrolysis, and reverse osmosis (Balasubramanian *et al*., 2009; Kim *et al*., 2006;

Kumari *et al*., 2006). Most of these methods suffer from some disadvantages because of which it is not very popular (Sharma and Sohn, 2009).

Among these methods, use of aquatic plants to absorb metals from surrounding water is extremely efficient. Biosorption has been treated as a potential technology for removal of toxic heavy metals from industrial wastewater using microbial biomass (Veglio and Beolchini, 1997).

A plant-based phytoremediation approach to remove heavy metals uses plant roots to extract, the vascular system to transport and the leaves as a sink to concentrate the elements above ground for harvest and processing of metals (Natarajan *et al*., 2008; Gratao *et al*., 2005; Jadia and Fulekar, 2009). Contaminants such as metals, pesticides, solvents, explosives and crude oil and its derivatives, are being removed by phytoremediation projects worldwide (Greipsson, 2011). Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites. Phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology, as opposed to mechanical cleanup methods such as soil excavation or pumping polluted groundwater. Over the past two decades, this technology has become increasingly popular and has been employed in situ in soil and water, contaminated with lead, uranium, and arsenic. However, one major disadvantage of phytoremediation is that, it requires a long-term commitment, as the process depends on plant growth, tolerance to toxicity, and bioaccumulation capacity (Salt *et al*., 1995; Glick, 2004). The use of hydroponic culture treatment has been considered as a means of assessing the plant tolerance to the toxic elements or its efficiency in mineral utilization. The bio removal process using aquatic plants contains two uptake processes such as (i) biosorption which is an initial fast, reversible, and metal-binding process and (ii) bioaccumulation which is a slow, irreversible, and ion-sequestration step. At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented in the literature to accumulate high levels of metals in leaves. In the last decade, extensive research has been conducted to investigate the importance of metal in biology. Some macrophytes are found to remove different concentrations of arsenic ions, which make them suitable to act as bio-monitors for metals, and have ability to act as biological filters of the aquatic environment (Chiu *et al*., 2005; Fayiga *et al*., 2005; Huang *et al*., 2004; Keith *et al*., 2006; Mishra *et al*., 2008).

Phytoremediation, the use of plants and their associated microbes, offers an effective, low cost and sustainable means to achieve the desired results (Hannink *et al*., 2001). It is a general term

for several ways in which plants are used to remediate sites by removing pollutants from soil and water. Plants can degrade organic pollutants or contain and stabilize metal contaminants by acting as filters or traps. Some of the methods that are being tested are described: **Phytoaccumulation/extraction:** Phytoextraction, also called phytoaccumulation, refers to the plant uptake metal contaminants through the roots and moves them into the upper portion of the plant (stems and the leaves) (Salt *et al*., 1995; Saraswat and Rai, 2009; Maine *et al*., 2004; Ampiah-Bonney *et al*., 2007). **Phytodegradation by plants:** Phytodegradation, also called phytotransformation, is the breakdown of contaminants taken up by plants through metabolic processes within the plant. **Rhizosphere biodegradation:** The breakdown of contaminants in the soil through microbial activity and is enhanced by the presence of the rhizosphere are called enhanced rhizosphere biodegradation, phytostimulation, or plant-assisted bioremediation/degradation. **Rhizofiltration:** Rhizofiltration is the adsorption or precipitation onto plant roots or absorption of contaminants into the roots which are in solution surrounding the root zone (Walton and Anderson, 1990). **Phytostabilization:** Plants prevent contaminants from migrating by reducing runoff, surface erosion, and ground water flow rates (Dary *et al*., 2010). **Phytovolatization:** Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant (Di Lonardo *et al*., 2011). The main advantages of this technique are environmentally friendly, cost-effective, aesthetically pleasing. In view of the above facts, it is considered worthwhile to use a plant species which are very commonly available and have potential to effectively remove the desired anions from water environments.

Eichhornia crassipes is common macrophyte which is abundant in wetlands, lakes and ditches. As it has a high growth-rate, fibrous root system and broad leaves along with tendency to tolerate high metal concentration, it is considered as an important species to be used in phytoremediation technique (Alvarado *et al*., 2008; Misbahuddin and Fariduddin, 2002; Snyder, 2006; Low and Lee, 1990). Arsenic (III) and chromium (VI) absorption from aqueous solution using *Eichhornia crassipes* attempt has been made in this work to develop the phytoremediation potential of this plant. These two species extracted from *Eichhornia crassipes* shoot biomass using different extracting solution by complete microwave assisted extraction method. These hazardous ions absorption and extraction using this floating water hyacinth has not been work elsewhere. Hence the present work was undertaken by using the *Eichhornia crassipes.*

Bioremediation is another technique which involves the use of microorganisms for the degradation of hazardous ions and chemicals from soil, sediments, water, or other contaminated materials. Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass (*Harms et al., 2011; Silar et al., 2011*). Alternatively, the contaminants may be enzymatically transformed to metabolites that are less toxic or innocuous (Iwamoto and Nasu, 2001). There are at least five critical factors that should be considered when evaluating the use of bioremediation for site cleanup. These factors are: (a) Magnitude, toxicity, and mobility of contaminants, (b) Proximity of human and environmental receptors, (c) Degradability of contaminants, (d) Planned site use and (e) Ability to monitor properly. Different bioremediation techniques are employed depending on the degree of saturation and aeration of an area (Tarangini *et al*., 2009; Qaiser *et al*., 2009). **In situ bioremediation:** In this remediation involves treating the contaminated material at the site. The most important in situ bioremediation treatments are bioventing, In situ biodegradation, biosparging and bioaugmentation. **Ex situ bioremediation:** In this remediation involves the removal of the contaminated material to be treated elsewhere. The most important ex situ bioremediation treatments are land farming, composting, biopiles and bioreactors.

Biosorption of metal /metalloids ions are examples of the wide variety of potential and actual applications of bioremediation technique in waste water treatment (Mungasavalli *et al*., 2007; Hansen *et al*., 2006). Biosorption is proven to be quite effective for the removal of heavy metal/metalloids ions from contaminated effluent in a low cost and environment friendly manner. The biosorption is a passive process which utilizes cell wall of biomass to sequester the metal ions from aqueous solutions. Mechanisms of cell surface sorption are independent of cell metabolism which is based on physico-chemical interactions between metal and functional groups of the cell wall. The cell wall of microorganism mainly consists of polysaccharides, lipids and proteins that serve binding sites for metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination. *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Citrobacteia*, *Klebsilla*, and *Rhodococcus* are organisms that are commonly used in bioremediation mechanisms. The main advantages of this technique are low cost and high efficiency, and regeneration of biosorbents and possibility of metal recovery.

Bacillus cereus (MTCC NO: 1305) of microbial type culture and collection is obtained from the Institute of Microbial Technology, Chandigarh, India to undertake the study. The identified species of *B. cereus* is a gram-positive, rod shaped bacterium. Many active researches are going on throughout the globe to find a standard method including the bioremediation technique by different living microorganism. The biosorption of the two hazardous ions using the above mention microorganism has not been worked elsewhere. Hence it was considered the best choice to use this microorganism for biosorption of these two anions. In view of the above facts, an attempt has been made in this work for biosorption of arsenic (III) and chromium (VI) from aqueous solution using living cells of *Bacillus cereus*. The novelty of this work is to use a new microorganism for the removal of two hazardous ions and the results are compared with a model to find the validity of the experimental results.

Artificial Neural Network (ANN) is categorized as an artificial intelligence modelling technique due to their ability to recognize patterns and relationships in historical data and subsequently make inferences concerning new data. ANNs can be used for two broad categories of problems: data classification and parameter prediction (Aleboyeh *et al*., 2008). (i) Data classification problems, the ANN uses a specified algorithm to analyze data cases or patterns for similarities and then separates them into a pre-defined number of classes. (ii) Parameter prediction problems, the ANN learns to predict accurately the value of an output parameter when information is given with sufficient input parameter (Yetilmezsoy and Demirel, 2008; Schalkoff, 1997). The main applications of the ANN technique in the water treatment industry are in the development of process models and model-based process-control and automation tools (Shetty and Chellam, 2003). Estimation of sorption efficiency using mathematical and analytical tools is involved because the physical phenomenon for removal of arsenic (III) and chromium (VI) by living cells of *Bacillus cereus* is complex one. Therefore, artificial neural network (ANN) has been attempted in this work for prediction purpose because ANN has the capacity to map inputs and outputs efficiently in complex situations.

Removal of metal ions using activated carbons is one of the examples of the wide variety of potential and actual application of adsorption technique in waste water treatment (Kimbrough *et al*., 1999). Adsorption is a conventional but efficient technology for the removal of toxic pollutants from wastewaters. So, there is a need to develop low cost and easily available activated carbon adsorbents for the removal of heavy metal ions from the aqueous environment.

The main advantages of this technique are the reusability of material, low operating cost, improved selectivity for specific metals of interest, removal of heavy metals from effluent irrespective of toxicity, short operation time. Activated carbon prepared from *Eichhornia crassipes* root biomass, and its use for the removal of chromium (VI) ions from water has not been reported. In view of the above facts it is worth to prepare the activated carbon from the root and subsequently use the material to remove the chromium (VI) from water.

In view of above facts, an attempt has been made in this work to evaluate the phytoremediation potential of the aquatic plant *Eichhornia crassipes* for arsenic (III) and chromium (VI) from aqueous solution. Similarly bioremediation technique has been used to remove the arsenic (III) and chromium (VI) from aqueous solution using living cells of *Bacillus cereus* biomass. To ascertain the mechanism of the process, the plant biomass was characterized by HPLC-ICP-MS, SEM, EDX, FTIR and XRD techniques. The biosorption of arsenic (III) and chromium (VI) from aqueous solution by living cells of *Bacillus cereus* biomass. *Bacillus cereus* biomass is characterized, using SEM-EDX, AFM and FTIR. To ascertain the validity of the experimental results, the data of both the biosorption process for both the hazardous ions collected from laboratory scale experimental set up will be used to train a back propagation (BP) learning algorithm having 4-7-1 architecture. The model uses tangent sigmoid transfer function at input to hidden layer whereas a linear transfer function is used at output layer. Many researchers throughout the globe are using modeling to validate the experimental data. The species used in this work for both the technique have not been used elsewhere hence It is assumed as a small step to approach to solve a big problem.

Chapter-2

2. Aims and Objectives

Water is one of the most important resources of nature which cover almost three-quarters of the planet. About 97 % of world's water resources are locked-up in the oceans and seas which are too saline to be used directly. Almost 2.4 % is trapped in giant glaciers and polar ice-caps. Thus not even 1 % of the total world's water resources are available for exploitation by man for domestic, agricultural and industrial purposes. The water is called elixir of life because of its multiple uses. Indiscriminate use and misuse of water is making it unfit for human consumption. A survey of literature reveals that toxic pollutants are removed successfully by different green floating plants and microorganisms from water using phytoremediation and bioremediation techniques respectively. Hence an attempt has been made in the present study to remove two priority pollutants under the topic "Removal of arsenic (III) and chromium (VI) from the water using phytoremediation and bioremediation techniques". To achieve the desired result, the present work was started with following objectives:

1. To evaluate the phytoaccumulation potential from different concentration of arsenic (III) and chromium (VI) ions (mg kg^{-1}) by the plant *Eichhornia crassipes* from aqueous solution. This objective has the following sub headings.
    - To study the relative growth, bio-concentration factor and toxicity of treated plants along with the temperature, TDS, DO of water.
    - To study the efficiency of arsenic and chromium extraction processes by complete microwave wet digestion and analysis by HPLC-ICP-MS.
    - To study the mechanism of the process.

2. To study the biosorption feasibility of arsenic (III) and chromium (VI) using living cells of the *Bacillus cereus* biomass, by varying the various process parameters like initial concentration, pH, biosorbent dose, contact time and temperature by batch mode.
    - To evaluate the mechanism of arsenic (III) and chromium (VI) biosorption using various biosorption isotherms, kinetic and thermodynamic study.
    - To study the reusability of the biosorbent by desorption and regeneration study.
    - To characterized the *Bacillus cereus* biomass before and after sorption of arsenic (III) and chromium (VI) using SEM-EDX, AFM, FTIR etc.
3. To predict and compare the removal efficiency of arsenic (III) and chromium (VI) from aqueous solution by biosorption process using a multilayer feed forward neural network model.
4. To use the activated carbon prepared from *Eichhornia crassipes* root biomass, for removal of chromium (VI) from aqueous solution by batch mode to know the practical applicability.
    - To evaluate the mechanisms.
    - To characterize the activated carbon by standard methods.
    - To understand the column study and breakthrough analysis to know the practical applicability.

Chapter-3

3. Literature Review

The development of future generation will be the result of research planned today. The knowledge of related literatures of the past studies is very much essential for any research for the formulation of sound methodology which acts as the guiding force during the advancement of research. New areas of research can be inferred from literature. The review of literature related to the present research is organized and presented as follows.

3.1. Heavy metals/ metalloids

The term 'heavy metal/metalloid' is the used to cover a diverse range of elements which constitute an important class of pollutants. These metals/metalloids are major pollutants in ground water, industrial effluent, marine water, and even treated waste water (Hogan, 2012). The important toxic metals/metalloids (i. e. Cd, Hg, As, Cr, Zn, and Pb) find its way to the water bodies through waste water (Agarwal, 2009; Merrill *et al*., 2007). Heavy metals/metalloids enter into the environment mainly via three routs: (i) deposition of atmospheric particulates, (ii) disposal of metal and metalloid enriched sewage sledges and sewage effluents, and (iii) by-product from metal mining processes and other processing industries. The other sources of metals and metalloids pollution are by irrigation of agricultural fields and uses of pesticides and fertilizers (Hani and Pazira, 2011). Due to the non-biodegradability and persistence nature, these metals and metalloids can enter in to the food chain, and thus may pose significant danger to human health other organism and plants.

3.1.1. Arsenic

Arsenic (atomic number 33) is ubiquitous and ranks 20th in natural abundance, comprising about 0.00005% of the earth's crust, 14th in the seawater, and 12th in the human body. It's concentration in most rocks ranges from 0.5 to 2.5 mg/kg, though higher concentrations are found in finer grained argillaceous sediments and phosphorites (Mandal and Suzuki, 2002). It is a silver-grey brittle crystalline solid with atomic weight 74.92 g mol $^{-1}$; specific gravity 5.73, melting point 817 ◦C (at 28 atm), boiling point 613 ◦C and vapor pressure 1mm Hg at 372 °C. Since its isolation in 1250 A.D. by Albertus Magnus (Mandal and Suzuki, 2002), this element has been a continuous center of controversy.

Arsenic exists in the −3, 0, +3 and +5 oxidation states (Smedley *et al*., 2002). The various forms of arsenic in the environment include arsenious acids (H_3AsO_3), arsenic acids (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$), arsenites, arsenates, methylarsenic acid, dimethylarsinic acid, arsine, etc. Arsenic (III) exist a hard acid and preferentially complexes with oxides and nitrogen. Conversely, arsenic (V) behaves like a soft acid, forming complexes with sulfides. Arsenic is uniquely sensitive to mobilization (pH 6.5-8.5) under both oxidizing and reducing conditions among heavy metalloids (Smedley and Kinniburgh, 2005). Two forms are common in natural waters: arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}), referred to as arsenic (III) and arsenic (V). Pentavalent (+5) or arsenate species are (AsO_4^{3-}, $HAsO_4^{2-}$, $H_2AsO_4^-$) oxyanions (Ranjan *et al.*, 2009) while trivalent (+3) arsenites include $As(OH)_3$, $As(OH)_4^-$, AsO_2OH^{2-} and AsO_3^{3-} (Mohan and Pittman Jr. 2007). Pentavalent species predominate and are stable in oxygen rich aerobic environments. Trivalent arsenites predominate in moderately reducing anaerobic environments such as groundwater. Arsenic is well known highly toxic metalloids and is a cumulative poison to living being and green environment.

3.1.1.1. Sources of arsenic

Arsenic is considered as one of the most significant pollutant in many countries of world. It can be easily solubilized in groundwater depending on pH, redox conditions, temperature, and solution composition (Jackson *et al*., 2012; Matschullat, 2000). There are many possible routes of human exposure to arsenic from both natural and anthropogenic sources. Arsenic is mobilized by natural weathering reactions, biological activity, mining activity, geochemical reactions, and volcanic eruptions (Dogan and Dogan, 2007). Soil erosion and leaching contribute to 612×10^8 and 2380×10^8 g/year of arsenic, respectively, in dissolved and suspended forms in the oceans

(Mackenzie *et al*., 1979). Most environmental arsenic problems are the result of mobilization under natural conditions. Arsenic from weathered rocks and soils dissolves in groundwater. Arsenic concentrations in groundwater are particularly high in areas with geothermal activity. Inorganic arsenic derived from rocks such as arsenic trioxide (As_2O_3), orpiment (As_2S_3), arsenopyrite (AsFeS) and realgar (As_4S_4) is most prevalent (Smedley and Kinniburgh, 2005).

Large quantities of arsenic are released by anthropogenic activity from combustion of fossil fuels, use of arsenic pesticides, herbicides, and crop desiccants and use of arsenic additives to livestock feed create additional impacts. Metallic arsenic is processed in lead or copper alloys, to increase hardness. The extremely toxic arsenic gas AsH_3 plays an important role in microchip production. Copper arsenate (Cu_3 $(AsO_4)_2.4H_2O$) is applied as a pesticide in viticulture. Paxite ($CuAs_2$) is an insecticide and fungicide. Other arsenic compounds are applied as a wood preservative, in glass processing, in chemical industries, or in semiconductor technique with gallium and indium (Garelick *et al*., 2008).

3.1.1.2. Uses of arsenic

Arsenic is highly poisonous to most life and there are only a few species of bacteria that are able to use arsenic compounds safely. The following are some of the most common uses for arsenic in the world today (Benner, 2010).

- The arsenic is used as pigment in paints. Specially as a white extender pigment in white paints where whiteness was important. It is also used to enhance green pigments.
- The main use of metallic arsenic is for strengthening the alloys of copper and lead to use in batteries and other purposes.
- Arsenic is also used in numerous pesticides, herbicides and insecticides though this practice is becoming less common as most of these products are banned.
- It is used as a wood preservative because of its toxicity to insects, bacteria and fungi.
- Arsenic is used in the medical treatment of cancers such as acute promyelocytic leukemia.
- Arsenic-74 an isotope is being used as a way to locate tumors within the body. It produces clearer pictures than that of iodine.

3.1.1.3. Toxicity of arsenic in water

The toxicity and deleterious effects of arsenic on living being as well as green environments are well documented. Arsenic occurrence in the environment, its toxicity, health hazards, and the techniques used for speciation analysis are well known and has been reviewed (Bissen and Frimmel, 2003; Jain and Ali, 2000; WHO, 1981). Long term drinking water exposure causes skin, lung, bladder, cardiovascular diseases, and kidney cancer as well as pigmentation changes, skin thickening (hyperkeratosis), neurological disorders, muscular weakness, loss of appetite, and nausea (Zhao *et al*., 2012; Wang *et al*., 2007; Guha Mazumder, 2007; Kapaj *et al*., 2006; Kitchin and Wallace, 2008). Toxicity differs between various arsenic compounds, for example, monomethyl arsenic acid and inorganic arsenide have a higher toxicity level than arsenic choline. Acute toxicity is generally higher for inorganic arsenic compounds than for organic arsenic compounds (Sharma and Sohn, 2009). Oral intake of more than 100 mg is lethal. The lethal dose of arsenic trioxide is 10-180 mg, and for arsenide this is 70-210 mg. The mechanism of toxicity is binding and blocking sulphur enzymes. Symptoms of acute arsenic poisoning are nausea, vomiting, diarrhoea, cyanosis, cardiac arrhythmia, confusion and hallucinations. Symptoms of chronic arsenic poisoning are less specific (http://www.epha.org/a/2750; Ng *et al*., 2003). These include depression, numbness, sleeping disorders and headaches. Arsenic related health effects are usually not acute, but mostly encompass cancer, mainly skin cancer (Wang *et al*., 2007).

Arsenic in natural waters is a worldwide problem. Arsenic pollution has been reported recently in the USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, New Zealand, Japan and India. The WHO provisional guideline of 10 ppb (0.01 mg/L) has been adopted as the drinking water standard. However, many countries have retained the earlier WHO guideline of 50 ppb (0.05 mg/L) as their standard or as an interim target including Bangladesh and China. In 2001, US-EPA published a new10 ppb (0.01 mg/L) standard for arsenic in drinking water, requiring public water supplies to reduce arsenic from 50 ppb (0.05 mg/L) to 10 ppb (USEPA, 2001), which are in effect from January 2006.

Arsenic toxicity is known to interfere with sulfhydryl groups in cells of most plants. Hence, plants those are not tolerant to arsenic shows toxic symptoms such as a decrease in plant growth, plasmolysis, wilting and necrosis of leaf tips, and decrease in photosynthetic capacity (Thomas *et al*., 2007; Natarajan *et al*., 2008). Arsenic in plant cells disrupts ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits

pyruvate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD^+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure probably from necrotic cell death (Vigo and Ellzey, 2006).

3.1.2. Chromium

Chromium (atomic number 24) is a steel-gray, lustrous, hard crystalline metal. It occupies the 24th position in the Periodic Table and belongs to transition group VI-B of with a ground-state electronic configuration of Ar $3d^5 4s^1$ along with molybdenum and tungsten. It comprises about 0.037 percent of the earth's crust and therefore ranks 21st in relative natural abundance (Shanker *et al*., 2005; Saha *et al*., 2011). Chromium (Cr) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Vauquelin. The atomic weight is 51.99 g.mol $^{-1}$; specific gravity 7.18 to 7.20; melting point 1857 ◦C and boiling point 2672 °C (Becquer *et al*., 2003).

The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) species; although there are various others valence states which are unstable and short lived in biological systems. Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions (Kabay *et al*., 2003; Kotas and Stasicka, 2000; Beaubien *et al*., 1994). Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer *et al*., 2003; Chen *et al*., 2011). The speciation of chromium in contaminated environments becomes critical for understanding its fate and exposure. The hydrolysis behavior of Cr(III) is complicated, and it produces mononuclear species $CrOH^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_4^-$, and $Cr(OH)_3$ and the polynuclear species $Cr_2(OH)_2$ and neutral species $Cr_3(OH)_4$ (Beaubien *et al*., 1994). The hydrolysis of chromium (VI) produces only neutral and anionic species. The predominate species, are CrO_4^{2-}, $HCrO_4^{2-}$, and $Cr_2O_7^{2-}$. At pH value less than 6.5 and at high chromium concentration; $Cr_2O_7^{2-}$ predominates, whereas CrO_4^{2-} predominates at pH value greater than 6.5 (Saçmac *et al*., 2012; Megharaj *et al*., 2003). Chromium(VI) compounds are found to be more toxic than Cr(III) compounds because of the high solubility and mobility of Cr(VI) in water.

3.1.2.1. Sources of chromium

Cr (III) and Cr (VI) are released to the environment primarily from stationary point sources resulting from human activities. Chromium is released due to environmental as well as occupational sources (Rosane Alves *et al*., 2012). Environmental sources of chromium includes airborne emissions from chemical plants and incineration facilities, cement dust, contaminated landfill, effluents from chemical plants, asbestos lining erosion, road dust from catalytic converter erosion and asbestos brakes, tobacco smoke (Ahluwalia and Goyal, 2007). Occupational sources of chromium includes anti-algae agents, antifreeze, cement, chrome alloy production, chrome electroplating (soluble Cr[VI]), copier servicing, glassmaking, leather tanning (soluble Cr[III]), paints/pigments (insoluble Cr[VI]), photoengraving, porcelain and ceramics manufacturing, production of high-fidelity magnetic audio tapes, textile manufacturing, welding of alloys or steel, and wood preservatives (Kotas and Stasicka, 2000).

The estimates of atmospheric chromium emissions in 1976 and 1980 in the Los Angeles, CA and Houston, TX areas indicate that emissions from stationary fuel combustion are about 46-47% of the total emission, and emissions from the metal industry range from 26 to 45% of the total (ATSDR, 2000).Coal and oil combustion contribute an estimated 1,723 metric tons of chromium per year in atmospheric emissions; however, only 0.2% of this chromium is Cr (VI). In contrast, chrome-plating sources are estimated to contribute 700 metric tons of chromium per year to atmospheric pollution, 100% of which is believed to be Cr (VI) (ATSDR, 2000). Cr (III) in the air does not undergo any reaction. Cr (VI) in the air eventually reacts with dust particles or other pollutants to form Cr (III). However, the exact nature of such atmospheric reactions has not been studied extensively (EPA, 1990).

3.1.2.2. Uses of chromium

Chromium has a wide range of uses in metals, chemicals, and refractories industries. The followings are some of the more common uses of chromium in the world today (Wugan and Tao, 2012; Sardohan *et al*., 2010).

- Magnetic tape (used in audio cassettes and high-class audio tapes) is made from a magnetic compound of chromium.
- Wood preservative by using salts of chromium (VI).

- Different compounds of chromium are used to make different colored pigments and dyes.
- Stainless steel, used in many applications, an alloy of iron with chromium.
- Alloys of iron, nickel and chromium are very strong and handle very high temperatures which are used in jet engines and gas turbines.
- Chromium is fairly hard and is resistant to corrosion. Therefore, many things are coated with chromium.

3.1.2.3. Toxicity of chromium in water

Chromium is unique among regulated toxic elements in the environment is due to different species of chromium. Specifically chromium (III) and chromium (VI) are regulated in different ways based on their differing toxicities. According to the United States Environmental Protection Agency (USEPA) the maximum permissible limit for Cr (VI) for discharge into inland surface waters is 0.1 mg L^{-1} and in potable water is 0.05 mg L^{-1} (EPA, 1990). Other problems related to excessive amounts of chromium in the body include liver problems (elevated hepatic enzymes), thrombocytopenia (low blood platelets), renal failure (kidney failure), rhabdomyolysis (breakdown of muscle fibers that can lead to kidney damage), hemolysis (breakdown of red blood cells), changes in thought processes, chest pain, gastrointestinal disorders, erythema/flushing/rash, headache, dizziness, and agitation (Megharaj *et al*., 2003; ATSDR, 2000).

Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr concentration (3.8×10^{-4} µM), Cr is toxic to higher plants at 100 µM. kg^{-1} dry weight. The impact of Cr contamination in the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system. Cr toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis (Becquer *et al*., 2003; Shanker *et al*., 2003; Mei *et al*., 2002).

3.2. Conventional methods for treatment of water

A variety of conventional treatment technologies, based on the principle of precipitation, ion exchange, electrolysis, solvent extraction, reverse osmosis, membrane and biosorption process

have been proposed and is tested for removal efficiency of different pollutants from potable water as well as industrial effluent (Baciocchi *et al*., 2005; McNeill and Edwards, 1997; Balasubramanian *et al*., 2009; Kumari *et al*., 2006; Tiravanti, *et al*., 1997; Moussavi and Barikbin, 2010; Seaman *et al*., 1999). Each technique provides a different and unique approach and perhaps provides certain advantages over others for a particular situation. But these process are not very popular because one or more disadvantage. However, when large volumes of water containing toxic elements are to be treated, it would, be of great advantage if the method would provide reliable results without involving much cost and working efforts.

3.2.1. Precipitation

Chemical precipitation of heavy metals and metalloids as their hydroxides using lime or sodium hydroxide is widely used in this process. Lime is generally used for precipitation purpose due to its low cost and easy control pH in the range of 8-10. The efficiency of the process depends on a number of factors, which include the ease of hydrolysis of metal and metalloids ions, nature of the oxidation state, pH, and presence of complex forming ions, standing time and filtering characteristics of the precipitate. This method has been used for the removal of metals and metalloids such as iron, copper, chromium, arsenic, cadmium and zinc from the effluents of the industries (Leupin and Hug, 2005; Dutta *et al*., 2005; Zaw and Emett, 2002; Ramkrishnaiah and Prathima, 2012; Park *et al*., 2010). Carbonate precipitation used to precipitate metal and metalloids ions using calcium or sodium carbonate is very limited. Patterson *et al*. (1977) reported improved results for carbonate precipitation of cadmium and lead from electroplating effluents.

3.2.2. Chemical reduction

Certain ions such as arsenite, chromate and dichromate have least tendency for precipitation and cannot be removed efficiently by any other removal technology. On the other hand, adsorption is a feasible process but there isn't many adsorbent available for removal of arsenite and dichromate ions since arsenite and dichromate are very selective towards bio-adsorption. To overcome these difficulties, researchers are working on chemical reduction methods. In recent years chemical methods of arsenite and chromate reduction using zero-valent metal like iron

(Fe ^{0}), aluminium (Al ^{0}) and magnesium (Mg ^{0}) have been studied intensively. In reducing waters, arsenite is found in some form of arsenious acid which ionizes according to the equations (Kartinen and Martin, 1995):

$H_3AsO_3 \rightarrow H^+ + H_2AsO_3^-$ pKa = 9.22 (3.1a)

$H_2AsO_3^- \rightarrow H^+ + HAsO_3^{2-}$ pKa = 12.3 (3.1b)

In oxygenated waters, generally surface waters, arsenate should strongly dominate over arsenite at least on the basis of thermodynamic (Cullen and Reimer, 1989). Pentavalent arsenic is normally found in water as arsenic acid which ionizes according to the equations (Kartinen and Martin, 1995):

$H_3AsO_4 \rightarrow H^+ + H_2AsO_4^-$ pKa = 2.2 (3.1c)

$H_2AsO_4^- \rightarrow H^+ + HAsO_4^{2-}$ pKa = 7.08 (3.1d)

$HAsO_4^{2-} \rightarrow H^+ + AsO_4^{3-}$ pKa = 11.5 (3.1e)

Arsenite and arsenate acid-base reactions can be assumed to occur instantaneously; however, time periods for the changes between oxidation states are uncertain in natural waters. The presence of manganese oxide, chlorine, permanganate and other oxidants can directly transform arsenite to arsenate in the absence of oxygen. The reduction of arsenate to arsenite in anaerobic conditions may require bacterial mediation since the conversion is chemically slow (Edwards, 1994; Jiang, 2001).

Reduction of hexavalent chromium can be accomplished with electrochemical process. In electrochemical reduction process, consumable iron electrodes and electrical current are used to generate ferrous ions which react with hexavalent chromium to give trivalent chromium.

$3\ Fe^{2+} + CrO_4^{2-} + 4\ H_2O \rightarrow 3\ Fe^{3+} + Cr^{3+} + 8\ OH^-$

Cr^{3+} is precipitated as Cr (III) hydroxide under slightly alkaline conditions (Thomas and Theis, 1976).

$Cr^{3+} + 3OH^- \rightarrow Cr(OH)_3$

Another example of the application of reduction process is the use of sodium borohydride, which is found to be effective for the removal of arsenic, mercury, cadmium, lead, silver and gold (Kiff, 1987).

3.2.3. Cementation

Cementation is the displacement of a metal/ metalloids from solution by a metal higher in the electromotive series. It offers an attractive possibility for treating any wastewater containing reducible metallic ions. In practice a considerable spread in the electromotive force between metals is necessary to ensure adequate cementation capability. Cementation in wastewater treatment include the precipitation of silver from photo processing discharges, the precipitation of copper from printed etching solutions and the reduction of Cr(VI) in chromium plating and chrome inhibited cooling water discharges has been reported (Case, 1974).

3.2.4. Solvent extraction

Solvent extraction is recommended a suitable method for the removal of heavy metals/ metalloids from the waste waters of the chemical and electronic industries. Solvent extraction involves on organic and an aqueous phase. The aqueous solution containing the metal/ metalloid of interest is mixed intimately with the appropriate organic solvent and the metal passes into the organic phase. Liquid- liquid extraction of metals from solution on a large scale has experienced phenomenal growth in recent years due to introduction of selective complexing agents (Iberhan and Wisniewski, 2003; EI-Hefny, 2009). In addition to hydro metallurgical applications, solvent extraction has gained widespread usage for waste reprocessing and effluent treatment. Di-n-pentyl sulphoxide has been evaluated as an extractant for the removal of Cr(VI), Fe(III), Co(II) and Ni(II) from aqueous solution (Reddy and Sayi, 1977). The extraction of Cr(VI) from aqueous solutions of 0.1 M ionic strength by trioctyl methyl ammonium compound, in a mixture of kerosene and xylene indicates that the extraction efficiency of Cr(VI) in acidic aqueous solution is good. The removal of arsenic from copper electrolytes by solvent extraction with tributylphosphate has also been reported (Navarro and Alguacil, 1996).

3.2.5. Electrodeposition

Some metals/metalloids found in waste solutions can be recovered by electrodeposition technique using insoluble anodes. Issabayeva *et al*. (2006) reported the electrodeposition of lead and copper ions into palm shell activated carbon electrodes. Kongsricharoem and Polprasert (1995) have reported Cr(VI) removal from an electroplating wastewater using the electrochemical precipitation process (ECP). The ECP process was found to be feasible in treating wastewater with a high concentration of Cr (VI). Ribeiro *et al*. (2000) reported

electrolytic removal of Cu, Cr, and As from chromated copper arsenate-treated timber waste. Hansen *et al*. (2007) reported electro coagulation in wastewater containing arsenic: comparing different process designs.

3.2.6. Reverse osmosis

Reverse osmosis is pressure driven membrane process in which a feed stream under pressure is separated into a purified stream and a concentrated stream by selective permeation of water through a semi-permeable membrane (Applegate, 1984; Gooding, 1985). Reverse osmosis enjoys wide spread popularity in the treatment of numerous diverse wastewaters. Ning (2002) reported arsenic removal from aqueous solution by reverse osmosis. Grampton (1982) reported the use reverse osmosis for the recovery of plating chemicals from rinse water as well as purification of mixed wastewater to allow its reuse. In plating chemical recovery application reverse osmosis units separate the valuable metal salts from rinse solutions, yielding a concentrated metal solution, which can be recycled to the plating bath. Reverse osmosis has also been successfully demonstrated for the removal of Cr, Pb, Fe, Ni, Cu and Zn from vehicle wash-rack water (Ameri *et al*., 2008; Kumari *et al*., 2006). This process is very costly and refines regularly maintenance.

3.2.7. Electrodialysis

Electrodialysis is accomplished by placing cation and anion selective membranes alternatively across the path of an electric current (Ribeiro *et al*., 2000). When current is applied, the electrically attracted cations pass through the cation-exchanging membranes in one direction and the anions pass through the anion-exchange membranes in the other direction. The result is that salinity decreases between one pair of membranes and increases between the next pair. Water can then pass through several such membranes until the required salinity is removed (Paul *et al*., 2007; Grebenyuk and Grebenyuk, 2002). Dermentzis *et al*. (2011) have reported removal of nickel, copper, zinc and chromium from synthetic and industrial wastewater by electro coagulation.

3.2.8. Biosorption/Adsorption

The term “biosorption or adsorption” includes the uptake a gaseous or liquid components of mixtures from the external and/or internal surface of porous solids biosorbents or adsorbents. In

chemical engineering, biosorption or adsorption is called the separation process during which specific components of one phase of a fluid are transferred onto the surface of a solid biosorbent or adsorbent (Hamdi Karaoglu *et al*., 2010; Deng *et al*., 2009). The bio adsorption of various substances on solids is due to the increased free surface energy of the solids due to their extensive surface (Mungasavalli *et al*., 2007; Hansen *et al*., 2006; Brum *et al*., 2010). According to the second law of thermodynamics, this free energy has to be reduced. This is achieved by reducing the surface tension via the capture of extrinsic substances. The term "sorption" is used to describe every type of capture of substances from the external surface of solids, liquids, or mesomorphs as well as from the internal surface of porous solids or liquids. Most of the heavy metals are efficiently removed by adsorption method (Connell *et al*., 2008).

Adsorption is a conventional but efficient technology for the removal of toxic pollutants from wastewaters. So, there is a need to develop low cost and easily available activated carbon adsorbents for the removal of heavy metal ions from the aqueous environment. The main advantages of this technique are the reusability of material, low operating cost, improved selectivity for specific metals of interest, removal of heavy metals from effluent irrespective of toxicity, short operation time. Removal of chromium (VI) ions from water was reported to be efficient by activated carbon prepared from saw dust, rice husk, raw rice bran, ethylenediamine-modified rice hull, coconut husk fibers, hazelnut shell, modified saw dust, maple saw dust, sugarcane bagasse, agricultural waste, cow dung, activated sludge, cow dung, fly ash, coconut shell charcoal, coniferous leaves, pine needles etc (Karthikeyan *et al*., 2005; Guo *et al*., 2003;Oliverira *et al*., 2005;Tang *et al*., 2003; Huang and Wu, 1977; Kobya, 2004; Sharma and Forster, 1994; Yu *et al*., 2003; Wartelle and Marshall, 2005; Mohan *et al*., 2005; Selvaraj *et al*., 2003; Das *et al*., 2000; Di Natale *et al*., 2007; Babel and Kurniawan, 2004; Ayoama *et al*., 1999; Dakiky *et al*., 2002). *Eichhornia crassipes* (family Pontederiaceae) commonly known as water hyacinth, is an aquatic weed found abundantly. The disposal of this weed is a major problem all over the world because of its vigorous growth in water bodies. Activated carbon prepared from *Eichhornia crassipes* root biomass, and its use for the removal of chromium (VI) ions from water has not been reported. In view of the above facts it is worth to prepare the activated carbon from the root and subsequently use the material to remove the chromium (VI) from water. Depending on the type of bonding involved, sorption can be classified as follows.

3.2.8.1. Physical sorption

In physical sorption (or physisorption), no exchange of electron is observed; rather, intermolecular attraction between favorable energy sites take place and are therefore independent of the electronic properties of the molecules involved. The biosorbate is held to the surface by relatively weak van der Waals forces and multiple layers may be formed with approximately the same heat of biosorption. The heat of biosorption for physisorption is at most a few kcal/mole and therefore this type of adsorption is stable only at temperature below 50 °C.

3.2.8.2. Chemical sorption

Chemical sorption (or chemisorptions) involves an exchange of electron between specific surface sites and solute molecules, and as a result, a chemical bond is formed. Chemisorption is characterized by interaction energies between the surface and adsorbate comparable to the strength of chemical bonds (tens of Kcal/mol), and is consequently much stronger and more at high temperatures than physisorption. Generally, only a single molecular layer can be adsorbed.

3.2.8.3. Electrostatic sorption (ion exchange)

This is a term reserved for Coulomb attractive forces between ions and charged functional groups and is commonly classified as ion exchange. Ion exchange is a process in which solid material takes up charged ions from a solution and release an equivalent amount of other ions into the solution (Sahu *et al*., 2009). The ability to exchange ions is due to the properties of the structure of the materials. The exchanger consists of a matrix, with positive or negative excess charge. This excess charge is localized in specific locations in the solid structure or in functional groups. The charge of the matrix is compensated by the counter ions, which can move within the free space of the matrix and can be replaced by other ions of equal charge sign (Helfferich, 1962). The pores sometimes contain not only counter ions but also solvent. When the exchanger is in contact with the liquid phase, the solvent can travel through the exchanger and cause "swelling" to an extent that depends on the kind of counter ions. Some electrolytes can also penetrate into the exchanger along with the solvent. As a result, there are additional counter ions, called co-ions, which have the same charge sign as fixed ions. Normally, exchanger has many open areas of variable size and shape that are altogether called "pores." Only a few inorganic exchangers contain pores of uniform cross section. So, the exchangers exhibit a three-

dimensional structure since a substance is captured by a solid in both processes, there is a characteristic difference between them: ion exchange is a stoichiometric process in contrast to sorption (Helfferich, 1962). It means that in the ion-exchange process, for contrast to other sorption, no replacement of the solute takes place. Ion exchange can be seen as a reversible reaction involving chemically equivalent quantities.

The removal of anionic or cationic constituents present in water by exchange with ions of the resin. When the resin bed becomes saturated, they are regenerated using acid or alkali. Ion-exchange resins are available selectively for certain ions. However, in the presence of large quantities of competing mono and divalent ions such as sodium and calcium, efficiency of ion-exchange process decreases. Ion removal by solids could involves more phenomena, as for example in inorganic natural materials where ion uptake is attributed to ion exchange and adsorption processes or even to internal precipitation mechanism (Inglezakis *et al.*, 2004). Inglezakis *et al.* (2005) reported the effects of competitive cations NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+} and Li^+ and co-anions Cl- and Br- on ion exchange of heavy metals Pb^{2+}, Fe^{3+}, Cr^{3+} and Cu^{2+} on clinoptilolite and found the selectivity of clinoptilolite for heavy metals to be $Pb^{2+} > Fe^{3+} > Cr^{3+} \geq Cu^{2+}$. Baciocchi *et al*. (2005) reported ion exchange equilibrium of arsenic in the presence of high sulphate and nitrate concentrations.

3.2.9. Disadvantages of conventional methods

Most of conventional methods suffer from some disadvantages, such as high capital and operational cost, high cost of reagent, limited tolerance to pH change, incomplete metal removal, require technically skilled manpower for operation, requires a large area of land, provides a low treatment efficiency and energy requirements (Sharma and Sohn, 2009).

3.3. Water treatment using phytoremediation techniques

Phytoremediation is a novel strategy that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (Sarma, 2011). The term phytoremediation is a combination of two words-phyto, which means plant, and remediation, which means to remedy (Salt *et al.*, 1995; Mohanty and Patra, 2012). The term was first coined in 1991 to describe the use of plants to accumulate metals from soil and groundwater. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established

treatment methods used at hazardous waste sites (Kramer, 2010; Raskin *et al*., 1994). Although microorganisms have also been tested for remediation potential, plants have shown the greater ability to withstand and accumulate high concentrations of toxic metals and chemicals (Ali and Zulkifli Hj, 2010). To date, phytoremediation efforts have focused on the use of plants to accelerate degradation of organic contaminants, usually in concert with root rhizosphere microorganisms, or remove hazardous heavy metals from soils or water. Seven aspects of phytoremediation are described in this chapter: phytoextraction, phytodegradation, rhizosphere degradation, rhizofiltration, phytostabilization and phytovolatization (Braud *et al*., 2009; Malik, 2007; Wang and Lewis, 1997; Zhu *et al*., 1999). However, the major focus is on phytoextraction.

3.3.1. The role of genetics

Genetic engineering are powerful methods for enhancing phtoremediation capabilities, or for introducing new capabilities into plants. Genes for phytoremediation may originate from micro-organism or may be transferred from one plant to anther variety better adapted to the environmental conditions at cleanup site (Verbruggen *et al*., 2009; Kotrba and Najmanova, 2009). Microorganisms are very diverse, they includes bacteria, fungi, green algae and protists. The mechanisms involved in bacterial metal resistance result from either the active efflux pumping of the toxic metal out of the cell, or the enzymatic detoxification (generally redox chemistry) converting a toxic ion into a less toxic or less available metal ions. Occasionally, bioaccumulation or sequestration of the metal in a physiologically in accessible form is used (Silver, 1992). The genes encoding these resistance systems are most often located on plasmids or transposons.

Detoxification of metals/metalloids by the formation of complexes is used by most eukaryotes. Metallothioneins (MTs) or phytochelatins (PCs) are low molecular weight (6 -7 kDa), cysteine-rich proteins found in animals, higher plants, eukaryotic microorganisms and some prokaryotes (Hamer, 1986).They are divided into three different classes on the basis of their cysteine content and structure. The Cys-Cys, Cys-X-Cys and Cys-X-X-Cys motifs (in which X denotes any amino acid) are characteristic and invariant for metallothioneins (Robinson *et al*., 1993). No aromatic amino acids or histidines are found in MTs. MTs found in yeast, cyanobacteria and a few higher plants are also low molecular weight proteins with high cysteine content. The biosynthesis of MTs is regulated at the transcriptional level and is induced by several factors

such as hormones, cytotoxic agents and metals/metalloids including Cd, zinc (Zn), mercury (Hg), copper (Cu), gold (Au), silver (Ag), cobalt (Co), nickel (Ni), arsenic (As) and bismuth (Bi). Non-essential metals, such as Cd, Hg, lead (Pb), Bi, Ag, Au and platinum (Pt), are sequestered by MTs (Kagi, 1991).

3.3.2. Mechanisms involved in phytoremediation

A relatively small group of hyperaccumulator plants is capable of sequestering heavy metals in their shoot tissues at high concentrations. In recent years, major scientific progress has been made in understanding the physiological mechanisms of metal uptake and transport in these plants. However, relatively little is known about the molecular bases of hyperaccumulation. The current progresses on understanding cellular/molecular mechanisms of metal tolerance/hyperaccumulation by plants are reviewed (Prasad *et al*., 2010; Prasad *et al*., 2007b). The major processes involved in hyperaccumulation of trace metals from the water to the shoots by hyperaccumulators include: (a) bioactivation of metals in the rhizosphere through root-microbe interaction; (b) enhanced uptake by metal transporters in the plasma membranes; (c) detoxification of metals by distributing to the apoplasts like binding to cell walls and chelation of metals in the cytoplasm with various ligands, such as phytochelatins, metallothioneins, metal-binding proteins; (d) sequestration of metals into the vacuole by tonoplast-located transporters. Once the rate-limiting steps for uptake, translocation, and detoxification of metals in hyperaccumulating plants are identified, more informed construction of transgenic plants would result in improved applicability of the phytoremediation technology. Possible mechanisms of plant roots and soil microbes and their interaction can improve metal bioavailability in rhizosphere through secretion of proton, organic acids, phytochelatins (PCs), amino acids, and enzymes are presented in Fig. 3.1. The prevailing mechanism is chelation through the induction of metal-binding peptides and the formation of metal complexes. The members of the third class of MTs, the PCs, are responsible for the formation of complexes with heavy metals in plants (Robinson, *et al.*, 1993; Zenk, 1996). These peptides are enzymatically derived and synthesized on exposure of the cell to toxic metals. The structure of PCs is (γ -Glu-Cys) nX, in which X is Gly, γ-Ala, Ser or Glu and n = 2-11 depending on the organism, although the most common forms have 2-4 peptides (Robinson, *et al.*, 1993). The biosynthesis of PCs is induced by many metals including Cd, Hg, As, Ag, Cu, Ni, Au, Pb and Zn; however, Cd is by far the strongest

inducer (Grill *et al.*, 1987). The metal binds to the constitutively expressed enzyme, γ-glutamylcysteinyl dipeptidyl transpeptidase (PC synthase), thereby activating it to catalyse the conversion of glutathione (GSH) to phytochelatin (Zenk, 1996) (Fig. 3.2). Glutathione, the substrate for PC synthase, is synthesized from its constituent amino acids in two steps. The first step is catalysed by γ -glutamyl-Cys synthetase (γ -ECS) and the second by glutathione synthetase (GS). γ-ECS is feedback regulated by glutathione and is dependent on the availability of cysteine. Plant MT-like genes have been isolated from several plant species including maize, soybean, rice, wheat, tobacco and *Brassica napus* (Nedkovska and Atanassov, 1998) although their role in metal detoxification has yet to be established.

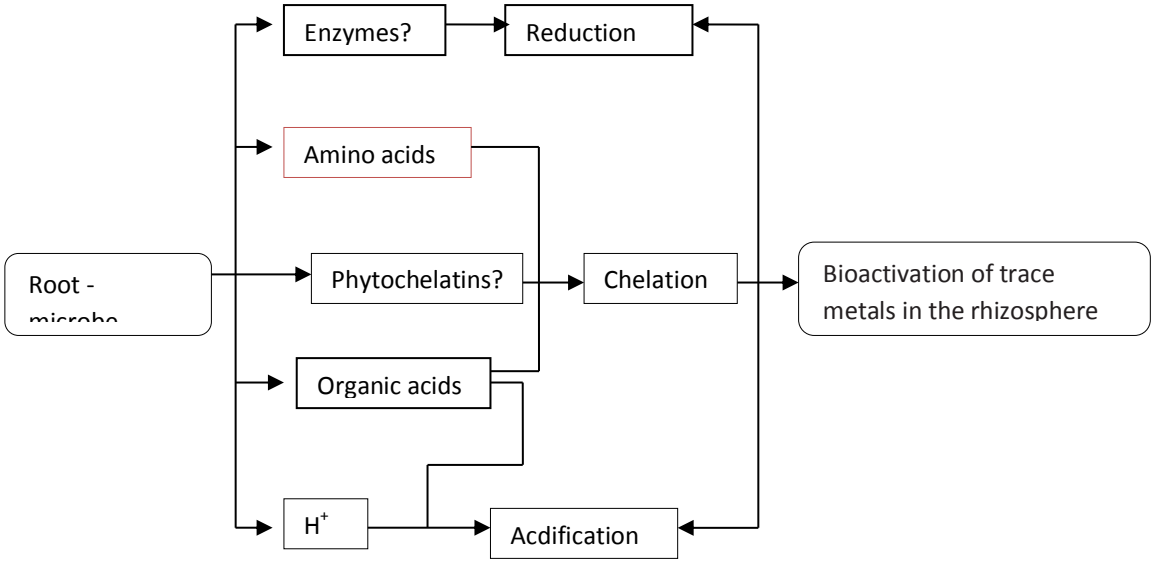


Fig.3.1. Processes possibly involved in heavy metal mobilization in the rhizosphere by root–microbe interaction.

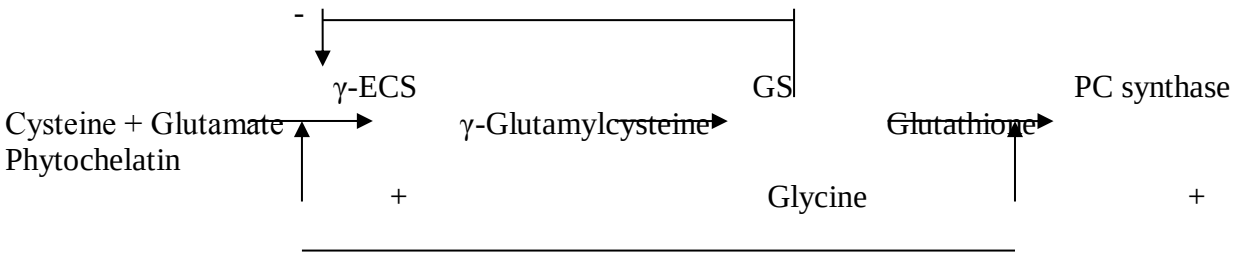


Fig. 3.2. The synthesis of phytochelatins (PCs) in plants.

Some macrophytes are found to remove different concentrations of arsenic and chromium ions, which make them suitable to act as bio-monitors for metals, and considered as biological filters of the aquatic environment (Chiu *et al*., 2005; Fayiga *et al*., 2005; Huang *et al*., 2004; Keith *et al*., 2006; Mishra *et al*., 2008). *Eichhornia crassipes* (family Pontederiaceae) is common macrophyte which is abundant in water logging area. It has a high growth-rate and fibrous root system along with tendency to tolerate high metal concentration, it is considered as an important species to be used in phytoremediation. Many results have been documented the phytoremediation ability of the free-floating *Eichhornia crassipes* for nutrient-rich waters (Alvarado *et al*., 2008; Misbahuddin and Fariduddin, 2002; Snyder, 2006; Low and Lee, 1990). In all the reported methods cited above, different plant species are being used to remove a number of cations and anions from soil and water environments with variation of variable parameters to obtain the optimum conditions. However it is not reported that the *Eichhornia crassipes* has been used ever for the removal of arsenic (III) and chromium (VI). Hence in the present study, *Eichhornia crassipes* was used for two specific purpose (a) to remove the metal ions from water (b) then metal ions is extracted from this plant biomass using specific method.

3.3.3. Advantages and limitations of phytoremediation techniques

- **Advantages:**
  - Environmentally friendly, cost-effective, and aesthetically pleasing.
  - Metals absorbed by the plants may be extracted from harvested plant biomass.
  - May reduce the entry of contaminants into the environment by preventing their leakage into the groundwater systems.
  - It is potentially the least harmful method because it uses naturally occurring organisms and preserves the environment in a more natural state.
- **Limitations**:
  - Slow growth and low biomass require a long term commitment.
  - With plant-based system of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater.
  - The survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil.

3.4. Water treatment using bioremediation techniques

Bioremediation is a technology that utilizes the metabolic potential of microorganisms to clean up contaminated environments (Tripathi *et al*., 2011). By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms (Pal and Vimala, 2011). It uses naturally occurring bacteria and fungi or detoxify substances hazardous to human health and/or the environment (Tripathi *et al*., 2011; Iwamoto and Nasu, 2001; Watanabe and Baker, 2000; Srinath *et al*., 2002). The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation, the process is known as bioaugmentation (Parameswari *et al*., 2009). For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Leavitt and Brown, 1994).

3.4.1. Mechanism involved in bioremediation

The complex structure of microorganisms implies that there are many ways for the metal/metalloid to be taken up by the microbial cell which are presented in Fig. 3.3. The bioremediation mechanisms are very complex and are not fully understood. They may be classified according to various criteria.

- ❖ According to the dependence on the cell's metabolism
    - Metabolism dependent and non -metabolism dependent
- ❖ According to the location where the metal removed from solution is found
    - Extra cellular accumulation/ precipitation
    - Cell surface sorption/ precipitation and intracellular accumulation

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of accumulation may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. In non metabolism dependent process, metal uptake

is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups.

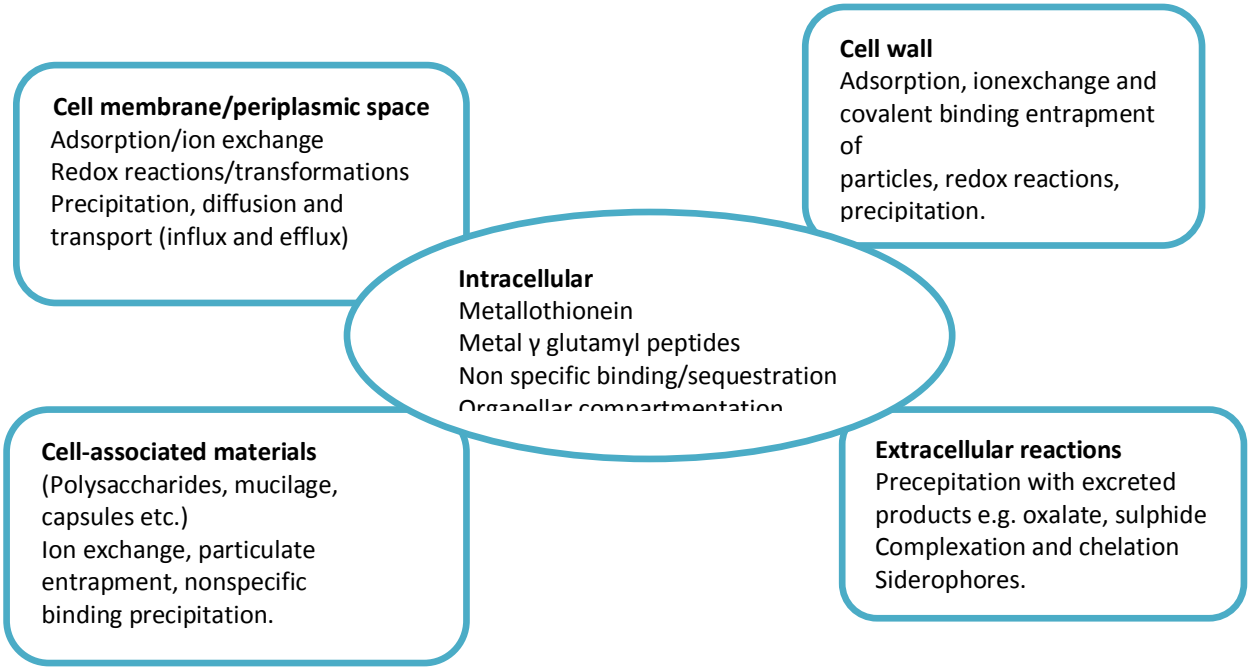


Fig. 3.3. Bioremediation mechanisms by microorganisms.

The non-metabolism dependent process is relatively rapid and can be reversible (Prasad *et al*., 2011). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Shroff and Vaidya, 2012). Further, it may be dependent on the cell's' metabolism, if in the presence of toxic metals, the microorganism produces compounds that favor the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

3.4.2. Biosorption and Bioaccumulation

Bioaccumulation is defined as the phenomenon of living cells; whereas, biosorption mechanisms are based on the use of dead biomass (Nirmal Kumar *et al*., 2009). Biosorption

possesses certain inherent advantages over bioaccumulation processes, which are presented below.

3.4.2.1. Biosorbent materials

Any biological material which exhibits its affinity and concentrates the heavy metals even in very dilute aqueous solutions is called as biosorbent material. Strong biosorbent behavior of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties. Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. The waste mycelia available from fermentation processes, olive mill solid residues (Pagnanelli, *et al*., 2002), activated sludge from sewage treatment, biosolids (Norton *et al*., 2003), aquatic macrophytes (Keskinkan *et aI*., 2003), etc. has been used successfully. Norton *et aI*. 2003 used dewatered waste activated sludge from a sewage treatment plant for the biosorption of zinc from aqueous solutions. The adsorption capacity was determined to be 0.564 mM/g of biosolids. Keskinkan *et al*., 2003 studied the adsorption characteristics of copper, zinc and lead on submerged aquatic plant. *Myriophyllum spicatum*. Pagnanelli, *et al*. 2000 have carried out a preliminary study on the use of olive mill residues as heavy metal sorbent material. The results revealed that copper was maximally adsorbed in the range of 5.0 to 13.5 mg/g under different operating conditions.

3.4.2.2. Bacterial biosorption

Early in 1980 it was observed that the capability of some microorganisms to accumulate metallic elements. Numerous research reports have been published from toxicological points of view, but these were concerned with the accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cell and the consequences of accumulation on the food chain (Tripathi and Garg, 2010; Basha *et al*., 2006). However, further research has revealed that inactive/dead microbial biomass can passively bind metal ions through various physicochemical mechanisms. Researchers have understood and explained that biosorption depends not only on the type or chemical composition of the biomass, but also on the

external physicochemical factors and solution chemistry. Many investigators have been able to explain the mechanisms responsible for biosorption, which may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and micro precipitation (Veglio and Beolchini, 1997; Volesky and Schiewer, 1999).

3.4.3. Mechanism of bacterial biosorption

The bacterial cell wall is the first component that comes into contact with metal ions where the solutes can be deposited on the surface or within the cell wall structure (Ray *et al*., 2005; Pan *et al*., 2006). Since the mode of solute uptake by dead/inactive cells is extracellular, the chemical functional groups of the cell wall play vital roles in biosorption. Due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphonate, amine and hydroxyl groups (van derWaal *et al*., 1997). As they are negatively charged and abundantly available, carboxyl groups actively participate in the binding of metal cations. Several dye molecules, which exist as dye cations in solutions, are also attracted towards carboxyl and other negatively charged groups. Golab and Breitenbach (1995) reported that the carboxyl groups of the cell wall peptidoglycan of *Streptomyces pilosus* were responsible for the binding of copper. Also, amine groups are very effective for removing metal ions, as it is not only chelates cationic metal ions, but also adsorbs anionic metal species or dyes via electrostatic interaction or hydrogen bonding. Kang *et al.* (2007) observed that amine groups protonated at pH 3 and attracted negatively charged chromate ions via electrostatic interaction. In general, increasing the pH increases the overall negative charge on the surface of cells until all the relevant functional groups are deprotonated, which favors the electrochemical attraction and adsorption of cations. Anions would be expected to interact more strongly with cells with increasing concentration of positive charges, due to the protonation of functional groups at lower pH values. The solution chemistry affects not only the bacterial surface chemistry, but the metal/dye speciation as well. Metal ions in solution undergo hydrolysis as the pH increases. The extent of which differs at different pH values and with each metal, but the usual sequence of hydrolysis is the formation of hydroxylated monomeric species, followed by the formation of polymeric species, and then the formation of crystalline oxide precipitates after aging. The different chemical species of a metal occurring with pH changes will have variable charges and adsorb ability at solid–liquid interfaces. In many instances, biosorption experiments conducted at

high alkaline pH values have been reported to complicate evaluation of the biosorbent potential as a result of metal precipitation (Selatnia *et al*., 2004b).

Different microorganisms are found to remove different concentrations of arsenic and chromium ions from aquatic environments ((Pokhrel Viraraghavan, 2006; Kumari *et al*., 2006; Sari and Tuzen, 2009). In all the reported methods cited above, different microorganisms are being used to remove a number of cations and anions from waste water with variation of variable parameters to obtain the optimum conditions. However it is not reported that the *Bacillus cereus* has been used ever for the removal of arsenic (III) and chromium (VI). Hence in the present study, *Bacillus cereus was* used for two specific purposes (a) to remove the metal ions from water using bath mode method (b) the reusability of the biosorbent by desorption and regeneration study.

In the context of present situation, both the technique phytoremediation and bioremediation are potential methods to be used as an environmental friendly technique to combat the menace of pollution in the water and soil environments. Many successful case studies are reported for the removal of hazardous chemicals and toxic ions. In the last decade significance attention has been drawn by the researchers and R&D laboratories of many prestigious and reputed organizations. Keeping all the above in minds the present research work was undertaken to remove the arsenic (III) and chromium (VI) from water. After successful removal of both these ions by the plant species *Eichhornia crassipes*, it was thought to use the activated carbon prepared from the use as an adsorbent to remove these ions. The results of the work were published in a peer reviewed journal which tempted us to put the research finding in the thesis.

3.4.4. Advantages and limitations of bioremediation techniques

- **Advantages of bioremediation**
  - Low cost and high efficiency
  - Minimization of chemical and biological sludge
  - Regeneration of biosorbents and possibility of metal recovery
- **Limitations of bioremediation**
  - It is difficult to extrapolate from pilot-scale studies to full-scale field operations.
  - Bioremediation often takes longer than other treatment.
  - Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation.

3.5. Artificial neural network modeling for environmental chemistry

The ANN modelling technique holds several advantages over mechanistic modelling that make it particularly suitable to process modelling in the drinking water treatment industry (Despagne and Massart, 1998). ANN models can handle non-linear relationships, and can provide predictions of output parameters in real time in response to simultaneous and independent fluctuations of the values of model input parameters. Data patterns where the value of one or more of the model inputs are missing can be incorporated into model building if necessary, although model prediction will improve if only complete data patterns are used. Similarly, when completed models are applied to new data patterns where values are missing, the value of model outputs can still be predicted. Yetilmezsoy and Demirel, (2008) observed that artificial neural network (ANN) can be applied for modeling of Pb(II) adsorption from aqueous solution by *Antep pistachio* (*Pistacia vera* L.) shells. Salari *et al*. (2005) observed that application of artificial neural networks for modeling of the treatment of wastewater contaminated with methyl tert-butyl ether (MTBE) by UV/H_2O_2 process. Strik *et al*. (2005) have used to predict of trace compounds in biogas from anaerobic digestion using the MATLAB neural network toolbox. Many user-friendly ANN software packages exist, offering the user a myriad of modelling options and allowing the user to customize the modelling process to suit his or her knowledge of modelling heuristics. Hamed *et al*. (2004) proposed the prediction of wastewater treatment plant performance using artificial neural networks. Aber *et al*. (2009) proposed the removal of Cr (VI) from polluted solutions by electrocoagulation: modeling of experimental results using artificial neural network. Several researchers have worked on wastewater treatment using artificial neural network modeling (Turan *et al*., 2011; Gob *et al*., 1999; Aksu, 2002; Patra *et al*., 1997; Peng *et al*., 1992; Park *et al*., 2004). In present study the artificial neural network approach for modeling of arsenic (III) and chromium (VI) biosorption from aqueous solution by living cells of *Bacillus cereus* biomass. The results of the work were published in a peer reviewed journal which tempted us to put the research finding in the thesis.

3.5.1 Types of ANN models

ANN models of drinking water treatment process can assume two distinct forms: (i) process models and (ii) inverse process models. (i) The process model predicts the value of one or more process outputs, if given the values of the process input parameters. An example of this type of model is the prediction of clarifier effluent turbidity using influent water parameters and

operational parameters. (ii) The inverse process model predicts the value of one or more process inputs, if given the values of the remaining process inputs and process output(s). This type of model is often used to predict the value of an operational parameter required to reach a target effluent quality (Patra *et al*., 1997; Peng *et al*., 1992).

3.5.2. Key components of ANN models

There are several key components to ANN models that are collectively referred to as the ANN architecture (Patra *et al*., 1997; Peng *et al*., 1992). Processing units or neurons perform primitive operations such as scaling data, summing weights, and amplifying or thresholding sums. Neurons are organized into layers with each layer performing a specific function. The input layer serves as an interface between the input parameter data and the ANN model. Most models also contain one or two hidden layers, although more are possible. These layers perform most of the iterative calculations within the network (Fernandes and Lona, 2005; Lek and Guegan, 1999). The output layer serves as the interface between the ANN model and the end-user, transforming model information into an ANN-predicted value of the output parameter(s). Each neuron is connected to every neuron in adjacent layers by weights; links that represent the 'strength' of connection between neurons. Each ANN model has a propagation rule that defines how the weights connected to a neuron are combined to produce a net input. The propagation rule is generally a simple summation of the weights. As discussed, the input layer serves as an interface between input parameter data and the model. In this layer, a scaling function is used to scale data from their numeric range into a range that the network deals with efficiently, typically 0 to 1. The hidden and output layers contain an activation function that defines how the net input received by a neuron is combined with its current state of activation to produce a new state of activation. The most common activation function used in process modelling is the sigmoidal activation function (Yetilmezsoy, 2006; Chu, 2003). Finally, each network has a learning rule that defines how the weights are modified in order to minimize prediction error.

3.5.3. ANN modeling requirements

The key requirement of the ANN modeling approach is the availability of relevant data to describe the process being modeled. Data must be available in a useable electronic format for each of the process input and output parameters. The data used in model development must be representative of plant operations, spanning the range of operating conditions that may be encountered during both routine operations and process upset conditions. Model development

requires the use of appropriate ANN software (Math works Incorporation, 2005). With respect to hardware requirements, recent advances in computing technology have made even the most modest new home computing system capable of performing the modeling calculations. To ensure optimal performance however, a 500 MHz processor and 128 M of RAM should be considered to be the minimum system requirements.

Chapter-4

4. Materials and methods

4.1. Experimental procedure of phytoremediation techniques

4.1.1. Preparation of standards and reagent

All the chemicals used in the studies are of analytical grades (Merck chemicals, Germany) which are used without any further purification. In all experiments, deionized water (Milli-Q Millipore 18.2 MΩ cm^{-1} conductivity) is used for the preparation, dilution and analytical purposes of solutions. A stock chromium (VI) solution of 1000 mg/L is prepared by dissolving 2.828 g of anhydrous potassium dichromate ($K_2Cr_2O_7$; MG7M571737) and by adding 1.5 mL 1M HNO_3 in a 1000 mL of deionized water. A stock arsenic (III) solution of 1000 mgL^{-1} is prepared by dissolving 1.320 g of arsenite (As_2O_3; Merck-HC622890, Chemicals, Germany) containing 4 g 1M NaOH in 1 L of deionized water (Greenberg *et al*., 2005). The stock solutions are preserved with 1% trace metal grade nitric acid for one month. Subsequently, different working solutions of required concentrations for chromium (VI) and arsenic (III) are prepared by proper dilution up to mark in a measuring flask. For the analysis of arsenic (III), 500 mL $NaBH_4$ solution is prepared by dissolving 2.5 g NaOH and 2.0 g $NaBH_4$, in deionized water and diluting up to mark. The $NaBH_4$ reagent is always prepared immediately before use. Sodium tetrahydroborate solution is dispensed into the acidic test sample solution. The reaction of sodium tetrahydroborate in acidic solution and the simultaneous reduction of the hydride forming element can be described as represented.

$$BH^-_4 + H_3O^+ + 2H_2O \rightarrow H_3BO_3 + 4H_2\uparrow$$

and

$3BH^-_4 + 3H^+ + 4H_3AsO_3 \rightarrow 4AsH_3\uparrow + 3H_2O + 3H_3BO_3$

4.1.2. Selection of plant materials

Young aquatic plants *Eichhornia crassipes* (Class-*Liliopsida*, Family- *Pontederiaceae*) were collected from ponds near civil township, Rourkela. The plants were placed in cement tanks with tap water under natural sunlight for one week to allow them to adapt the new environment. The plants having approximately weight, 250g, 10 to 11 cm. root length and 6 to 7cm. shoot length were selected for further experimentation. The modified Hoagland nutrient solutions are required for nourishment of *Eichhornia crassipes* plant during the experiment. The modified 0.25 N Hoagland nutrient solution are prepared which consists of 4.0mM $CaNO_3$, 2.0mM $MgSO_4$, 4.0mM KNO_3, 0.4mM $(NH_4)_2SO_4$, 2µM $MnSO_4$, 0.3µM $CuSO_4$, 0.8µM $ZnSO_4$, 30µM NaCl, 0.1µM Na_2Mo_4, 1.43µM KH_2PO_4, 10 µM H_3BO_3 and 20 µM Fe-Na-EDTA.

4.1.3. Experiment setup and procedure

The experiments were conducted in a series of rectangular glass container with dimension (32 x 20 x 18 cm) containing 200 mL/L of nutrient solutions at a temperature ranges of 15 ± 2^{o}C to 45 ± 2^{o}C. The rectangular glass container was kept in a growth room at desired temperature lighted with cool, fluorescent lamp (200 µmol.m^{-2} s^{-1}) under16 h photoperiod. The pH (Orion two stars, USA) of the nutrient solution was adjusted to 6.8 -7.2 for better growth of the plants using required amount of 0.1 M HCl or 0.1M NaOH. The solution was changed regularly at intervals of 03 days to maintain the desired pH. The plants were maintained in the specified container separately for the removal of As (III) and Cr (VI) supplemented with 0, 0.01, 0.025, 0.05, and 0.10 mgL^{-1} arsenic (III) solutions and 0, 5, 10, 15, and 20 mgL^{-1} chromium (VI) solutions respectively. The plants were harvested after 0, 3, 9 and 15 days. Fig. 4.1 schematically summarizes details experimental procedure of absorption of arsenic (III) and chromium (VI) from water by phytoremediation process. Deionized water was added daily to compensate the water loss through plant transpiration, sampling and evaporation. After the completion of each test duration, *Eichhornia crassipes* were separated into shoots and roots for the analysis of relative growth, arsenic (III) and chromium (VI) accumulation, toxicity and bio-concentration factor. The total dissolved solid (TDS) and dissolved oxygen (DO) of the treated water are also

measured to know the effect of accumulation on the plant. In addition, the residual arsenic (III) and chromium (VI) concentration in the test solution was measured to assess the removal potential of *Eichhornia crassipes*.

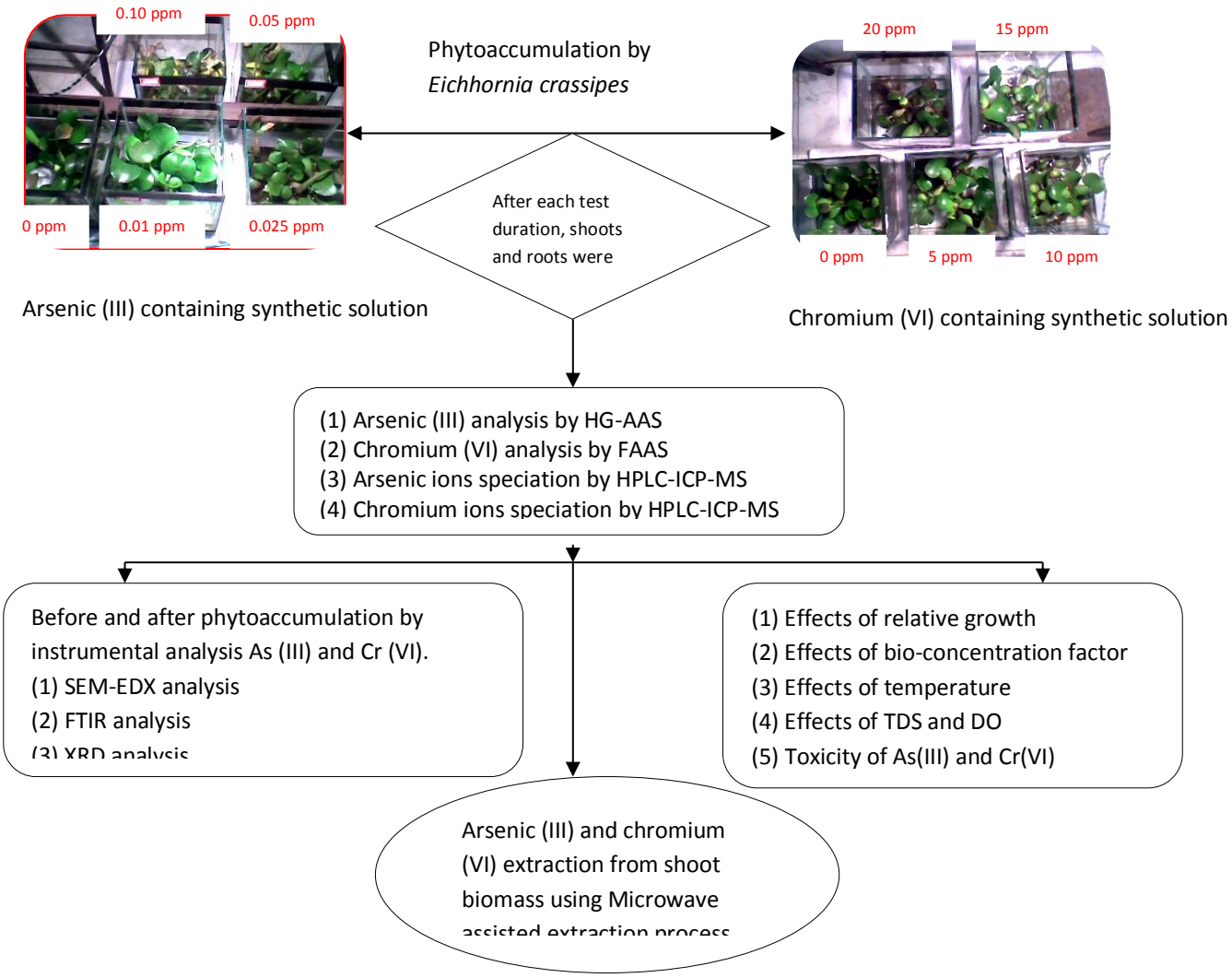


Fig. 4.1. Schematic diagram for details experimental procedure of phytoremediation process for the absorption of arsenic (III) and chromium (VI) from water by *Eichhornia crassipes*.

4.1.4. Relative growth

The relative growth of plant species is a major factor for contributing to invasion. Relative growth of control and treated plants was calculated as represented below (Lu *et al*., 2004; James

and Drenovsky, 2007). The results are expressed as increase of biomass per unit mass per day (g. $g^{-1}.d^{-1}$).

$$\text{Relative growth} = \frac{\text{Final fresh weight of treated plants}}{\text{Initial fresh weight of treated plant}}$$

4.1.5. Bio-concentration factor (BCF)

The bio-concentration factor (BCF) provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. The result of bio-concentration factor was calculated as follows (Lu *et al*., 2004).

$$\text{BCF} = \frac{\text{Concentration of metal ions in plant tissue } (\frac{\text{mg}}{\text{Kg}})}{\text{Initial concentration of metal ions in external solution } (\frac{\text{mg}}{\text{L}})}$$

4.1.6. Total dissolved solids and dissolved oxygen (TDS and DO)

100 mL of water sample was taken and was filtered it by using Whatmann 42 filter paper. The filtrate was placed in a clean, dry and weighted crucible which was evaporated to dryness in an oven at 98°C and subsequently dried for one hour at 103-105 °C. The crucible with residue was cooled in desiccators and weighted till a constant weight.

$$\text{TDS (mg/L)} = \frac{\text{Weight of residue of filtrate (mg)}}{\text{Volume of sample (mL)}} \text{ x } 1000$$

$$= \frac{W_2 - W_1 \text{ X } 10^3}{V} \text{ x } 1000$$

Weight of crucible = W_1 g, Weight of residue + crucible = W_2 g, Weight of residue = (W_2-W_1) g

Dissolved oxygen is required for living organism to maintain their biological processes. DO is also important in precipitation and dissolution of inorganic substances water. Dissolved oxygen is determined in water sample by Winkler's method.

Total dissolved solids (TDS) are the measure of the combined content of all inorganic and organic substances contained in a liquid in: molecular, ionized or micro-granular suspended form. The two principal methods of measuring total dissolved solids are available: gravimetry and conductivity. Gravimetric methods are the most accurate which involve the weighing of

mass of residues left after evaporating the liquid solvent. Dissolved oxygen (DO) analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement), and as a waste product of photosynthesis. In the present study, the results of the effects of treatment by *Eichhornia crassipes* on total dissolved solids (TDS) and dissolved oxygen (DO) without and with nutrient solution of control with arsenic (III) and chromium (VI) containing water tank at different days results are presented in Table 4.1.

Table 4.1. Results of TDS and DO without and with nutrient solution of control, arsenic(III) and chromium(VI) containing water tank at different days treatment.

	3 days		9 days		15 days	
	TDS (ppm)	DO (ppm)	TDS (ppm)	DO (ppm)	TDS (ppm)	DO (ppm)
Without metal/metalloid ion and nutrient solution container	74	4.52	75	4.54	77	4.57
Control(nutrient solution)	762.43	5.78	750.23	5.73	755.11	5.75
With nutrient solution container						
Arsenic(III)	769.00	6.23	771.12	6.75	785.45	6.84
Chromium(VI)	755.32	6.42	762.42	6.64	781.23	6.74

TDS may be consists of $CaNO_3$, $MgSO_4$, KNO_3, $(NH_4)_2SO_4$, $MnSO_4$, $CuSO_4$, $ZnSO_4$, NaCl, Na_2Mo_4, KH_2PO_4, H_3BO_3 and Fe-Na-EDTA. There is an increase in the growth of *Eichhornia crassipes* with the increase in TDS value containing As (III) and Cr (VI) ions. It may be due to the presence of suitable cations and anions that have influencing effect on the nutrient uptake. The comparison of dissolved oxygen without and with As (III) and Cr (VI) reveals that uptake of metal/metalloids ions increases with increasing dissolved oxygen. It may be due to the enhancement in the binding capacity to the site of metals/metalloids by molecular oxygen. Goto *et al*. (1996) were reported that *Lettuce* growth experiments were carried out to study the effect of dissolved oxygen (DO) concentration on plant growth in a floating hydroponic system and reported similar results.

4.1.7. Temperature

Temperature influences most plant processes, including photosynthesis, transpiration, and respiration. Depending on the situation and the specific plant species, the effect of temperature can either speed up or slow down this transition. In the present study, the effect of temperature on the absorption of arsenic (III) and chromium (VI) ions onto *Eichhornia crassipes* with initial arsenic (III) concentration of 0.010, 0.025, 0.05 and 0.10 mgL^{-1}, while initial chromium (VI) concentration 0.75, 1.50, 2.50 and 4 mg/L and 15 days exposure times and the results are presented in Table 4.2. It can be clearly seen from the results from Table 4.2 that the removal of arsenic (III) and chromium (VI) decreases slowly with increase in temperature (25 ^{o}C to 45 ^{o}C).

Table 4.2. The effects of temperature of *E. crassipes* at different concentrations of arsenic (III) and chromium (VI) and 15 days exposure times (Mean ± S.D.).

(The standard deviation has been obtained for n=3, "n" stands for the number of experiment replicates.)

It is evident that rapid absorption rate occurs at the beginning of absorption process may be due to availability of more number of active metal/metalloid binding sites on the root biomass. Téllaz *et al*. (2008) were reported the effects of water hyacinth, *Eichhornia crassipes*:

	Temperature (° C)		
	25°C	35°C	45°C
As_2O_3 concentration (mg/L)			
0.010	61.80 ± 0.11	55.35 ± 0.24	53.13 ± 0.16
0.025	66.68 ± 0.16	59.23 ± 0.43	54.25 ± 0.42
0.050	79.04 ± 1.22	71.51 ± 0.75	64.90 ± 1.06
0.100	85.96 ± 0.24	75.12 ± 0.29	69.60 ± 0.06
$K_2Cr_2O_7$ concentration (mg/L)			
0.75	88.95 ± 1.02	85.25 ± 1.12	80.24 ± 0.04
1.50	93.40 ± 0.08	88.21 ± 1.11	85.10 ± 0.02
2.50	94.40 ± 0.15	91.30 ± 0.27	87.12 ± 0.24
4.0	98.26 ± 0.43	94.07 ± 0.33	90.16 ± 0.13

an invasive plant in the Guadiana river basin (Spain). From the above it indicates the absorption process of arsenic (III) and chromium (VI) by *Eichhornia crassipes* was controlled by an exothermic process. As the maximum removal occurs at 25 ^{o}C, the room temperature was selected at 25 ± 2 ^{o}C at 15 days experiment for further absorption experiments.

4.2. Experimental procedure of bioremediation techniques

4.2.1. Selection of microorganism

Bacillus cereus (MTCC NO: 1305) of microbial type culture and collection was obtained from the Institute of Microbial Technology, Chandigarh, India to undertake the study. The identified species of *B. cereus* is a gram-positive, rod shaped bacterium. The surface properties of *Bacillus cereus* cells indicated that, there is formation of bio film due to biosorption. The extracellular polymeric substances proteins are function as non-specific adhesions during bio film formation. The extracellular polymeric substances (EPS) are implicated in imparting bio films with structural stability and resistance to cleaning heavy metals/metalloids. Because of these properties, the species is selected for the biosorption of the heavy metals. Bacterial strain was first grown on agar petri dish containing agar medium, which consisted of beef extract (3.0 g), peptone (5.0 g), agar (20.0 g), NaCl (5.0 g) in 1 L deionized water, and the pH is adjusted to 7.2 ± 0.3 with 10% (w/v) NaOH and 10% (w/v) HCl. After the incubation of cultures at 30 ºC for 24 h in agar plates, the bacteria are inoculated from the plates onto the agar slants and stored at 4 ºC until needed for further experiments. Fig. 4.2 schematically summarizes details experimental procedure of biosorption of arsenic (III) and chromium (VI) from water by bioremediation process.

4.2.2. Bacterial growth and preparation

Before the beginning of each experiment, strains are enriched by transferring one loop of cells from the agar slants to 100 mL of previously sterilized liquid nutrient medium in 250 mL flasks and incubated at 30 ºC for 24 h by shaking at 160 rpm in an orbital shaker. The liquid medium contained the same components described above in agar medium except agar, and the pH value is also adjusted to 7.2 ± 0.3 in the same way mentioned above. The cells grown in liquid nutrient medium are centrifuged at 7000 rpm for 30 min at 4 ºC. The supernatant is discarded and the cell pellets were washed six times with deionized water and suspended in phosphate buffer (1/15

mol/L NaH_2PO_4, 1/15 mol/L Na_2HPO_4, pH 7) before used in experiments. The experiments are conducted in presence of mineral liquid medium for the survival of Bactria. The components of the mineral liquid medium are KH_2PO_4 0.5 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $CaCl_2$ 0.1 g/L, NaCl 0.2 g/L, $MnSO_4.H_2O$ 0.01 g/L, NH_4NO_3 1.0 g/L. For experiment, 5 ml of the above solution are added to culture along with the solution of arsenic (III) and chromium (VI) in required concentrations separately.

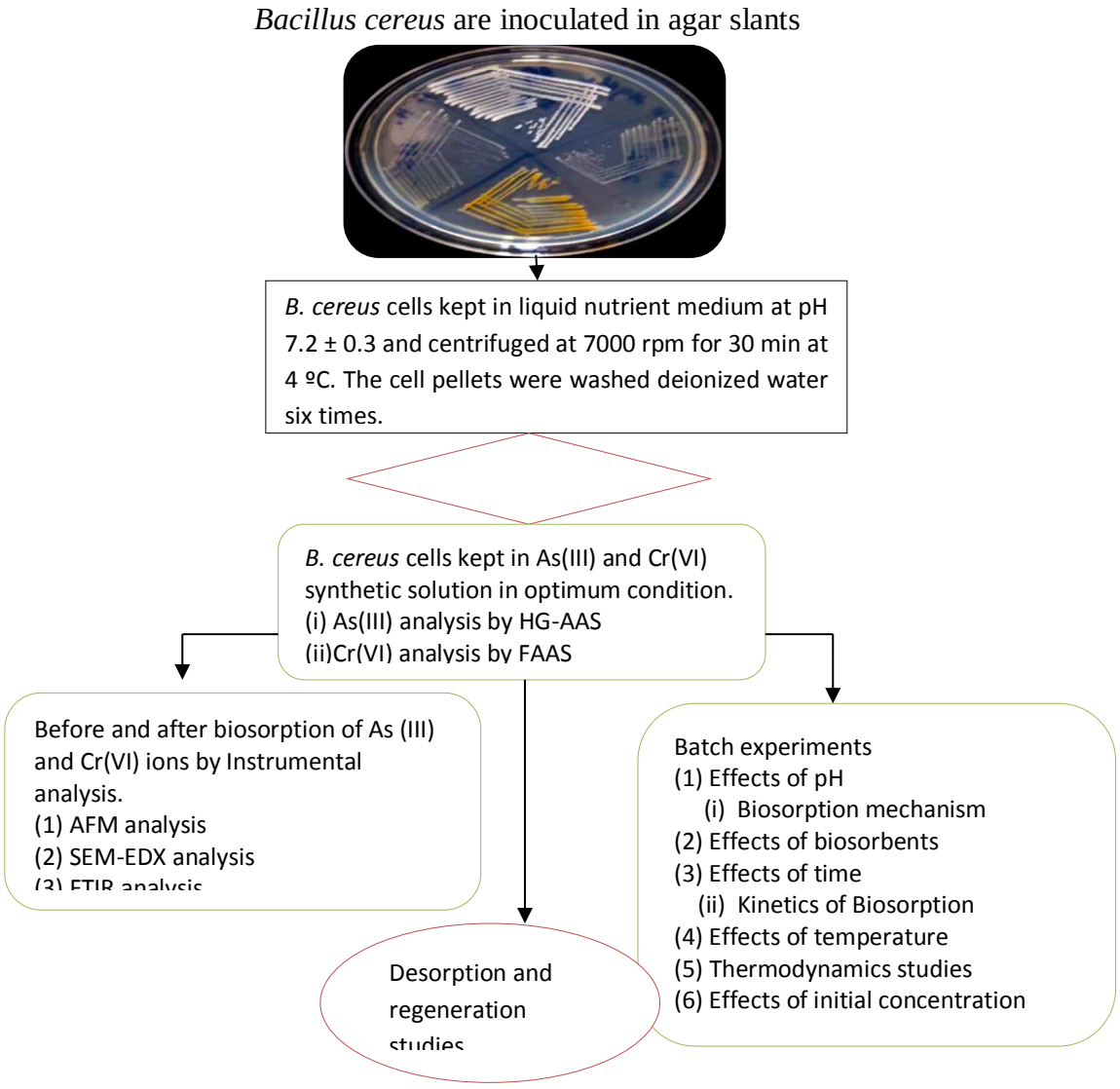


Fig. 4.2. Schematic diagram for details experimental procedure of bioremediation process for the biosorption of arsenic (III) and chromium (VI) from water by living cells of *Bacillus cereus*.

4.2.3. Batch experiments

The arsenic(III) sorption experiments from its aqueous solution on living cells of *Bacillus cereus* were carried out using standard 1 mg/l, 5 mg/L and 10 mg/L As^{3+} solution in absence of other competing ions. Optimum sorption conditions are determined as a function of pH, initial arsenite concentration, biomass loading, contact time, and temperature affecting the biosorption of arsenic (III) in the batch mode process. The biosorption experiments were carried out in series of 100 mL Borosil conical flask with stopper by adding 0.50 - 15 g/L of the biosorbent in 50 mL of synthetic arsenic (III) solution. Stoppers were provided to avoid change in concentration due to evaporation. All the biosorption experiments were carried out at ambient temperature (30 ± 2 °C). Necessary amount of the biomass is added and contents in the flasks are shaken at 120 rpm for the desired period of contact time in an electrically thermostatic reciprocating shaker. The time required for reaching the equilibrium condition is estimated by drawing samples at regular intervals of time till equilibrium is reached. The contents of the flasks are filtered through blue band filter paper and the filtrate is analyzed for arsenic (III) concentration by using hydride generated system HG-AAS (Perkin-Elmer P 200, USA). The chromium (VI) sorption experiments are conducted with similar procedure as discussed in arsenic (III) sorption experiment. But the concentration of chromium (VI) is measured by using FAAS (Perkin-Elmer P 200, USA). The biosorption capacity of the metal is expressed the arsenic (III) and chromium (VI) sorbed per gram of sorbent (mgg^{-1}), and is calculated as follows:

$$q_e\,(\mathrm{mg/g}) = (C_i - C_f)\,V/M$$

The amount of arsenic (III) and chromium (VI) sorbed percentage is calculated as per the equation:

$$\text{Sorption}\ (\%) = (C_i - C_f)/C_i \times 100$$

Where C_i and C_f are the initial and final concentrations of the arsenic (III) and chromium (VI) in the aqueous solution (mgL^{-1}), respectively. V is the volume (L) of test solution; and M is the mass of biosorbent in (g) used.

4.2.3.1. Estimation of arsenic (III) and chromium (VI) ions after biosorption

Estimation of arsenic (III) was done by using standard method given in user's manual of (Perkin-Elmer 200) hydride generated system (HG-AAS). Estimation of chromium (VI) was done by using standard method given user's manual of (Perkin-Elmer 200) flame atomic spectroscopy (FAAS). Three standards were prepared for both arsenic (III) and chromium (VI) that bracket the expected concentration range of sample which differs in concentration by factor ten. Measurements were done by taking 10 µL of each standard and sample in separate 100 mL beaker. All the sample and standards were maintained at same temperature to avoid interference due to difference in temperature.

4.2.3.2. Effect of biosorbent dose

The effect of biosorbent dose on biosorption was studied by using 50 mL of arsenic (III) solution of initial concentration of 1 mg/L prepared by serial dilution of stock solution (1000 mg/L). The solutions were taken in a series of conical flasks (Borosil 100 mL with stopper) containing 0.05g, 0.10g, 0.15g, 0.20 g, 0.25g, 0.30g, 0.35g, 0.40g, 0.45g, 0.50g, 0.55g, 0.60g , 0.65g , 0.70g, 0.75g and 0.80 g of *Bacillus cereus* biomass. Similarly, 50 mL of arsenic (III) solution of initial concentrations, 5 mg/L and 10 mg/L were prepared by serial dilution of stock solution and were added to a series of conical flask containing 0.05g, 0.10g, 0.15g, 0.20 g, 0.25g, 0.30g, 0.35g, 0.40g, 0.45g, 0.50g, 0.55g, 0.60g, 0.65g, 0.70g, 0.75g and 0.80 g of *Bacillus cereus* biomass. All the studies were carried out at pH 7.5 for arsenic (III). The contents of conical flask were stirred at 120 rpm at ambient temperature (30 ± 2 ℃) till equilibrium is achieved (30 minutes). Then the contents of the conical flasks were filtered through blue band filter paper and filtrates were analyzed for residual arsenic (III) concentration by standard method using AAS. The effects of biosorbent dose on biosorption of chromium (VI) are studied by the similar procedure as discussed in arsenic (III) experiment.

4.2.3.3. Effect of pH

The effect of pH on biosorption was studied by varying the pH from 3.5 to 9.5 (3.5, 4.5, 5.5. 6.5, 7.5, 8.5, and 9.5) keeping the other variable constant. pH was measured by using Orion two star pH meter. 50 mL of arsenic (III) solutions with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L were prepared by serial dilution of arsenite stock solution (1000 mg/L). The pH of the test

solutions is adjusted using required amount of 1M HNO_3 and 1M NaOH solution. The solutions of metal were added to conical flasks (Borosil 100 mL with stopper) containing optimum biosorbent dose (0.30 g of *Bacillus cereus* biomass). The contents of conical flask were stirred at 120 rpm at ambient temperature (30 ± 2 °C) till equilibrium is achieved (30 minutes). After the desired period, the contents of the conical flasks were filtered through blue band filter paper and filtrates were analyzed for residual arsenic (III) concentration by standard method using AAS. The effects of pH on chromium (VI) bisorption are done following similar procedure as discussed above in arsenic (III) experiment.

4.2.3.4. Effect of contact time

The effect of contact time was studied at ambient temperature of 30 ± 2°C. Arsenic (III) solution of initial concentration 1 mg/L was prepared by proper dilution of stock solution (1000 mg/L). 50 mL of the above mentioned solution was added to conical flasks containing optimum biosorbent dose (0.30 g of *Bacillus cereus* biomass) for different contact time (5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min and 60 min). pH adjustments were made by adding required amount of 1M HNO_3 and 1M NaOH solution and all the studies were carried out at pH 7.5 for arsenic(III). After predetermined time interval the content of the flasks were taken and filtered through blue band filter paper. The filtrate was analyzed for residual arsenic (III) concentration in the solutions by standard method using AAS. Similar 5 mg/L and 10 mg/L arsenic (III) solution were also prepared by serial dilution of stock solution (1000 mg/L). And all the above processes were repeated. The effects of contact time on biosorption of chromium (VI) are studied by the similar procedure as discussed in arsenic (III) experiment.

4.2.3.5. Effect of temperature

To study the effect of temperature, arsenic (III) solution of initial concentration 1 mg/L was prepared by proper dilution of stock solution (1000 mg/L). 50 mL of the solution was taken in series of conical flasks and were maintained at different temperatures (10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C and 60 °C). To above solutions, maintained at appropriate temperature, optimum adsorbent dose (0.30 g of *Bacillus cereus* biomass) were added and were stirred at 120 rpm till equilibrium was achieved (30 minutes). All the

experiments were carried out at pH 7.5 for arsenic (III). After predetermined time interval, the content of the flasks were filtered through blue band filter paper and the filtrate was analyzed for residual arsenic (III) concentration using AAS. Similarly 5 mg/L and 10 mg/L arsenic (III) solution were also prepared by proper dilution of stock solution (1000 mg/L) and were maintained at different temperatures. To the above solutions optimum biosorbent doses (0.30 g of *Bacillus cereus* biomass) were added and were stirred, filtered and analyzed for residual arsenic (III) as above. The effects of temperature on biosorption of chromium (VI) are studied by the similar procedure as discussed in arsenic (III) experiment.

4.2.3.6. Effect of initial concentration

Batch mode experiments were performed with optimum amount of *Bacillus cereus* biomass and varying initial concentration of 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L, 5 mg/L, 6 mg/L, 7 mg/L, 8 mg/L, 9 mg/L and 10 mg/L. The solutions of different initial arsenic (III) concentration were taken in Borosil conical flask with stopper of 100 mL capacity and optimum biosorbent doses (0.30 g of *Bacillus cereus* biomass) were added and stirred at 120 rpm till equilibrium was achieved (30 minutes). pH adjustments were made and all the studies were carried out at pH 7.5 for arsenic(III). After predetermined time interval the content of the flasks were filtered through blue band filter paper. The filtrate was analyzed for residual arsenic (III) concentration using by standard method using AAS. The study was carried out at ambient temperature (30 ± 2 °C). The effects of initial concentration on biosorption of chromium (VI) are studied by the similar procedure as discussed in arsenic (III) experiment.

4.2.3.7. Desorption and regeneration studies

For the sustainability of biosorption process, the biosorbents should have good desorption and reuse potential. For this 50 mL of 1 mg/L arsenic (III) solution, was transferred separately into a beaker and 10mL of buffer solution was added. After a fast shaking, 6 g/L of living cells of *Bacillus cereus* was added and the mixture was shaken again for 90 min at 150 rpm. The solution was filtered with blue band filter paper and separated. Then the residues are washed with deionized water. In order to elute the sorbed analytes onto *Bacillus cereus*, 10mL of 1M HCl and 10mL of 1M HNO_3 is used separately. The filtered biosorbent was retreated with 100 mL deionized water and adjusted to different pH with the help of 1M HNO_3 and 1M NaOH solution.

It was stirred for 24 hours. The residual arsenic (III) concentration was measured using AAS by standard method. The study was carried out at ambient temperature (30 ± 2 °C). Desorption and regeneration studies of chromium (VI) are studied by the similar procedure as discussed in arsenic (III) experiment.

4.3. Experimental procedure of adsorption study

4.3.1. Adsorbent preparation

Young aquatic weeds *Eichhornia crassipes* are collected from nearby ponds. The weeds are placed in cement tanks with tap water under natural sunlight for one week to allow them to adapt the new environment. The root and shoot parts of selected *Eichhornia crassipes* weeds are separated. Fig 4.3 schematically summarizes details preparation procedure of activated carbon from root biomass.

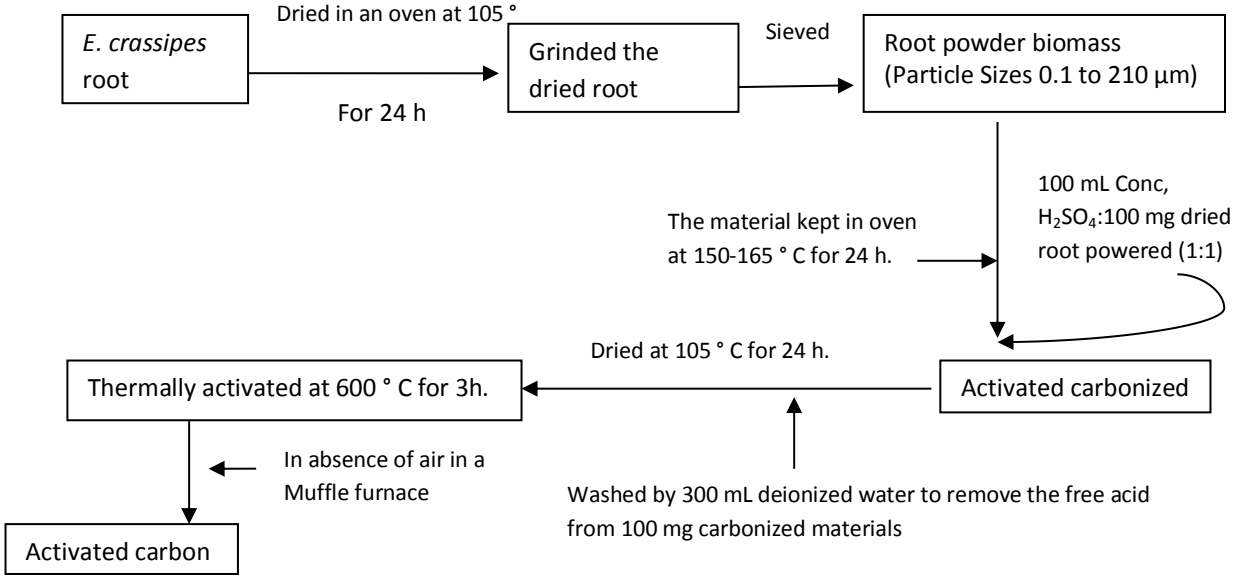


Fig. 4.3. Schematic diagram of *Eichhornia crassipes* root biomass-derived activated carbon.

The material was activated at higher temperatures of 600-1000 °C which form (H-AC) 'H' activated carbon, and develops basic surface sites which increase the pH value (Sinha *et al*., 2003). These materials adsorb acids and exhibit a positive zeta potential (Sinha *et al*., 2003;

Mattson and Mark, 1971). The desired product is cooled and sieved to the desired particle sizes (0.1 to 210 µm). Finally, the product is stored in vacuum desiccators until required. The analysis, characterization, and batch-to-batch reproducibility of the materials are strictly controlled.

4.3.2. Characterization of activated carbon

4.3.2.1. Physico-chemical parameters

The physico-chemical parameters of the activated carbon are analyzed using standard methods and results are summarized in Table 4.3.

Table 4.3. Physico-chemical parameters of the activated carbon.

Parameter	Value
Carbon particle size (µm)	0.1 to 210
pH	3.5
Moisture contained (%)	9.45
Conductivity (µS cm^{-1})	28.23
Specific gravity	0.66
Porosity (%)	70
Bulk density (g/mL^{-1})	0.75
Ash contained (%)	1.92
Ion exchange capacity (meq./g)	0.87
Water soluble matter (%)	1.46
Acid soluble matter (%)	5.21
Volatile matter (%)	47
Surface area (m^2/g)	109.23
Fixed carbon (%)	70.38
O (w/w) (%)	22.27

H (w/w) (%)	2.14
N (w/w) (%)	4.20
Cl (w/w) (%)	5.39
K (w/w) (%)	2.92
Mg (w/w) (%)	1.00
Na (w/w) (%)	0.93
Ca (w/w) (%)	0.88
P (w/w) (%)	0.83

4.3.2.1. Determination of Zeta potential at different pH value

The surface charge density (σ) of sorbent was determined by a potentiometric titration method. The following equation-1 was used to determine the surface charge density

$\sigma_o = ((C_A - C_B + [OH^-] - [H^+])F) / m$

Where C_A and C_B are the molar concentrations of acid and base needed to reach a point on the titration curve, $[H^+]$ an $[OH^-]$ were the concentrations of H^+ and OH^-, F was the Faraday constant (96,490 C/mol), m (g/L) was the concentration of the sorbent. Fig. 4.4 show Zeta potential at different pH value

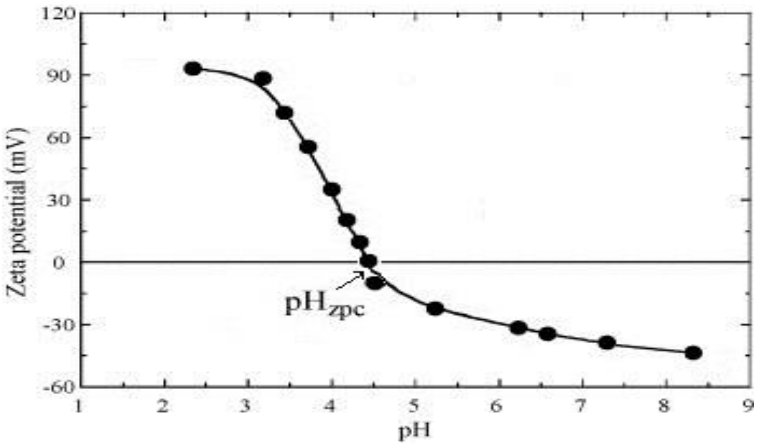


Fig. 4.4. Zeta potential at different pH values of activated carbon.

4.3.3. Batch adsorption experiment

Adsorption experiments are optimized at the desired pH value, contact time and adsorbent dosage level using the necessary adsorbents in a 250mL stopper conical flask containing 50mL of test solution. A number of experimental parameters such as adsorbent dose, pH, initial Cr (VI) concentration and temperature affecting the adsorption of chromium (VI) have been studied to optimize in the batch mode process. Necessary amount of the adsorbents is then added and contents in the flask are shaken for the desired contact time in an electrically thermostatic reciprocating shaker at 120 rpm. The experiments are repeated at 25 °C to 55 °C. The time required for reaching the equilibrium condition is estimated by drawing samples at regular intervals of time till equilibrium is reached. Adsorption experiments for the effect of pH are conducted by using a solution having 10 mg/L, 50 mg/L and 100 mg/L of chromium (VI) concentration with a biomass dosage of 7 g/L. Throughout the study, the contact time is varied from 5 to 90 min, the pH from 1.5 to 8.5, chromium (VI) concentration from 10 to 100 mg/L, and the adsorbent dosage from 0.5 to 15 g/L. All adsorption studies are carried out at a constant ionic strength of 0.01M maintained with NaCl. After stirring, the solutions are allowed to settle for 10 min and the samples are centrifuged at 3000 rpm for 20 min and filtered through Whatman 42 filter paper. The filtrate is used for the analysis of residual chromium (VI) concentration in the solution. For each experiment, the concentrations of the metals before and after adsorption are determined by atomic absorption spectrophotometer (AAS). The adsorption capacity, expressed as the chromium (VI) adsorbed per gram of adsorbent (mg g^{-1}), is calculated as follows:

$$q_e\,(\text{mg/g}) = \frac{(C_\text{i} - C_\text{f})V}{M}$$

The adsorbed percentage of chromium (VI) is calculated as per the equation:

$$\text{Adsorption (\%)} = \frac{(C_\text{i} - C_\text{f})}{C_\text{i}} \text{ x } 100$$

Where C_i and C_f are the initial and final concentrations of the chromium (VI) in the aqueous solution (mg L^{-1}), respectively. *V* is the volume in liter (L) of test solution; and *M* is the mass of adsorbent in (g) used.

4.3.4. Desorption experiment

The sample volume of 50mL, containing 100 mg/L of chromium (VI) at pH 4.5, is transferred into a beaker; and 10mL of buffer solution is added to maintain the pH value. After shaking for few minutes, 7 g/L of activated carbon is added and the mixture is shaken with a mechanical shaker at 120 rpm again for 90 min. The content is filtered with Whatman 42 filter paper. Then the constituents in the residue are washed with distilled water several times. In order to elute the adsorbed analytes by activated carbon, 100 mL of 5N H_2SO_4 is used. Analyte contents of the final solution are determined by AAS. The same procedure is applied to the blank solution. In order to use the activated carbon for the next experiment, the activated carbon is washed with excess of 1M H_2SO_4 solution and distilled water. It is again used as an adsorbent in subsequent cycles to evaluate the reuse potential of the adsorbent.

4.3.5. Column studies

Adsorption isotherms have traditionally been used for preliminary investigations and fixing the operating parameters. The isotherms cannot provide accurate scale-up data in a fixed-bed system, so to know the practical applicability of materials, column operations has also been investigated to obtain a factual design model. The column tests are performed in a 1.20 cm diameter column. The column is filled with a 200 mg sample of the activated carbon (particle size 0.1-210 µm). This weighed quantity of adsorbent is made into slurry with hot water and fed slowly into the column, displacing a below of water. The bed height is 6 cm, and the bed volume is 20 mL. The

inlet concentration is 10 mg/L solution and pH is 4.5. The flow rate is 1.2 mL/min. The results are used to build the breakthrough curve and to determine the breaking point**.**

4.4. Instrumental Analysis

4.4.1. Arsenic (III) and chromium (VI) analysis using AAS

The freeze-dried samples were ground to fine powder using a ceramic mortar and pestle. About 0.5 g the powder was taken in a 50 mL conical flask. Plant samples were heated to dry matter by heating at 120 °C for 24 hours in a hot air oven and the ash was digested with 10 mL nitric acid and filtered into a volumetric flask with the help of Whatman -42 filter paper. The final volume was made up with de-ionized water and the analysis of arsenic (III) and chromium (VI) concentration was carried using AAS standard method given is user's manual of (Perkin-Elmer 200).

4.4.2. Microwave assisted extraction procedure

Microwave digestion with closed vessel of microwave assisted extraction is conducted with Shanghai Sineo MAS-II oven delivering a maximum power of 1000 W. To optimize the microwave-assisted extraction of arsenic and chromium ions from shoot parts of plant material, a factorial experiment was conducted consisting of heating of different temperatures at 40, 60 and 80 ℃ and for heating times of 5, 15 and 25 min for each of the three different extraction solutions. The three extracting solutions used were 10% (v/v) tetramethylammonium hydroxide (TMAH), deionized water and a modified protein extracting solution for arsenic (III) and 0.02 M ethylenediaminetetraacetic acid (EDTA), deionized water and HCl for chromium (VI). Each treatment was replicated three times. Freeze dried *Eichhornia crassipes* shoot biomass with arsenic (III) concentration of 32.1 ± 0.05 mg kg^{-1} and *Eichhornia crassipes* shoot biomass with chromium (VI) concentration of 260 ± 0.05 mg kg^{-1} was weighed (0.50 g) into the reaction vessels and 10 mL of each extractant was added to each sample. The microwave system was programmed to digest the sample to a specified temperature, for a specified period. After microwave digestion, the samples were allowed to cool to room temperature followed by filtering through a Whatman no. 42. Total arsenic and chromium in the filtrate were then measured by flow injection of ICP-MS.

4.4.2.1. Total arsenic and chromium analysis by ICP-MS

Total arsenic in the shoot part of plant samples were determined by digesting the plant material in concentrated nitric acid using the Frypan method. Dry plant material (0.50 g) was weighed into a 50 mL conical flask to which 10 mL of concentrated nitric acid was added. The digested mixture was then digested at 60 °C for 30 min and filtered into a volumetric flask with the help of Whatman 42 filter paper. The flasks were allowed to cool to room temperature and made up to a final volume (10 mL) with deionized water. In each analytical batch, at least two reagent blanks and one internationally certified reference material (2 replicates) were included. The accuracy of the method was checked by analysis of the standard reference material 1575 Pine needles (NBS certified arsenic concentrations: 0.21 ± 0.04 $\mu g g^{-1}$. The measured arsenic concentration: 0.20 ± 0.04 $\mu g g^{-1}$; n= 4, values represents mean and 95% confidence limits. To measure total arsenic concentration in digested samples, 1 mL of digest was mixed with 9 mL of reducing solution consisting of 1.5% (w/v) potassium iodide, 1.5% (w/v) ascorbic acid and 10% (v/v) hydrochloric acid. This mixture was then heated at 40 °C for 1 h. Total arsenic in digests was determined by a hydride generation-ICP-MS. The carrier solution was 10% (v/v) hydrochloric acid, and the reductant solution consisted of 0.2% (w/v) sodium borohydride and 0.05% (w/v) sodium hydroxide. The carrier solution was 1% (v/v) nitric acid; samples were made up in solution of 10 µg rhodium L^{-1} which served as an internal standard.

Total chromium in the shoot part of plant samples were determined by digesting the plant material in concentrated nitric acid using the frypan method. Dry plant material (0.50 g) was weighed into a 50 mL conical flask to which 10 mL of concentrated nitric acid, 2.0 mL hydrogen peroxide, and 3.0 mL water was added. The digested mixture was then heated at 60 °C for 30 min and filtered into a volumetric flask with the help of Whatman 42 filter paper. The flasks were allowed to cool to room temperature and made up to a final volume (10 mL) with deionized water. Total chromium in digests was determined by ICP-MS. The carrier solution was 1% (v/v) nitric acid; samples were made up in solution of 10 µg rhodium L^{-1} which served as an internal standard.

4.4.2.2. Arsenic and chromium ions speciation analysis using HPLC-ICP-MS

For arsenic speciation, 0.5 g samples were ultrasonically extracted with 5 ml of 1:1 methanol / water for 2 h. The samples were then centrifuged; the supernatant was decanted into a 50 mL volumetric flask. The procedure was repeated with the residual pellet and the two extracts were combined. The residue was rinsed three times with 5 mL of water, and all supernatants were

combined. Mobile phase was 20 mM ammonium phosphate ($NH_4H_2PO_4$) buffer at pH 6.0 and flow rate 1.5mLmin^{-1}.

For chromium speciation, the mobile phase was prepared by dissolving the appropriate amounts of 1 mM tetrabutylammonium hydroxide (TBAH) and the 0.60 mM dipotassium salt of ethylenediaminetetraactic acid (EDTA) in deionized water with a flow rate of 1.5mLmin^{-1}. The pH was then adjusted with nitric acid to 6.90 and methanol was added to make 2% methanol solution. The extract was then diluted to the 50 ml mark with water and then filtered using 0.45 PTFE syringe filters. The filtrate was directly subjected to HPLC-ICP-MS and experiments were performed using an anion exchange column (Hamilton PRP x100 250mm×4.6 mm).

4.5. Instrumental analysis of arsenic (III) and chromium (VI) ions

4.5.1. Inductively coupled plasma-mass spectroscopy (ICP-MS)

The mass spectrometry is an instrumental technique of analysis based on separation, and the identification and the quantification of the components of a sample are made according to their mass. It is supported on the coupling of a plasma generating ions and of a quadrupolar mass spectrometer which separates these ions accordingly to mass, and a model PE ELAN 6000 inductively coupled plasma mass spectrometry (ICP-MS) instrument (Perkin-Elmer, Norwalk, CT, USA) is used for this purpose.

4.5.2. Hydride generation atomic absorption spectroscopy (HG-AAS)

Atomic absorption spectroscopy (AAS) is one of the commonest instrumental methods for analyzing the metals and some metalloids ions. But because of interferences, poor reproducibility, and poor detection limits an alternative method for detection some metalloids like arsenic, antimony, bismuth, selenium has been developed, which are called Hydride generation atomic absorption spectroscopy (HG-AAS, Perkin-Elmer P 200, USA). Instrument calibration standards for transfer of 2.00, 5.00, 10.00 and 15.00 mL standard solution of As (III) to 100 mL volumetric flasks and bring to volume with water containing the same acid concentration (2 to 5 mL conc. HNO_3/L) used for sample preservation. So the standard solutions are respectively 2, 5, 10 and 15 µg/L. Details preparation procedure of As(III) standards solution has been using manual's of Perkin-Elmer P 200. A calibration curve of As(III) standard solutions are presented in Fig. 4.5.

4.5.3. Flame atomic absorption spectroscopy (FAAS)

Flame atomic absorption spectroscopy is one of the commonest instrumental methods for analyzing for metals ions (FAAS, Perkin-Elmer P 200, USA). This is used in the present study. Instrument calibration standards for transfer of 5.00, 10.00, 15.00 and 20.00 mL standard solution of Cr(VI) to 100 mL volumetric flask, add appropriate amount of matrix modifier and dilute to volume with water. So the standard solutions are respectively 5, 10, 15 and 20 µg/L. Details preparation procedure of Cr(VI) standards solution has been using manual's of Perkin-Elmer P 200. A calibration curve of Cr(VI) standard solutions are presented in Fig. 4.6.

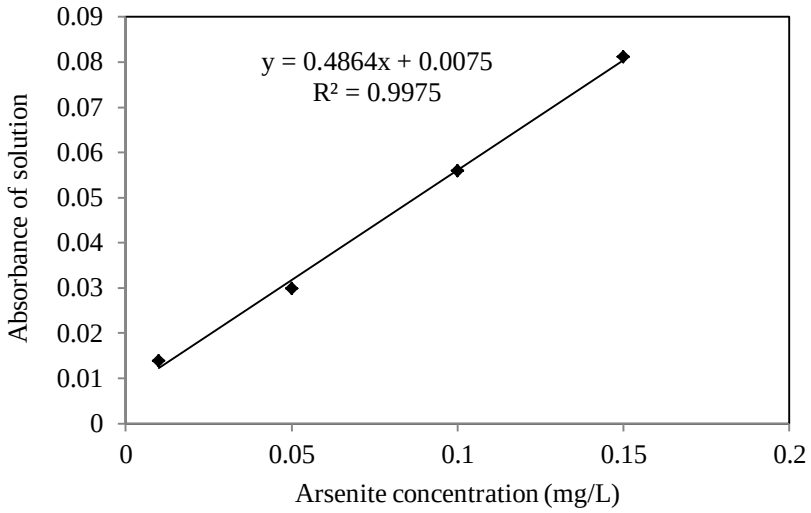


Fig. 4.5. A calibration curve of As(III) standards solution.

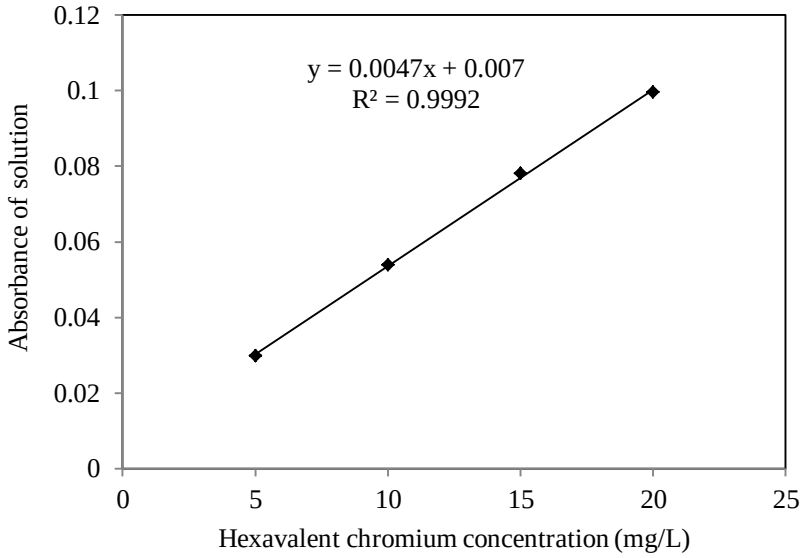


Fig. 4.6. A calibration curve of Cr(VI) standards solution.

4.5.4. High performance liquid chromatography (HPLC)

High-performance liquid chromatography is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. HPLC-ICP-MS experiments were performed using an anion exchange column (Hamilton PRP x100 250mm×4.6 mm).

4.5.5. Scanning electron microscopy-Energy dispersive X-ray (SEM-EDX)

Scanning electron micrographs – Energy dispersive X-ray (EDX) of the sample was obtained by JEOL JSM – 6480 LV (Japan) scanning electron microscope. The sample was coated with platinum for 30 seconds at a current of 50 mA before the SEM-EDX micrograph was obtained.

4.5.6. FTIR study

FTIR of the sample was obtained by KBr pellet method using Perkin Elmer FTIR spectrophotometer SPECTRUM RX - 1. A homogenous mixture of sample and KBr in the ratio of 1:50 was made. The pellet was prepared by taking the mixture in a KBr Die and a pressure of 5 Ton was applied using a hydraulic pressure for 2 minutes. Then the pellet was gently removed and was placed in a pellet holder. The instrument was switched on and background scan was obtained without placing the pellet. The data was plotted using standard software provided with the instrument.

4.5.7. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) (Digital Instruments, Santa Barbara, CA, USA) is a very high-resolution type of scanning probe microscopy, with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The standard V-shaped silicon nitride (Si_3N_4) cantilevers (with integral tips, Model OTR8-35) of different stiffness and tip sharpness were used for imaging. AFM scan size detection limit of 130 x 130 μm is used in the present study.

4.5.8. X-Ray diffraction study

The powder X-ray diffraction (XRD) of sample was determined by using Philips X' Pert X-ray diffractometer (model PW 1710) with Cu Kα (35 kV and 30 mA) radiation at a scan rate of 2^{o}/min and is analyzed using standard software provided with the instrument.

4.6. Prediction of arsenic (III) and chromium (VI) ions by biosorption process using artificial neural network (ANN) modeling

In recent years, considerable advancement in artificial intelligence techniques has been used to predict the responses in complex and difficult situations using MATLAB 7.0 is used for simulation purpose. Such techniques can enhance predicting capability of the model when mathematical or statistical methods are difficult to formulate and fails to predict with desired accuracy. In the present work, estimation of sorption efficiency using mathematical and analytical tools is involved because the physical phenomenon for removal of arsenic (III) and chromium (VI) by living cells of *B. cereus* is complex one. Therefore, artificial neural network (ANN) has been attempted in this work for prediction purpose because ANN has the capacity to map inputs and outputs efficiently in complex situations.

4.6.1. Back propagation neural network architecture (BPNNA)

A back propagation neural network (BPNN) architecture consisting of three layers such as input layer, hidden layer and output layer is considered. Functioning of neural network proceed in two stages viz., learning or training and testing or inferences (Yetilmezsoy and Demirel, 2008; Schalkoff, 1997). The network architecture is represented as *l-m-n* where *l* neurons are present at input layer (equal to the number of inputs in the models), *m* neurons at the hidden layer (optimized through experimentation), and *n* neurons at the output layer depending on number of outputs desired from the model. Input layer receives information from the external sources and passes this information to the network for processing. Hidden layer receives information from the input layer, does all the information processing and output layer receives processed information from the network, and sends the results out to an external receptor. The input signals are modified by interconnection weight known as weight factor (W_{ji}), which represents the interconnection of i^{th} node of the first layer to j^{th} node of the second layer. The sum of modified signals (total activation) is then modified by a sigmoid transfer function *(f)*. Similarly, outputs signal of hidden layer are modified by interconnection weight (W_{ji}) of k^{th} node of output layer to

j^{th} node of hidden layer. The sum of the modified signal is then modified by sigmoid transfer *(f)* function and output is collected at output layer (Aleboyeh *et al*., 2008; Giri *et al*., 2011).

Let $I_p = (I_{p1}, I_{p2}, \ldots\ldots\ldots I_{pl})$, $P = 1, 2 \ldots. N$ is the P^{th} pattern among N input patterns. Where W_{ji} and W_{kj} are connection weights between i^{th} input neuron to j^{th} hidden neuron, and j^{th} hidden neuron to k^{th} output neuron, respectively.

Output from a neuron in the input layer is,

$$O_{pi} = I_{pi},\ i = 1, 2 \ldots\ldots l$$

$$O_{pj} = f\left(\sum_{i=0}^{l} W_{ji}\, O_{pi}\right), j = 1,2 \ldots m$$

Output from a neuron in the output layer is,

$$O_{pk} = f\left(\sum_{j=0}^{m} W_{kj}\, O_{Pj}\right), k = 1,2 \ldots n$$

Sigmoid transfer function (*f*) is a bounded, monotonic, non-decreasing, S-shaped function that provides a graded nonlinear response (Yetilmezsoy, 2006; Park *et al*., 2004). The logistic sigmoidal function is given as

$$f(x) = \frac{1}{1+e^{-x}}$$

Sigmoid transfer function is recommended for obtaining activation signals from input and hidden layer neurons whereas linear transfer function is used to get the same at output layer neurons. Metal concentration, biosorbent dosage, different temperature and time are used as inputs to the ANN model. Sorption efficiency (%) is desired from the network as output. One hundred seventy one experimental data are used to develop the ANN model. The complete experimental are divided into two sets - training (75% of data) and test sets (25% of data). All data are normalized in the 0.1-0.9 range to avoid the scaling effect of parameter values. Therefore, all of the data (x_i) are converted to normalize values (x_{norm}) as follows.

$$X_{norm} = 0.8 \times \left(\frac{X_i - X_{min}}{X_{max} - X_{min}}\right) + 0.1$$

where X_i is i^{th} input or output variable X.

X_{min} and X_{max} are minimum and maximum value of variable X.

4.6.2. Learning or training in back propagation neural network

Initially, the connection weights are generated randomly in the range of -1 to +1. Batch mode type of supervised learning has been adopted in the present case where interconnection weights are adjusted using delta rule algorithm after sending the entire training sample to the network. During training, the predicted output is compared with the desired output, and the mean square error is calculated. If the mean square error is more than a prescribed limiting value, it is back propagated from output to input, and weights are further modified till the error or number of iterations is within a prescribed limit. Mean square error, E_p for pattern *p* is defined as

$$E_p = \sum_{i=1}^{n} \frac{1}{2}(D_{pi} - O_{pi})^2$$

where, D_{pi} is the target output, and O_{pi} is the computed output for the i^{th} pattern.

Weight change at any time *t*, is given by

$\Delta W(t) = -\eta E_p(t) + \alpha \times \Delta W(t-1)$

η = *learning rate i.e* $0 < \eta < 1$

α = momentum coefficient i.e $0 < \alpha < 1$

4.6.3. Testing of back propagation neural network

Entire experimental data set is divided into training set and testing set. The mean square error is monitored during the training phase. The error usually decreases during the initial phase of training. However, when the network begins to over fit the data, the error on the training set will typically begin to rise. When the training error starts increasing for a specified number of iterations, the training is stopped and the weights at the minimum value of the training error are returned. The testing data is then fed to the trained network to check the percentage variation of predicted output in comparison to the actual one.

4.7. Statistical analysis

4.7.1. F-statistics

F-test can be regarded as a comparison of two variances, but the specific case being discussed in this work is that of two populations, where the test statistic used is the ratio of two sample variances. Let $X_1 \ldots . X_n$ and $Y_1 \ldots . Y_m$ be independent and identically distributed samples from two populations which each have a normal distribution. The expected values for the two populations can be different, and the hypothesis to be tested is that the variances are equal. Let

$$\overline{X} = \frac{1}{n}\sum_{i=1}^{n} X_i \text{ and } Y = \frac{-1}{m}\sum_{i=1}^{m} Y_i$$

be the sample means. Let

$$S_X^2 = \frac{1}{n-1}\sum_{i=1}^{n}(X_i - X)^2 \text{ and } S_Y^2 = \frac{1}{m-1}\sum_{i=1}^{m}(Y_i - Y)^2$$

Be the sample variances. Then the test statistic has an F-distribution with *n*-1 and *m*-1 degrees of freedom if the null hypothesis of equality of variances is true.

$$F = \frac{S_X^2}{S_Y^2}$$

4.7.2. p-value

The p-value measures consistency by calculating the probability of observing the results from samples of data or a sample with results more extreme, assuming the null hypothesis is true. The smaller the p-value, the greater the inconsistency.

4.7.3. Data analysis and calculations

Experimental measurements always have some random error, so no conclusions can be drawn with complete certainty. However, statistical methods give us tools to accept conclusions that have a high probability of being correct and to reject those are not. Various inbuilt statistical tools of Microsoft Excel 2007 like Pearson's correlation coefficient, plotting the best straight line etc. were extensively used in the present research work. Microsoft Excel 2007 proved to be very beneficial for calculating the values of various constants, plotting graph, calculating pearson's correlation coefficient, finding the best straight line, and getting slope and getting slope and intercept of the straight line. Syntax of various functions is below.

4.7.3.1. Slope

Slope is often used to describe the measurement of the steepness, incline, gradient, or grade of a straight line. A higher slope value indicates a steeper incline. The slope of a line in the plane containing the *x* and

y axes is defined as the change in the *y* coordinate divided by the corresponding change in the *x* coordinate, between two distinct points on the line. The slope is the vertical distance divided by the horizontal distance between any two points on the line, which is the rate of change along the regression line.

The equation for the slope of the regression line is:

$$b = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2}$$

where 'x' and 'y' are the sample means AVERAGE (known x's) and AVERAGE (known y's).

4.7.3.2. Intercept

Intercept of a straight line is the point at which a line will intersect the y-axis by using existing x-values and y-values. The intercept point is based on a best fit regression line plotted through the known x-values and known y-values. Intercept function is used when it is necessary want to determine the value of the dependent variable when the independent variable is 0 (zero).

The equation for the intercept of the regression line, a, is

$$a = \bar{y} - b\bar{x}$$

where the slope, b, is calculated as:

$$b = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2}$$

and where x and y are the sample means AVERAGE (known x's) and AVERAGE (known y's).

4.7.3.3. Pearson's correlation coefficient (R)

In probability theory and statistics, correlation, (often measure as a correlation coefficient), indicates the strength and direction of a linear relationship between two random variables. In general statistical usage, correlation or co-relation refers to the departure of two variables from independence. Pearson's correlation coefficient, r, a dimensionless index that ranges from -1.0 to 1.0 inclusive and reflects the extent of a linear relationship between two data sets.

The equation for the correlation coefficient, R, is:

$$R = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 \sum(y-\bar{y})^2}}$$

where x and y are the sample means AVERAGE(array1) and AVERAGE (array 2).

Chapter-5

5. Results and discussion

5.1. Accumulation of arsenic (III) and chromium (VI) on *Eichhornia crassipes* using Phytoremediation techniques.

In the present work, studies on the removal of arsenic (III) and chromium (VI) was carried out by phytoremediation technique using water floating macrophytes *Eichhornia crassipes*. The technique used in this process is called more appropriately phyto-accumulation technique, which is a part of phytoremediation. The phytoremediation studies were performed as a function of relative growth, bioconcentration factor, total dissolved solids, dissolved oxygen, accumulation and toxicity. High-performance liquid chromatography in conjunction with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to measure arsenic and chromium speciation in plant material using microwave extraction processes.

5.1.1. Effects on relative growth

Relative growth is considered to be the most widely used method for estimating plant growth. It is measured as the increase in biomass per day, with unit as g. $g^{-1}.d^{-1}$ (Hoffmann and Poorter, 2002; Sarma, 2011). In the present study, the effects of different concentrations and exposure times of arsenic (III) and chromium (VI) ions on relative growth of *Eichhornia crassipes* were studied and the results are presented in Fig. 5.1 and Fig. 5.2, respectively. The relative growth of control plants significantly increased ($P< 0.05$) with the passage of time. As indicated from Table 5.1 the plants treated with arsenic (III), the relative growth significantly increased ($P < 0.05$) in 0.010, 0.025, 0.05 and 0.10 mgL^{-1} treatments. As indicated from Table 5.1 the plants treated with $K_2Cr_2O_7$, the relative growth significantly increased ($P<0.05$) in 0.75, 1.50, 2.50 treatments but decreased in 4 mg/L. The statistical analysis of treated plants at different concentration of arsenic (III), chromium (VI) and exposure days have been conducted at 0.05 levels and presented in Table 5.2. The highest values of relative growth were $1.34g.g^{-1}.d^{-1}$ treated with arsenic (III) at 0.1

mg L^{-1} and 1.33g.g^{-1}.d^{-1} treated with chromium (VI) at 2.50 mgL^{-1} solution after 15 days treatments respectively.

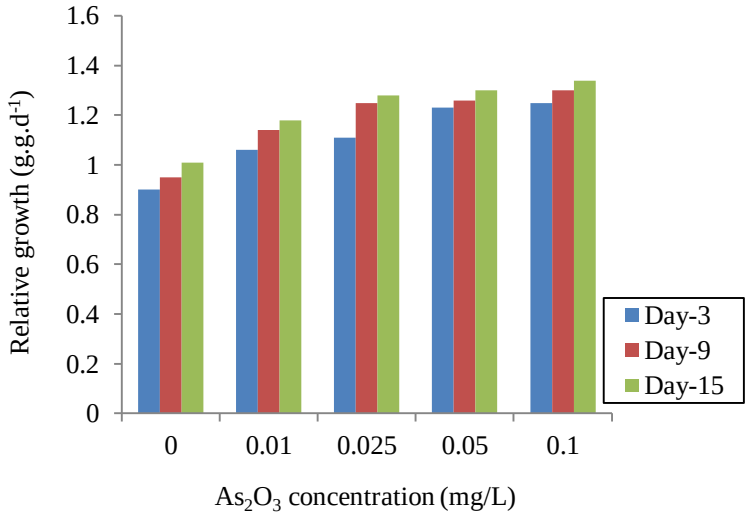


Fig. 5.1. The effects of arsenic (III) on relative growth of *Eichhornia crassipes* at different concentrations and exposure time.

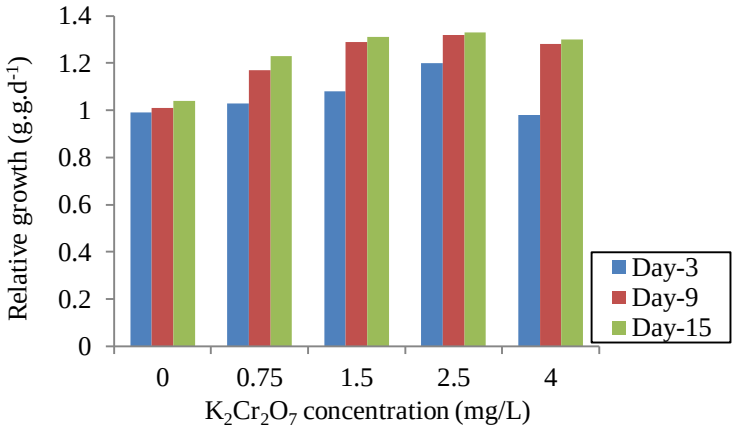


Fig. 5.2. The effects of chromium (VI) on relative growth of *Eichhornia crassipes* at different concentrations and exposure time.

Table 5.1. The relative growth of *E. crassipes* at different concentration of arsenic(III) and chromium(VI) and exposure times (Mean ± S.D.).

Relative growth (g. g^{-1}.d^{-1})	Mean ± S.D.

As_2O_3 concentration (mg/L)	Days		
	3	9	15
0.010	1.06 ± 0.01	1.14 ± 0.05	1.18 ± 0.01
0.025	1.11 ± 0.03	1.25 ± 0.03	1.28 ± 0.04
0.050	1.23 ± 0.02	1.26 ± 0.04	1.30 ± 0.03
0.10	1.25 ± 0.01	1.30 ± 0.02	1.34 ± 0.06
$K_2Cr_2O_7$ concentration (mg/L)			
0.75	1.03 ± 0.03	1.17 ± 0.04	1.23 ± 0.13
1.50	1.08 ± 0.01	1.29 ± 0.11	1.31 ± 0.08
2.50	1.20 ± 0.04	1.32 ± 0.08	1.33 ± 0.04
4.0	0.98 ± 0.01	1.28 ± 0.05	1.30 ± 0.12

(The standard deviation has been obtained for n=3, "n" stands for the number of experiment replicates.)

Table 5.2. ANOVA table for relative growth of *E. crassipes* at different concentration of arsenic (III) and chromium (VI) and exposure times.

Source	Degree of Freedom (df)	Sum of Squares (SS)	Mean Squares (MS) = SS/df	F-Statics	P-value
As_2O_3 concentration (mg/L)					
Different concentration	3	0.00645	0.00215	20.59	0.001
Different days	2	0.00345	0.0017	16.53	0.004
Error	6	0.0063	0.000104		
Total	11	0.01053			

$K_2Cr_2O_7$ concentration (mg/L)					
Different concentration	3	0.00431	0.00143	4.13	0.046
Different days	2	0.01593	0.00796	22.84	0.002
Error	6	0.00209	0.000349		
Total	11	0.02233			

Lu *et al*., 2004 reported the relative growth were 0.85 $g.g^{-1}.d^{-1}$ and 0.89 $g.g^{-1}.d^{-1}$ for *Eichhornia crassipes* treated with Cd at 4 mg/L and Zn at 40 mg/L, respectively. Zaranyika and Ndapwadza, (1995) were reported that Cr (VI) tolerance and accumulation in selected *Eichhornia crassipes* growth are mainly by suppressing development of new roots and reducing relative growth rates to about 15% of those controls. Several researchers have reported similar results **(Stratford *et al*., 1984;** Gupta *et al*., 2009; Murányi and Ködöböcz, 2008**)**. The best way of long term strategy for improving phytoextraction is to understand and exploit the biological processes involved in metal/metalloid acquisition, transport and shoot accumulation. This is due to higher concentrations level of heavy metal/metalloid ions in solution and also have inhibitory effects on plant metabolic activity, alternatively reduced growth of plants, leaf necrosis and inhibits the plant physiology systems. It appears that low concentration could stimulate plants growths.

5.1.2. Effects of bio-concentration factor

Bio-concentration factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals/metalloids and this value was calculated on dry weight basis. The ambient metal/metalloid concentration in water was the major factor influencing the metal/metalloid uptake efficiency. In general, when the metal/metalloid concentration in water increases, the amount of metal/metalloid accumulation in plants increases, accordingly the BCF values also increases (Wang and Lewis, 1997; Karimi *et al*., 2009). In the present study, the effects of bio-concentration factor values of arsenic (III) and chromium (VI) at different concentration and exposure times were presented in Fig. 5.3 and Fig. 5.4, respectively.

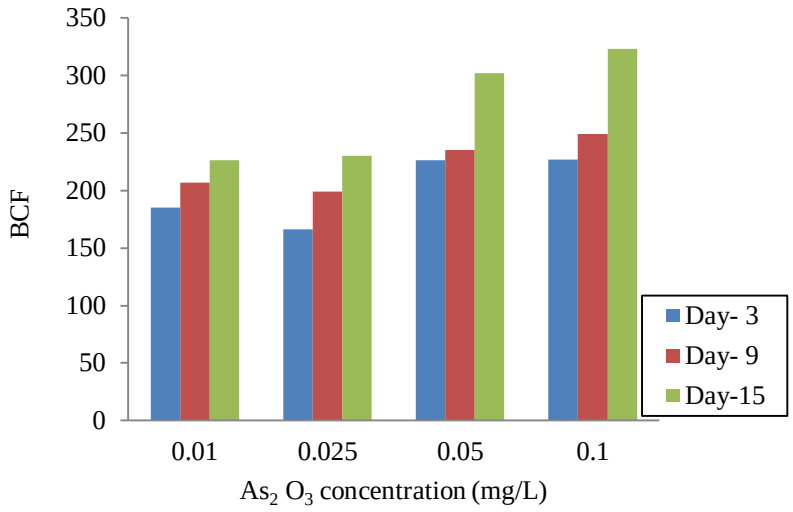


Fig. 5.3. The effects of arsenic (III) on bio-concentration factor of *Eichhornia crassipes* at different concentrations and exposure time.

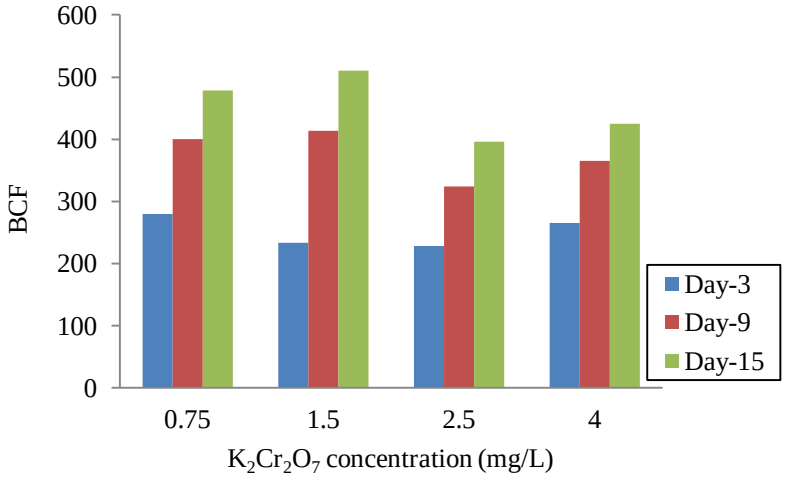


Fig. 5.4. The effects of chromium (VI) on bio-concentration factor of *Eichhornia crassipes* at different concentrations and exposure time.

As expected the bio-concentration factor for arsenic (III) and chromium (VI) significantly increased ($P < 0.05$) with passage of time. In plants treated with arsenic (III), the bio-concentration factor significantly increased ($P < 0.05$) in 0.010, 0.025, 0.05 and 0.10 mgL^{-1} treatments and results are presented in Table 5.3. In plants treated with $K_2Cr_2O_7$, the bio-

concentration factor significantly increased (P<0.05) in 0.75, 1.50 mg/L treatments but decreased in 2.50 and 4.0 mg/L and results were presented in Table 5.3.

Table 5.3. The bio-concentration (BCF) values of *Eichhornia crassipes* at different concentrations of arsenic (III) and chromium (VI) and exposure times (Mean ± S.D.).

Bio-concentration factor Mean ± S.D.			
As_2O_3 concentration (mg/L)	Days		
	3	9	15
0.010	185 ± 1.24	207 ± 1.45	226 ± 0.65
0.025	166 ± 0.54	199 ± 0.62	230 ± 1.32
0.050	226 ± 1.44	235 ± 1.11	302 ± 2.05
0.10	227 ± 0.37	249 ± 2.21	323 ± 1.25
$K_2Cr_2O_7$ concentration (mg/L)			
0.75	280 ± 1.11	400 ± 2.32	478.23 ± 1.21
1.50	233.33 ± 2.10	413.32 ± 1.34	510.03 ± 2.31
2.50	228 ± 0.65	324 ± 0.88	395.55 ± 1.41
4.0	265 ± 1.06	365 ± 2.13	425.23 ± 0.21

(The standard deviation has been obtained for n=3, "n" stands for the number of experiment replicates.)

The bio-concentration factor of 249.20 was obtained in plants treated with 0.10 mg/L of As_2O_3 and 413.33 was obtained in plants treated with 1.50 mg/L of $K_2Cr_2O_7$ after 9 days treatments. The maximum bio-concentration factor values for arsenic (III) were 323.12 in plants treated with 0.10 mg/L of As_2O_3 on day 15, indicating that *Eichhornia crassipes* can be used for effective phytoremediation. The maximum BCF of 510.03 was obtained in plants treated with 1.50 mg/L of $K_2Cr_2O_7$ after 15 days treatments. The statistical analysis of treated plants at different concentration of arsenic (III), chromium (VI) and exposure days have been conducted at 0.05 levels and results are presented in Table 5.4**.** Zhu *et al*. (1999) reported that the bio-

concentration factor values of *Eichhornia crassipes* are very high for Cd, Cu, Cr and Se at low external concentration, and they are found to decreases with the increase in external concentration (Mohanty *et al*., 2012; Wang and Lewis, 1997). The maximum BCF values for As (III) and Cr (VI) were 323.12 and 510.03, respectively indicating that *Eichhornia crassipes* can be used for effective phytoremediation. It represents a cost-effective plant-based technology for the removal of metals from the environment and has great potential for future applications.

Table 5.4. ANOVA table for bio-concentration factor of *Eichhornia crassipes* at different concentrations of arsenic (III) and chromium (VI) and exposure times.

Source	Degree of Freedom (df)	Sum of Squares (SS)	Mean Squares (MS) = SS/df	F-Statics	P-value
As_2O_3 concentration (mg/L)					
Different concentration	3	0.03612	0.012042	25.70	0.001
Different days	2	0.033008	0.016504	35.23	0.000
Error	6	0.002811	0.00046		
Total	11	0.071944			
$K_2Cr_2O_7$ concentration (mg/L)					
Different concentration	3	0.020547	0.006849	91.01	0.000
Different days	2	0.108262	0.054131	719.88	0.000
Error	6	0.000451	0.0000752		
Total	11	0.1292609			

5.1.3. Arsenic (III), chromium (VI) accumulation and remediation mechanism

5.1.3.1. Translocation of arsenic (III) ions and accumulation mechanism

Movement of metal/metalloids containing sap from the root to the shoot, termed translocation is primarily controlled by two processes root pressure and leaf transpiration. Some metals are

accumulated in roots, probably due to the some physiological barriers against metal/metalloid transport to the aerial parts (Rahman *et al.*, 2008; Bose *et al.*, 2008). The results of arsenite ions accumulation by *Eichhornia crassipes* at 25 ^{o}C of different concentrations and exposure times are presented in Table 5.5.

Table 5.5. The accumulation of arsenic (III) ions at pH 6.8 in shoots and roots of *Eichhornia crassipes* at different arsenite concentration and exposure times.

(Mean ± S.D.)			
As_2O_3 concentration (mg/L)	Days		
	3	9	15
Shoot (mg/kg)			
0.010	1.42 ± 0.04	1.61 ± 0.03	1.74 ± 0.12
0.025	3.05 ± 0.08	4.47 ± 0.11	5.47 ± 0.06
0.050	9.58 ± 0.13	10.21 ± 0.06	12.21 ± 0.22
0.10	19.53 ± 0.24	21.3 ± 0.28	32.1 ± 0.05
Root (mg/kg)			
0.010	0.43 ± 0.02	0.46 ± 0.01	0.52 ± 0.03
0.025	1.11± 0.05	1.25 ± 0.08	1.32 ± 0.05
0.05	1.82 ± 0.11	2.52 ± 0.04	3.18 ± 0.14
0.10	4.21 ± 0.15	5.62 ± 0.02	9.20 ± 0.12

(The standard deviation has been obtained for n=3, "n" stands for the number of experiment replicates.)

From the data in Table 5.5 it is inferred that, there is an increase in the arsenic (III) accumulation in shoots and roots when arsenite concentration and exposure times were increased ($P < 0.05$). The statistical analysis of treated plants at different concentration of arsenic (III) and exposure times have been conducted at 0.05 levels and presented in Table 5.6. Plants treated with 0.10 mgL^{-1} of arsenite for 15 days accumulated the highest arsenic (III) in shoots (32.1 mg kg^{-1},

dry weight; Fig. 5.5) and in roots (9.2 mg kg^{-1}, dry weight; Fig. 5.6). Arsenic once accumulated inside the plant, the arsenic ions must be translocated through the symplast at high concentrations in a manner that does not disrupt cytoplasmic function, which gives the considerable phytotoxicity of arsenic species. Finally, the arsenic is stored at very high concentrations in the shoots.

Table 5.6. ANOVA table for arsenic (III) ions absorption efficiency of shoots and roots.

Source	Degree of Freedom (df)	Sum of Squares (SM)	Mean Squares MS = SS/df	F-Statistics	P-value
Shoots					
Different concentration	3	2.30222	0.01904	523.25	0.000
Different days	2	0.03808	0.76741	12.98	0.007
Error	6	0.00880	0.00147		
Total	11	2.34910			
Roots					
Different concentration	3	1.2672	0.4224	3210.5	0.000
Different days	2	0.0178	0.00891	67.72	0.000
Error	6	0.00078	0.00013		
Total	11	1.28587			

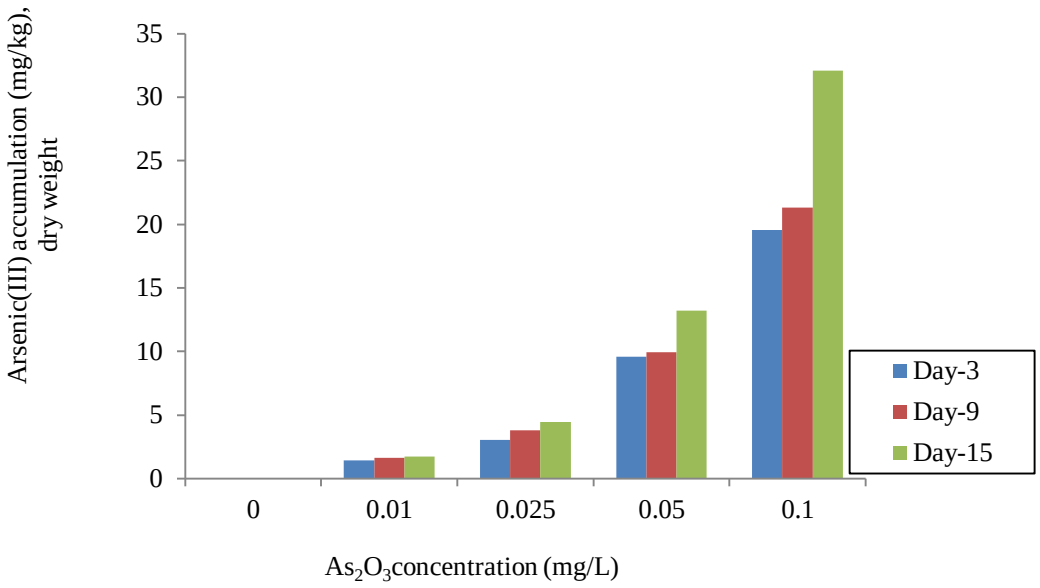


Fig. 5.5. The accumulations of arsenic (III) ions shoot part of *Eichhornia crassipes* at different concentrations and exposure time.

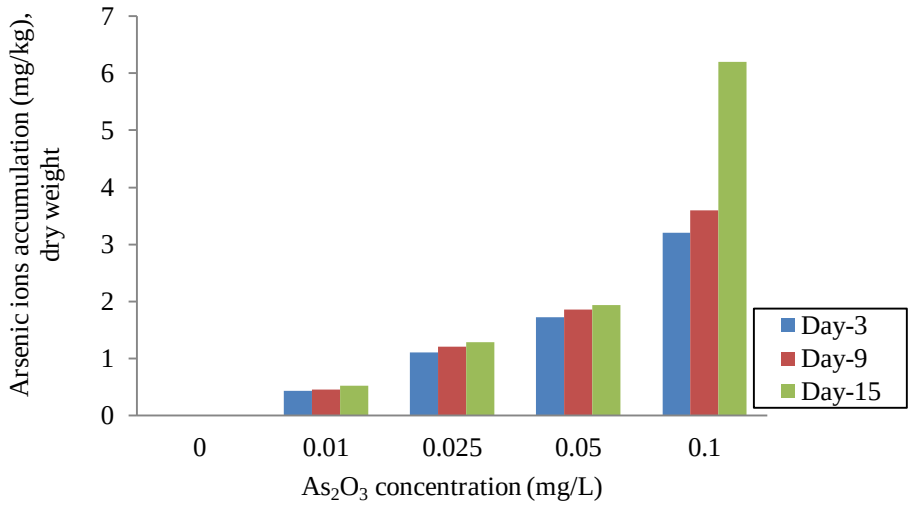
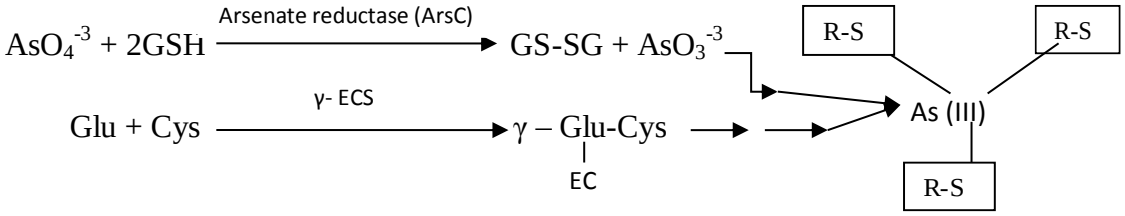


Fig. 5.6. The accumulations of arsenic(III) ions root part of *Eichhornia crassipes* at different concentrations and exposure time.

The metals accumulation increased linearly with the solution concentration in the order of leaves > stems > roots in *Eichhornia crassipes* (Feng *et al*., 2009; Alvarado *et al*., 2008; Snyder, 2006; Low and Lee, 1990; Vamerali *et al*., 2009; Misbahuddin and Fariduddin, 2002; Mishra *et al*., 2008; Keith *et al*., 2006). Metalloid ions penetrated plants by passive process, mostly by

exchange of cations which occurred in the cell wall. All heavy metals were taken up by plants through absorption, translocation and released by excretion. It can be proposed that the roots reached saturation during the period and there exists some mechanism in roots that could detoxify heavy metals or transfer them to aerial parts.

The most efficacious remediation of arsenic requires that plants extract arsenic from water and accumulate in shoot parts. To accomplish this, the electrochemical species of arsenic must be changed in different parts of the plant. The bacterial arsenate reductase (ArsC) catalyzes the electrochemical reduction of arsenate to arsenite. The bacterial γ-glutamylcysteine synthetase (γ-ECS) catalyzes the formation of γ-glutamylcysteine (γ-EC) from the amino acids glutamate and cysteine and is the committed step in the synthesis of glutathione (GSH) and phytochelatine (PCs) (indicated by three arrows which represents the interaction of three thiols groups) (Meharg and Hartley-Whitaker, 2002; Zhu and Rosen, 2009). Reduced arsenite can bind organic thiols (RS) such as those in γ- EC, GSH, and PCs through the replacement of oxygen by organic sulfur species. The scheme of reaction mechanism is as follows (Dhankher and Meagher, 2005).



5.1.3.2. Translocation of chromium (VI) ions and accumulation mechanism

Phytoremediation methods using aquatic plants to absorb metals/metalloids from their surrounding waters are highly efficient (Dutton and Fisher, 2011). The bio removal process using aquatic plants contains two uptake processes; (i) biosorption which is an initial fast, reversible, and metal-binding process and (ii) bioaccumulation which is a slow, irreversible, and ion-sequestration step (Braud *et al*., 2009; Keskinkan *et al*., 2003; Mohanty and Patra, 2012; Delgado *et al*., 1993).

Table 5.7 The accumulation of chromium (VI) ions at pH 6.8 in shoots and roots of *Eichhornia crassipes* at different hexavalent chromium concentration and exposure times.

(Mean ± S.D.)

$K_2Cr_2O_7$ concentration (mg/L)	Days		
	3	9	15
Shoot (mg/kg)			
0.75	10.11 ± 0.04	40.44 ± 0.43	50.45 ± 0.12
1.50	50.23 ± 0.08	80 ± 1.11	101 ± 0.16
2.50	70.24± 0.13	120 ± 0.62	140 ± 0.42
4.0	90.22 ± 0.24	240 ± 0.18	260 ± 0.05
Root (mg/kg)			
0.75	200 ± 0.42	260 ± 1.01	280 ± 1.03
1.50	300± 2.05	540 ± 0.08	560 ± 2.05
2.50	500 ± 0.11	790 ± 1.04	820 ± 1.14
4	570 ± 2.15	1220 ± 3.02	1320 ± 3.12

(The standard deviation has been obtained for n=3, "n" stands for the number of experiment replicates.)

In the present study, the accumulation of hexavalent chromium ions by *Eichhornia crassipes* at 25 ^{o}C with different concentrations and exposure times was analyzed and were presented in Table 5.7. From the data in Table 5.7 it is clear that there is an increase in the chromium (VI) accumulation in shoots and roots when chromium (VI) concentration and exposure times are increased ($P<0.05$). The statistical analysis of treated plants at different concentration of chromium (VI) and exposure times have been conducted at 0.05 levels and presented in Table 5.8.

Table 5.8. ANOVA table for chromium (VI) absorption efficiency of shoots and roots.

Source	Degree of Freedom (df)	Sum of Squares (SM)	Mean Squares MS = SS/df	F-Statistics	P-value

Shoots					
Different concentration	3	1.0574	0.3525	30.02	0.001
Different days	2	0.4442	0.2221	18.92	0.003
Error	6	0.0704	0.0117		
Total	11	1.5721			
Roots					
Different concentration	3	0.60391	0.20130	64.65	0.000
Different days	2	0.16080	0.08040	25.82	0.001
Error	6	0.01868	0.00311		
Total	11	0.78340			

Plants treated with 4.0 mg/L of Cr (VI) after day 15 accumulated the highest level of metal in shoots (260 mg/kg, dry weight; Fig. 5.7) and roots (1320 mg/kg, dry weight; Fig. 5.8). In the presence of excessive oxygen, chromium (III) oxidizes into chromium (VI), which is highly toxic and more soluble in water than the other forms. Chromium (VI) can easily cross the cell membrane, whereas the phosphate-sulphate carrier also transports the chromite anions. Fe, S, and P are known also to compete with Cr for carrier binding (Hadad *et al*., 2011; Mei *et al*., 2002; Wang and Lewis, 1997). Metal ions penetrated plants by passive process, mostly by exchange of cations which occurred in the cell wall. The metals accumulation in *Eichhornia crassipes* increases linearly with the solution concentration in the order of leaves < stems < roots (Maine *et al*., 2004; Qian *et al*., 1999; Keith *et al*., 2006). It can be proposed that the roots reached saturation during the period and there exists some mechanism in roots that could detoxify heavy metals or transfer them to aerial parts.

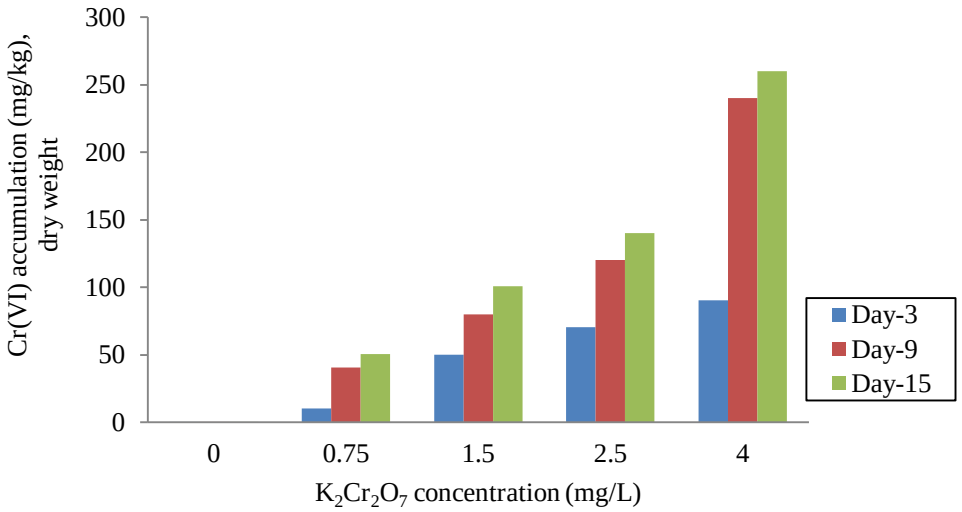


Fig. 5.7. The accumulation of chromium (VI) ions in shoot part of *Eichhornia crassipes* at different concentration and exposure time.

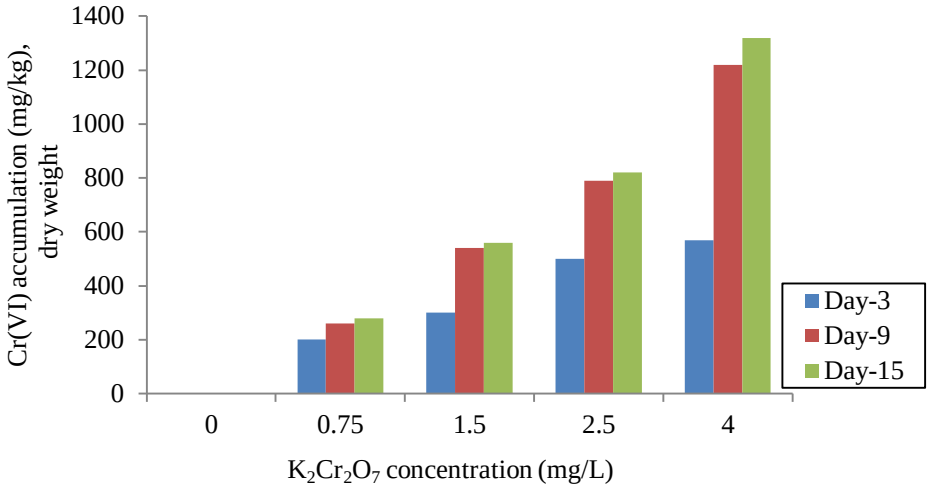


Fig. 5.8. The accumulation of chromium (VI) ions in root part of *Eichhornia crassipes* at different concentration and exposure time.

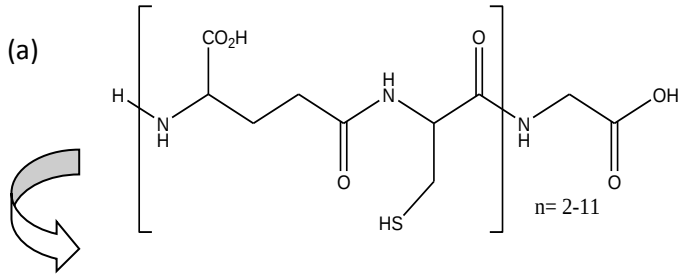
5.1.4. Toxic effects of arsenic and chromium ions in plants

5.1.4.1. Arsenic ions detoxification and remediation mechanism

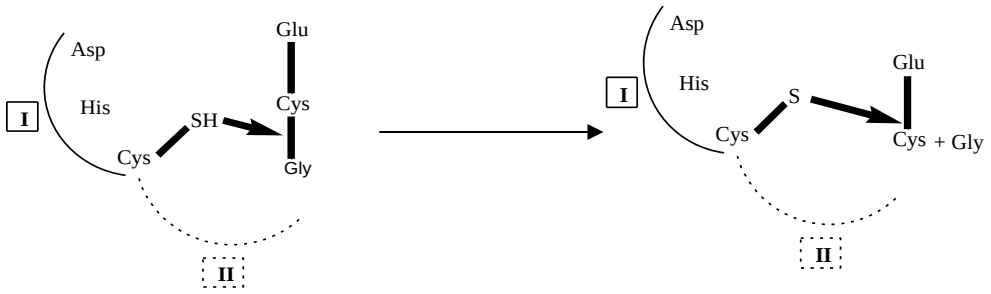
The toxic effects of arsenic ions mainly depend on the metal speciation, which decides its uptake, translocation and accumulation mechanism. A strategy for cells to detoxify non-essential metal ions and an excess of essential metal ions is the synthesis of high-affinity binding sites to suppress binding to physiologically important functional groups. The high reactivity of metal

ions with thiol, amino or hydroxyl groups makes the molecules carrying these functional groups as metal chelators. The best-known and presumably most effective chelators for arsenic ions are small Cys-rich proteins (metallothioneins, MTs) and Cys-containing peptides glutathione (GSH) and phytochelatins (PCs). PCs peptides of general structure $(\gamma\text{-Glu-Cys})_n$ -Gly (n= 2-11) has been represented in Fig. 5.9(a) (Meharg and Hartley-Whitaker, 2002). They are enzymatically synthesized by a specific transpeptidase, the phytochelatin synthase, which is activated by the presence of metal ions and uses glutathione as substrate. Their detoxifying function depends on the ability to bind metals to form stable complexes, which effectively reduce the intracellular concentration of potentially toxic free metal ions.

A detoxification pathway for arsenate (AsO_4^{3-}) by conversion to arsenite (AsO_3^{3-}) upon its uptake into roots has been proposed. Arsenite (AsO_3^{3-}) transformation and detoxification system in root cells is responsible for the phytochelatins and oxidized to form arsenate (Sneller *et al*., 1999). Arsenate can be reduced to arsenite enzymatically by arsenate reductase as shown *in vitro* and non-enzymatically by glutathione (GSH) (Delnomdedieu *et al*., 1994) followed by the formation of an arsenite-thiol (AsO_3^{3-}-SH) complex. Acylation of binding site (Fig. 5.9(b)) I (step 1) occurs at a cysteine that is 100% conserved in all known phytochelatin synthases and phytochelatin synthase-like proteins. The cysteine together with a histidine and an aspartate forms the catalytic triad typical for cysteine proteases. Glycine is cleaved off (step 2) and the resulting γ-glutamylcysteine dipeptide is transferred onto another glutathione (or a PC molecule). Binding site II remains to be identified and is possibly not present in bacterial phytochelatin synthase-like proteins (Mukhopadhyay *et al*., 2000). They catalyse steps 1 and 2, resulting in the degradation of glutathione to γ-glutamylcysteine and glycine. Steps 1 and 2 are metal ion-independent. Acylation of site II and peptide transferase activity require metal ion activation and/or the binding of a metal-glutathione complex.



(b)



Step-1 Step-11

Fig. 5.9 (a) The structure of phytochelatins, metal-binding peptides synthesized non-ribosomally from glutathione. **(b)** Acylation of site II and peptide transferase activity require metal ion activation and/or the binding of a metal-glutathione complex.

5.1.4.2. Chromium detoxification and remediation mechanism

The toxic effects of Chromium are primarily dependent on the metal speciation, which determines its uptake, translocation and accumulation mechanism. The oxidation state of chromium strongly influences the rate of chromium uptake. Chromium (VI) can easily cross the cell membrane and the phosphate sulphate carrier transports the chromate anions. It forms a number of stable oxyacids and anions, including $HCrO_4^-$ (Hydrochromate), $Cr_2O_7^{2-}$ (dichromate), and CrO_4^{-2} (chromate).The chromate ion has a large ionic potential and have the potential for tetrahedral coordination and acts both as strong acid and an oxidizing agent. The toxic properties of Cr (VI) originate from the action of this form which as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr (VI) to Cr (III) inside the cell. Induction and activation of superoxide dismutase (SOD) and of antioxidant catalase are some of major metal detoxification mechanisms in plants as shown in Fig. 5.10.

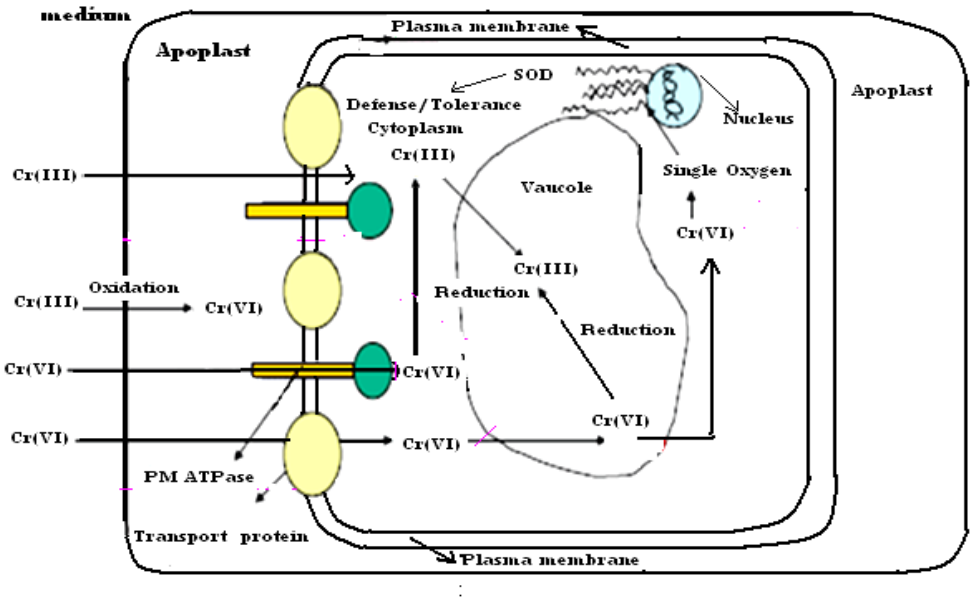


Fig. 5.10. Hypothetical model of chromium ions transport and toxicity in *Eichhornia crassipes* plant root cell.

SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of reactive oxygen species (Puntarulo *et al.*, 1988). The SODs remove O_2• by catalyzing its dismutation, one O_2• being reduced to H_2O_2 and another oxidized to O_2. It has been noted that O_2• can undergo protonation to give up a strong oxidizing agent, HO_2• in negatively charged membrane surfaces, which directly attack the polyunsaturated fatty acids (Halliwell, 2006; Elstner, 1987) Furthermore, O_2• can also donate an electron to chromium (Cr^{6+}) to yield a reduced form of chromium (Cr^{+3}) which can then reduce H_2O_2, produced as a result of SOD led dismutation of O_2• and $OH^{\bullet}$. The reactions through which O_2•, H_2O_2 and chromium rapidly generate $OH^{\bullet}$ which is called the Haber-Weiss reaction (Scarpeci *et al.*, 2008).

$$O_2\bullet + Cr^{6+} \rightarrow {}^1O_2 + Cr^{+3}$$

$$2O_2\bullet + 2H^{+} \xrightarrow{\text{SOD}} O_2 + H_2O_2\ Cr^{6+}$$

$$Cr^{+3} + H_2O_2 \rightarrow Cr^{6+} + OH^{-} + OH\bullet \text{ (Fenton reaction)}$$

5.1.5. Arsenic and chromium speciation in *Eichhornia crassipes* shoot biomass using complete microwave assisted extraction by HPLC-ICP-MS

5.1.5.1. Extraction efficiency of arsenic ions

Extraction is an important step for separation of constituents from the plant material (adsorbed). Microwave heating technique is a simple, inexpensive and valuable tool used in applied chemistry which requires lesser amount of solvent, simplified manipulation and higher purity of final product with lower cost (Ammann, 2007; Quaghebeur *et al*., 2003). Microwave extraction is becoming the choice for the extraction of solid matrices for organic analyte analysis by HPLC-ICP-MS techniques. In the present study, extraction of 32.1 ± 0.05 $mgkg^{-1}$ arsenic (III) ions from *Eichhornia crassipes* shoots biomass using 10% (v/v) tetramethylammonium hydroxide (TMAH), deionized water, and a modified protein extracting solution has been conducted at different time temperatures and times durations and the results are presented in Fig. 5.11, Fig. 5.12 and Fig. 5.13, respectively.

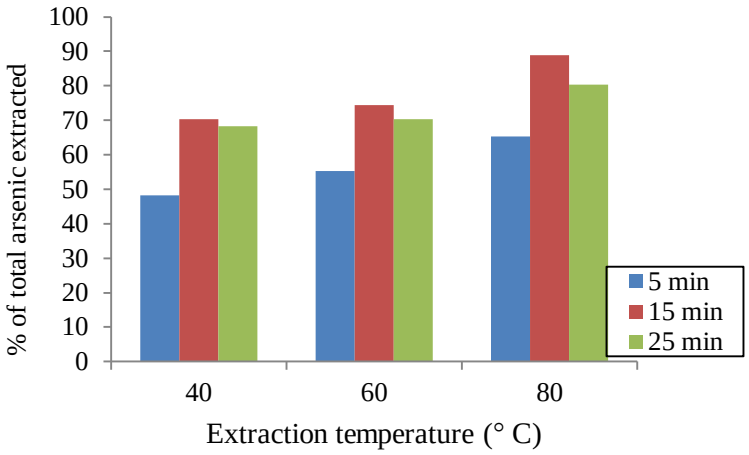


Fig. 5.11. Total arsenic extraction efficiency for *Eichhornia crassipes* shoot biomass using a modified protein extracting solution at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

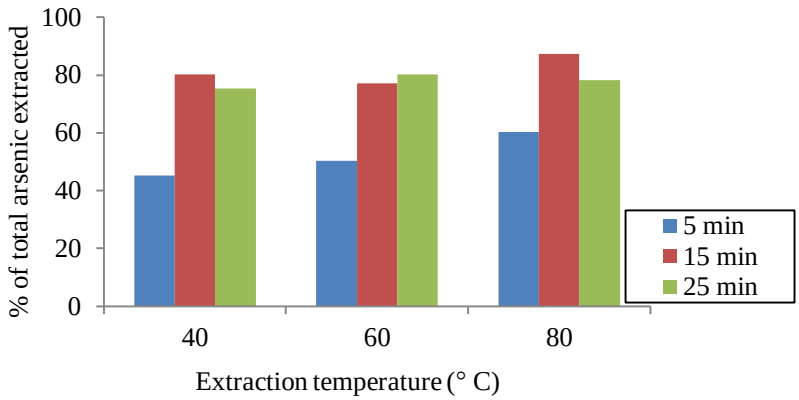


Fig. 5.12. Total arsenic extraction efficiency for *Eichhornia crassipes* shoot biomass using deionized water at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

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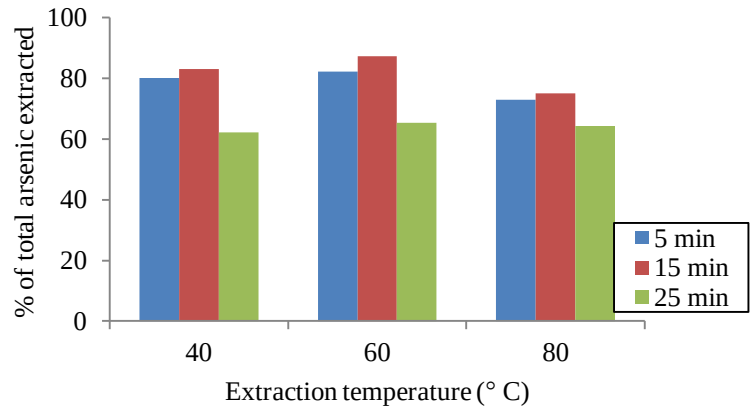


Fig. 5.13. Total arsenic extraction efficiency for *Eichhornia crassipes* shoot biomass using 10% TMAH solution at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

The efficiency of the extraction of arsenic ions increased with increasing temperature in both modified protein extracting solution and deionized water (Fig. 5.11 and Fig. 5.12) but in TMAH the extraction efficiency decreased with increase in temperature with the lowest value at a temperature of 80 °C (Fig. 5.13). This is because at high temperature, TMAH is expected to break the strong As-SH bonds present in plant material and can extract inorganic arsenic successfully even at low temperatures. It is evident from the results that TMAH extracted around 95% of the arsenic from *Eichhornia crassipes* shoot biomass at 60 °C, which was significant compared to deionized water (87.24%) at the same temperature. The extraction time significantly

affected arsenic extraction efficiencies (Table 5.9). Longer extraction times improved arsenic recoveries in all extractants at 40 °C and in the modified protein extracting solution and TMAH at 60 °C. However, the extraction time did not significantly affect extraction efficiencies at 80 °C in any of the 3 extractants (Table 5.9). Ackley *et al*. 1999 have reported that TMAH can able to extract 95% of the total arsenic from DORM-2 reference material heated in a microwave at 50 °C compared a recovery of 74% when water was used as a solvent. Quaghebeur *et al.* 2003 have reported the modified protein extracting solution extracted arsenic from shoot and root materials of *Brassica napus, Holcus lanatus, Arabidopsis thaliana* and *Senna planitiicola* by microwave heating the sample at 90 °C for 20 min and the extraction efficiency was 104 ± 16 %.

Table 5.9. Analysis of variance of arsenic extraction efficiency for *Eichhornia crassipes* shoots biomass with extractant, heating temperature and duration as main factors.

Source	Degree of Freedom (df)	Sum of Squares (SM)	Adj. Sum Squares (SM)	Adj. Mean Squares MS = SS/df	F-Statistics	P-value
Extractant	2	0.0000558	0.0000558	0.0000279	50.46	0.000
Temperature	2	0.0001564	0.0001564	0.0000782	141.48	0.000
Time	2	0.0000599	0.0000599	0.0000300	54.18	0.000
Extractant xTemp.	4	0.0001937	0.0001937	0.0000484	87.61	0.000
Extractant xTime	4	0.0000060	0.0000060	0.0000015	2.70	0.108
Temp. xTime	4	0.0000099	0.0000099	0.0000025	4.48	0.034
Error	8	0.0000044	0.0000044	0.0000006		
Total	26	0.0004862				

In order to validate statistical importance of each influencing parameter on response, analysis of variance has been conducted and the data of statistical analysis are presented in Table 5.9 at confidence level of 95% for arsenic (III). The results from the above table indicate that there is a

significant variation in the results. It is observed from the table that the factors extractant, temperature and time are statistically significant as the p-value is less than 0.05. From the data of Table 5.9, it is revealed that interactions of extractant × temperature have largest influence on response as p-value is 0.000. The interaction extractant × time may be treated as having moderate influence on response as p-value is observed as 0.108. To establish the fact, DOE has proceeded in a reasonably good manner; normal probability plot of residuals is shown in Fig. 5.14. Because the data points roughly follow the straight line, it is concluded that the data are from a normally distributed population.

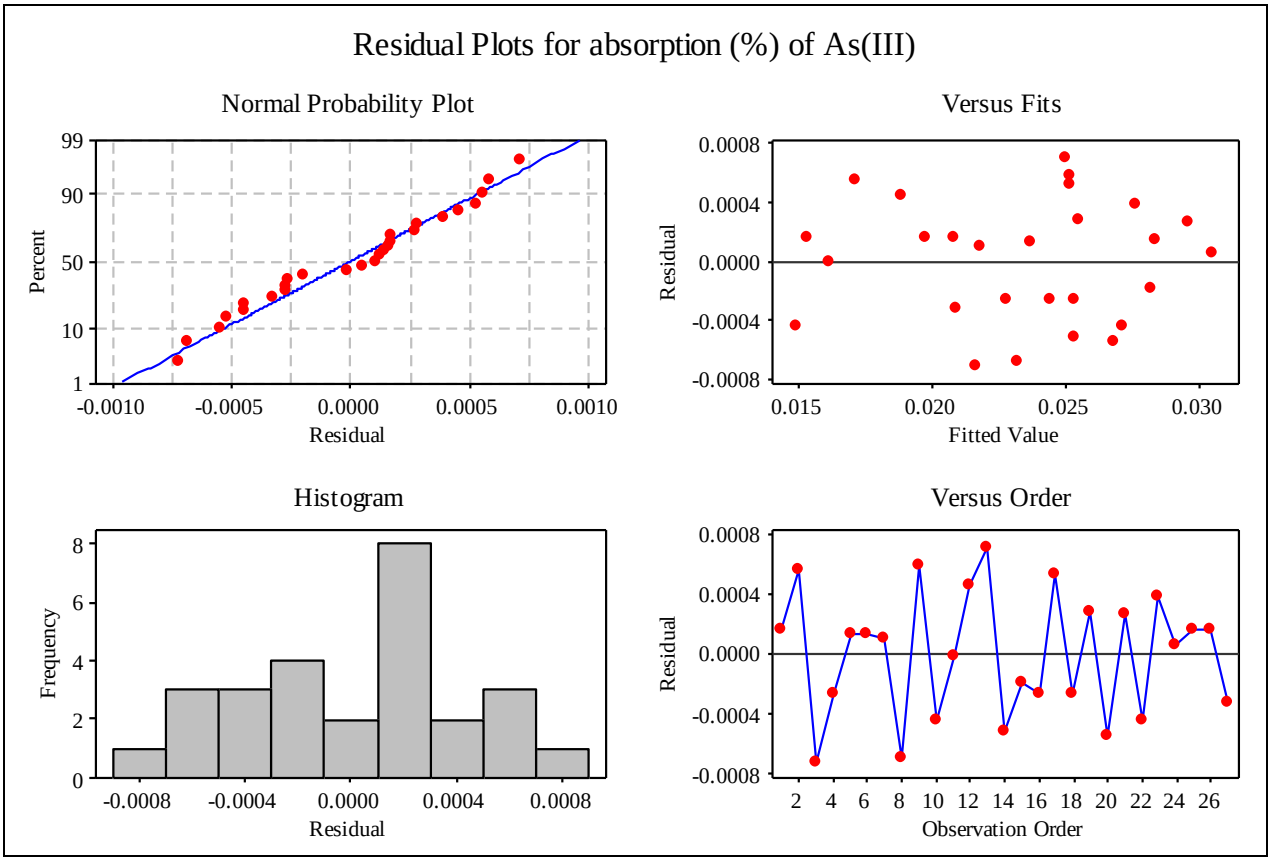


Fig. 5.14. Residual plots for arsenic (III) extraction efficiency from *Eichhornia crassipes* shoot biomass.

5.1.5.2. Stability of arsenic species during the extraction procedure

Eichhornia crassipes shoot biomass was spiked with mixture of four arsenic species [As (III), As (V), DMA and MMA] and was taken through the extraction procedure as described. 0.15 g of the

plant material was weighed and 500 µL of a stock solution containing 1000 μgL^{-1} of the species and 9500 µL of the extracting solution were added. The extracts were subjected to only one heating temperature (60 °C) and heating duration of 25 min. The experiments are conducted plant materials, the 3 extractants (water, modified protein extracting solution, and 10% (v/v) TMAH) separately. After microwave heating, the samples were allowed to cool and were filtered through Whatman 42 filter paper. The arsenic species were measured by HPLC-ICP-MS. Before injection, the samples were filtered through a 0.45 µm low protein binding Durapore disposable syringe filter. Before injecting TMAH solutions into the chromatographic column, the pH was adjusted to 5.0 by adding 0.1 mL concentrated acetic acid and 0.133 mL acetate buffer solution to 1 mL of sample. Chromatogram of a solution containing a mixture of As(III), As(V), DMA and MMA standards at concentration of 25 μgL^{-1} each and presented in Fig. 5.15.

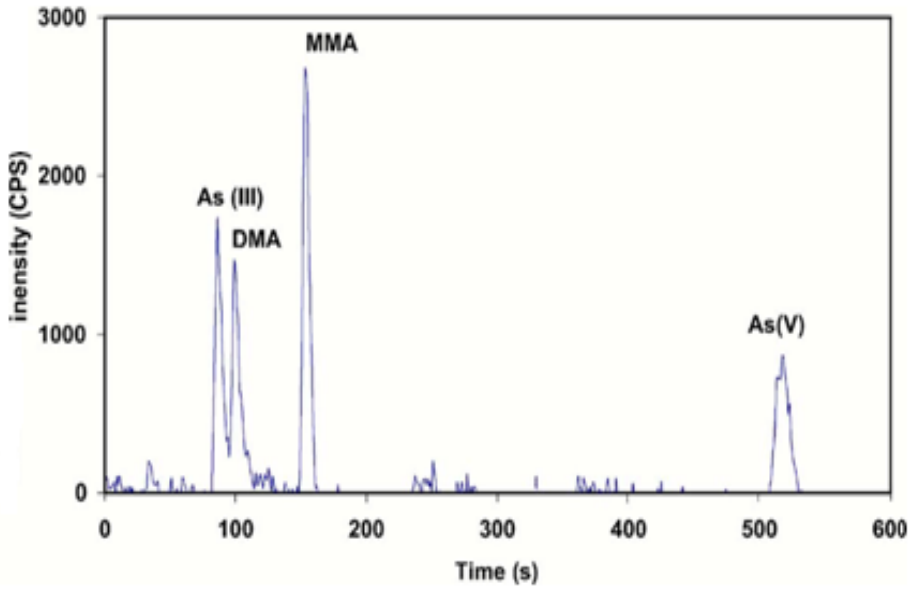
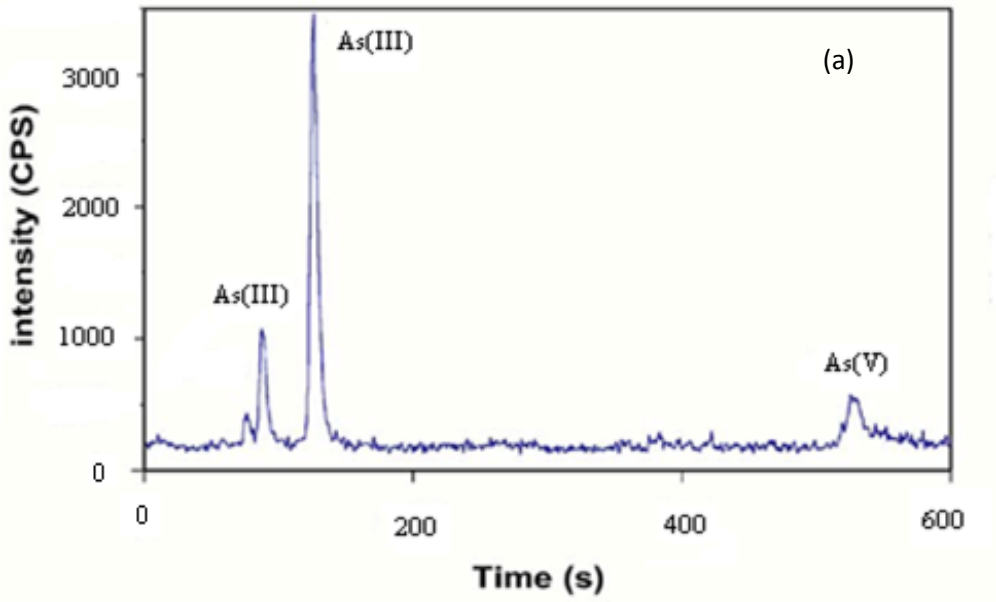


Fig. 5.15. Chromatogram of a solution containing a mixture of As(III), DMA, MMA and As(V) standards at concentrations of 25 µg AsL^{-1} each.

Arsenic species were separated on the Hamilton PRP x 100 anion-exchange column. In present study, the plant material was used to evaluate the stability of arsenic species during the extraction procedure. Heitkemper *et al*. 2001 extracted 97 or 23% of total arsenic from two different rice grain samples using methanol by accelerated solvent extraction. Francesconi *et al.* 2002 extracted 22-93% of total arsenic from different fern parts (fronds, petioles and rhizoids) which contained various amounts of arsenic (88-5230 mg kg^{-1}) using water in combination with

sonication. Therefore, the method adopted in this study was tested on shoot biomass of *E. crassipes* containing 32.1 ± 0.05 $mgkg^{-1}$ arsenic (III) ions. Extraction of arsenic from plant materials using three extractant solutions: (i) Extracted by 10% (v/v) tetramethylammonium hydroxide (TMAH) with yield of 95.14%, (ii) Extracted by double deionized water with yield of 87.24% and (iii) Extracted by a modified protein extracting solution with yield of 88.92%. Chromatograms are obtained for arsenic species in plant shoot biomass with modified protein extraction and TMAH extract solution by using HPLC-ICP-MS, and are shown in Fig. 5.16a and Fig. 5.16b, respectively**.** *Eichhornia crassipes* consisted only inorganic arsenic species as it is indicated from the above figures. Arsenic (III) are present in maximum quantity, arsenic (V) in minimum quantity and the organic arsenic like monomethylarsonic (MMA) and dimethylarsinic acid (DMA) are absent. Recent studies have described the formation of As-phytochelatin complexes in several terrestrial plants upon exposure to arsenate (Sneller *et al*., 1999; Hartley-Whitaker *et al*., 2002; Schmoger *et al*., 2000; Aydina and Soylaka, 2010; Ambushe *et al*., 2009). Phytochelatins (PCs) are thiol (SH) - rich peptides derived from glutathione (GSH) and are considered to be involved in the mechanism of detoxifying heavy metals in higher plants. Arenic (III) readily forms complexes with thiol groups which is supported by the results.



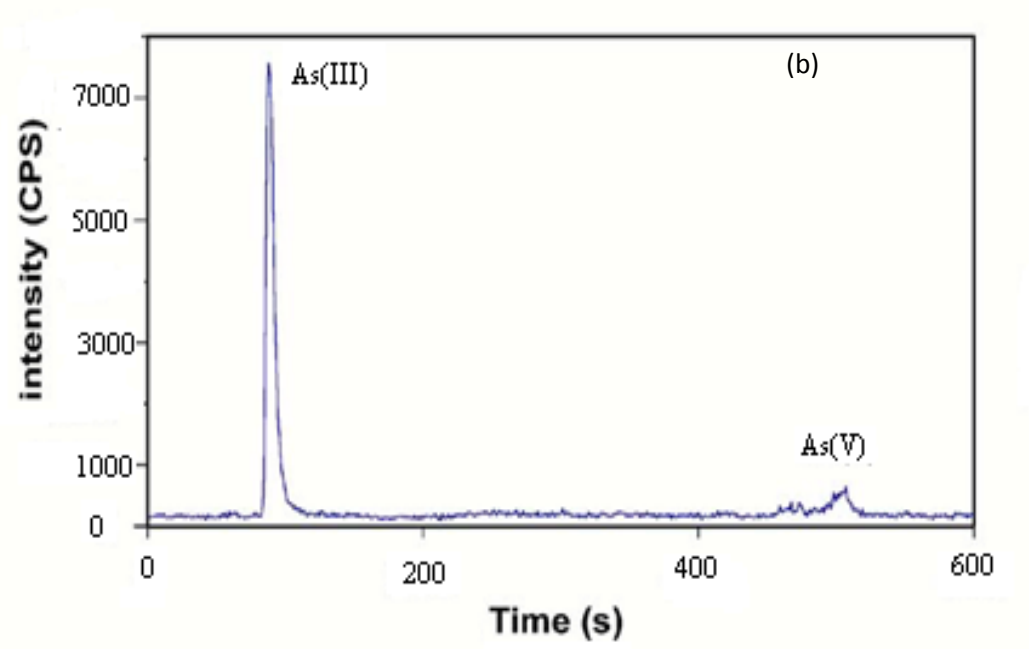


Fig. 5.16. Hamilton PRP-X100 anion-exchange column Chromatogram of (a) a modified protein extraction solution, and (b) TMAH extract of freeze-dried *Eichhornia crassipes* shoot biomass

5.1.5.3. Extraction efficiency of chromium ions

In this work, microwave assisted extraction as a method of sample preparation for determination of a range of chromium ions in plant samples was studied. Extraction of 260 ± 0.05 $mgkg^{-1}$ chromium (VI) ions from *Eichhornia crassipes* shoots biomass using 0.02 M ethylenediaminetetraacetic acid (EDTA), deionized water and hydrochloric acid. The percentage of chromium extracted with respect to temperatures and time durations are presented in Fig. 5.17, Fig. 5.18 and Fig. 5.19**,** respectively. The extraction of chromium ions efficiency increased with increasing temperature for all the extranct: ethylenediaminetetraacetic acid (EDTA), deionized water and hydrochloric acid with maximum extraction efficiency at 60 ° C for a time duration of 15 min. EDTA extracted around 97% of the chromium ions from *Eichhornia crassipes* shoot biomass at 60 °C, which was significantly compared to deionized water (88.22%) at the same temperature. The extraction time significantly affected chromium extraction efficiencies (Table 5.10).

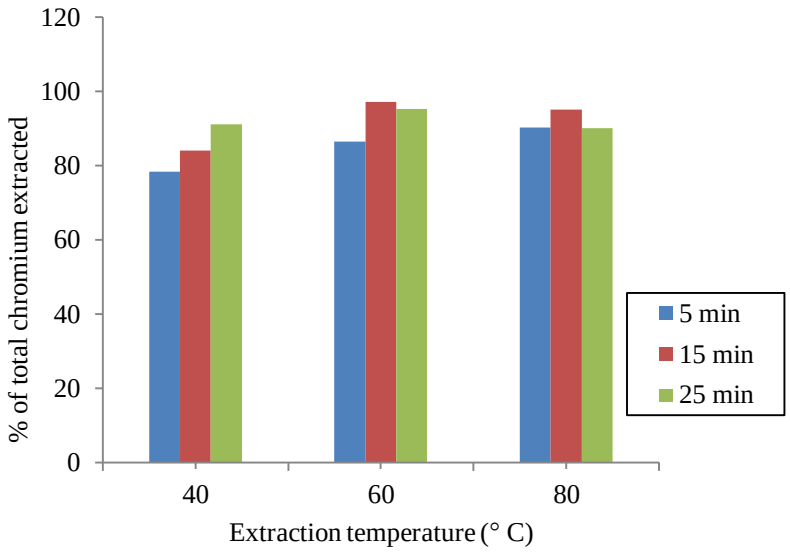


Fig. 5.17. Total chromium extraction efficiency for *Eichhornia crassipes* shoot biomass using 0.02 M EDTA solutions at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

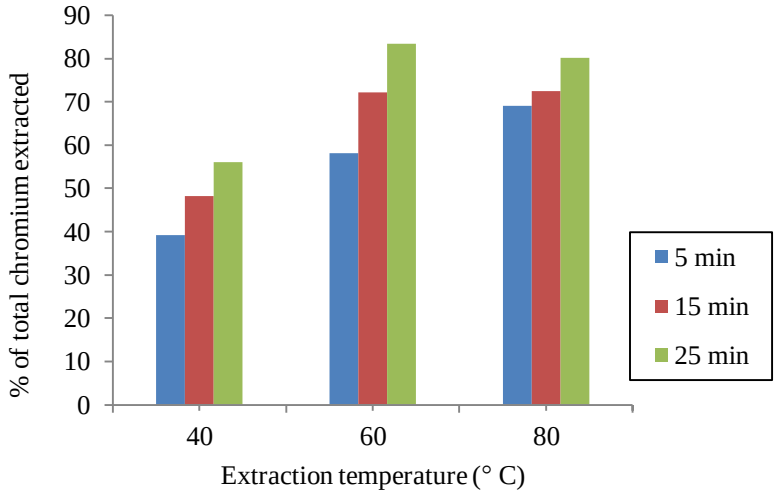


Fig. 5.18. Total chromium extraction efficiency for *Eichhornia crassipes* shoot biomass using deionized water at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

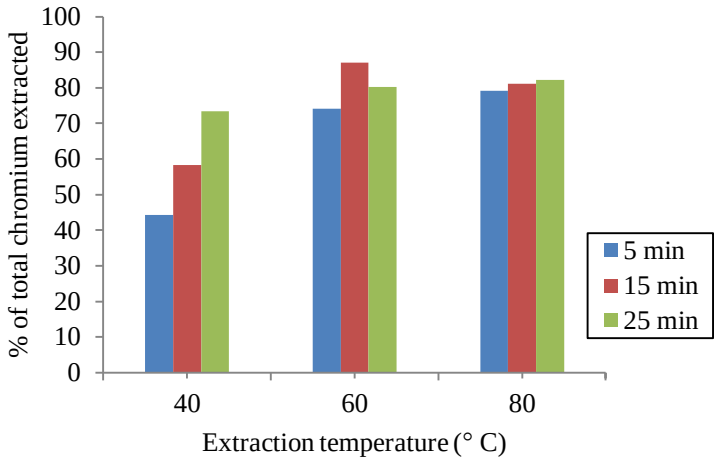


Fig. 5.19. Total chromium extraction efficiency for *Eichhornia crassipes* shoot biomass using HCl solution at different temperature and times. Data represents the mean ± S.D (n=3, "n" stands for the number of experiment replicates.)

Table 5.10. Analysis of variance of chromium extraction efficiency for *Eichhornia crassipes* shoots biomass with extractant, heating temperature and heating duration as main factors.

Source	Degree of Freedom (df)	Sum of Squares (SM)	Adj. Sum Squares (SM)	Adj. Mean Squares MS = SS/df	F-Statistics	P-value
Extractant	2	0.0121410	0.0121410	0.0060705	80.38	0.000
Temperature	2	0.0125336	0.0125333	0.0062668	82.97	0.000
Time	2	0.0070470	0.0070470	0.0035235	46.65	0.000
Extractant xTemp.	4	0.0067935	0.0067935	0.0016984	22.49	0.000
Extractant xTime	4	0.0008996	0.0008996	0.0002249	2.98	0.088
Temp. xTime	4	0.0005002	0.0005002	0.0001250	1.66	0.252
Error	8	0.0006042	0.0006042	0.0000755		

Total	26	0.0405191				

Longer extraction times improved chromium recoveries in ethylenediaminetetraacetic acid (EDTA) and deionized water at 60 °C, but HCl at 80 °C. However, the extraction time did not significantly affect extraction efficiencies at 80 °C in any of the 3 extractants (Table 5.10). The statistical analysis of efficiency differences between various extractants, extraction temperatures and durations are presented in Table 5.10. The results from the above table indicate that there is a significant variation in the results. In order to validate statistical importance of each influencing parameter on response, analysis of variance has been conducted and the data are presented in Table 5.10 at confidence level of 95% for chromium (VI). It is observed from the table that the factors extractant, temperature and time are statistically significant as the p-value is less than 0.05. From Table 5.10, it is revealed that interactions of extractant × temperature have largest influence on response as p-value is 0.000. The interaction extractant × time may be treated as having moderate influence on response as p-value is observed as 0.088. To establish the above fact, DOE has proceeded in a reasonably good manner; normal probability plot of residuals is shown in Fig. 5.20**.** Because the data points roughly follow the straight line, one can conclude that the data are from a normally distributed population.

5.1.5.4. Stability of chromium species during the extraction procedure

Chromium exists primarily in two forms, trivalent and hexavalent. Trivalent chromium is present in cationic forms as Cr^{+3} and is an essential nutrient, but hexavalent chromium is toxic and exists as an anion, either as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$). A common problem with chromium speciation is the well-known interconversion of Cr^{+3} and Cr^{+6}. The focus of this work is to explore chromatographic and instrumental analysis and parameters necessary to distinguish Cr^{+3} from Cr^{+6} in samples using HPLC-ICP-MS (Wolf *et al*., 2007; Sheehan *et al*., 1992). 1000 mg/L stock solutions for chromium standards for Cr^{+3} and Cr^{+6} were prepared from [$Cr(NO_3)_3.9H_2O$ and $[(NH_4)_2Cr_2O_7]$ respectively. Intermediate 2 mg/L solutions of each species were prepared in deionized water. Standards of different chromium concentrations were made by appropriate dilution of the intermediate standards with mobile phase. Fig. 5.21 shows a chromatogram of a mixture of Cr^{+3} and Cr^{+6} (10µg/L each) acquired while monitoring ions.

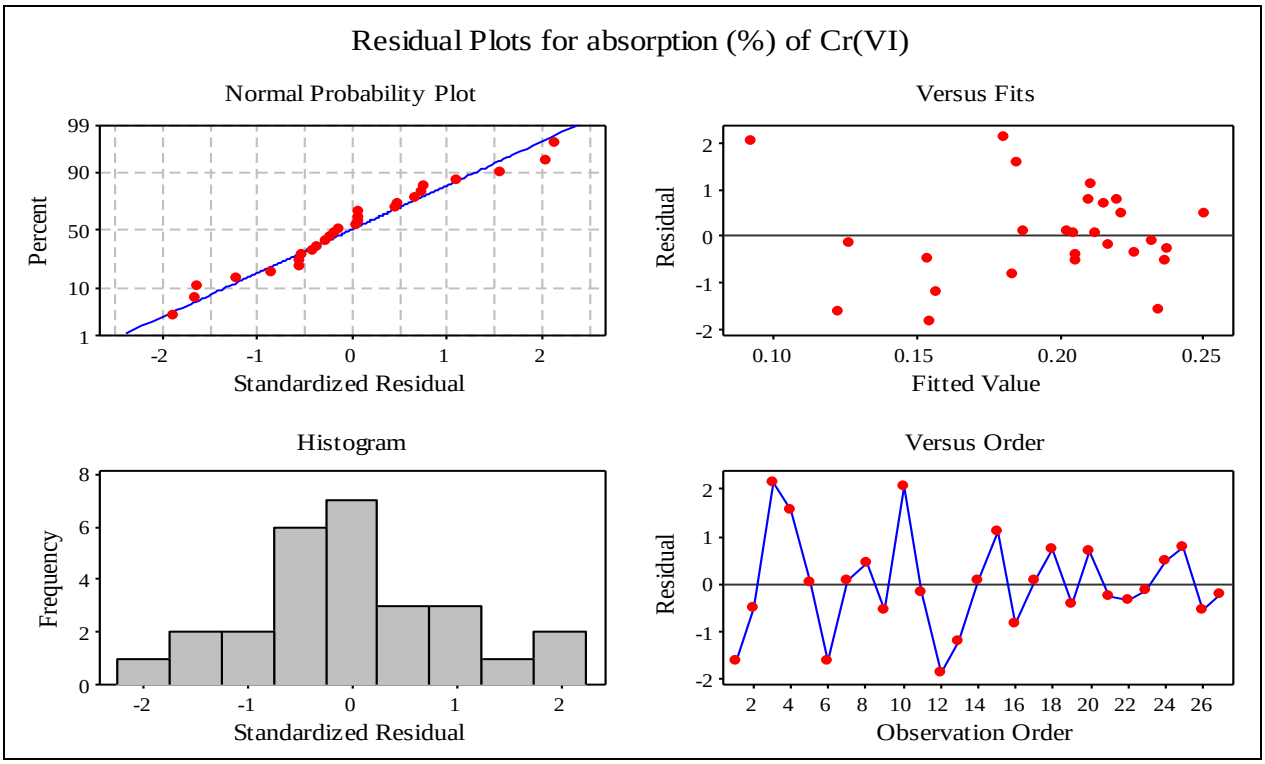


Fig. 5.20. Residual plots for chromium (VI) extraction efficiency from *Eichhornia crassipes* shoot biomass.

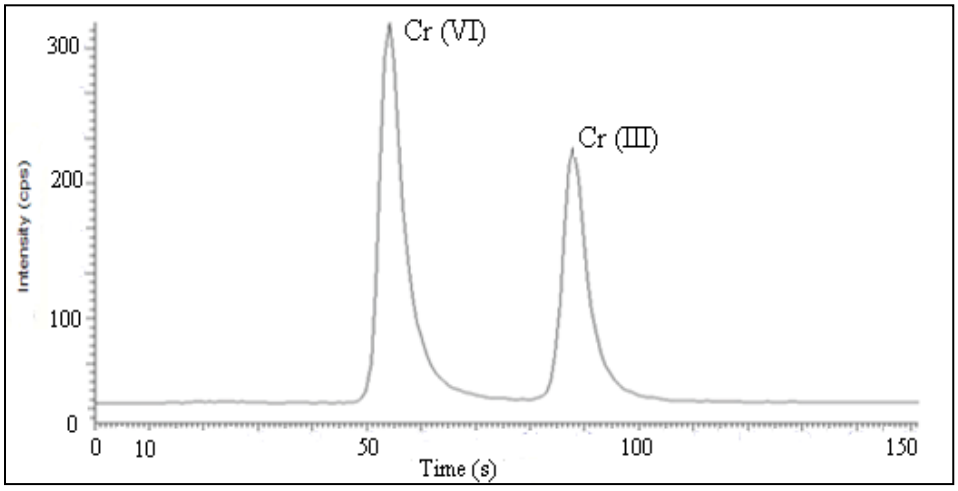
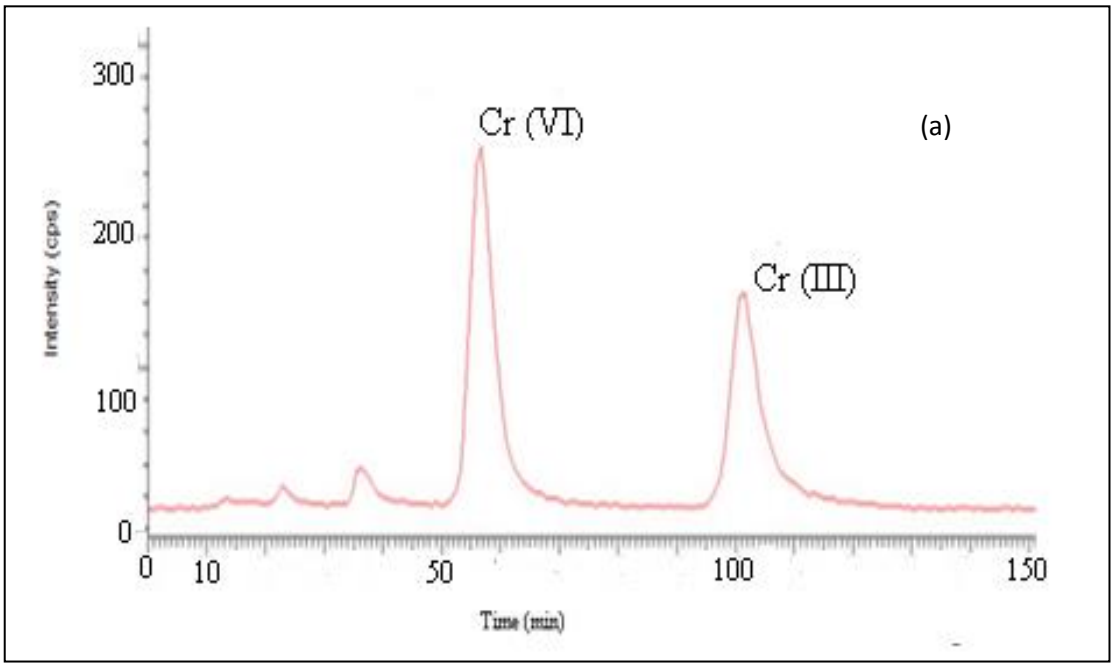


Fig. 5.21. Chromatogram of a solution containing a mixture of Cr (III) and Cr (VI) standards at concentrations of 10 µg Cr L^{-1} each.

The separation is accomplished by interaction of the chromium species with the different components of the mobile phase. The Cr^{+3} forms a complex with the EDTA is retained on the

column and Cr^{+6} exist in solution as dichromate. The negative charge of the chromium-EDTA complex and the negative charge of the dichromate interact with positive charge of the tetrabutylammonium hydroxide (TBAH) (Wolf *et al*., 2007). Therefore, the method developed in this study was tested on shoot biomass of *Eichhornia crassipes* containing 260 ± 0.05 $mgkg^{-1}$ chromium (VI) ions. Extraction of chromium from plant materials using three extractant solutions: (i) Extracted by 0.02 M ethylenediaminetetraacetic acid (EDTA), with yield of 97.24%, (ii) Extracted by double deionized water with yield of 72.21% and (iii) Extracted by HCl solution with yield of 87%. All extraction was done in similar condition at 60 °C and duration of 15 minutes. Chromatograms are obtained for chromium species in plant shoot biomass 0.02 M ethylenediaminetetraacetic acid (EDTA) and HCl extract solution by using HPLC-ICP-MS, and are shown in Fig. 5.22a and Fig. 5.22b, respectively**.** The results in figures clearly indicate that chromium species in the *Eichhornia crassipes* consisted only Cr^{+3} and Cr^{+6}, chromium (VI) are present in maximum quantity compare with chromium (III) ions.



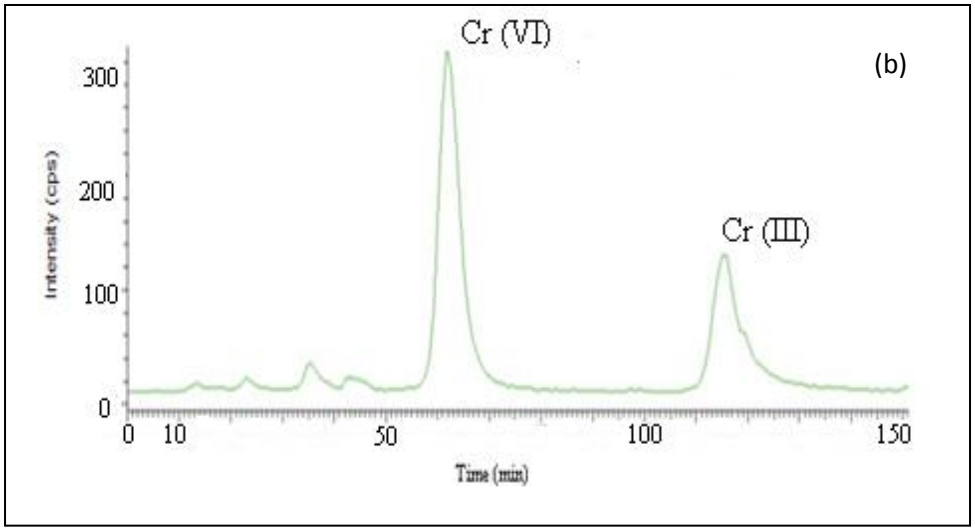


Fig. 5.22. Hamilton PRP-X100 anion-exchange column Chromatogram of (a) 0.02 M ethylenediaminetetraacetic acid (EDTA) and (b) HCl extract of freeze-dried *Eichhornia crassipes* shoot biomass.

5.1.6. Characterizations of *Eichhornia* crassipes shoot biomass before and after absorption of arsenic (III) and chromium (VI) ions.

5.1.6.1. SEM-EDX analysis

The surface morphology of *Eichhornia* crassipes shoot biomass without and with removal of arsenic (III) and chromium (VI) ions during absorption process was observed with the help of SEM-EDX (JOEL model JSM-6480LV, Japan) and presented in Fig. 5.23. It clearly reveals the surface texture and pores in the species without absorption of arsenic (III) and chromium (VI) ions. Fig. 5.24 and Fig. 5.25 show the morphological changes with respect to shape and size of the materials after absorption of arsenic (III) and chromium (VI) ions respectively. It can be clearly observed that the surface of materials shape has been changed into a new shiny bulky particle and whitish patches structure the adsorption of after arsenic (III) and chromium (VI) ions. The EDX spectra of arsenic (III) and chromium (VI) ions are shown in Fig. 5.23 and arsenic (III) and chromium (VI) loaded of extract materials are shown in Fig. 5.24 and Fig. 5.25, respectively. So, it was concluded that, arsenic (III) and chromium (VI) ions were adsorbed on the surface of the extract materials. These results are further confirmed with the results of FTIR spectra analysis.

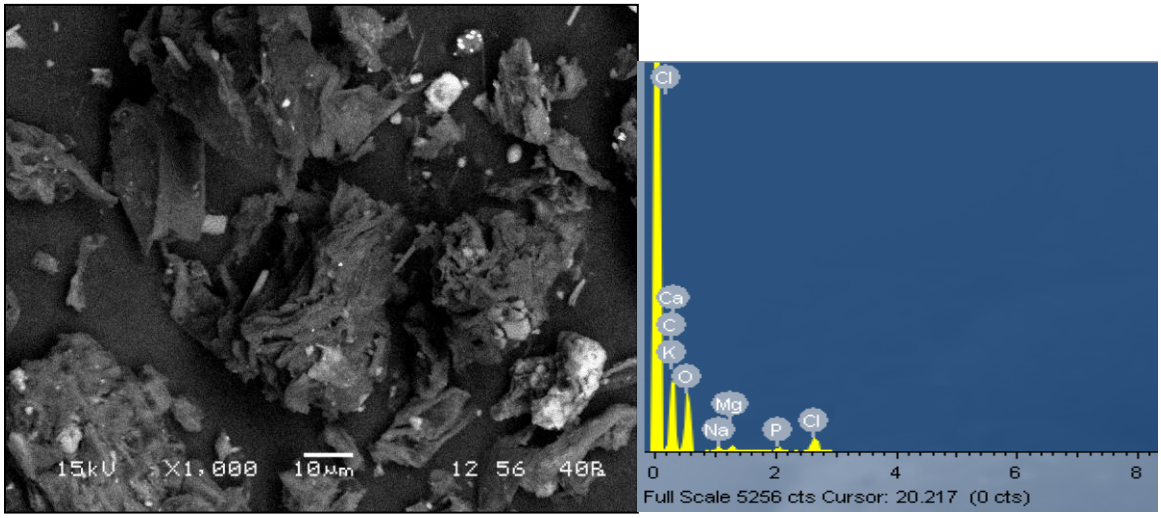


Fig. 5.23. SEM-EDX images of *Eichhornia crassipes* shoot biomass without absorption of arsenic (III) and chromium(VI) ions.

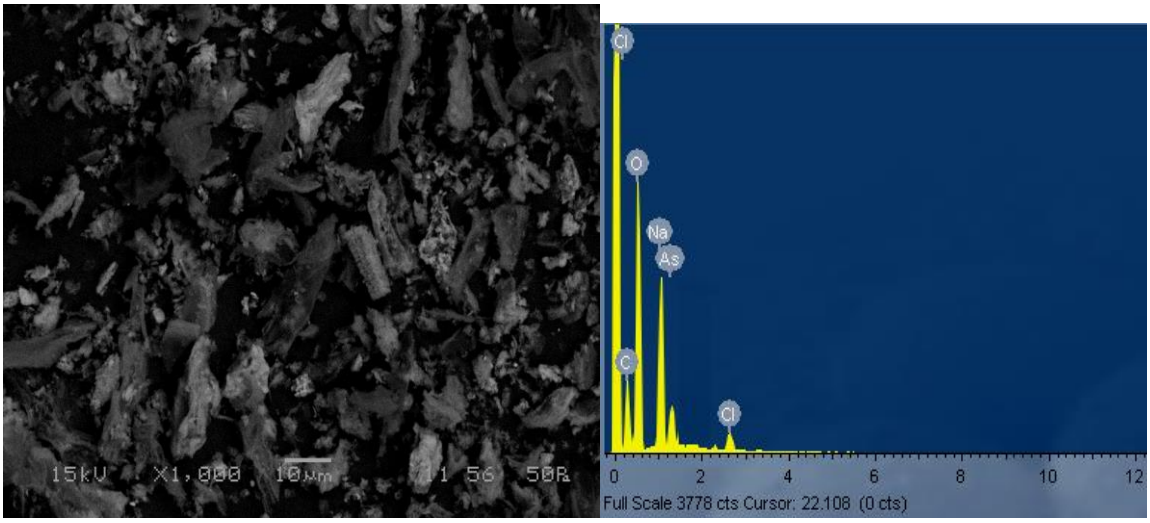


Fig. 5.24. SEM-EDX images of *Eichhornia crassipes* shoot biomass with absorption of arsenic(III) ions.

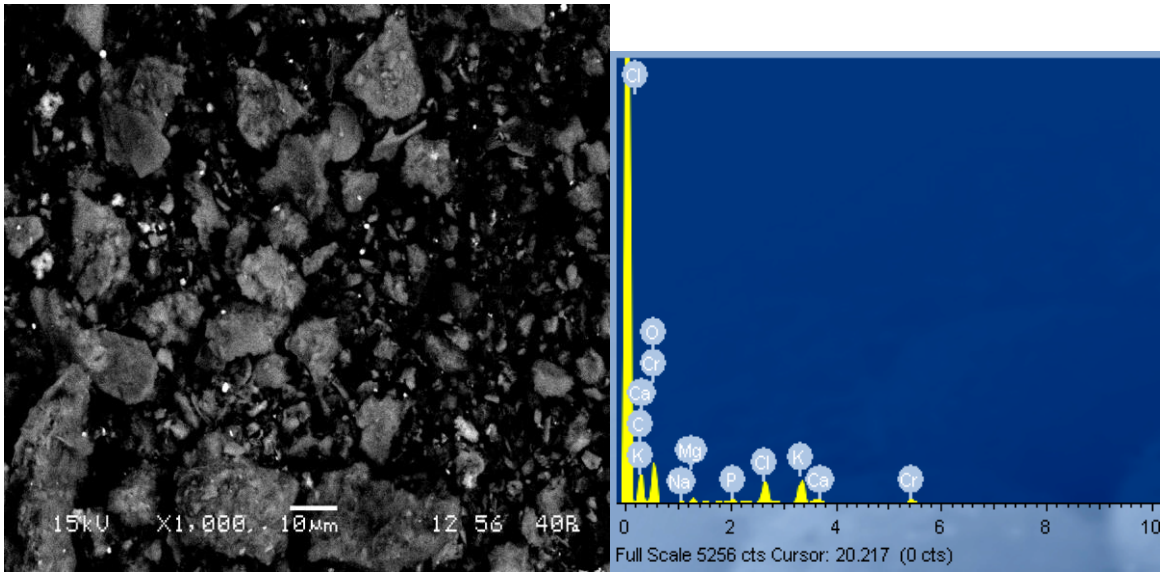


Fig. 5.25. SEM-EDX images of *Eichhornia crassipes* shoot biomass with absorption of chromium(VI) ions.

5.1.6.2. FTIR analysis

Infrared spectra (Perkin Elmer Spectrum RX-I) of the *Eichhornia crassipes* shoot biomass without arsenic and chromium ions loaded are obtained to determine which functional groups may have contributed to the arsenic and chromium ions adsorption are presented in Fig. 5.26. The FTIR spectra of the shoot biomass loaded with arsenic ions are presented in Fig. 5.27 which displays a number of absorption peaks, indicating the complex nature of the biomass. The spectra of loaded with arsenic and without are compared and found the following shift is observed in spectra. The spectra of extract materials exhibits a broad absorption band at 3225.78 cm^{-1} due to bonded –OH stretching vibration which is shifted to 3195.91 cm^{-1} may be due to complexation of –OH groups with metal. The band at 2,918.50 cm^{-1} has been shifted insignificantly. The new peak at 2,351.75 cm^{-1} may be due to the complexation of –SH group with arsenic ions (Coates, 1996). The next absorption peak at 1,638.96 cm^{-1} may be due to the presence of amide group (N-H stretching and C=O stretching vibration) is shifted to higher frequency and appeared at 1,645.03 cm^{-1} may be due to the complexation of amide group with arsenic ions (Bang *et al*., 2005). Another peak at1, 319.75 cm^{-1} has been shifted insignificantly. Another shift was observed from 1,163.54 cm^{-1} to 1,169.27 cm^{-1} and 1,022.47 cm^{-1} to 1,023.65 cm^{-1} may be due the interaction of nitrogen from amino group with arsenic ions (Roddick-Lanzillota *et al*., 2002; Castaldi *et al*., 2010). The other weak absorption peak shifted from 780.91 cm^{-1} to 780.64 cm^{-1}

and 670.09 cm^{-1} to 668.44 cm^{-1} corresponding to the thiol or sulfhydryl group with arsenic ions (Coates, 1996). The above changes in the spectra may be attributed to the interaction of arsenic ions with the hydroxyl, amide, thiol and amino groups present on the shoot biomass.

The spectra of the chromium (VI) loaded biomass, when compared with that of without biomass represented in Fig. 5.28. There is a significant shift of few absorption peaks indicate the coordination of metal to biomass. The band at 2918.50 cm^{-1} has been shifted insignificantly. The peaks at 1645.17 cm^{-1} have been shifted to 1638.96 cm^{-1} may be due to the complexation of carboxylic group with Cr (VI). Another shift was observed from 1418.96 cm^{-1} to 1319.75 cm^{-1}, corresponding to the complexation of nitrogen with chromium from the N-H group. The next shift was observed from 1172.97 cm^{-1} to 1163.54 cm^{-1} and 1008.50 cm^{-1} to 1022.47 cm^{-1} may be due the interaction of nitrogen from amino group with chromium. The other weak absorption peak shifted from 632.40 cm^{-1} to 630.18 and 520.23 cm^{-1} to 522.34 cm^{-1}, corresponding to the O-C-O scissoring vibration of polysaccharide. The above changes in the spectra may be attributed to the interaction of Cr (VI) with the carboxyl, hydroxyl and amino groups present on the surface of the *Eichhornia crassipes* biomass. This clearly manifests the binding chromium to the biomass.

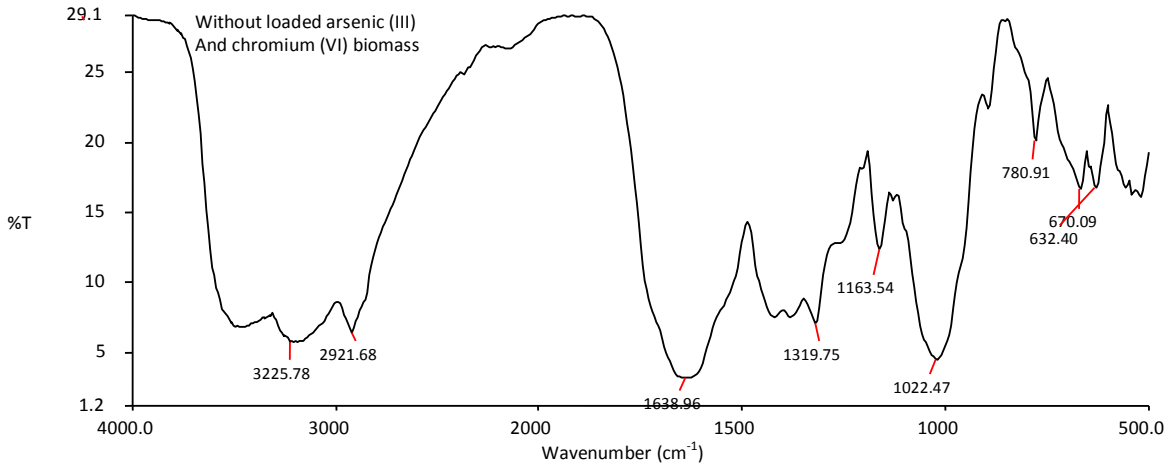


Fig. 5.26. FTIR spectra of *Eichhornia crassipes* shoot biomass without absorption of arsenic(III) and chromium(VI) ions.

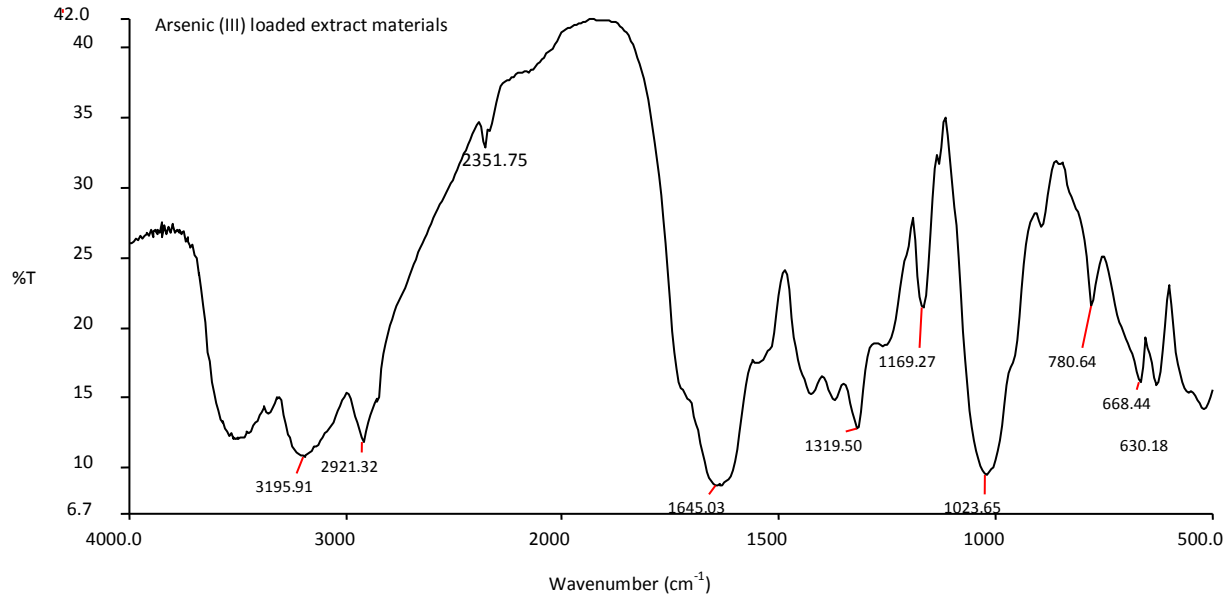


Fig. 5.27. FTIR spectra of *Eichhornia crassipes* shoot biomass with absorption of arsenic(III) ions.

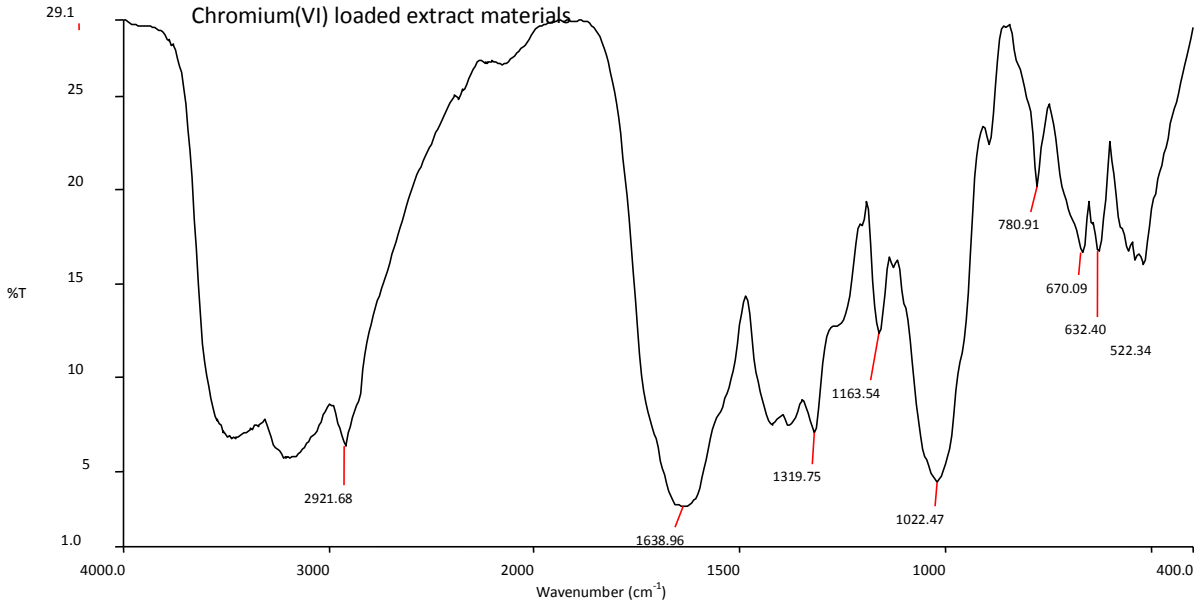


Fig. 5.28 FTIR spectra of the *Eichhornia crassipes* shoot biomass with absorption of chromium(VI) ions.

5.1.6.3. X-Ray diffraction analysis

XRD pattern of the arsenic and chromium extract materials is shown in Fig. 5.29 and Fig. 5.30, respectively. Few sharp peaks are observed indicating the sample is crystalline in nature. Fig.5.29 represents the XRD (JCPDS No- 37-1129) pattern of arsenic ions loaded plant materials. The phases of $AlAsO_4$, As_2O_3 and $As(OH)_3$ are found in the recovered arsenic ions. So it is concluded that, some of the arsenic ions are converted into $AlAsO_4$, and As_2O_3, some are converted into $As(OH)_3$ and finally get adsorbed over the surface of plant materials (Jia *et al*., 2007; Lim *et al*., 2009). Fig. 5.30 represents the XRD (JCPDS No- 34-0756) pattern of chromium ions loaded plant materials. The phases of $K_2Cr_2O_7$, $K_3Er\ (CrO_4)_3$, and Al_8Cr_4 Dy are found in the recovered chromium ions, So it is concluded that, some of the chromium ions are converted into $K_2Cr_2O_7$ and $K_3Er\ (CrO_4)_3$, some of the converted into Al_8Cr_4 Dy and finally get adsorbed over the surface of plant materials (Wang *et al*., 2006).

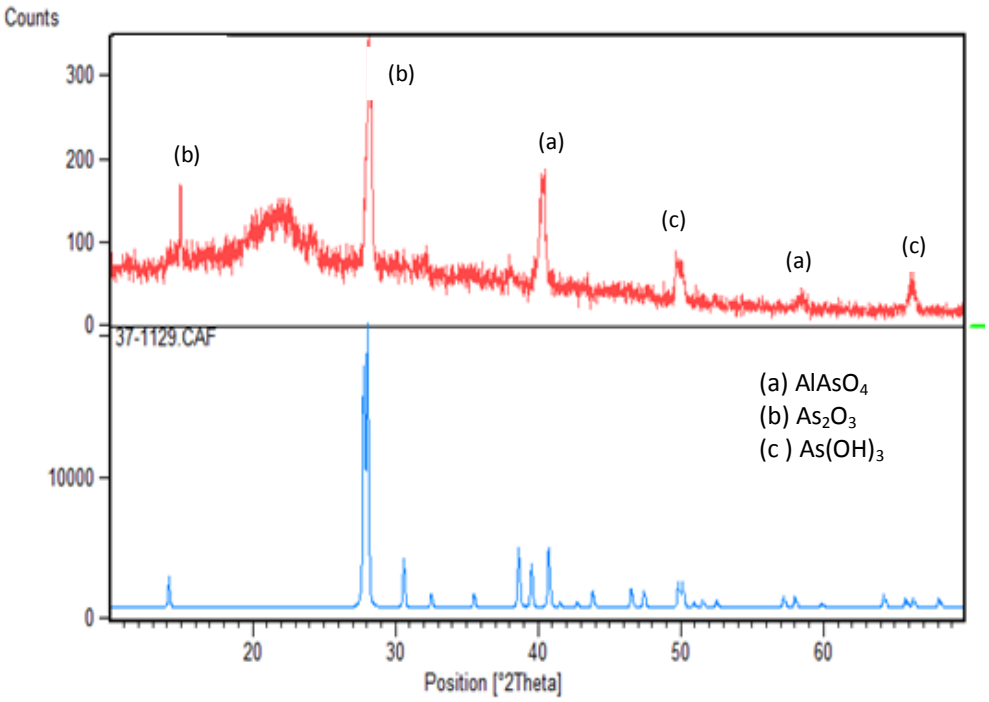


Fig. 5.29. XRD pattern of arsenic ions loaded materials.

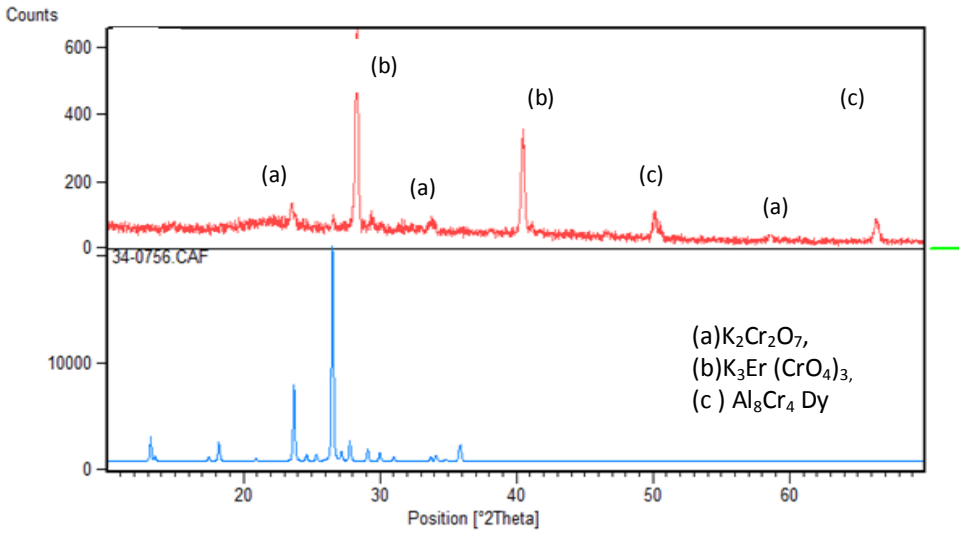


Fig. 5.30. XRD pattern of chromium ions loaded materials.

5.2. Biosorption of arsenic (III) and chromium (VI) ions by living cells of *Bacillus cereus* biomass using bioremediation techniques.

The arsenic(III) and chromium(VI) sorption experiments from its aqueous solution on living cells of *Bacillus cereus* were carried out using standard 1 mg/l, 5 mg/L and 10 mg/L As^{3+} and Cr^{6+} solution in absence of other competing ions. Optimum sorption conditions are determined in batch mode process as a function of pH, initial arsenite concentration, biomass loading, contact time, and temperature affecting the biosorption of arsenic (III) and chromium (VI). The biosorption experiments were carried out in a series of 100 mL Borosil conical flask with stopper by adding 0.5 - 15 g/L of the biosorbent in 50 mL of synthetic arsenic (III) and chromium (VI) solution in distilled water. Stoppers were provided to avoid change in concentration due to evaporation. All the biosorption experiments were carried out at ambient temperature (30 ± 2 °C).

5.2.1. Effects of biosorbent dosage on arsenic (III) and chromium (VI) removal

Biosorbent dose is an important parameter which determines the capacity of biosorbent for an initial concentration of the sorbate. The effect of biosorbent dose on the biosorption of arsenic (III) and chromium (VI) ions are studied at ambient temperature 30 ± 2°C and contact time of 60

min for initial arsenic(III) and chromium(VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L and the results are represented in Fig. 5.31 and Fig. 5.32, respectively.

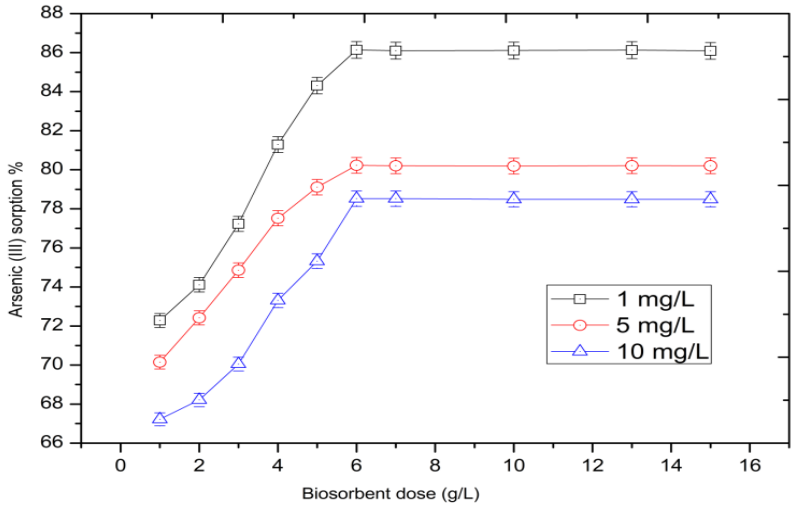


Fig. 5.31 Effect of biosorbent dose on the biosorption of arsenic (III) with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

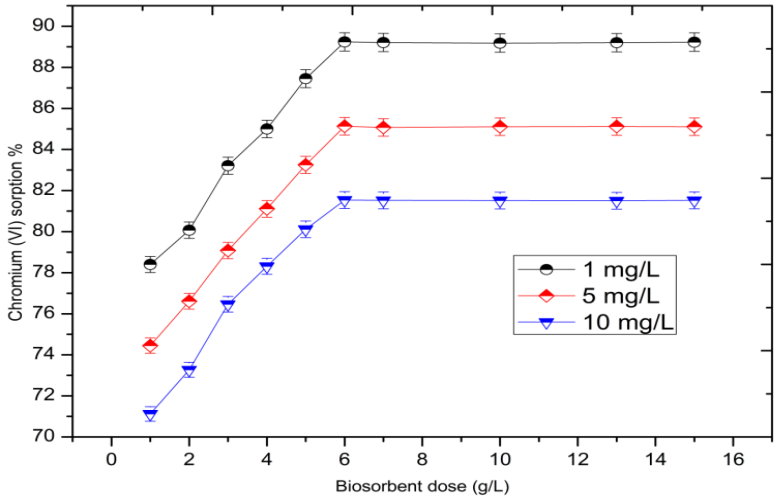


Fig. 5.32 Effect of biosorbent dose on the biosorption of chromium(VI) with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

Experimental results showed that with the increase in biosorpbent dose of living cells of *Bacillus cereus* from 1g/L to 6 g/L, the percentage removal of arsenic (III) increased from72.28 % to 86.14 %, 70.15% to 80.23% and 67.22% to 78.54% for 0.05 to 0.5 g/50 mL of synthetic solution of initial arsenic (III) concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively.

Similarly, with the increase in biosorpbent dose of living cells of *Bacillus cereus* from 1g/L to 6 g/L, the percentage removal of chromium (VI) increased from 78.40 % to 89.24 %, 74.45% to 85.13% and 71.12% to 81.54%, for 0.05 to 0.5 g/50 mL of synthetic solution of initial chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively. This result is expected because for a fixed initial biosorbent concentration, increase in total biosorbent doses provides a greater biosorption sites and increases the biosorption potential. Several researchers have reported similar results (Singh *et al*., 2012; Davis *et al*., 2003; Hussein *et al.*, 2004; Beolchini *et al*., 2009; Bai and Abraham, 2002; Qaiser *et al*., 2009; Kim *et al*., 2004; Radhika *et al*., 2006; Pokhrel and Viraraghavan, 2006). However it is observed that after 0.3g/50 ml of *Bacillus cereus* biomass, there is no significant change in percentage of removal of arsenic (III) and chromium (VI) , may be due to the higher dosage could produce a 'screening effect' on the cell wall, protecting the binding sites, causes lower arsenite and hexavalent chromium sorption. So, 0.3 g of *Bacillus cereus* biomass in 50 mL of arsenic (III) and chromium (VI) solution was considered as optimum dose and was used for further study. The initial and final pH of the solutions was measured after biosorption using optimum biosorbent dose and the results are presented in Table 5.11.

Table 5.11. Initial and final pH of arsenic(III) and chromium(VI) solution of 1 mg/L, 5 mg/L and 10 mg/L concentration in biosorption process.

Initial concentration in (mg/L)	Arsenic(III) biosorption		Chromium(VI) biosorption	
	Initial pH	Final pH	Initial pH	Final pH
1	7.51	7.21	7.50	6.25
5	7.53	7.28	7.54	6.20
10	7.52	7.30	7.51	6.22

It was observed that there was slight decrease in pH of the solution after treatment. It is due exchange of H^+ ion from biomass by arsenic and chromium ion, which increase the H^+ ion concentration in the solution thereby decreasing the pH. The maximum percentage removal of arsenic (III) by *Bacillus cereus* biomass was found to be 86.14 %, 80.23%, and 78.54% for initial concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively. Whereas the maximum percentage removal of chromium(VI) by *Bacillus cereus* biomass was found to be 89.24 %, 85.13% and 81.54%, for initial concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively.

5.2.2. Effects of pH on arsenic (III) and chromium (VI) removal

The pH is one of the important factors affecting the biosorption process and thus the role of hydrogen ion concentration on the biosorption was studied with initial arsenic (III) and chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L of synthetic solution at different pH ranges of 2.5 to 9.5 (2.5, 3.5, 4.5, 6.5, 7.5, 8.5 and 9.5) with prepared biosorbents dose of 0.3 g of *Bacillus cereus* in 50 mL at an ambient temperature of 30 ± 2°C and contact time 60 minutes by batch mode. The results of the above studies are presented in Fig. 5.33 for arsenic (III) and Fig. 5.34 for chromium (VI), respectively. The percentage removal of arsenic (III) was found to increase from 38% to 76%(10mg/L), 43% to 82%(5mg/L), 45% to 86%(1mg/L) and increased from 53% to 80%(10mg/L) , 55% to 85%(5mg/L) ,58% to 89%(1mg/L) for chromium (VI) by *Bacillus cereus* biomass, with the variation of pH from 2.5 to 9.5. The results in Fig. 5.33 clearly indicate that the sorption efficiency was increased with the increase of pH from 2.5 to 7.5 than decreases slightly with further increase in pH up to 9.5 for arsenic (III). The results in Fig. 5.34 clearly indicate that, the sorption efficiency was increased with the increase in pH from 2.5 to 7.5 than remain almost constant with further increase in pH up to 10.5 for chromium (VI).

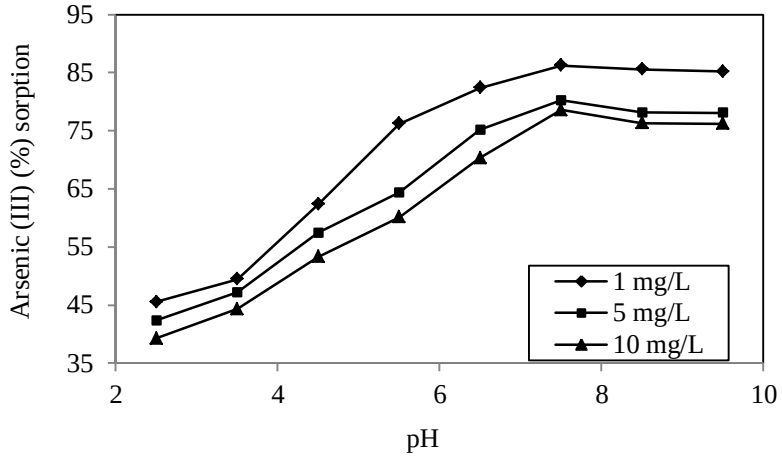


Fig. 5.33 Effect of pH on the biosorption of arsenic (III) ions with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

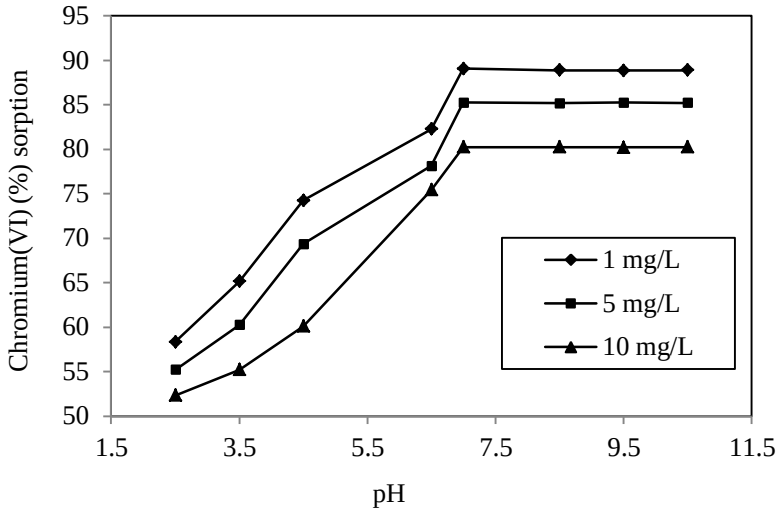


Fig. 5.34 Effect of pH on the biosorption of chromium (VI) ions with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

At low pH (2.0-6.0), the surface of living *Bacillus cereus* biomass is highly protonated and as a result, a strong attraction exists between oxyanion and positively charged surface of the biomass (Boddu *et al*., 2008). The further decreases in arsenic (III) and chromium (VI) uptake with increase in pH (7.5 - 10.5) may be due to the fact that at higher pH, the substrate may be negatively charged by adsorbing hydroxyl ions on the surface or by ionization of very weak acidic functional groups of the living cells of *Bacillus cereus* biomass. A repulsive force may develop between the negatively charged surface and the anions. At lower pH, the process of regeneration predominates over the process of removal. The process of conversion of biosorbent into its H^+ form plays an important role leaving behind arsenic (III) and chromium(VI) ions in the aqueous solution (Costa *et al.* 2001). The above results are supported by decrease in pH of the solution. In the solution having pH above 7 the predominant species is $rO_4^{-\,-}$. A suitable mechanism is proposed which explains the decrease in pH during removal of arsenic (III) and chromium (VI) ions, which is in line with the mechanism proposed by Igwe *et al*., 2005 and Manning and Goldberg, 1997.

$$MOH + H_3AsO_3 \rightarrow MH_2AsO_3 + H_2O \qquad (5.2.2.a)$$

$$MOH + H_3AsO_3 \rightarrow MHAsO_3^- + H_2O + H^+ \qquad (5.2.2b)$$

$$MOH + H_3AsO_3 \rightarrow MAsO_3^{-2} + H_2O + 2H^+ \qquad (5.2.2c)$$

$$MOH + K_2Cr_2O_7 \rightarrow MCrO_4^- + H_2O + H^+ \text{ (5.2.2d)}$$

5.2.3. Mechanism of arsenic (III) and chromium (VI) removal

The mechanism of any biosorption process is an important component in understanding the process as well as to know the characteristics of the material which help to design a new biosorbent for future applications. A mechanism for the biosorption of arsenic (III) and chromium (VI) ions by ion-exchanger *Bacillus cereus* biomass has been proposed by taking the results obtained from the experimental investigations. At lower pH of the medium, surface sites are positively charged and, therefore, attract negatively charged arsenite ion and hexavalent chromium ion, by an electrostatic interaction process (Mashitah *et al*., 1999; Hansen *et al*., 2006). The materials under hydration, the *Bacillus cereus* biomass surface completes the coordination shells with the available OH group. On the variation of pH, these surface active OH groups may further bind or release H^+ where the surface remains positive due to the reaction:

$MOH + H_3O^+ \longrightarrow MOH_2^+ + H_2O$

Thus, when pH < 7.00, the overall arsenite and hexavalent chromium, sorption mechanism can be represented in three different forms: (i) electrostatic interaction between positively charged center (nitrogen, OH) and negatively charged arsenite and dichromate, in solution, (ii) electrostatic attraction between positively charged surface hydroxyl group and AsO_3^{3-} , $Cr_2O_7^{2-}$ and (iii) ion-exchange reaction between positively charged biomass center and AsO_3^{3-}, $Cr_2O_7^{2-}$.

$MOH + H_3O^+ + AsO_3^{3-}, Cr_2O_7^{2-} \longrightarrow MOH_2^{+\cdots\cdots} AsO_3^{3-}, Cr_2O_7^{2-} + H_2O$

(Electrostatic attraction)

$MOH + H_3O^+ + AsO_3^{3-}, Cr_2O_7^{2-} \longrightarrow M^{+\cdots\cdots} AsO_3^{3-}, Cr_2O_7^{2-} + 2H_2O$ (ion-exchange)

Further, when the pH of the medium remains relatively in a neutral range, (pH = 7.00), the arsenic (III), sorption onto the neutral biosorbent surface can be described by a ligand or ion exchange reaction mechanism, which is represented as:

$MOH + AsO_3^{3-}, Cr_2O_7^{2-} \longrightarrow M^{+\cdots\cdots} AsO_3^{3-}, Cr_2O_7^{2-} + OH^-$

The modeling of the specific sorption of AsO_3^{3-}, $Cr_2O_7^{2-}$ on any material surface depends on a number of external factors such as temperature, pH, AsO_3^{3-} , $Cr_2O_7^{2-}$ concentration, as well as the density of surface functional groups available for coordination. In light of the above mentioned mechanism of biosorption, it may be further noted that *Bacillus cereus* biomass showed

biosorption capacity at normal pH range of 6.5-7.5, which could be useful for commercial exploitation purpose.

5.2.4. Effect of contact time on arsenic (III) and chromium (VI) removal

Removal of arsenic (III) and chromium (VI) also depends on its contact time with the biosorbents. Biosorption of arsenic (III) and chromium (VI) at different contact time was studied for initial arsenite and hexavalent chromium concentration of 1 mg/L, 5 mg/L and 10 mg/L at pH 7.5 for arsenic(III) and chromium(VI) keeping all other parameters constant. Batch biosorption experiments were conducted with living cells of *Bacillus cereus* biomass at 30 ± 2°C by varying the contact time from 5 to 60 minutes (5min, 10 min, 20min, 30min, 40min, 50min and 60 min). The results are presented in Fig.5.35 and Fig. 5.36. It is clear from the Figure 5.35 and 5.36 that maximum removal takes place within few minutes and equilibrium is reached 30 min. The percentage removal with living cells of *B. cereus* biomass was found to increase from 47.32 % to 86.24%, 43.02% to 85% and 40% to 80.44% for 5 min to 60 min of contact time, for initial arsenic (III) concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively.

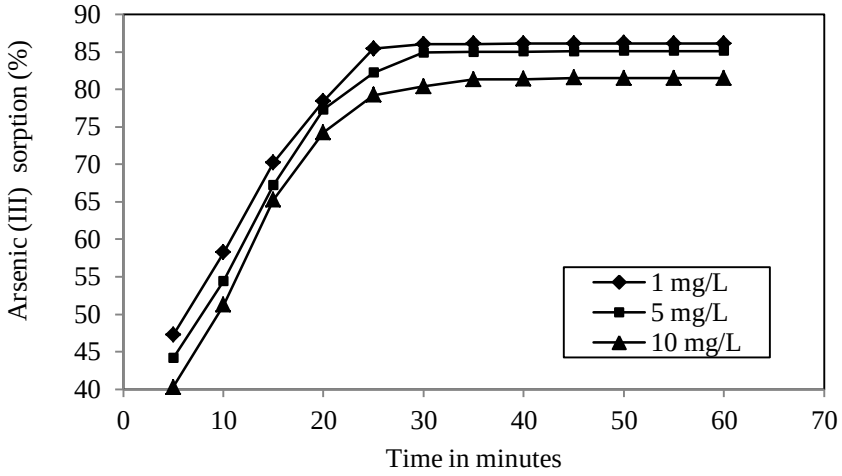


Fig. 5.35. Effect of contact time on the biosorption of arsenic (III) ions with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

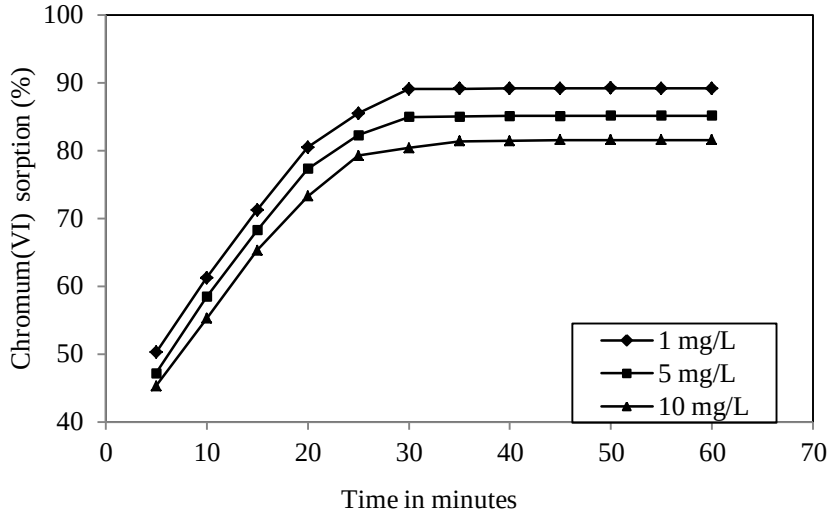


Fig. 5.36 Effect of contact time on the biosorption of chromium (VI) with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

And the percentage removal with living cells of *Bacillus cereus* biomass was found to increase from 50.11% to 90%, 45.33% to 85.32% and 43.16% to 80.11% for 5 min to 60 min of contact time, for initial chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively. The change in the rate of removal might be due to the fact that initially all sorbent sites are vacant and also the solute concentration gradient was high. As time passes, the number of sites on the sorbent filled up by the sorbate also increases. At equilibrium, when all the sites are filled, the rate of biosorption is equal to the rate of desorption. So, after equilibrium, it is found that there was no further increase in the removal of metal ion with increases in contact time. At higher concentrations, metals need to diffuse to the sorbent surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of arsenic (III) and chromium (VI) ions on the outer surface. In the present work, a steady increase in percentage removal was observed up to a contact time of 30 minutes, after that there wasn't any further increase in percentage removal with increase in time. The results in this study are also similar to the results reported in the removal of arsenic and chromium from aqueous solution by microorganisms (Kotiranta *et al*., 2000; Kang *et al*., 2007; Preetha and Viruthagiri, 2007; San and Dönmez, 2012), use of modified *A. niger* biomass (Pokhrel and Viraraghavan, 2006).

5.2.5. Biosorption kinetics

The prediction of biosorption rate gives important information for designing batch biosorption systems. The experimental data are applied to pseudo-first-order, pseudo-second-order and Intraparticle diffusion rate constant (Weber-Morris equation) models to clarify the sorption kinetics of arsenic (III) and chromium (VI) ions onto living cells of *Bacillus cereus* biomass. Biosorption of arsenic (III) and chromium (VI) ions are rapid for the first 30 minutes and its rate slowed down and it approaches towards equilibrium.

5.2.5.1. Lagergren's rate equation

Determination of efficiency of biosorption process requires an understanding of the kinetics of uptake of sorbate by biosorbent or the time dependence of the concentration distribution of the solute in both bulk solution and solid biosorbent and identification of the rate determining step. The study of kinetics of biosorption describes the solute uptake rate. The rate constants for the biosorption from arsenic (III) and chromium (VI) from aqueous solution on living cells of *B. cereus* biomass were determined using Lagergren's rate equation (Lagergren, 1898, Aksu, 2002).

Pseudo-first-order rate depends on the concentration of only one reactant. The rate constant K_1 for biosorption of arsenic (III) and chromium(VI) are studied by Lagergren rate equation for initial arsenite and hexavalent chromium concentration of 1 mg/L, 5 mg/L and 10 mg/L.

$$\log (q_e - q_t) = \log q_e - K_1 \left(\frac{t}{2.303}\right)$$

where q_e and q_t (both in mg/g) are the amounts of arsenic(III) and chromium(VI) ions sorbed at equilibrium (mg/g) and at time 't' (min), respectively, and k_1 is the rate constant of the equation (min^{-1}). The sorption rate constants (k_1) was calculated from the slope of the linear plot of log (q_e - $q_{t)}$ vs t and the results are presented in Fig. 5.37 and Fig 5.38, respectively**.** The values of R^2 (correlation coefficient) calculated shows good correlation. The biosorption rate constant (K_1), calculated from the slope of the above plot and standard error were presented for arsenic (III) and chromium (VI) in Table 5.12. The plots of log (q_e - $q_{t)}$ vs t for the pseudo-first-order model were almost linear, indicates the validity of Lagergren rate equation of first order kinetics (Prasad *et al*., 2011). The results of this investigation are matching well with the results obtained in the biosorption of arsenic (III) using rice polish (Ranjan *et al*., 2009), modified *A. niger* biomass (Pokhrel Viraraghavan, 2006), shelled *Moringa oleifera* (Kumari *et al*., 2006), and macrofungus (*Inonotus hispidus*) biomass (Sari and Tuzen, 2009). The results noted in biosorption of Cr (VI) on methylated yeast biomass (Seki *et al*., 2005), red, green and brown

seaweed biomass (Murphy *et al*., 2008), and acid-treated green alga *Oedogonium hatei* (Gupta and Rastogi, 2009) were also found to obey first order kinetics, comparable with the present investigation.

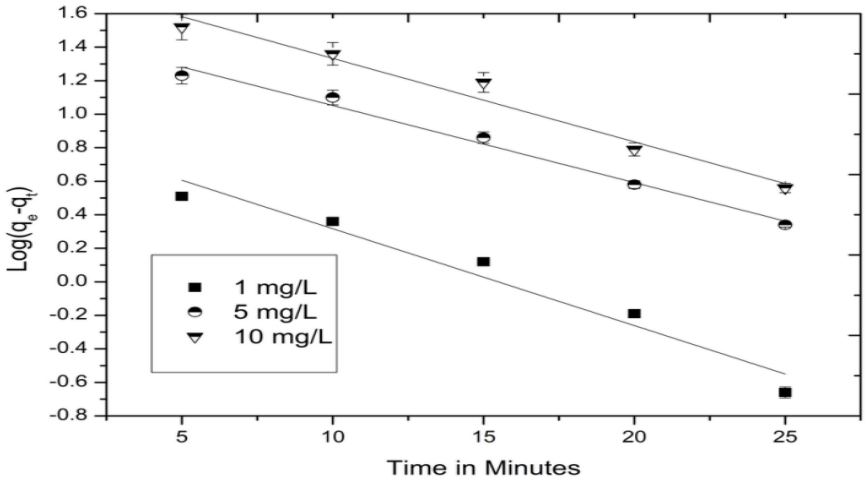


Fig. 5.37. Linear plot of Lagergren rate equation using living cells of *Bacillus cereus*, time vs. log (q_e-q_t) with initial arsenic (III) ions concentration of 1 mg/L, 5 mg/L and 10 mg/L.

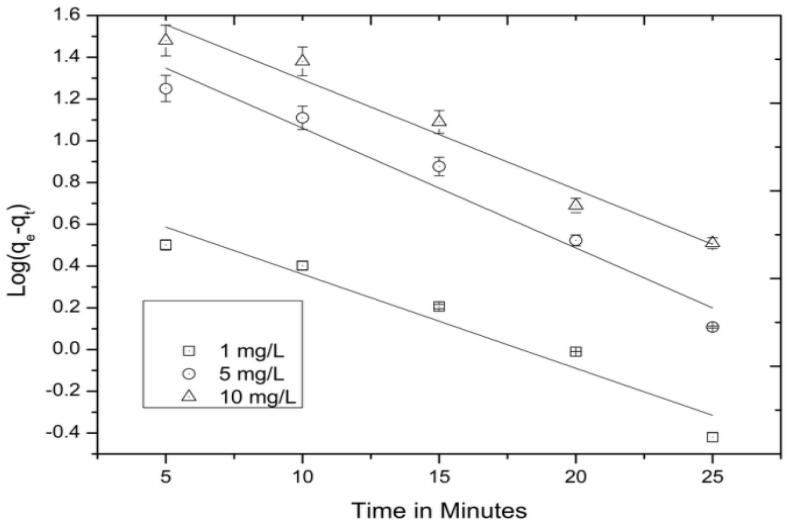


Fig. 5.38. Linear plot of Lagergren rate equation using living cells of *Bacillus cereus*, time vs. log(q_e-q_t) with initial chromium(VI) ions concentration of 1 mg/L, 5 mg/L and 10 mg/L.

5.2.5.2. Second order rate equation

Pseudo-second-order depends on the concentrations of one second-order reactant, or two first-order reactants. Experimental data were also tested by the pseudo-second-order kinetic model which is given in the following form (Ho and McKay, 1999; Singh *et al*., 2012):

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e}\ t$$

where k_2 (g/mg /min) is the rate constant of the second-order equation for sorption of initial arsenite and hexavalent chromium concentration of 1 mg/L, 5 mg/L and 10 mg/L, q_t (mg/g) is the amount of arsenic(III) and chromium(VI) sorped per unit gram of biosorbent (in mg/g) at time 't' and q_e is the maximum biosorption capacity (mg/g) for the second-order biosorption. The kinetic plots of t/q_t versus t for arsenic (III) and chromium (VI) sorption at ambient temperature (30 ± 2°C) are presented in Fig. 5.39 and Fig. 5.40. Values of K_2, q_o, R^2 and standard error were calculated from the plot of t/q_t versus t and the results are presented in Table 5.12. The correlation coefficient (R^2) for second order rate constant are in the range of 0.98-0.99 for the bisorption of the arsenic (III) and chromium (VI). The data obtained from the study at different time interval fits the second order rate equation better than the Lagergren's rate equation. Fig. 5.39 and Fig. 5.40 shows the linear plot of t/q_t vs t for the pseudo-second-order model is more likely to predict kinetic behavior of arsenic(III) and chromium(VI) sorption with chemical sorption being the rate-controlling step (Yan *et al*., 2010).

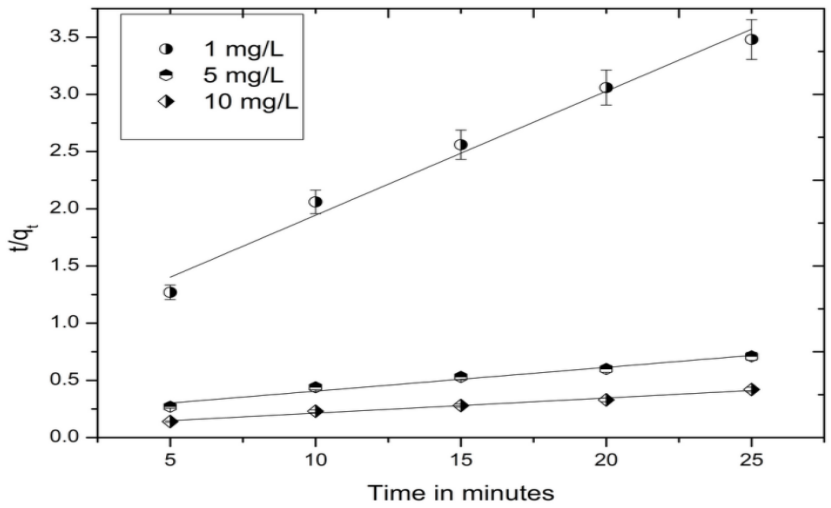


Fig. 5.39. Linear plot of second order rate equation using living cells of *Bacillus cereus*, time vs. t/q_t with initial arsenic (III) ions concentration of 1 mg/L, 5 mg/L and 10 mg/L.

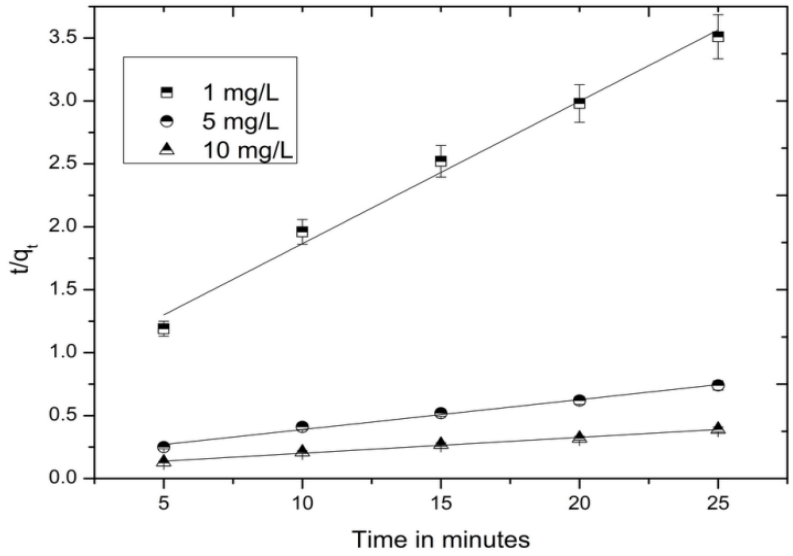


Fig. 5.40. Linear plot of second order rate equation using living cells of *Bacillus cereus*, time vs. t/q_t with initial chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L.

Table 5.12. Kinetic parameters from pseudo-first-order and pseudo-second-order for arsenic (III) and chromium(VI) ions biosorption onto living cells of *Bacillus cereus* at different initial concentration.

		Pseudo-first-order					Pseudo-second-order				
	Slope	Intercept	K_1 (min^{-1})	*SD*	R^2	Slope	Intercept	q_0 (mg/g)	K_2 (g/mg min)	*SD*	R^2
Initial As(III) concentration (mg/L)											
1(mg/L)	-0.165	0.154	0.3799	0.0067	0.957	0.034	0.03355	29.23	0.03488	0.0080	0.985
5(mg/L)	-0.214	0.326	0.4928	0.0030	0.986	0.085	0.03822	11.68	0.19179	0.0208	0.984
10(mg/L)	-0.194	0.361	0.4467	0.0050	0.971	0.134	0.03200	7.434	0.56548	0.0132	0.974
Initial Cr(VI) concentration (mg/L)											
1(mg/L)	-	0.217	0.3339	0.0064	0.942	0.026	0.02365	38.16	0.02908	0.0065	0.998

	0.145										
5(mg/L)	-0.102	0.126	0.2349	0.0066	0.962	0.081	0.02812	12.26	0.23674	0.0011	0.998
10(mg/L)	-0.155	0.068	0.3569	0.0055	0.967	0.167	0.0221	5.970	1.26903	0.0005	0.997

5.2.5.3. Intraparticle diffusion rate constant (Weber-Morris equation)

Due to rapid stirring in batch reactors, there is a possibility of insertion of arsenic (III) and chromium (VI) species from the bulk into pores of the biosorbent as well as biosorption at outer surface of the biosorbent. The rate-limiting step may be either biosorption or intraparticle diffusion. The possibility of arsenic (III) and chromium (VI) species to diffuse into the tenor sites of the biosorbents was tested with Weber-Morris equation given as follows: (Allievi *et al*., 2011; Inbaraj and Sulochana, 2002).

$$q_e = K_p t^{1/2} + C$$

Where q_e is the amount of arsenic (III) and chromium (VI) biosorbed in mg, K_p is the intraparticle diffusion rate constant and t is the time (agitation) in minutes. In order to study the diffusion process, batch biosorption experiments were carried out with *Bacillus cereus* biomass at 30 ± 2°C and at pH 7.5 with initial arsenic (III) and chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L respectively. The results are presented in the Table 5.13 and graphically shown in the Fig. 5.41 and Fig. 5.42. The rate constant (K_p) for intraparticle diffusion for various initial concentration of arsenic (III) and chromium(VI) solution, for the different biosorbents were determined from the slope of respective plots drawn between square root of time and amount of biosorbate biosorbed and the results are presented in Table 5.13**.** The intercept of the plot reflects the boundary layer effect. The larger the intercept greater the contribution of the surface adsorption in the rate controlling step. If the regression of q_t verses $t^{1/2}$ is linear and passes through the origin, the intraparticle diffusion is the sole rate limiting step.

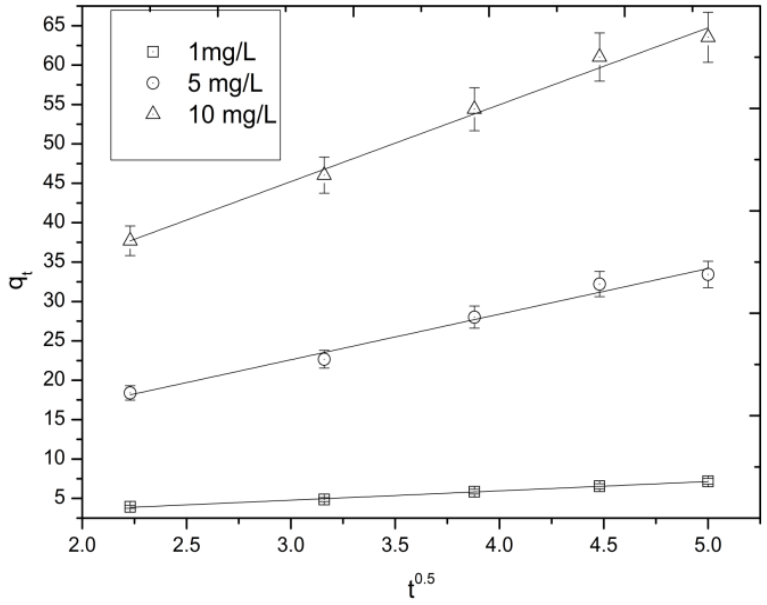


Fig. 5.41. Linear plot of Intraparticle diffusion rate equation using living cells of *Bacillus cereus*, $t^{0.5}$ vs. q_t with initial arsenic (III) ions concentration of 1 mg/L, 5 mg/L and 10 mg/L.

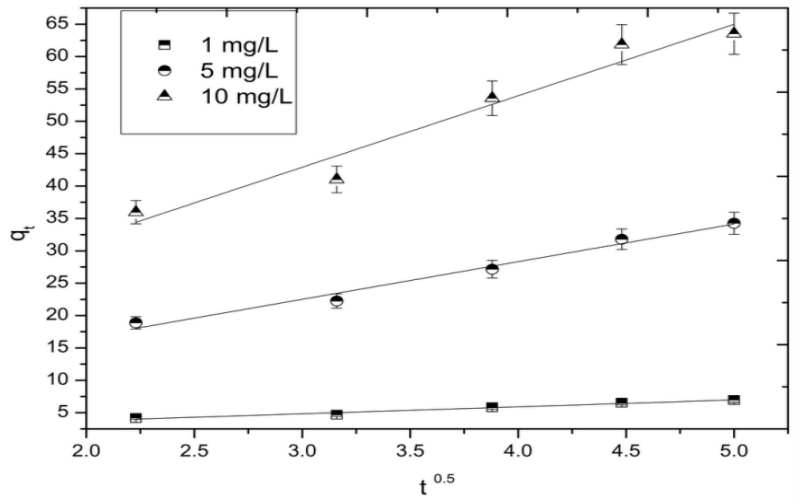


Fig. 5.42. Linear plot of Intraparticle diffusion rate equation using living cells of *Bacillus cereus*, $t^{0.5}$ vs. q_t with initial chromium(VI) ions concentration of 1 mg/L, 5 mg/L and 10 mg/L.

Table 5.13. Intraparticle diffusion rate constants obtained from Weber- Morris equation for different initial arsenic (III) and chromium (VI) concentration.

	Intraparticle diffusion rate constant

	Slope	Intercept	K_p (mgg^{-1} $min^{-0.5}$)	*SD*	R^2
Initial As(III) concentration (mg/L)					
1	0.1015	-1.5828	0.1015	0.0367	0.997
5	0.1701	-0.8318	0.1701	0.4212	0.984
10	0.8841	-1.0165	0.8441	0.5426	0.990
Initial Cr(VI) concentration (mg/L)					
1	0.0867	-0.6926	0.0867	0.1029	0.973
5	0.169	-0.7921	0.1690	0.4433	0.982
10	0.908	-1.373	0.9080	1.3340	0.958

However, the linear plots at each initial concentration did not pass through the origin which indicates that the intraparticle diffusion was not only the rate controlling step but the surface adsorption on activated adsorbent also contributes to the rate determining step (Chowdhury and Mulligan, 2011). In the present study the plots are straight lines but not passing through the origin and thus indicating that intraparticle diffusion is not the sole rate-limiting factor for the biosorption of arsenic (III) and chromium (VI) on various biosorbents (Gupta and Rastogi, 2009, Sari and Tuzen, 2009, Park *et al*., 2005 and Han *et al*., 2007). The results obtained by Mangaiyarkarasi *et al*., (2011), Deepa *et al*., (2006), and Kumari *et al*., (2006) were similar with the results of the present work. As evident from the results of Table 5.15 there is an increase in K_p values with the increase in arsenic (III) and chromium (VI) concentration (Tuzen *et al*., 2009).

5.2.6. Effects of initial concentration on arsenic (III) and chromium (VI) removal

The biosorption of arsenic (III) and chromium (VI) ions onto living cells of *Bacillus cereus* biomass is studied by varying arsenite and hexavalent chromium concentration using optimum

biosorbent dose (0.3g/50 mL) at ambient temperature and contact time of 60 min. The results are presented in graphical form as percentage removal verseus initial arsenic (III) and chromium (VI) concentration in Fig. 5.43 and Fig. 5.44. It is evident from initial arsenite concentration is decreased from 1 mg/L to 10mg/L and the corresponding removal gradually decreases from 86.14% to 79.24 % at pH 7.5, respectively. The initial chromium (VI) concentration increases from 1 mg/L to 10mg/L and the corresponding removal gradually decreases from 89.24% to 79.22 % at pH 7.5, respectively. It is clear from the results that more than 80 % sorption of arsenic (III) and chromium (VI) ions took place in first 30 min and equilibrium is established 30 min. At higher concentrations, metals need to diffuse to the biosorbent surface by intraparticle diffusion and highly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of arsenic (III) and chromium (VI) ions on the outer surface.

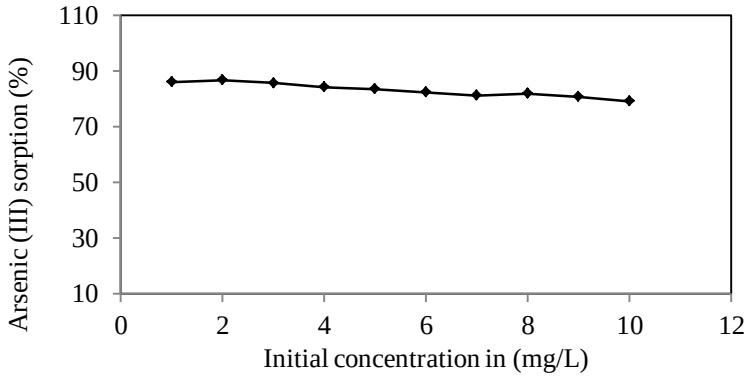


Fig. 5.43. Percentage removal of arsenic (III) by living cells of *Bacillus cereus* versus initial arsenite concentration.

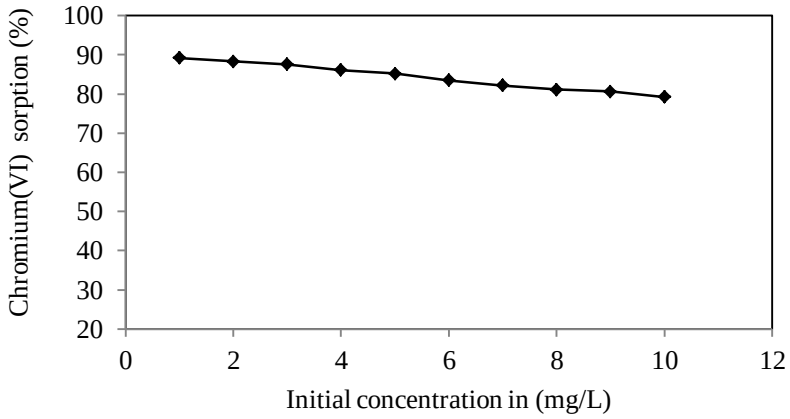


Fig. 5.44. Percentage removal of chromium (VI) by living cells of *Bacillus cereus* versus initial hexavalent chromium concentration.

5.2.7. Biosorption isotherms

It is important to have a satisfactory description of the equilibrium sate between the two phases in order to successfully represent the dynamic behavior of any biosorbate from solution to the solid (biosorbent) phase. Biosorption isotherm can be defined as a functional expression for the variation in biosorption of the biosorbate by the biosorbent in the bulk solution at constant temperature. The capacity of living cells of *Bacillus cereus* biomass can be described by equilibrium biosorption isotherm, which is characterized by certain constants whose values express the surface properties and affinity of the biomass (Velásquez and Dussan, 2009; Merroun *et al*., 2005; Allievi *et al*., 2011). The analysis of the isotherm data is important to develop an equation which accurately represents the results and could be used for design purpose. An analysis of the bisorption isotherm is important to develop and represent the results accurately for design purpose. Batch mode biosorption were carried out at 30 ± 2°C by varying the concentration of arsenic (III) and chromium (VI).

5.2.7.1. Langmuir biosorption isotherm

The Langmuir isotherm was developed by Irving Langmuir in 1916. The Langmuir biosorption isotherm describes quantitatively the buildup of a layer of molecules on a biosorbent surface as a function of the concentration of the biosorbed material in the liquid phase in which it is in contact. Langmuir assumed that a surface consists of a given number of equivalent sites where a species can physically or chemically stick. Physical biosorption through van der Waals interaction is called physisorption, whereas chemical biosorption through the formation of a covalent bond is called chemisorptions. It is important to realize that the processes of biosorption and the opposite process (desorption) is dynamic. A rate can be written for each processs, and when the rates become equal, an equilibrium sate will exist characterized by a constant fractional coverage of the original sites. The Langmuir isotherm is the simplest of all mechanistic models and it is based on the following assumptions:

1. Biosorption cannot proceed beyond monolayer coverage onto a surface containing finite number of biosorption sites. (2) All surfaces sites are equivalent (With uniform energies of biosorption) and can accommodate, at the most one molecular or atomic species of the adsorbate. (3) Adsorbate species on different sites do not interact with each other and there is no transmigration of adsorbate on the plane of the surface.

When the whole surface of the biosorbent is completely covered by a unimolecular layer of the adsorbate, further biosorption is not possible and it indicates a saturation of biosorption. The Langmuir equation correlates the amount of adsorbate adsorbed with the equilibrium aqueous solution. The Langmuir bisorption isotherm data for arsenic (III) and chromium (VI) biosorption on living cells of *Bacillus cereus* biomass are presented in the Table 5.14 and graphically represented in the Fig. 5.45 and Fig. 5.46.

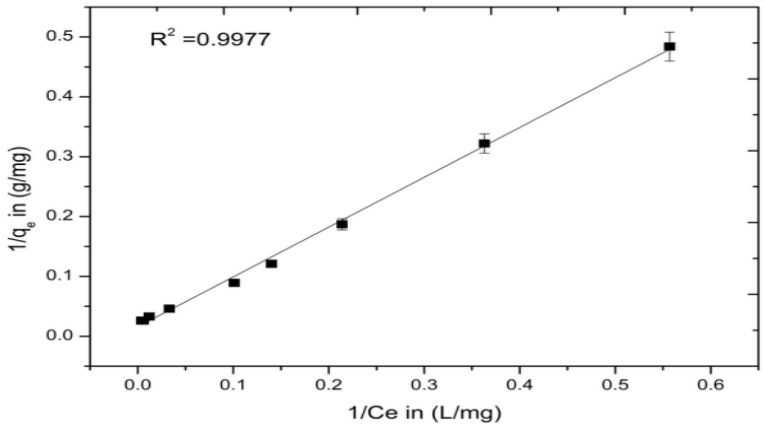


Fig. 5.45. Langmuir isotherm plot of $1/C_e$ versus $1/q_e$ for arsenic (III) biosorption.

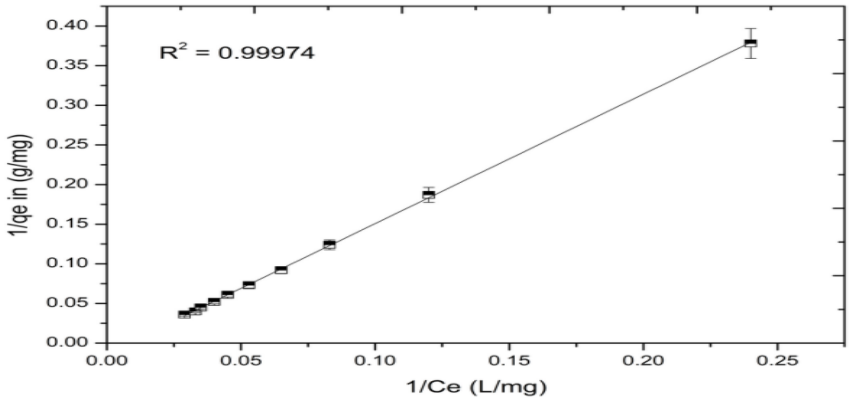


Fig. 5.46. Langmuir isotherm plot of $1/C_e$ versus $1/q_e$ for chromium(VI) biosorption.

A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the sorbent. This model can be written in linear form.

$$\frac{1}{q_e} = \frac{1}{q_0 bCe} + \frac{1}{q_0}$$

Where q_e is the amount adsorbed per unit mass of biosorbent (mg/g), C_e is the equilibrium arsenic (III) and chromium (VI) ion concentration in the solution (mg/L), q_0 is the monolayer biosorption capacity of the biosorbent (mg/g), and *b* is the Langmuir biosorption constant (L/mg) related with the free energy of biosorption. The values of 'q_o' and 'b' were calculated from intercept and slope of the graph (1/ C_e versus 1/ q_e) respectively. The Langmuir parameters (q_0, *b* and R^2) for arsenic (III) and chromium (VI) biosorption in 30 minutes of bisorption study at 30 ± 2°C are given in Table 5.14.

Table 5.14. Langmuir, Freundlich and Dubinin–Radushkevich isotherm constants on the adsorption of arsenic (III) and chromium (VI) ions from aqueous solution onto living cells of *Bacillus cereus* at ambient temperature (30 ± 2°C).

Langmuir isotherm					Freundlich isotherm							Dubinin–Radushkevich isotherm			
b (L/mg)	q_o (mg/g)	*SD*	χ^2	R^2	K_f (mg/g)	*1/n*	*SD*	χ^2	R^2	*K* (mol^2 kJ^{-2})	q_m (g/g)	*E* (kJ mol^{-1})	*SD*	χ^2	R^2
Arsenic (III)															
0.078	32.42	0.008	2.97	0.99	1.133	0.61	0.068	0.005	0.96	3.18 x 10^{-4}	0.003	12.65	0.69	0.003	0.99
Chromium(VI)															
0.098	39.06	0.001	0.27	0.99	4.042	0.62	0.066	0.005	0.97	16.64 x 10^{-4}	0.008	17.36	0.057	0.001	0.99

Table 5.15. Langmuir dimensionless equilibrium parameter of living cells of *Bacillus cereus* of arsenic (III) and chromium (VI) at different concentrations.

Biosorbents	Langmuir dimensionless equilibrium parameter (r)		
	1 mg/L	5 mg/L	10 mg/L
Arsenic(III) with living cells of *B. cereus*	0.927	0.719	0.561

Chromium(VI) with living cells of *B. cereus*	0.910	0.671	0.505

Table 5.16. Comparison of biosorption capacity of *Bacillus cereus* biomass for chromium (VI) and arsenic(III) with that of different biosorbents.

Biosorbent	Type of water	pH	Cr(VI) mg/g	References
Bacillus circulans	Aqueous solution	2.5	34.5	Srinath et al 2002
Bacillus megaterium	Aqueous solution	2.5	32.0	Srinath et al., 2002
Rhizopus nigricans	Aqueous solution	4.0	200	Bai and Abraham, 2001
Aspergillus flavus	Waste water	3.0	0.335	Deepa et al., 2006
Rhizopus arrhizus	Aqueous solution	-	5.1	Sag et al., 2001
Hazelnut shell	Aqueous solution	1.0	170	Kobya, 2004
Saw dust	Waste waters	2.0	39.7	Sharma and Forster, 1994
Maple saw dust	Aqueous solution	6.0	5.1	Yu et al., 2003
Sugarcane bagasse	Waste water	3.0	103	Wartelle and Marshall, 2005
Agricultural waste	Aqueous solution	2.0	22.29	Mohan et al., 2005
Bacillus cereus biomass	Aqueous solution	7.0	39.06	Present study
Biosorbent	Type of water	pH	As(III) (mg/g)	References
L. nigrescens	Aqueous solution	2.5	45.5	Hansen et al., 2006
A. niger biomass	Aqueous solution	3.5	0.20	Pokhrel and Viraraghavan, 2007
Tea fungal biomass	Ground water	7.20	4.95	Murugesan et al., 2006
P. purpurogenum	Aquous solution	5.0	35.5	Ridvan et al., 2003

Methylated biomass	Aqueous solution	6.5	3.75	Seki et al., 2005
I. hispidus	Aqueous solution	2.0	59.6	Sari and Tuzen, 2009
Chitosan-coated biosorbent	Aqueous medium	4.0	96.46	Boddu et al., 2008
M. oleifera seed powder	Aqueous solution	2.5	2.16	Kumari et al., 2006
Bacillus cereus biomass	Aqueous solution	7.5	32.24	Present study

Hall in 1966 has proposed dimensionless equilibrium parameters (r), to revel the essential characteristics of Langmuir isotherm by relating 'r' with Langmuir constant 'b' and the initial concentration of the adsorbate solution, C_0 (Singh *et al*., 2006; Manju *et al*., 1999).

$$r = \frac{1}{1+bC_0}$$

Value of 'r' indicates the shape of isotherm to be either unfavorable ($R_L > 1$) or linear ($R_L = 1$) or favorable ($0 < R_L < 1$) or irreversible ($R_L = 0$). The values of the dimensionless equilibrium parameters are presented in Table 5.15. The values of the dimensionless equilibrium parameter 'r' revealed that the process was favorable for initial arsenic (III) and chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L for *Bacillus cereus* biomass. The comparison of biosorption capacity of living cells of *B. cereus* biomass for arsenic (III) and chromium (VI) ions with that of different biosorbent in literature is presented in Table 5.16.

5.2.7.2. Freundlich biosorption isotherm

Herbert Max Finley Freundlich, a German physical chemist, presented an empirical bisorption isotherm for non ideal system in 1906. The Freundlich isotherm is the earliest known relationship describing the applicability of heterogeneous surface energy in the bisorption process. The empirical Freundlich equation (Freundlch, 1926) is

$$q_e = K_f C_e^{1/n}$$

This empirical equation when expressed in logarithmic form becomes a straight line equation with a slope of 1/n and y-intercept of log K_f. The linear form of Freundlich equation (Sumanjit and Prasad, 2001) is represented as

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$

Where q_e is the amount adsorbed per unit mass of biosorbent (mg/g), C_e is the equilibrium adsorbate concentration in solution (mg/L), K_f and 'n' are Freundlich constants related to the bisorption capacity and bisorption intensity respectively. Freundlich bisorption data for arsenic (III) and chromium (VI) onto living cells of *Bacillus cereus* biomass are given in Table 5.14 and graphically shown in Fig. 5.47 and Fig 5.48. Comparing the correlation coefficient values of Freundlich bisorption isotherm with those of Langmuir isotherm, It is clear that Langmuir isotherm fits better than Freundlich isotherm. The values of 1/n (>1) indicates unfavorable nature of the isotherm for arsenic (III) and chromium (VI) onto living cells of *Bacillus cereus* biomass. The values of *n* (intensity of adsorption) between 1 and 10 (i.e., 1/*n* less than represents a favorable biosorption. For the present study the value of *n* represented the same trend of a beneficial biosorption (François *et al*., 2012).

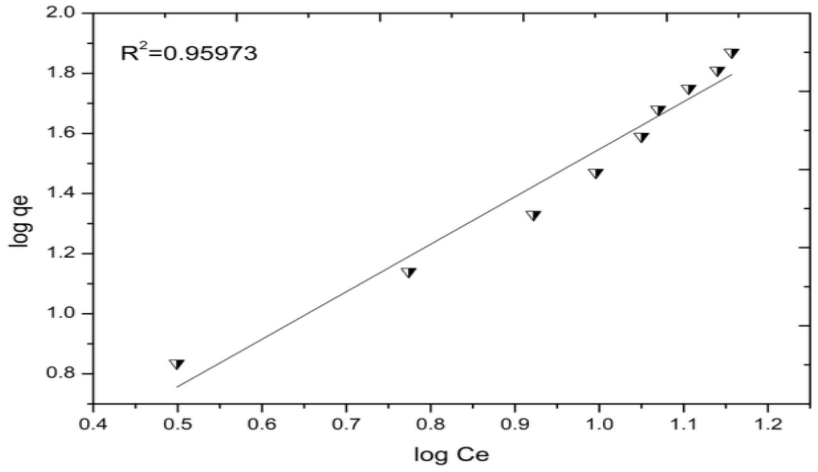


Fig. 5.47. Freundlich isotherm plot of log q_e vs. log C_e, for arsenic (III) biosorption.

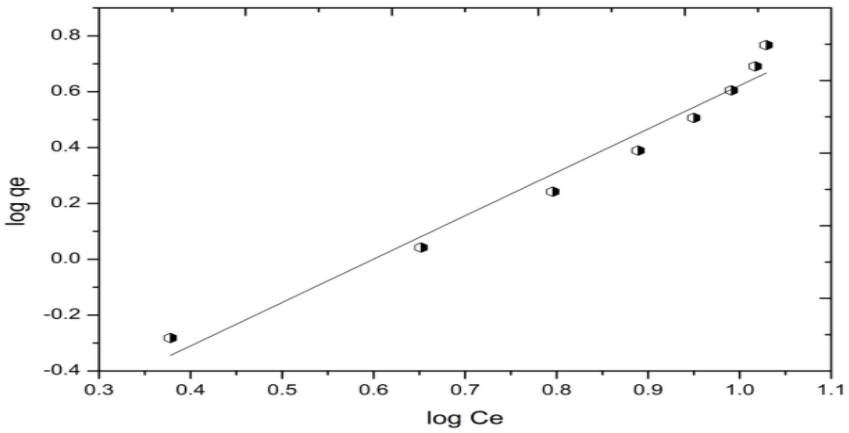


Fig. 5.48. Freundlich isotherm plot of log q_e vs. log C_e, for chromium(VI) biosorption.

5.2.7.3. Dubinin-Radushkevich isotherm

The development of Dubinin-Radushkevich equation was prompted by the failure to extend the theories governing adsorption on non-porous solids to that on real, porous solids such as active carbon, synthetic zeolites, and dehydrated inorganic gels (Dubinin, 1975). This lack of success led Dubinin and co-workers to focus their investigation on the problem of physical adsorption on porous materials and resulted in the Dubinin-Polanyi theory of micropore filling (also known as the theory of volume filling of micropores (TVFM)). This theory is based on the postulate that the mechanism for biosorption in micropores is that of pore filling rather than a layer-by layer formation of a film on the walls of the pores. The D-R equation is an adaptation of the earlier Polanyi potential theory of biosorption (Dubinin and Stoeckli, 1980; Gregg and Sing, 1982). The D-R equation has been effectively used to describe bisorption by microporous solids (Stoeckli *et al*., 1978). The D-R equation seems to be particularly useful in describing bisorption by microporous sorbents (Dubinin and Stoeckli, 1980).

It is known that the Langmuir and Freundlich biosorption isotherm constant do not give any idea about the biosorption mechanism. In order to understand the biosorption type, equilibrium data are tested with Dubinin–Radushkevich isotherm (Pokhrel and Viraraghavan, 2007). The non linear form of D-R isotherm equation is given below,

$$q_e = q_m e^{-K\varepsilon^2}$$

The linearized D. R. equation can be obtained by taking logarithm on both sides and written as

$$\ln q_e = \ln q_m - K\varepsilon^2$$

Where ε is Polanyi potential, and is equal to $RT \ln(1+ 1/C_e)$, q_e is the amount of arsenic(III) and chromium(VI) ions biosorbed per unit mass of biosorbent, q_m is the theoretical sorption capacity, C_e is the equilibrium concentration of arsenic(III) and chromium(VI) ions, K is the constant related to biosorption energy, R is the universal gas constant and T is the temperature in Kelvin.

Fig. 5.49 and Fig. 5.50 shows the plot of ln q_e against ε^2 of arsenic (III) and chromium (VI) onto living cells of *Bacillus cereus* biomass, which were almost linear with correlation coefficient (R^2), 0.997 and 0.981. D. R. isotherm constants K and q_m are calculated from the slope and intercept of the plot, respectively and the results are presented in Table 5.14. For arsenic (III),

the value of K is found to be 3.18 x 10^{-3} mol^2 kJ^{-2} and that of q_m is 0.0039g/g. And for chromium (VI), the value of K is found to be 16.64 x 10^{-4} mol^2 kJ^{-2} and that of q_m is 0.0083g/g.

The mean free energy of biosorption (E) was calculated from the constant K using the relation (Baciocchi *et al*., 2005).

$$E = (-2K)^{-1/2}$$

It is defined the free energy change when 1 mole of ions is transferred to the surface of the solid from infinity in solution. The value of E was found to be 12.65 kJ mol^{-1} for arsenic (III) and 17.36 kJ mol^{-1} for chromium (VI). The value of E is very useful in predicting the type of biosorption. If the value is less than 8 $kJmol^{-1}$, the biosorption process is of physical in nature due to weak vander Waals forces. If the magnitude of E is between 8 and 16 kJ mol^{-1}, then the sorption is due to exchange of ions (McNeill and Edwards, 1997). The value in the present study was found to be little greater than16 $kJmol^{-1}$. This is due to different chemical processes accompanying the ion exchange process (Tuzen *et al*., 2009a; Tuzen *et al*., 2009b).

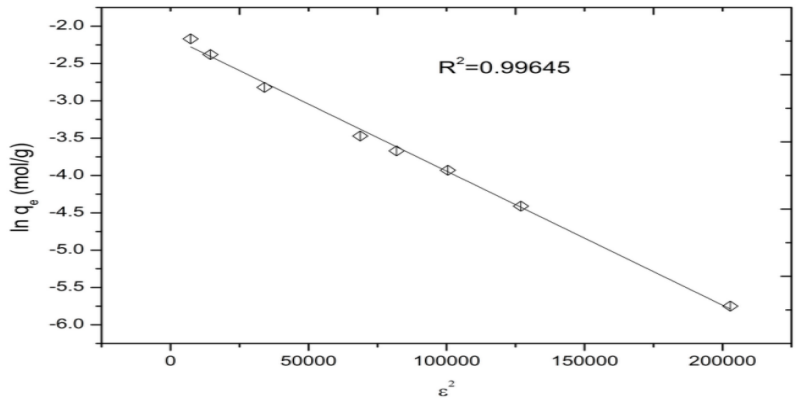


Fig. 5.49. Dubinin–Radushkevich (D–R) isotherm of ln qe versus ε^2, for arsenic (III) biosorption.

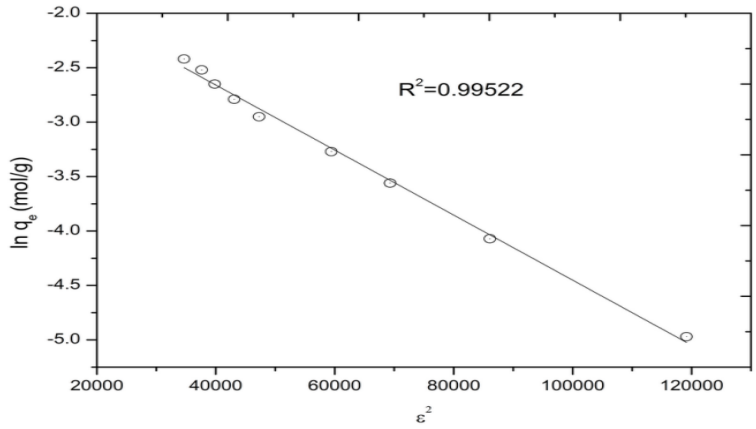


Fig. 5.50. Dubinin–Radushkevich (D–R) isotherm of ln *q*e versus ε^2, for chromium(VI) biosorption.

5.2.8. Effects of temperature

Temperature affects the biosrption rate by altering the molecular interaction and the solubility of the biosorbate (Singh and Srivastava, 2001). Batch biosorption studies were carried out at varying temperature (15 ºC to 40 ºC). The effects of temperature on the biosorption of arsenic (III) and chromium (VI) with initial concentration 1 mg/L, 5 mg/L and 10 mg/L onto living cells of *Bacillus cereus* biomass was studied using optimum biosorbent dose (0.3 g /50 mL) and are presented in Fig. 5.51 and Fig. 5.52.

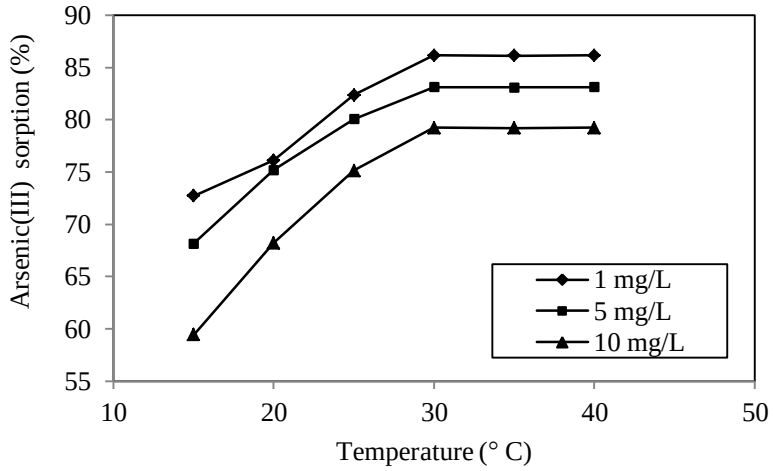


Fig. 5.51. Effect of temperature on the biosorption of arsenic (III) with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

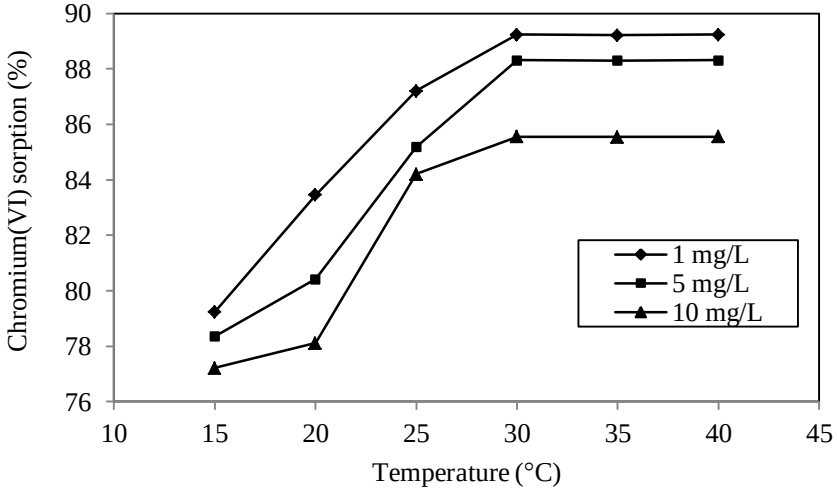


Fig. 5.52. Effect of temperature on the biosorption of chromium (VI) with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

The results are presented as percentage removal of arsenic (III) with initial concentration 1 mg/L, increased from 72.22% to 86.14%, the percentage removal of arsenic(III) ions of initial concentration 5 mg/L, increased from 68.16% to 83.12% and the percentage removal of arsenic(III) ions of initial concentration 10mg/L, increased from 59.44% to 79.24 % for 15-40 ºC. It can be clearly seen from the figures that, with the increase in temperature the percentage removal increases slowly and reached almost 86 %. The percentage removal of chromium (VI) increased from 77.22% to 85.56% with initial concentration of 10 mg/L, from 78.36% to 88.32% with initial concentration of 5 mg/L and from 79.24% to 89.24 % with initial concentration 1mg/Lin the range of temperature 15-40 ºC respectively. It can be clearly seen from the figure that, with the increase in temperature, the percentage removal increased slowly and reached almost 90%. The continuous increase in percentage removal with increase in temperature for arsenic (III) and chromium (VI) onto living cells of *Bacillus cereus* biomass indicated that the biosorption process was endothermic in nature. But many researchers have reported it and this may be probably due to a decrease in the escaping tendency of the adsorbate species from the surface of the bisorbent. The increase in metal/metalloid biosorption with increase in temperature could be due to dissociation of some compounds available in the biosorbent, which may provide more sites for metal adsorption. The H^+ ions biosorbent bond is important because biosorption of arsenic (III) and chromium (VI) involves displacement of H^+ ions from the biosorbent surface. Therefore it may be inferred that at higher temperatures, the increased in biosorption could be due to the weakening of H^+ biosorbent bond which consequently increases the percentage removal.

5.2.9. Thermodynamic parameters

It is a fundamental concept that any chemical system tends to attain a state of equilibrium from the state of non-equilibrium. In order to determine the thermodynamic parameters, experiments are carried out at different temperature. The change in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) of sorption are calculated using the following equation (Baciocchi *et al*., 2005; Murugesan *et al*., 2006).

$$\log K_c = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303RT}$$

$$\Delta G = \Delta H - T\Delta S$$

The *K*c value is calculated using the following equation.

$$K_c = C_1/C_2$$

Where K_c is the equilibrium constant, C_1 is the amount of arsenic (III) and chromium (VI) ions sorbed per unit mass of *Bacillus cereus* biomass and C_2 is the concentration of arsenic (III) and chromium (VI) ions in aqueous phase. *R* is the gas constant (8.314 J/mol K), *T* is the temperature in Kelvin and ΔG, ΔS and ΔH are the changes in Gibb's free energy, entropy and enthalpy of biosorption respectively.

Batch biosorption studies were carried out with arsenic (III) and chromium (VI) solution separately at varying temperature (15 °C to 40 °C) and with optimum biosorbent dose. A graph was plotted, 1000/T versus log K_c (Van't Hoff plots) for initial arsenic (III) and chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L and are presented in Fig. 5.53 and Fig. 5.54. Values of ΔH and ΔS were calculated from the slope and intercept of Van't Hoff plots respectively and results are presented in Table 5.17. It is evident from the figures and tables that for arsenic (III) and chromium (VI), ΔG values were negative in whole range of temperature. The value of ΔH and ΔS were positive. The positive value of entropy (ΔS) indicates the increase in randomness of the ongoing process and hence a good affinity of *Bacillus cereus* biomass. The positive values of ΔS indicate some structural changes in the biosorbent and also reflect the affinity of the biosorbent for arsenic (III) and chromium (VI) species. During the biosorption of arsenic (III) and chromium (VI), the H^+ ions, which are displaced by the As(III) and Cr(VI) ions, gain more entropy than is lost by sorbate species, thus allowing the prevalence of randomness in the system. Similar results were reported in the adsorption of basic dyes (Khatri and Singh, 1999), Cr (VI), Fe (II) and Hg (II) (Singh *et al*., 2001) and Cr (VI) (Murugan and Subramanian, 2003). For both arsenic (III) and chromium (VI) ions biosorption by *Bacillus cereus* biomass, positive value of (ΔH) indicates the endothermic nature of sorption process. The increase of percentage removal of arsenic (III) and chromium (VI) with increase in temperature can be explained by taking into account, the endothermic nature of the process. Negative value of Δ*G* at each temperature indicates the feasibility and spontaneity of ongoing sorption. A decrease in values of Δ*G* with the increase in temperature suggests more bisorption of arsenic (III) and chromium (VI) at higher temperature. Positive value of enthalpy (ΔH) suggests that entropy (ΔS)

is responsible for making the ΔG value negative. So, the biosorption process is spontaneous, since the entropy contribution is much larger than that of enthalpy (Sari *et al*., 2011).

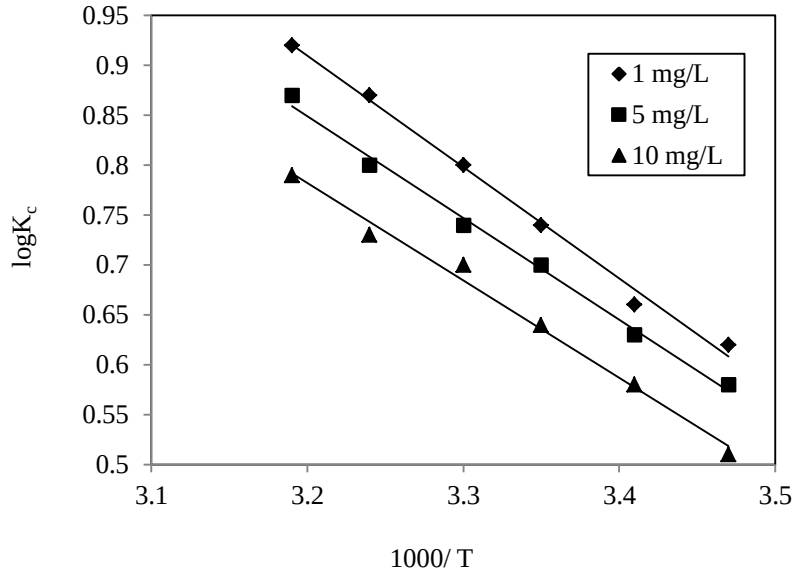


Fig. 5.53. Van't Hoff plots, log K_c vs. 1000/T for arsenic (III) biosorption with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

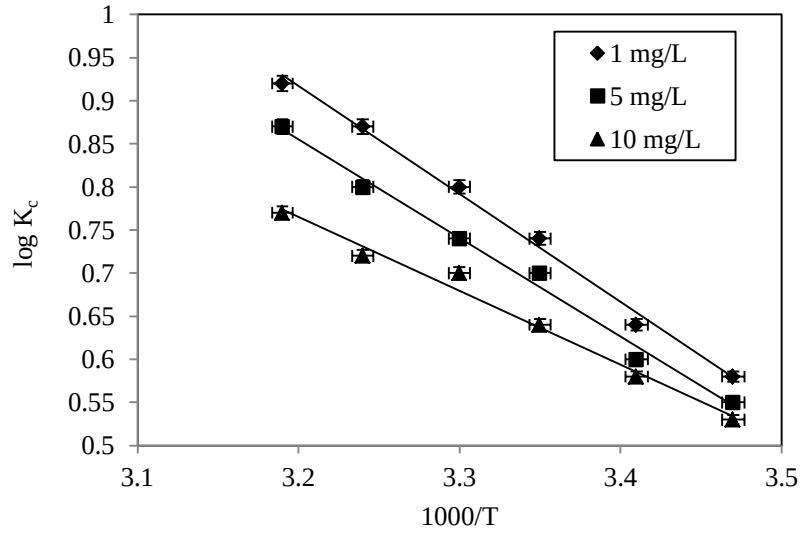


Fig. 5.54. Van't Hoff plots, log K_c vs. 1000/T for chromium (VI) biosorption with initial concentration of 1 mg/L, 5 mg/L and 10 mg/L.

Table 5.17. Thermodynamic parameters using arsenic (III) and chromium (VI) solution of 1mg/L, 5 mg/L, and 10 mg/L.

	ΔH (KJ mol^{-1})	ΔS (KJ/(Kmol))	ΔG (KJ mol^{-1})						R^2
			15 ° C	20 ° C	25 ºC	30 ºC	35 ºC	40 ° C	
Initial As(III) concentration (mg/L)									
1	10.301	0.04160	-1.679	-1.887	-2.095	-2.303	-2.511	-2.719	0.995
5	11.141	0.04362	-1.421	-1.639	-1.857	-2.075	-2.293	-2.512	0.994
10	12.023	0.04256	-0.234	-0.447	-0.659	-0.872	-1.085	-1.298	0.990
Initial Cr(VI) concentration (mg/L)									
1	12.331	0.05160	-2.529	-2.787	-3.045	-3.303	-3.561	-3.819	0.990
5	13.241	0.05362	-2.094	-2.469	-2.737	-3.005	-3.273	-3.542	0.982
10	14.423	0.05256	-0.714	-0.977	-1.239	-1.502	-1.765	-2.028	0.993

5.2.10. Desorption and regeneration studies

Desorption and regeneration studies were carried out in order to know the reusability and nature of biosorption (i. e. physical or chemical). Desorption of biosorbed analyte ions onto *Bacillus cereus* were also studied by using HCl and HNO_3 at various concentrations in Table 5.18. For these studies, 10mL of each eluent was used. Analyte ions were desorbed from *Bacillus cereus* with both 1M HCl and 1M HNO_3. The highest recovery for arsenic (III) ions was found to be 90.55% using 1M HNO_3 and 81.33% using 1M HCl. The highest recovery for chromium (VI) ions was found to be 90.23% using 1M HNO_3 and 87.21% using 1M HCl.

Table 5.18. Influence of various eluents on desorption of arsenic(III) and chromium (VI) ions from living cells of *Bacillus cereus* .

Eluent	Recovery (%)	Recovery (%)
	Arsenic(III)	Chromium(VI)
0.5 mol L^{-1} HCl	72.23±3	77.11±3
1mol L^{-1} HCl	81.33±3	87.21±3
0.5 mol L^{-1} HNO_3	78.34±3	84.55±2
1mol L^{-1} HNO_3	90.55±3	90.23±2

The effects of HNO_3 as also volume of 1M eluent were investigated in

the range of 5.0-10.0 mL. The highest recovery values (90%) were obtained for arsenic (III) and chromium (VI) ions after 8.0mL of 1M HNO_3. Subsequent elution with 10mL 1MHNO_3 readily strips the sorbed arsenic (III) and chromium (VI) ions from *Bacillus cereus* biomass (Sari and Tuzen, 2009, 2010; Lin and Puls, 2000). The high stability of *B. cereus* permitted ten times of sorption-elution process along the studies without a decrease about 10% in recovery of arsenic (III) and chromium (VI) ions and results are presented in Fig. 5.55.

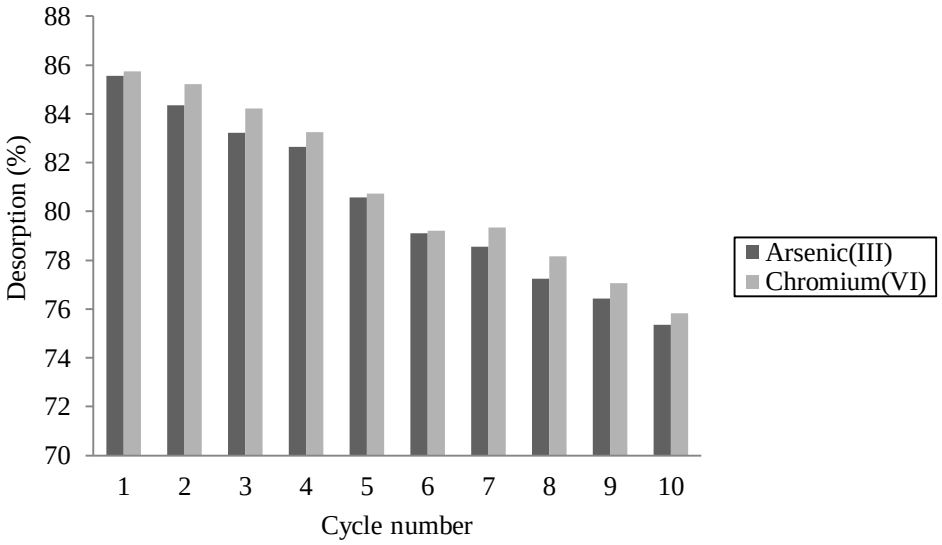


Fig. 5.55. Desorption efficiency of *Bacillus cereus* biomass with cycle number

5.2.11. Characterization of *Bacillus cereus* biomass before and after biosorption of arsenic (III) and chromium (VI) ions.

5.2.11.1. Atomic force microscopy (AFM) analysis

Atomic force microscopy (AFM) is one of the for most tools for imaging, measuring, and manipulating matter at the nanoscale and was developed by Gred Binning and Heinrich Rohrer in the early 1980s at IBM research-zurich. The present investigation for surface morphology of *Bacillus cereus* biomass without and with sorption of arsenic (III) and chromium (VI) during biosorption process is observed with the help of Atomic Force Microscopy (Digital Instruments, Santa Barbara, CA, USA). *Bacillus cereus* bacteria without arsenic (III) and chromium (VI) ions exposure in the control blank are rod-like in shape with a smooth surface (the dimension of these cells is about 4.0 µm long and 1.0 µm wide, on average, as shown in Fig. 5.56. After the arsenic (III) and chromium (VI) ions exposure and the ultra-structures mostly disconnected with the cells

adhering to each other randomly. It can be clearly observed that the biomass shape has changed into a spindle-like structure after arsenic (III) and chromium (VI) sorption and presented in Fig. 5.57 and Fig. 5.58. The morphological changes of the sample can be attributed to the interactions between arsenic (III) and chromium (VI) ions and the surface of *Bacillus cereus* cells. These agree with the results of FTIR spectra analysis.

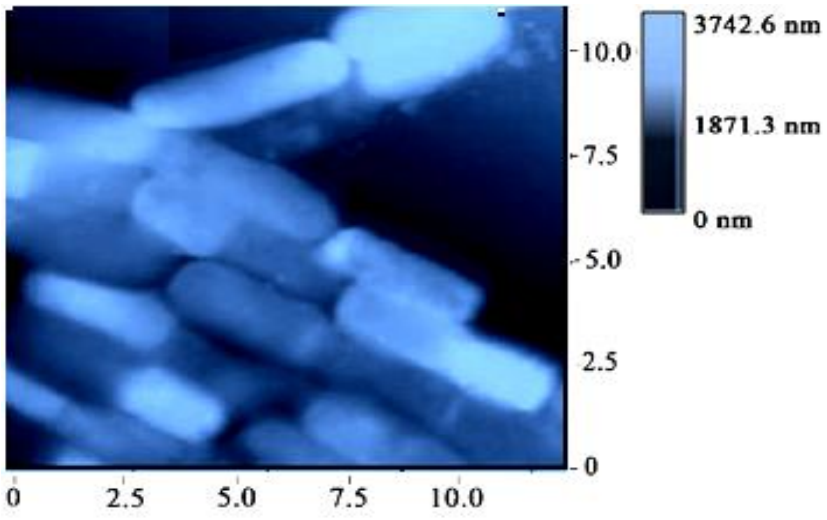


Fig. 5.56. AFM image of *Bacillus cereus* cells (ion strength 0.01 mol/L; pH 7.0) control blank of *Bacillus cereus.*

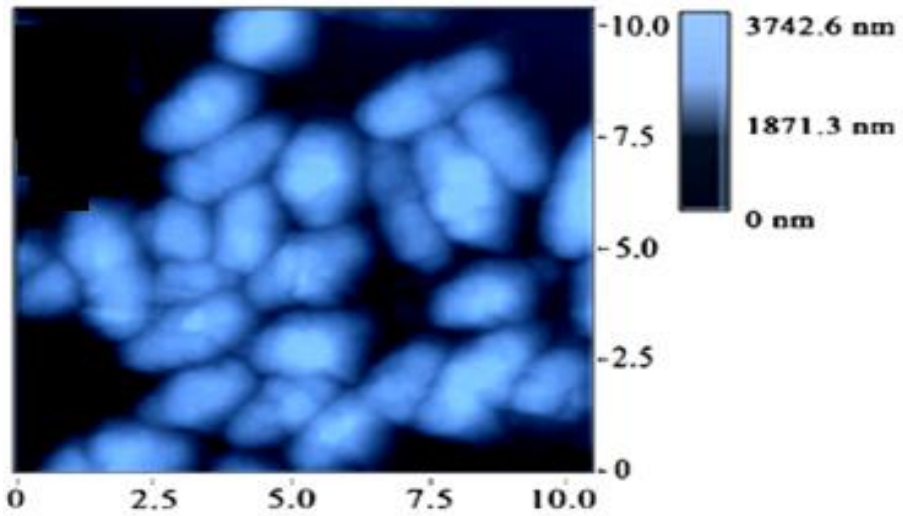


Fig. 5.57. AFM image of *B. cereus* cells (ion strength 0.01 mol/L; pH 7.0) with 1 mg/L As(III) ion-exposed cells.

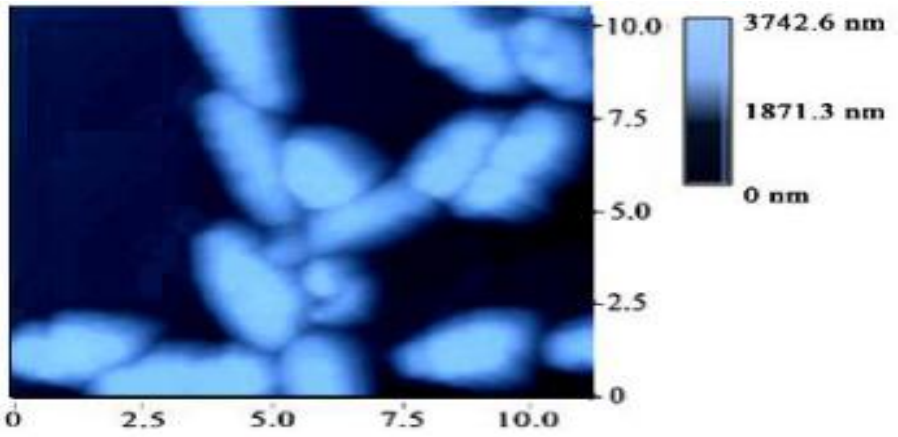


Fig. 5.58. AFM image of *B. cereus* cells (ion strength 0.01 mol/L; pH 7.0) with 1 mg/L Cr (VI) ion-exposed cells.

5.2.11.2. Scanning electron microscopy-Energy dispersive X-ray (SEM-EDX) analysis

The surface morphology of *Bacillus cereus* biomass without and with sorption of arsenic (III) and chromium (VI) ions during biosorption process is measured with the help of SEM-EDX (Jeol JSM-6480 LV electron microscope). Fig. 5.59 shows without sorption of arsenic (III) and chromium (VI) ions reveal rod-like shape with a smooth surface of *Bacillus cereus* cells (Ray *et al*., 2005).

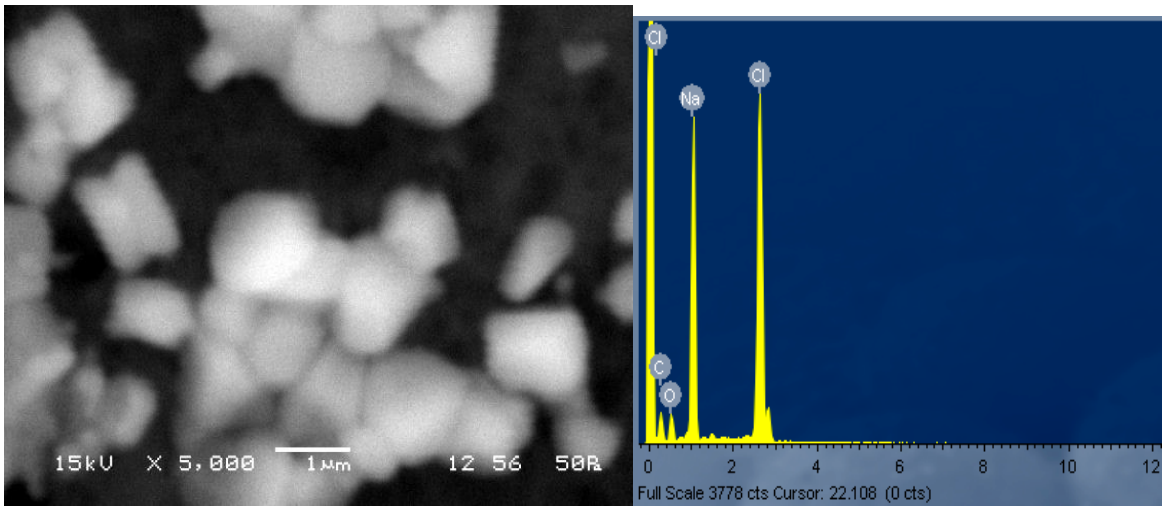


Fig. 5.59. SEM-EDX images of *B. cereus* biomass (ion strength 0.01 mol/L; pH 7.0) without sorption of arsenic (III) and chromium(VI) ions.

The morphological changes with respect to shape and size of the bacteria after sorption of arsenic (III) and chromium (VI) ions with *B. cereus* cells are presented in Fig. 5.60 and Fig. 5.61.

It can be clearly observed that the biomass shape has changed into a spindle-like structure after arsenic (III) and chromium (VI) ions sorption. The EDX spectra of arsenic (III) and chromium (VI) ions unloaded and loaded biomass obtained are shown in Fig. 5.59, Fig. 5.60 and Fig. 5.61, respectively. So, it is concluded that, arsenic (III) and chromium (VI) ions sorbed on the biosorbent.

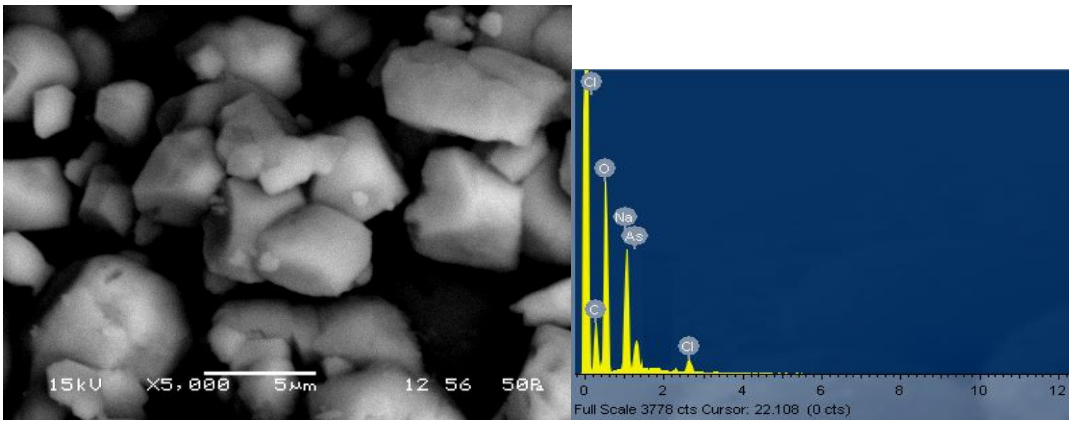


Fig.5.60. SEM-EDX images of *Bacillus cereus* biomass (ion strength 0.01 mol/L; pH 7.0) with sorption of arsenic (III) ions.

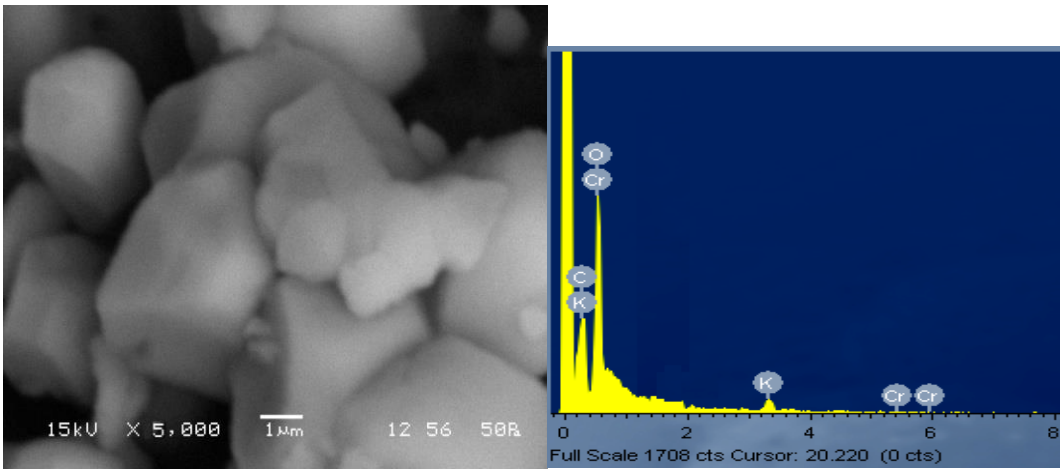


Fig.5.61. SEM-EDX images of *Bacillus cereus* biomass (ion strength 0.01 mol/L; pH 7.0) with sorption of chromium (VI) ions.

5.2.11.3. FTIR analysis

FTIR spectra of the *Bacillus cereus* biomass with and without arsenic (III) ions loaded are obtained to determine the probable functional groups which may have contributed to the arsenic (III) ions sorption. The FTIR spectra of the *Bacillus cereus* biomass without arsenic (III) and chromium (VI) ions loaded displays a number of absorption peaks, indicating the complex nature of the bacterial biomass and the spectra is presented in Fig. 5.62. The spectra of *Bacillus cereus* biomass loaded with arsenic (III) are presented in Fig. 5.63. Where both the spectra in Fig. 5.62 and Fig. 5.63 are compared than the following changes are observed. The spectra of sorbent exhibits a broad absorption band at 3,448.94 cm^{-1} due to bonded –OH stretching vibration which is shifted to 3,460.29 cm^{-1} may be due to possibly complexation of –OH groups with arsenic(III) ions (Pangnanelli *et al*., 2000; Ray *et al*., 2005). The absorption peaks at 2,920.11 cm^{-1} has not been shifted. The next absorption peaks at 2,343.50 cm^{-1} has been shifted to lower frequency and appears at 2,282.91 cm^{-1} possibly due to the complexation of -CH stretching vibration of alkyl chains. The next absorption peaks at 1655.83 cm^{-1} has been shifted to 1,652.62 cm^{-1} possibly due to the complexation of amide group (N-H stretching and C=O stretching vibration) with arsenic (III) ions (Seki *et al*., 2005). Another shift is observed from 1,407.37 cm^{-1} to 1,450.38 cm^{-1}, corresponding to the complexation of nitrogen N-H group with arsenic (III) ions of the (Kumari *et al*., 2006). **The** peaks at 1,074.06 cm^{-1} may be attributed to C-N stretching vibrations of amino groups which is shifted to higher frequency and appeared at 1,116.26 cm^{-1} due the interaction of nitrogen from amino group with arsenic(III) ions (Baciocchi *et al*., 2005; Giri *et al*., 2011). The other weak absorption peak is shifted from 889.44 cm^{-1} to 879.51 cm^{-1}, corresponding to the O-C-O scissoring vibration of polysaccharide (Pokhrel and Viraraghavan 2007).The above changes in the spectra may be attributed to the interaction of arsenic (III) ions with the hydroxyl, amide and amino groups present on the *Bacillus cereus* biomass.

Similarly the FTIR spectra of the *Bacillus cereus* biomass with and without chromium (VI) ions loaded displays a number of absorption peaks, indicating the complex nature of the bacterial biomass and the spectra are presented in Fig. 5.62 and Fig. 5.64, respectively. The spectra of sorbent exhibits a broad absorption band at 3,448.94 cm^{-1} due to bonded –OH stretching vibration which is shifted to 3,450.21 cm^{-1} may be due to complexation of –OH groups with chromium (VI) (Gardea-Torresdey *et al*., 2000). The next absorption peaks at 1655.83 cm^{-1} has been shifted to 1,648.97 cm^{-1} possibly due to the complexation of amide group (N-H stretching and C=O stretching vibration) with chromium (VI) (Nourbakhsh *et al*., 2002; Park *et al*., 2004).

Another shift is observed from 1,407.37 cm^{-1} to 1,412.83 cm^{-1}, corresponding to the complexation of nitrogen with chromium (VI) of the N-H group. **The** peaks at 1,074.06 cm^{-1} may be attributed to C-N stretching vibrations of amino groups is shifted to higher frequency and appeared at 1,118.07 cm^{-1} due the interaction of nitrogen from amino group with chromium(VI) (Mangaiyarkari *et al*., 2011). The other weak absorption peak is shifted from 889.44 cm^{-1} to 849.69 cm^{-1}, corresponding to the O-C-O scissoring vibration of polysaccharide (Seki *et al*., 2005). The above changes in the spectra may be attributed to the interaction of chromium (VI) with the hydroxyl, amide and amino groups present on the *Bacillus cereus* biomass.

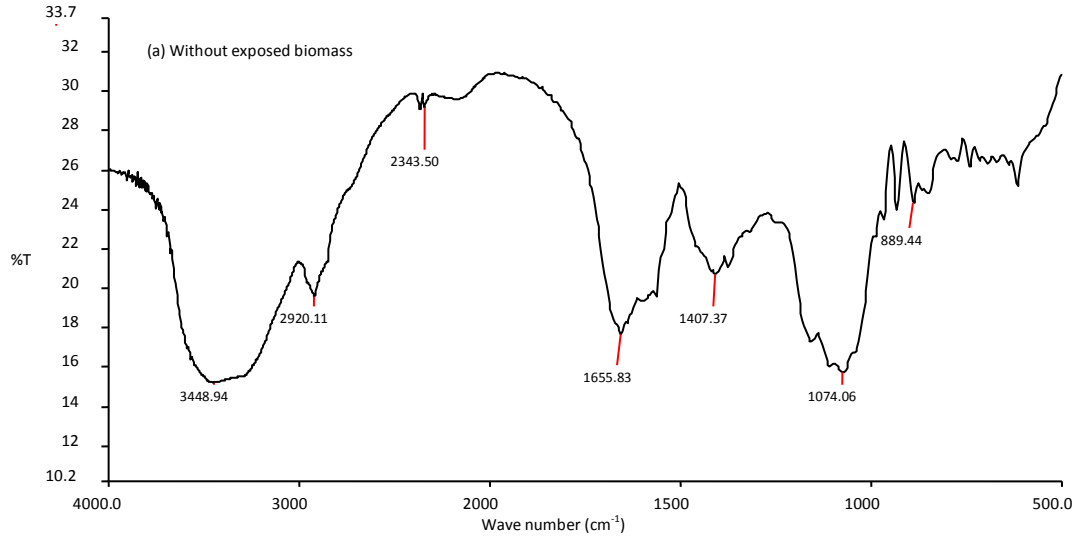


Fig. 5.62. FTIR spectra of *B. cereus* biomass without exposed of arsenic (III) and chromium(VI) ions.

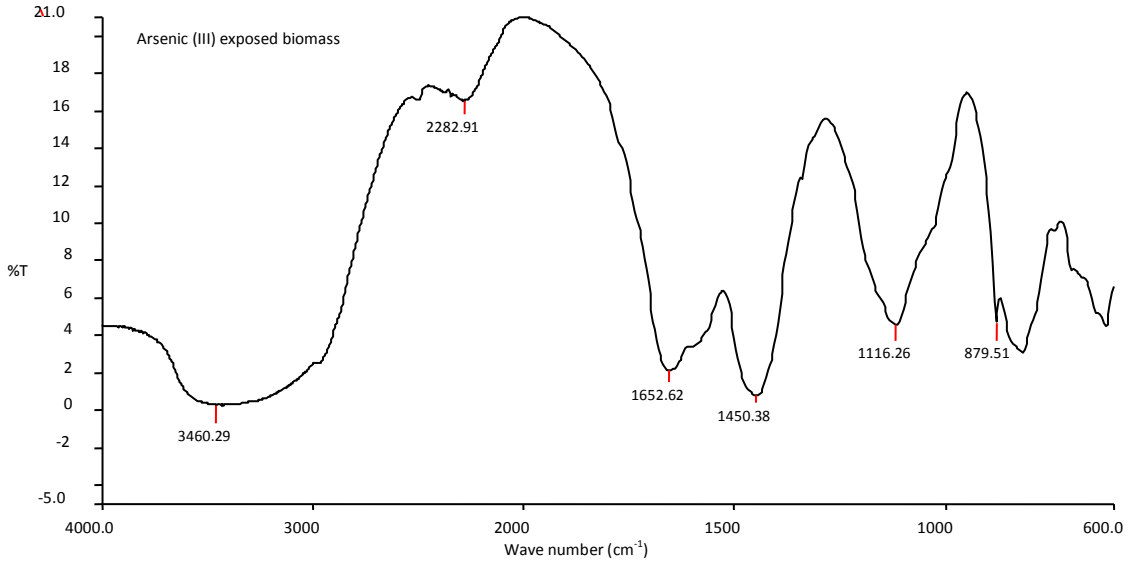


Fig. 5.63. FTIR spectra of *Bacillus cereus* biomass with exposed of arsenic (III) ions.

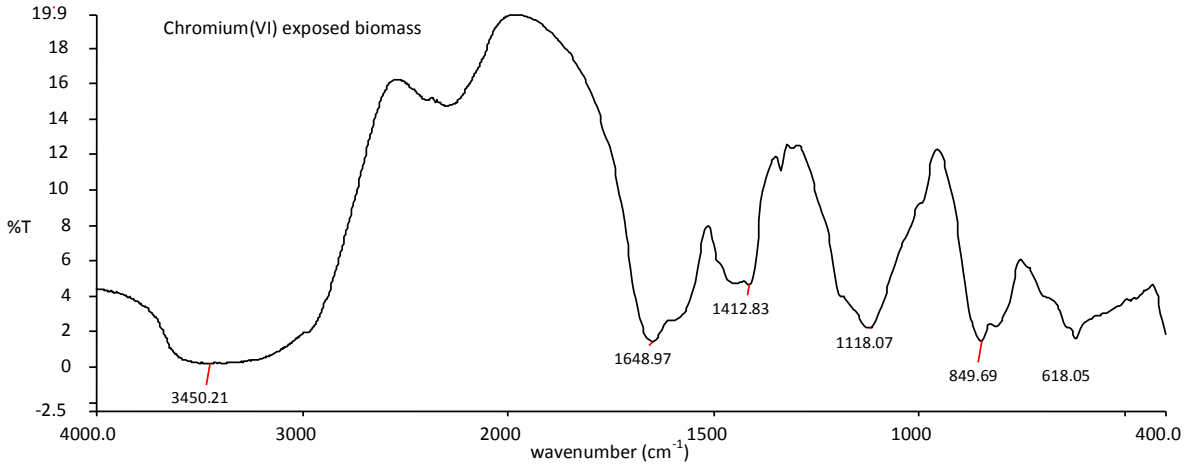


Fig. 5.64. FTIR spectra of *Bacillus cereus* biomass with exposed of chromium (VI) ions

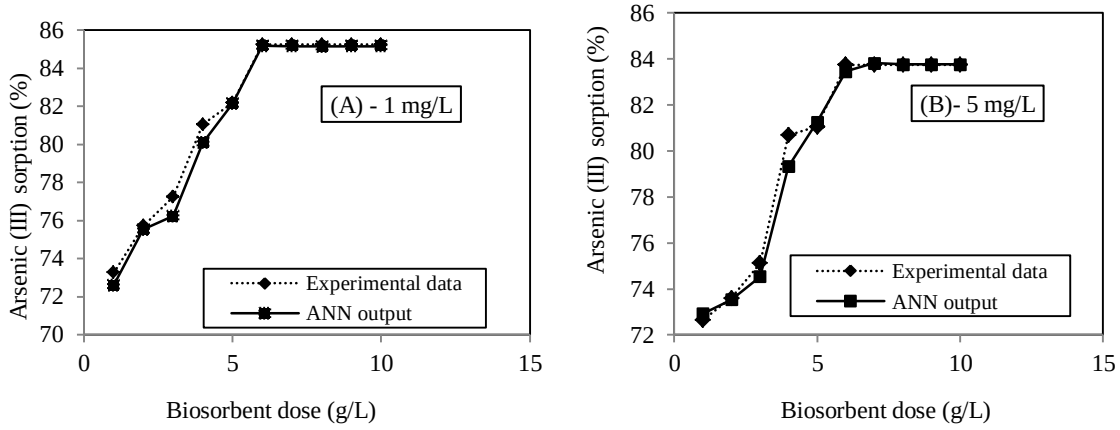
5.3. Artificial neural network (ANN) modeling of arsenic (III) and chromium (VI) ions biosorption by living cells of *Bacillus cereus* biomass.

Waste water treatment using biosorption process is a complex process because a large number of variables influence the removal efficiency. Modeling of such processes is difficult when standard procedure like simple linear multivariate correlation is used. Artificial neural network (ANN), being capable of mapping inputs and outputs efficiently, can recognize and reproduce the cause and effect relationships through training in a multiple input-output systems (Peng *et al*., 1992). Like any experimental investigation, study on biosorption by living cells demand

substantial amount of time, energy and materials. Hence, there is a need for a prediction tool to supplement to the experiments. Over the last two decades, ANNs have been used by many researchers for a variety of engineering applications. ANNs are a family of massively parallel architectures that solve difficult problems via the cooperation of highly interconnected but simple computing elements (or artificial neurons) arranged in layers. ANN represents a powerful tool for the identification of the relevant parameters and their interactions especially when relationships are very complex and highly non-linear. Prediction of biosorption of heavy metals from aqueous solution has been attempted in the past by many researchers using ANN to a reasonably good degree of success (Aleboyeh *et al*., 2008; Chu, 2003; Saha *et al*., 2010).

5.3.1. Effect of biosorbent dosage on the sorption efficiency

Biosorbent dose is an important parameter which determines the capacity of sorbent for an initial concentration of the sorbate. The effect of sorbent dose on the sorption of arsenic (III) and chromium is studied at ambient temperature (30±2°C) and contact time of 60 min for initial arsenic (III) and chromium(VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L and results are presented in Fig. 5.65 and Fig. 5.66, respectively. Experimental results details discussed in chapter 5 (Section 5.2.1.) Fig. 5.65 and Fig. 5.66 show a comparison between the ANN model predictions and the experimental data as a function of biosorbent dose. It can be seen that the ANN model satisfactorily predicts the trend of the experimental data.



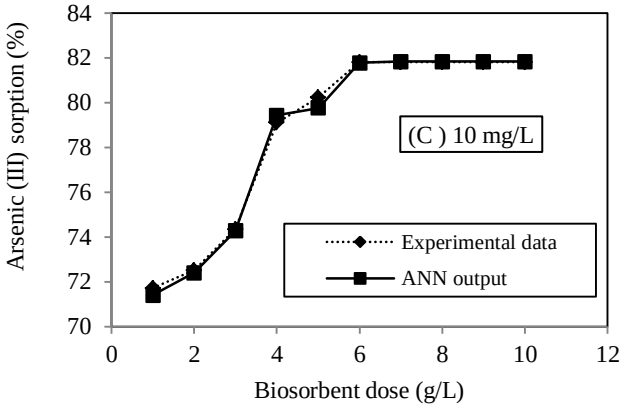


Fig. 5.65 Experimental data and ANN outputs as a function of biosorbent dose versus (%) removal of arsenic (III) by *Bacillus cereus* biomass.

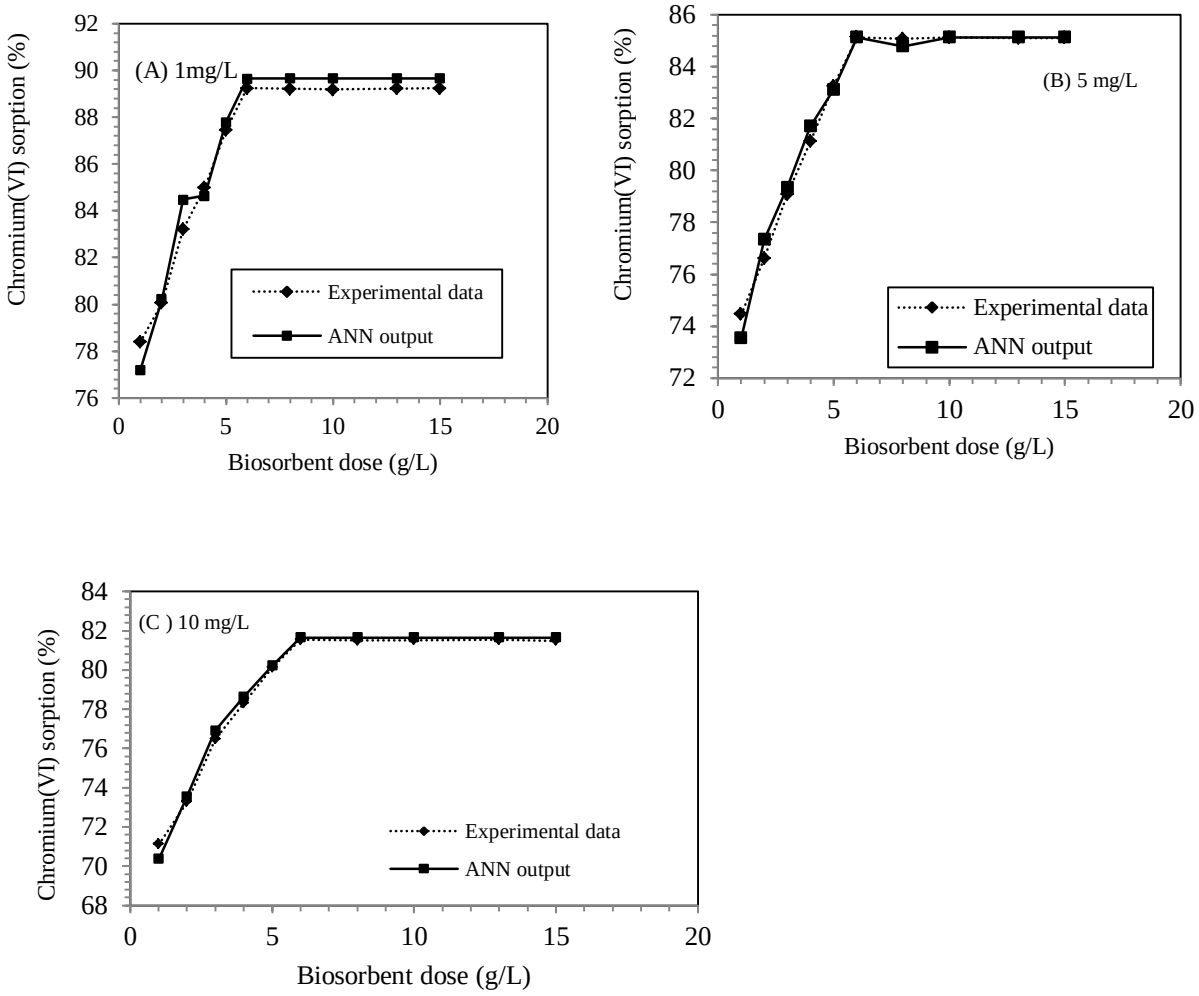


Fig. 5.66 Experimental data and ANN outputs as a function of biosorbent dose versus (%) removal of chromium (VI) by *Bacillus cereus* biomass.

5.3.2. Effect of contact time on the sorption efficiency

Biosorption of arsenic (III) and chromium (VI) at different contact time is studied for initial arsenic (III) and chromium (VI) concentration of 1 mg/L, 5 mg/L and 10 mg/L at pH 7.5 keeping all other parameters constant and results are presented in Fig. 5.67 and Fig. 5.68, respectively**.** Experimental results details discussed in chapter 5 (Section 5.2.4). Fig. 5.67 and Fig. 5.68 show a comparison between the ANN model predictions and the experimental data as a function of contact time. It can be seen that the ANN model satisfactorily predicts the trend of the experimental data.

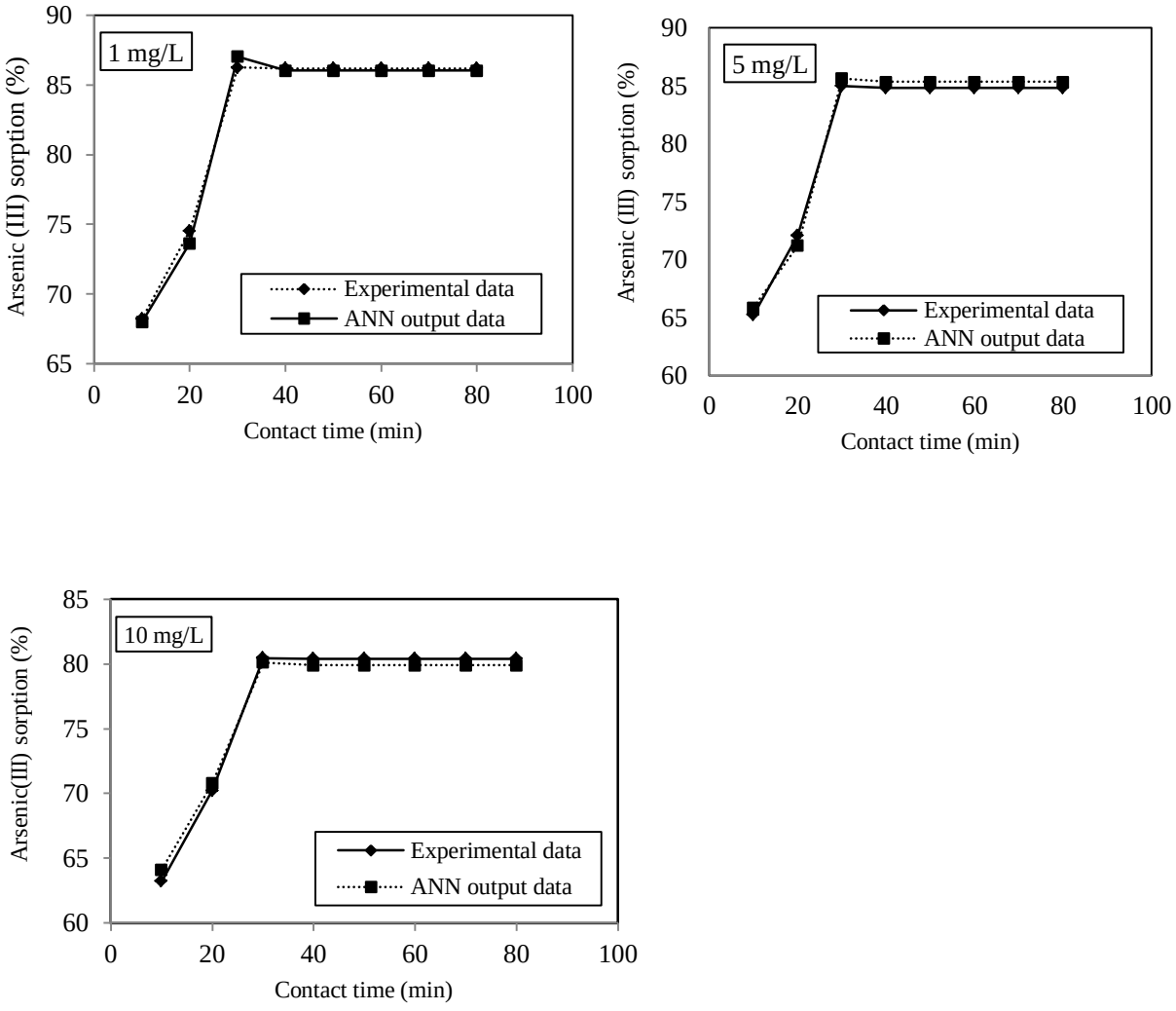


Fig. 5.67 Experimental data and ANN outputs as a function of contact time versus (%) removal of arsenic (III) by *Bacillus cereus* biomass.

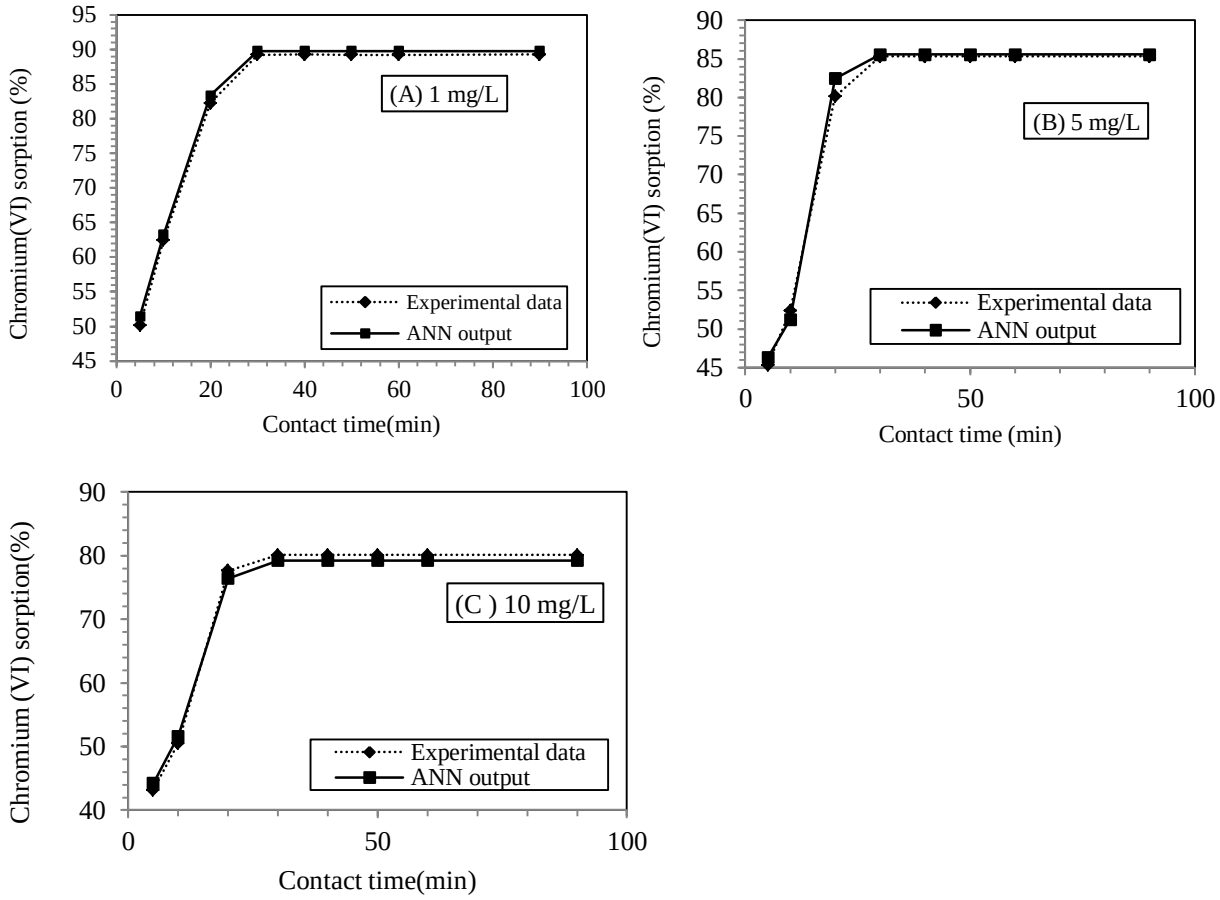


Fig. 5.68 Experimental data and ANN outputs as a function of contact time versus (%) removal of chromium(VI) by *B. cereus* biomass.

5.3.3. Effect of initial concentration on the sorption efficiency

The biosorption of arsenic (III) onto *B. cereus* is studied by varying initial arsenic (III) and chromium(VI) concentration using optimum adsorbent dose (0.3 g/50 mL) at ambient temperature (30±2º C) and contact time of 60 min. Experimental results details discussed in chapter 5 (Section 5.2.6). Fig. 5.69 and Fig. 5.70 show a comparison between the ANN model predictions and the experimental data as a function of initial concentration. It can be seen that the ANN model satisfactorily predicts the trend of the experimental data.

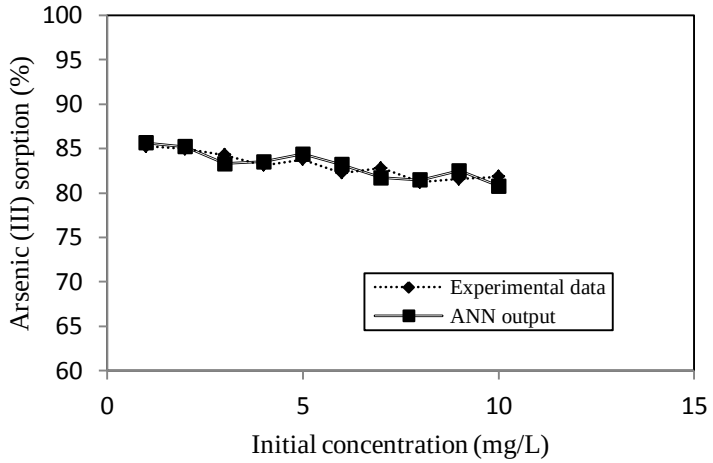


Fig. 5.69. Experimental data and ANN outputs as a function of initial concentration versus (%) removal of arsenic (III) by *Bacillus cereus* biomass.

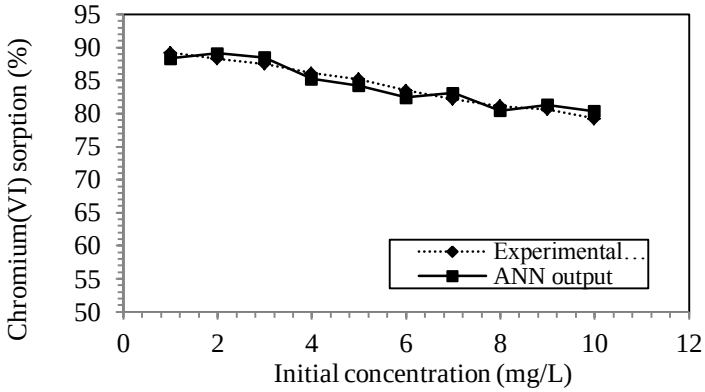


Fig. 5.70. Experimental data and ANN outputs as a function of initial concentration versus (%) removal of chromium(VI) by *Bacillus cereus* biomass.

5.3.4. Effect of temperature of sorption efficiency

The effect of temperature on the sorption of arsenic (III) and chromium(VI) with initial concentration 1mg/L, 5 mg/L and 10 mg/L is studied using optimum adsorbent dose (0.3 g /50 mL). The results are represented as percentage removal of arsenic (III) and chromium (VI) versus temperature. Experimental results details discussed in chapter 5 (Section 5.2.8). The ANN model predictions and the experimental data as a function of operating temperature are depicted in Fig.5.71 and Fig. 5.72. From this plot it can be seen that there is a good agreement between predictions of the ANN model and the experimental data.

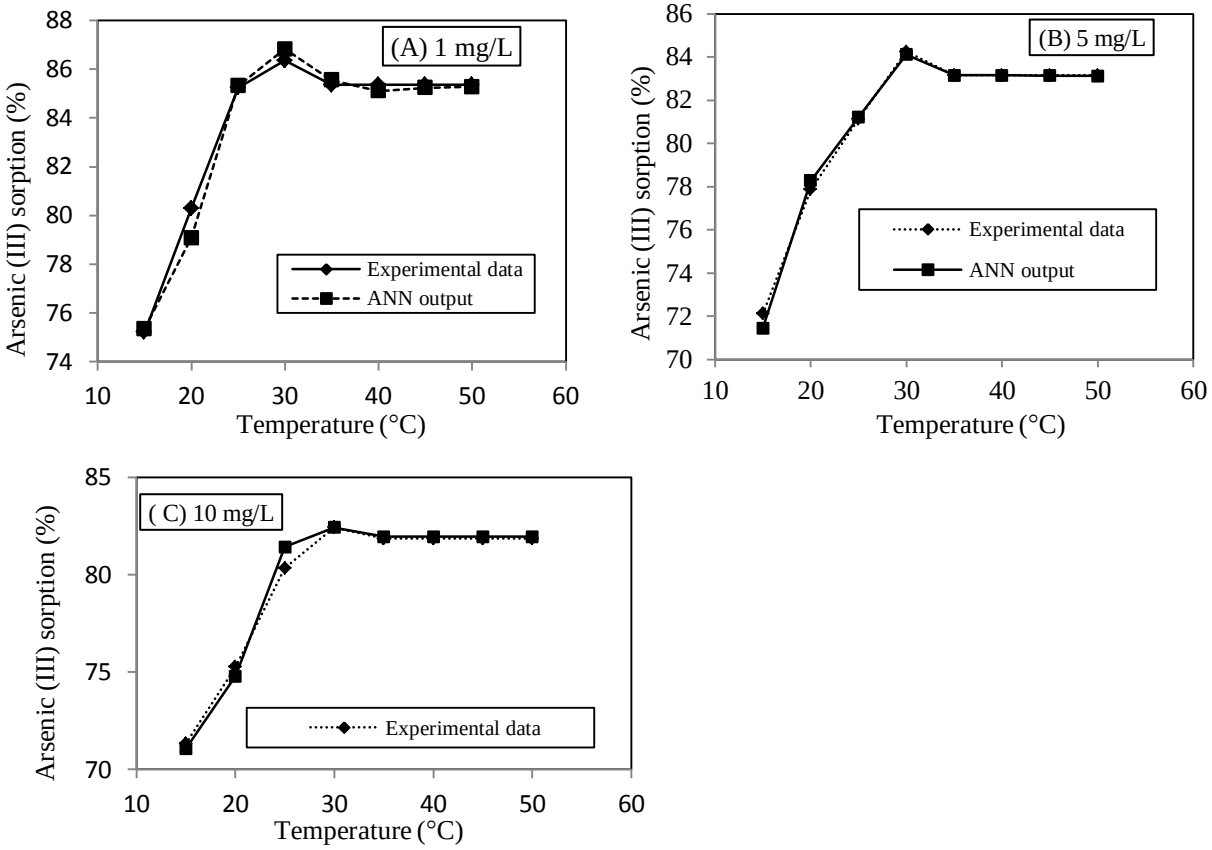
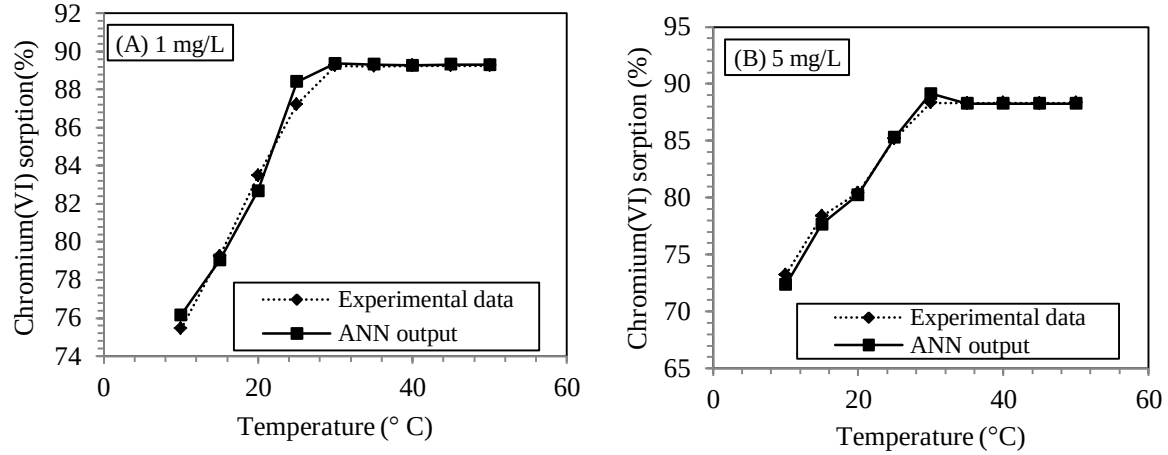


Fig. 5.71. Experimental data and ANN outputs as a function of temperature versus (%) removal of arsenic (III) by *Bacillus cereus* biomass.



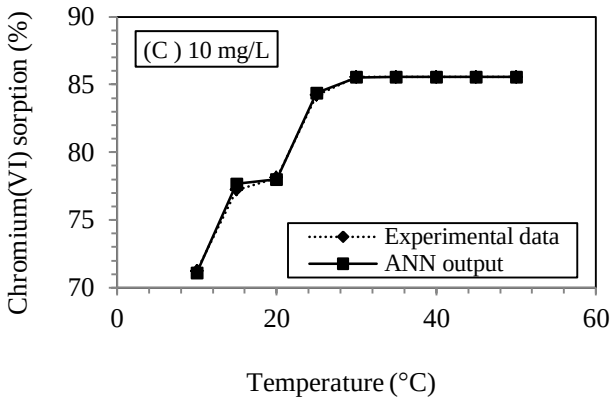
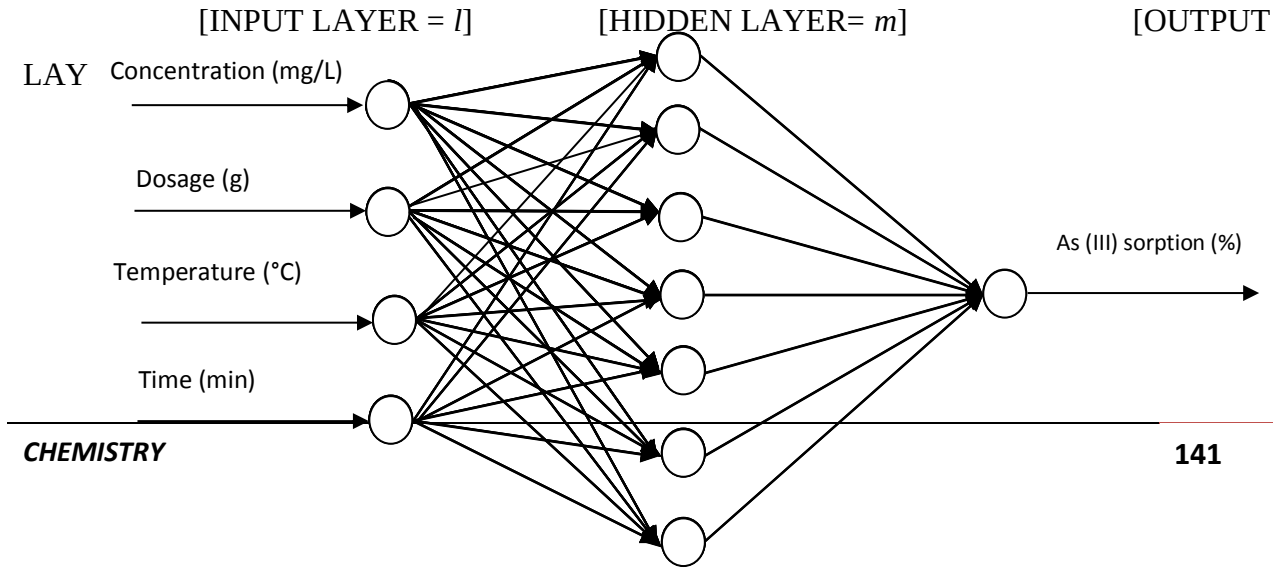


Fig. 5.72. Experimental data and ANN outputs as a function of temperature versus (%) removal of chromium (VI) by *Bacillus cereus* biomass.

5.3.5. Prediction of sorption efficiency using ANN model

The prediction of removal efficiency of arsenic(III) and chromium(VI) ions from aqueous system using *Bacillus cereus* biomass is a complex proposition and hence, a neural network approach is adopted. One hundred seventy one experimental data is divided into training and testing sets. Seventy five percent of data (131) is used as training set whereas twenty five percent of data (40) are used for testing for arsenic (III). And One hundred seventy one experimental data is divided into training and testing sets. Seventy five percent of data (131) is used as training set whereas twenty five percent of data (40) are used for testing for chromium (VI). Fig. 5.73 shows a BP algorithm with three-layer architecture (a single hidden layer) with a tangent sigmoid transfer function (*tansig*) at input and hidden layer and a linear transfer function (*purelin*) at output layer is used and run on MATLAB 7.0 using a Pentium IV PC (Chu, 2003).The distribution of output of training data is shown in Fig. 5.74 for arsenic (III) and Fig. 5.75 for chromium (VI). Initially, the training data set is presented to the network.



Cr(VI) sorption (%)

Fig. 5.73. The ANN Architecture

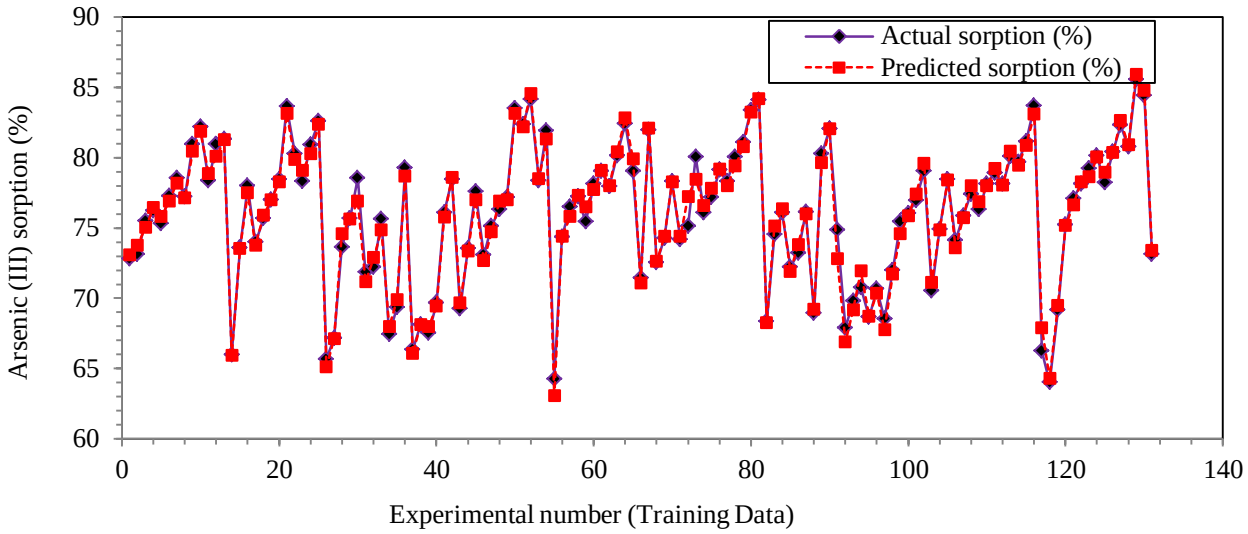


Fig. 5.74. Distribution of As (III) sorption percentage (Training data).

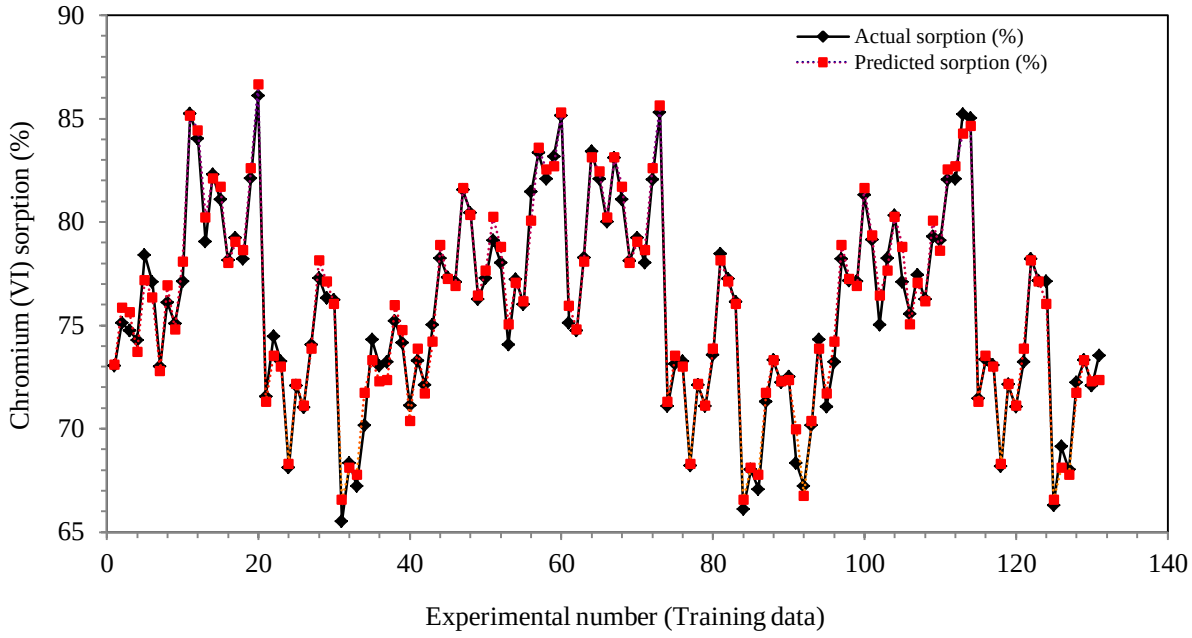


Fig. 5.75. Distribution of chromium (VI) sorption percentage (Training data).

The number of nodes in the hidden layer (H) is decided by the relation below:

$H = 2\sqrt{(I+1)}$

where I is the number of nodes at the input layer.

The network is tested with different number of neurons to find the optimal number of neurons at the hidden layer by observing the mean squared error (Gob *et al*., 1999; Turan *et al*., 2011a; Turan *et al*., 2011b). Seven neurons are selected in the hidden layer when mean square error starts decreasing. Learning and momentum parameters are set at 0.25 and 0.20 respectively during the training phase both arsenic (III) and chromium (VI). During training phase, the output vector is computed by a forward pass in which the input is propagated forward through the network to compute the output value of each unit. The output vector is then compared with the desired vector which resulted into error signal for each output unit. In order to minimize the error, appropriate adjustments were made for each of the weights of the network. After several such iterations, the network was trained to give the desired output for a given input vector. The network is trained till minimum root mean square error is observed. A root mean square error of 0.77 is observed at epoch number 23, 644, 85 for arsenic (III) and 0.68 is observed at epoch number 23, 835, 76 for chromium (VI). Training was stopped at this point and weights have been

frozen for network to undergo testing phase. A high degree of correlation between actual and predicted sorption efficiency (%) is observed as shown in Fig. 5.76 for arsenic (III) and Fig. 5.77 for chromium (VI). Coefficient of determination (R^2) of 0.986 is for arsenic (III) and 0.984 for chromium (VI) obtained for training data set. When the network is well trained, testing of the network with testing data set is carried out.

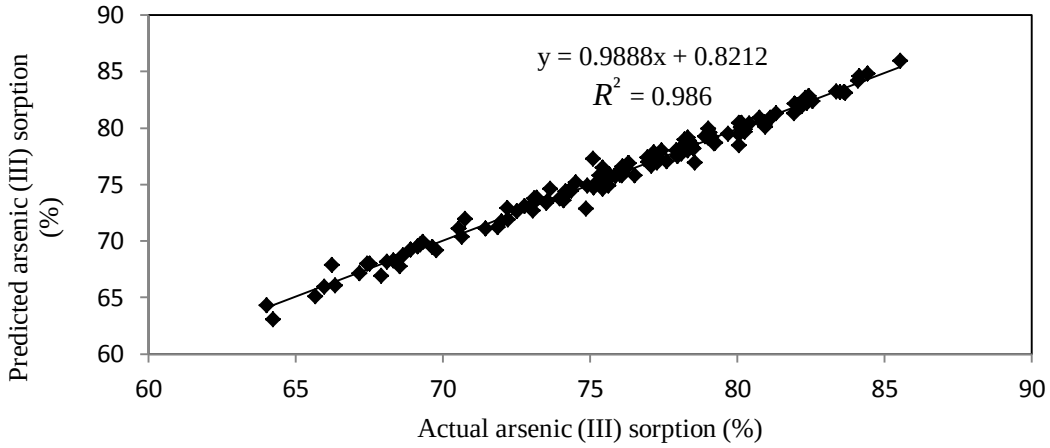


Fig. 5.76. Correlation of predicted and actual arsenic (III) sorption percentage (Training data).

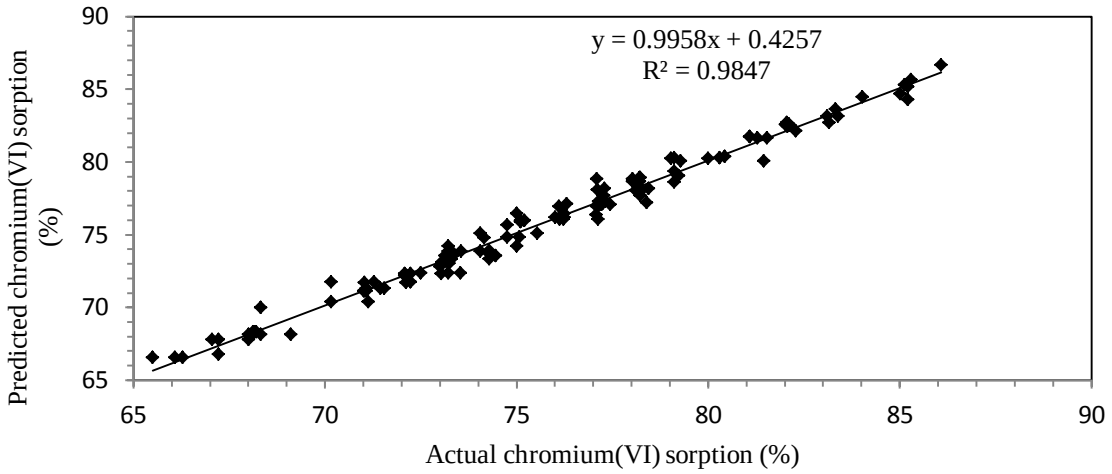


Fig. 5.77. Correlation of predicted and actual chromium (VI) sorption percentage (Training data).

The prediction ability of the developed network model for responses of experimental data not forming part of the training set. During testing phase, output of the data is not presented to the

network. The distribution of output of testing data is shown in Fig. 5.78 for arsenic (III) and Fig. 5.79 for chromium (VI), respectively.

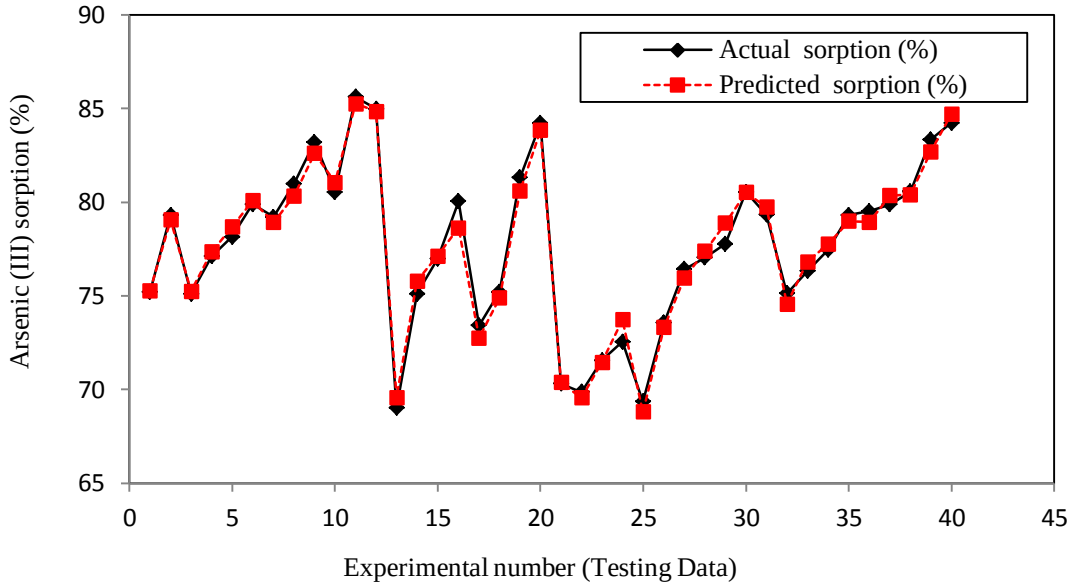


Fig. 5.78. Distribution of arsenic (III) sorption percentage (Testing data).

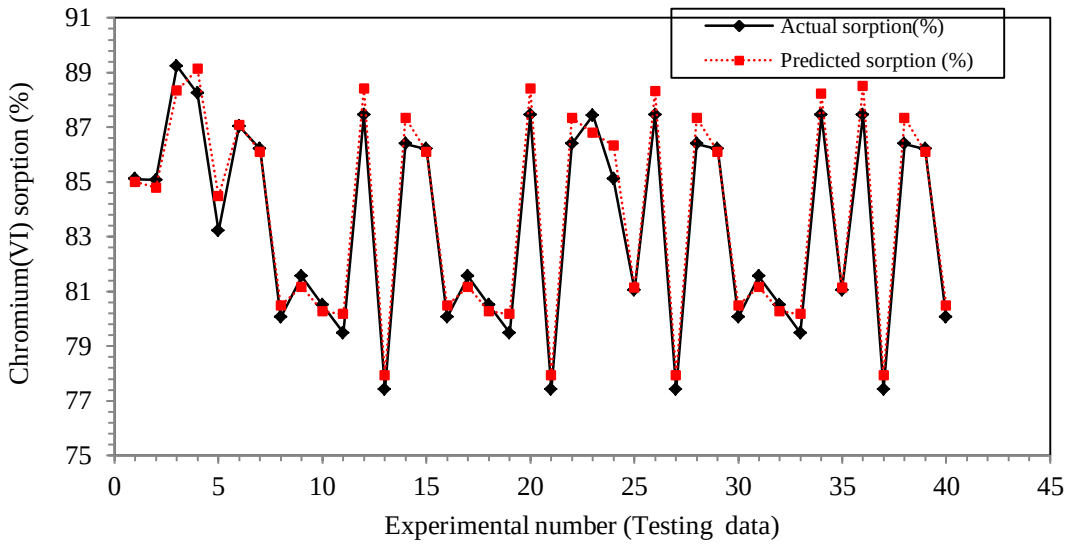


Fig. 5.79. Distribution of chromium (VI) sorption percentage (Testing data).

A high degree of correlation (R^2=0.9849) between actual and predicted arsenic(III) sorption efficiency (%) is observed as shown in Fig. 5.80 for testing data set. The mean absolute relative

percentage error for training data is obtained as 0.567 whereas mean absolute relative percentage error for testing data is found to be 0.563. The predicted and actual experimental values for testing data are shown in Table 5.19. The residuals for training data are plotted in Fig. 5.81. It can be observed that the residuals are distributed uniformly below and above zero line.

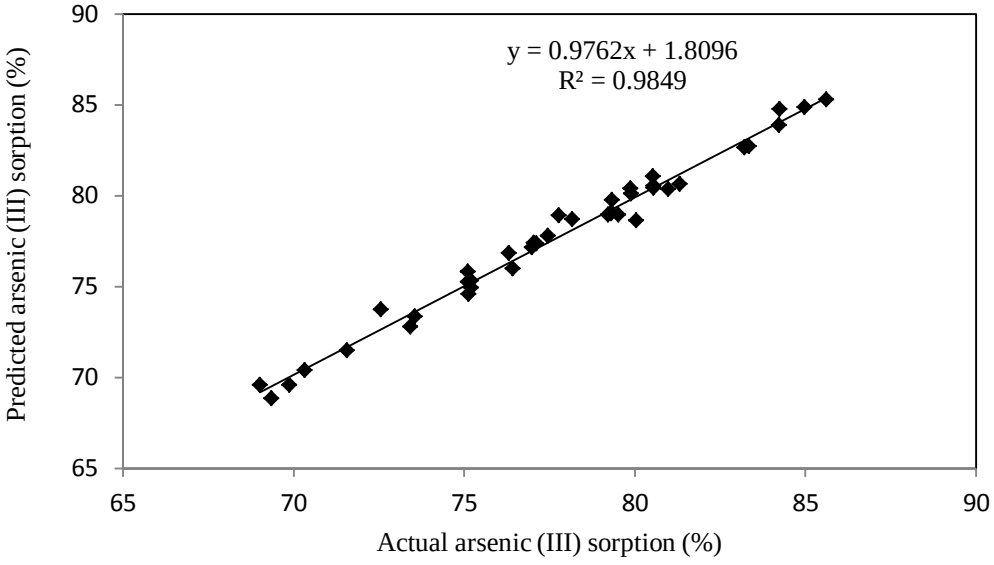


Fig. 5.80. Correlation of predicted and actual As (III) sorption percentage (Testing data).

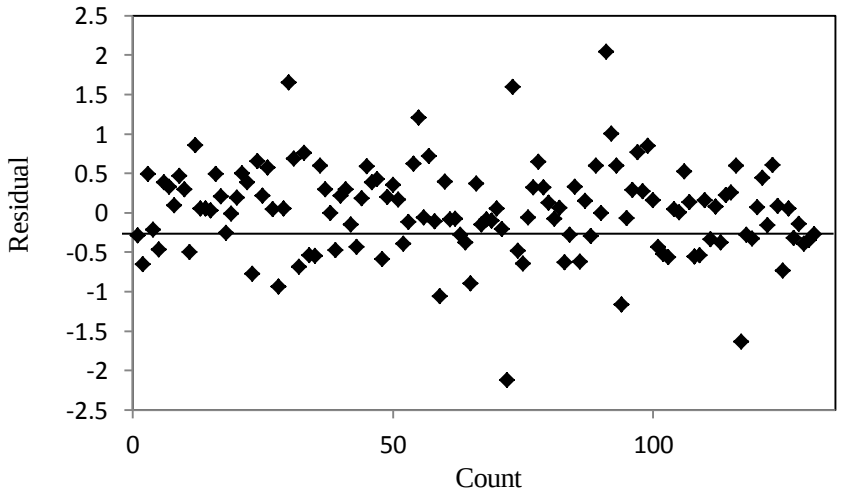


Fig. 5.81. Distribution of residuals (Training data).

Table 5.19. Comparison of experimental and predicted output for arsenic (III) (testing data).

Exp. No.	Concentra-tion (mg/L)	Biosorbent (g/L)	Temp (°C)	Time (min)	Actual sorption (%)	Predicted sorption (%)	Residual (%)	(%) error	Absolute error
1	10	6	40	30	73.11	73.38136	-0.27136	-0.36979	0.369794
2	10	6	20	30	75.21	75.29337	-0.08337	-0.11073	0.110727
3	10	6	30	30	79.32	79.06295	0.25705	0.325121	0.325121
4	10	6	40	30	75.12	75.24944	-0.12944	-0.17201	0.172015
5	5	2	20	30	77.11	77.35783	-0.24783	-0.32037	0.320368
6	5	2	30	30	78.16	78.68963	-0.52963	-0.67306	0.673062
7	5	2	40	30	79.89	80.09287	-0.20287	-0.25329	0.253293
8	5	4	20	30	79.22	78.93162	0.28838	0.365354	0.365354
9	1	4	30	30	80.99	80.34122	0.64878	0.807531	0.807531
10	1	4	40	30	83.21	82.6212	0.5888	0.71265	0.71265
11	1	6	20	30	80.54	81.05475	-0.51475	-0.63506	0.635065
12	1	6	40	60	85.62	85.26082	0.35918	0.421272	0.421272
13	5	4	20	60	84.98	84.84826	0.13174	0.155265	0.155265
14	5	4	30	60	69.02	69.57363	-	-	0.79574

							0.55363	0.79575	7
15	5	4	40	60	75.11	75.79496	-0.68496	-0.9037	0.903701
16	3	2	20	60	76.98	77.12617	-0.14617	-0.18952	0.189521
17	10	2	30	60	80.05	78.62916	1.42084	1.807014	1.807014
18	10	2	40	60	73.43	72.76161	0.66839	0.918603	0.918603
19	10	4	20	60	75.21	74.89846	0.31154	0.41595	0.41595
20	1	4	30	60	81.32	80.60181	0.71819	0.891035	0.891035
21	1	6	40	30	84.22	83.84704	0.37296	0.44481	0.44481
22	10	2	20	30	70.32	70.37903	-0.05903	-0.08387	0.083874
23	10	2	30	30	69.87	69.56259	0.30741	0.441919	0.441919
24	10	2	40	30	71.56	71.45126	0.10874	0.152188	0.152188
25	5	4	20	30	72.56	73.73324	-1.17324	-1.5912	1.591196
26	5	4	30	30	69.35	68.81047	0.53953	0.784081	0.784081
27	5	4	40	30	73.56	73.32636	0.23364	0.31863	0.31863
28	5	4	20	30	76.43	75.9723	0.4577	0.602456	0.602456
29	1	2	30	30	77.05	77.37918	-0.32918	-0.42541	0.425412
30	1	2	40	30	77.78	78.88525	-	-	1.40108

							1.10525	1.40109	6
31	1	6	20	30	80.55	80.52705	0.02295	0.0285	0.0285
32	10	6	30	90	79.34	79.7483	-0.4083	-0.51199	0.511986
33	10	6	40	90	75.13	74.56836	0.56164	0.753188	0.753188
34	10	6	20	90	76.32	76.82391	-0.50391	-0.65593	0.655929
35	10	6	30	90	77.45	77.75763	-0.30763	-0.39563	0.395627
36	10	6	40	90	79.31	78.99341	0.31659	0.40078	0.40078
37	1	4	20	90	79.52	78.93287	0.58713	0.743835	0.743835
38	1	4	30	90	79.88	80.3618	-0.4818	-0.59954	0.599539
39	1	4	40	90	80.56	80.40672	0.15328	0.190631	0.190631
40	1	4	20	90	83.34	82.69735	0.64265	0.777111	0.777111

A high degree of correlation (R^2=0.9773) between actual and predicted chromium(VI) sorption efficiency (%) is observed as shown in Fig. 5.82 for testing data set. The mean absolute relative percentage error for training data is obtained as 0.749 whereas mean absolute relative percentage error for testing data is found to be 0.673. The predicted and actual experimental values for testing data are shown in Table 5.20. The residuals for training data are plotted in Fig. 5.83. It can be observed that the residuals are distributed uniformly below and above zero line.

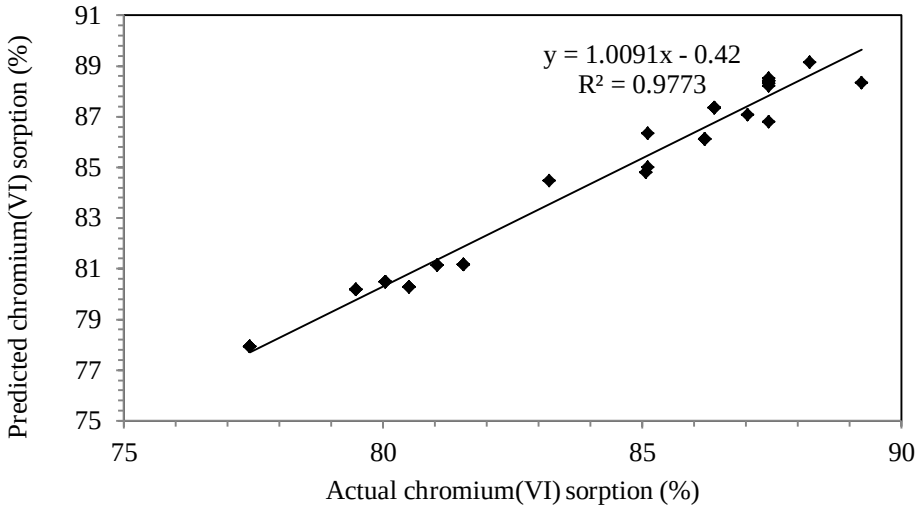


Fig. 5.82. Correlation of predicted and actual chromium(VI) sorption percentage (Testing data).

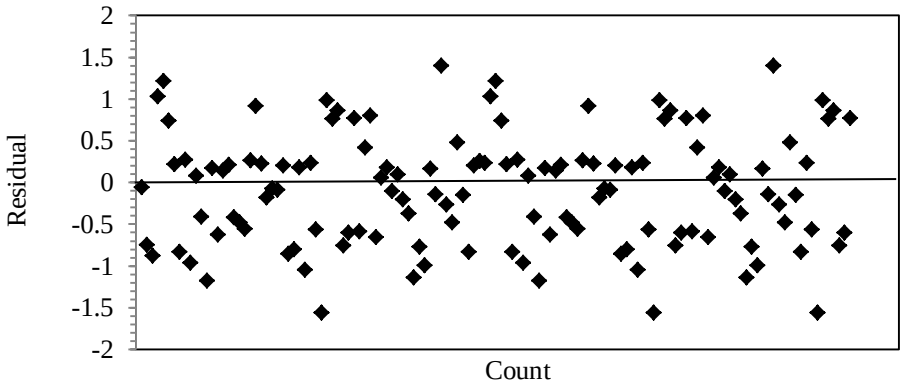


Fig. 5.83. Distribution of residuals (Training data).

Table 5.20. Comparison of experimental and predicted output for chromium(VI) (testing data).

Exp. No.	Concentra-tion (mg/L)	Biosorbent (g/L)	Temp (ºC)	Time (min)	Actual sorption (%)	Predicted sorption (%)	Residual (%)	(%) error	Absolute error
1	10	6	40	30	85.11	84.9976	0.1124	0.132064	0.132064
2	10	6	20	30	85.07	84.7944	0.2756	0.323968	0.323968

3	10	6	30	30	89.24	88.3401	0.8999	1.0084	1.0084
4	10	6	40	30	88.24	89.1302	-0.8902	-1.0088	1.0088
5	5	2	20	30	83.21	84.4803	-1.2703	-1.52661	1.52661
6	5	2	30	30	87.04	87.0811	-0.0411	-0.04722	0.04722
7	5	2	40	30	86.21	86.0977	0.1123	0.13026	0.13026
8	5	4	20	30	80.05	80.489	-0.4391	-0.54841	0.5484
9	1	4	30	30	81.55	81.1587	0.3913	0.479828	0.479828
10	1	4	40	60	80.51	80.2694	0.2406	0.298844	0.298844
11	1	6	20	60	79.48	80.1682	-0.6882	-0.8658	0.8658
12	1	6	40	60	78.45	79.5125	-1.0625	-1.37227	1.37227
13	5	4	20	60	77.426	77.9345	-0.5085	-0.65676	0.65676
14	5	4	30	60	76.395	77.3455	-0.9505	-1.24491	1.24491
15	5	4	40	60	75.364	75.7895	-0.4255	-0.56459	0.564592
16	5	2	20	60	74.333	73.3659	0.9671	1.301037	1.301037
17	10	2	30	60	73.302	73.2625	0.0395	0.053887	0.053886
18	10	2	40	60	72.271	72.621	-0.35	-0.48429	0.484229
19	10	4	20	60	71.24	71.8066	-0.5666	-0.79534	0.795343
20	1	4	30	30	70.209	69.4716	0.7374	1.050292	1.050292
21	1	6	40	30	69.178	70.3689	-1.1909	-1.7215	1.72152
22	10	2	20	30	84.21	84.9976	-0.7876	-0.93528	0.93528
23	10	2	30	30	87.44	86.7944	0.6456	0.738334	0.738334
24	10	2	40	30	85.11	86.3401	-1.2301	-1.44531	1.44531
25	5	4	20	30	81.05	81.1302	-0.0802	-0.09895	0.09895
26	5	4	30	30	82.35	82.4803	-0.1303	-0.15823	0.158227
27	5	4	40	30	82.419	81.0811	1.3379	1.62329	1.62329
28	5	4	20	30	80.488	80.0977	0.3903	0.484912	0.484912
29	1	2	30	30	81.457	81.489	-0.032	-0.03928	0.039284

30	1	2	40	30	79.426	79.1587	0.2673	0.336539	0.336539
31	1	6	20	30	82.395	82.2694	0.1256	0.152436	0.152436
32	10	6	30	90	74.164	74.1682	-0.00042	-0.00057	0.00057
33	10	6	40	90	75.333	76.5125	-1.1795	-1.56571	1.56571
34	10	6	20	90	73.402	73.9345	-0.5352	0.725457	0.725457
35	10	6	30	90	73.271	73.3455	-0.0745	-0.10168	0.101677
36	10	6	40	90	74.24	74.7895	-0.5495	-0.74017	0.74017
37	1	4	20	90	69.209	70.3659	-1.1569	-1.6716	1.67161
38	1	4	30	90	70.178	70.2625	-0.0845	-0.12041	0.12041
39	1	4	40	90	73.41	73.621	-0.211	-0.28748	0.28748
40	1	4	20	90	72.86	72.8066	0.0534	0.073291	0.073291

Therefore, it can be assumed that the errors are normally distributed and the model can be used for prediction purpose with reasonable accuracy. The development of the proposed ANN model is an effort towards the growing interest in applying ANN modeling technique to the area of biosorption of pollutants from water bodies (Aber *et al*., 2009; Hamed *et al*., 2004; Giri *et al*., 2011). Turan *et al*. (2011) have tested several ANN approaches to discover the optimum learning algorithm for modeling Zn(II) adsorption from leachate using a new biosorbent considering input parameters as initial pH, adsorbent dosage, contact time, and temperature. They have also suggested a design of experiment approach for finding out significant parameters. However, the present study is focused on modelling As(III) and Cr(VI) biosorption using a new biosorbent. Both the studies conclude that ANN approach is quite efficient in modelling complex biological phenomenon.

5.4. Adsorption of chromium(VI) ions from aqueous solution using activated carbon derived from *Eichhornia crassipes* root biomass

5.4.1. Characterization of the activated carbon before and after adsorption

5.4.1.1. SEM-EDX analysis

The surface morphology of activated carbon without sorption of chromium (VI) ions and with sorption of chromium (VI) ions during adsorption process is measured with the help of SEM-

EDX and is presented in Fig. 5.84 and Fig. 5.85, respectively. Fig. 5.84 clearly reveals the surface texture and porosity in the materials. It is evident that the carbon particles are in the form of spheres with a wide range of sizes (Sinha *et al*., 2003). Fig. 5.85 shows the morphological changes with respect to shape and size of the activated carbon after adsorption of chromium (VI) ion. It can be clearly observed that the surface of activated carbon has been changed into a new shiny bulky particles and whitish patches structure after chromium (VI) adsorption. The EDX spectra of chromium (VI) unloaded and loaded activated carbon obtained is shown in Fig. 5.84 and Fig. 5.85, respectively. So, it is concluded that, chromium (VI) are adsorbed on the surface of the adsorbent. These results are further confirmed with the results of FTIR spectra analysis.

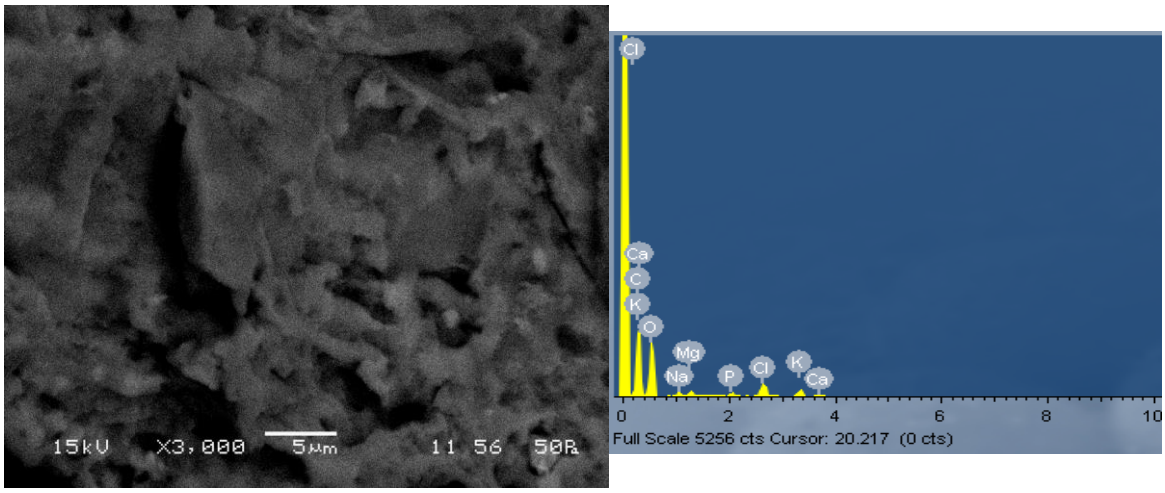


Fig.5.84. SEM-EDX images of activated carbon without sorption of chromium (VI) ions.

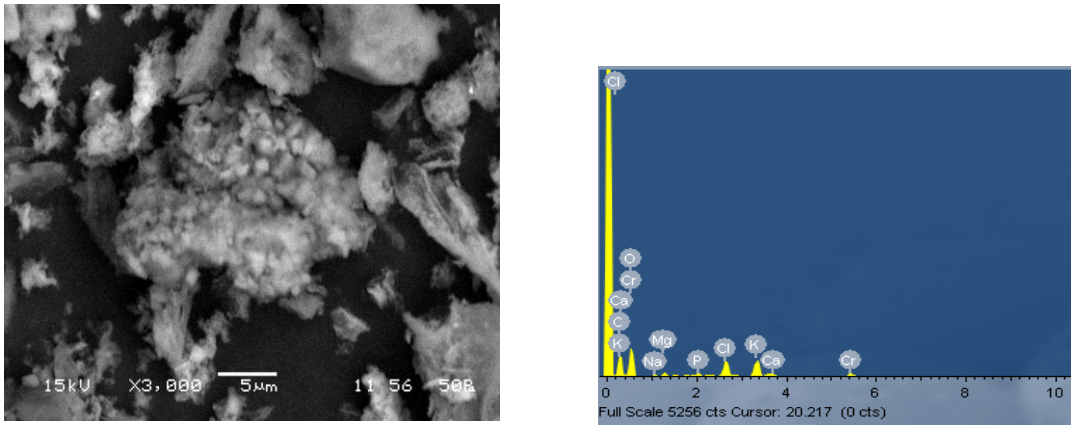


Fig.5.85. SEM-EDX images of activated carbon with sorption of chromium (VI) ions.

5.4.1.2. FTIR analysis

FTIR spectra of the activated carbon without chromium (VI) loaded displays a number of absorption peaks, presented in Fig. 5.86 indicates the complex nature of the activated carbon. The spectra of activated carbon loaded with chromium (VI), presented in Fig. 5.87. When the spectra are compared the following changes are observed. The spectra of activated carbon exhibits a broad absorption band at 3,146.34 cm^{-1} due to bonded –OH stretching vibration which is shifted to 3,225.78 cm^{-1} may be due to complexation of –OH groups with chromium (VI) ions (Gardea-Torresday *et al*., 2000). The peak at 2,918.50 cm^{-1} has been shifted insignificantly. The peak at 1,645.17 cm^{-1} has been shifted to 1,638.96 cm^{-1}, may be due to the complexation of carboxylic group with chromium (VI) (Basha *et al*., 2008). Another shift is observed from 1,418.96 cm^{-1} 1,319.75 cm^{-1}, corresponding to the complexation of N-H group with chromium (VI) (Bansal *et al*., 2009). The next shift was observed from 1,172.97 cm^{-1} to 1,163.54 cm^{-1} possibly due to the interaction of nitrogen from amino group with chromium (VI) (Vinodhini and Das, 2009). The other weak absorption peak shifted from 1,008.50 to 1,022.47 cm^{-1}, corresponding to the O-C-O scissoring vibration of polysaccharide. The above changes in the spectra may be attributed to the interaction of chromium (VI) with the hydroxyl, carboxyl and amino groups present on the surface of the activated carbon (Ning *et al*., 2009). This clearly manifests the binding of chromium (VI) to the activated carbon.

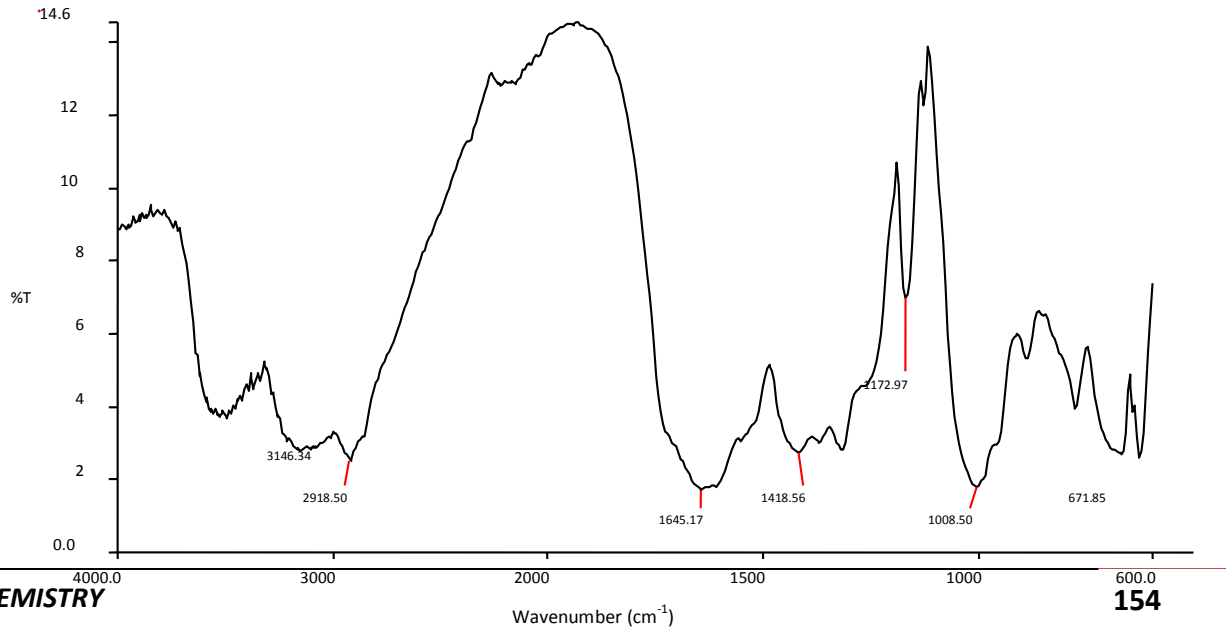


Fig. 5.86. FTIR spectra of the activated carbon without sorption of chromium (VI) ions.

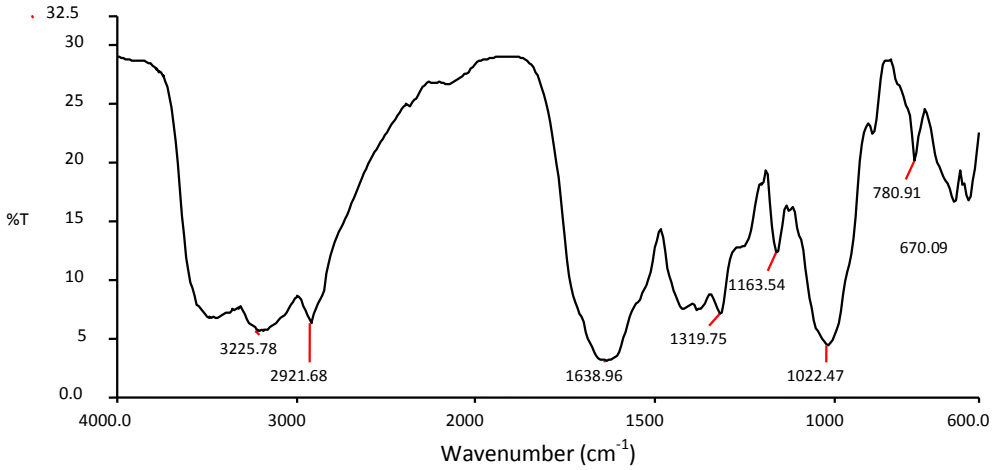


Fig.5.87. FTIR spectra of the activated carbon with sorption of chromium (VI) ions.

5.4.1.3. XRD analysis

XRD pattern of the activated carbon without treated with the chromium (VI) solution is shown in Fig. 5.88. Broad peaks were obtained instead of sharp peaks indicating the sample was poorly crystalline. The phases of $Cr_2O_7^{2-}$ and $HCrO_4^-$ are found in the recovered adsorbent as represented in Fig. 5.89. Some of the Cr (V) is converted into $Cr_2O_7^{2-}$ and some of the converted into $HCrO_4^-$ at pH 4.5. So it was concluded that, chromium (VI) ions finally get adsorbed over the surface of adsorbent (Ramkrishnaiah and Prathima, 2012).

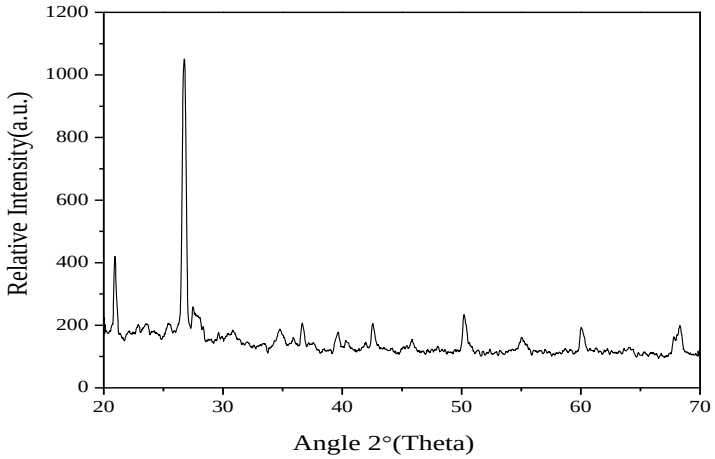


Fig. 5.88. XRD pattern of activated carbon without sorption of chromium (VI) ions.

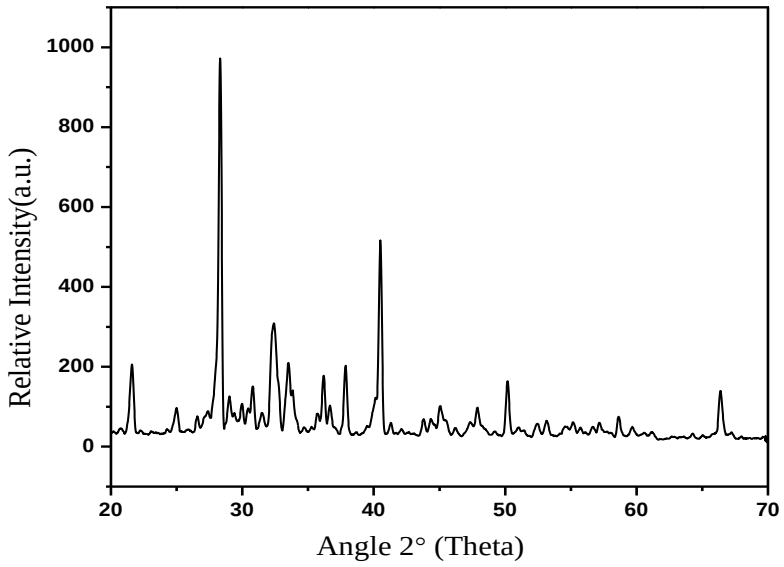


Fig. 89. XRD pattern of activated carbon with sorption of chromium (VI) ions.

5.4.2. Adsorption study of chromium (VI) batch experiments

5.4.2.1. Effects of adsorbent dosage on chromium (VI) removal

Adsorbent dose is an important parameter which determines the capacity of adsorbent for an initial concentration of the adsorbate. The effect of adsorbent dose on the adsorption of chromium (VI) is studied at pH 4.5, at ambient temperature (25 ± 2°C) and contact time of 60

min for initial chromium (VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L. Experimental results showed that as the adsorbent mass increased from 1g/L to 7 g/L, the percentage removal of chromium (VI) also increased from 75.22% to 85.56%, 77.45% to 88.32% and 81.70 % to 92.24% for 0.05-0.5 g/50 mL of activated carbon and initial Cr(VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L. It is observed that after dosage of 0.35 g/50 mL, there is no significant change in percentage of removal of chromium (VI). This may be due to the overlapping of active sites at higher dosage (Dakiky *et al*., 2002; Kyzas *et al*., 2009). So, there is not any appreciable increase in the effective surface area resulting due to the conglomeration of exchanger particles, thus in lower chromium (VI) uptake. Hence 0.35 gm/50 mL is considered as the optimum dose uses for further studies.

5.4.2.2. Effects of pH on chromium (VI) removal

The pH is one of the important factors which affect the adsorption of metal ions in the solution. Percentage removal of chromium(VI) at pH 1.5-8.5 was studied in batch experiments using 0.35 g of activated carbon in 50 mL synthetic solution, at ambient temperature (25 ± 2°C) and contact time of 60 min for initial chromium(VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L. The adsorption efficiency of chromium (VI) was found to increase from 41.22% to 85.52% for 10 mg/L, 45.34% to 89.23 % for 50 mg/L and 50.23% to 92.24 % for 100 mg/L with the increase of pH from 1.5 to 4.5 than decreases with further increase in pH up to 9.5 and presented in Fig. 5.90.

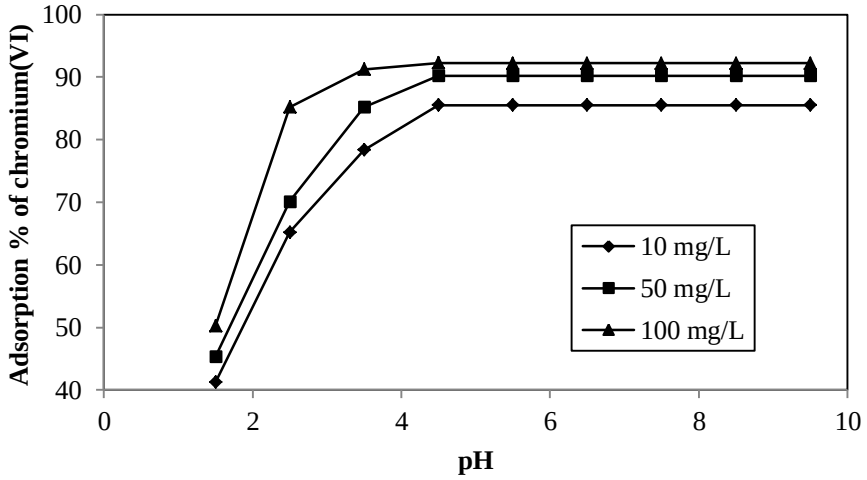


Fig. 5.90. Effect of pH on the activated carbon of chromium (VI) ions with initial concentration of 10 mg/L, 50 mg/L and 100 mg/L.

At low pH (1.5-4.5) the surface of activated carbon is highly protonated and as a result, a strong attraction exists between positively charged surface of the adsorbent and oxyanion (Heidmann *et al*., 2008; Huang *et al*., 2009). The further decrease in chromium (VI) uptake with increase in pH (4.0 - 9.5) may be due to the fact that at higher pH, the substrate may be negatively charged by adsorbing hydroxyl ions on the surface or by ionization of very weak acidic functional groups of the adsorbent, or both. A repulsive force may develop between the negatively charged surface and the anions. At lower pH, the process of regeneration predominates over the process of removal. And hence the process of conversion of adsorbent into its H^+ form plays an important role leaving behind chromium (VI) in the aqueous solution. The process of adsorption and regeneration is demonstrated in Fig. 5.91.

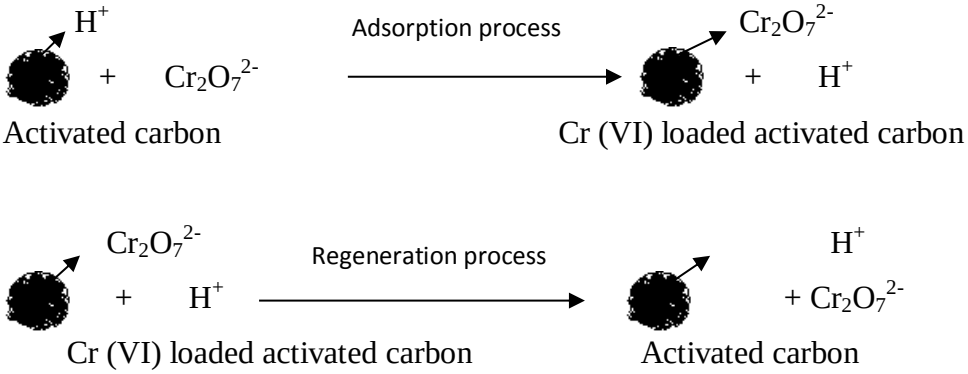


Fig.5.91. Adsorption and regeneration process of activated carbon with chromium (VI) ions.

5.4.2.3. Mechanism of chromium (VI) removal

The mechanism of any adsorption process is an important component in understanding the process as well as to know the characteristics of the material which help to design a new adsorbent for future applications. A mechanism for the adsorption of chromium (VI) by ion-exchanger activated carbon has been proposed by taking the results obtained from the experimental investigations. At lower pH 4.0 and 4.5 of the medium, surface sites are positively charged and, therefore, attract negatively charged $Cr_2O_7^{2-}$ by an electrostatic interaction process (Mashitah *et al*., 1999; Vinodhini and Das, 2009; Sengupta and Clifford, 1986). The materials under hydration, the activated carbon surface completes the coordination shells with the

available OH group. On the variation of pH, these surface active OH groups may further bind or release H^+ where the surface remains positive due to the reaction:

$MOH + H_3O^+ \longrightarrow MOH_2^+ + H_2O$ (5.4.2.3a)

Thus, when pH < 7.00, the overall chromium (VI) adsorption mechanism can be represented in three different forms: (i) electrostatic interaction between positively charged center (nitrogen) and negatively charged chromium molecule in solution, (ii) electrostatic attraction between positively charged surface hydroxyl group and $Cr_2O_7^{2-}$

$MOH + H_3O^+ + Cr_2O_7^{2-} \longrightarrow MOH_2^{+\cdots\cdots} Cr_2O_7^{2-} + H_2O$ (5.4.2.3b)

(Electrostatic attraction) and (iii) ion-exchange reaction between positively charged metal center and AsO_3^{3-}, $Cr_2O_7^{2-}$:

$MOH + H_3O^+ + Cr_2O_7^{2-} \longrightarrow M^{+\cdots\cdots} Cr_2O_7^{2-} + 2H_2O$ (ion-exchange) (5.4.2.3c)

The modeling of the specific adsorption of $Cr_2O_7^{2-}$ on any material surface depends on a number of external factors such as temperature, pH, initial $Cr_2O_7^{2-}$concentration, as well as the density of surface functional groups available for coordination. In light of the above-mentioned mechanism of adsorption, it may be further noted that activated carbon material showed adsorption capacity at a wide pH of acidic pH range, which could be useful for commercial exploitation purpose.

5.4.2.4. Effect of contact time on chromium (VI) removal

Adsorption of chromium (VI) at different contact time is studied for initial chromium(VI) at pH 4.5 concentration of 10 mg/L, 50 mg/L and 100 mg/L keeping all other parameters constant and results are presented in Fig. 5.92. It is clear from the figure that more than 90% removal takes place within 30 min and equilibrium is reached after 30 min. The change in the rate of removal might be due to the fact that initially all sorbent sites are vacant and also the solute concentration gradient is high. At higher concentrations, metals need to diffuse to the sorbent surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of chromium (VI) ions on the outer surface.

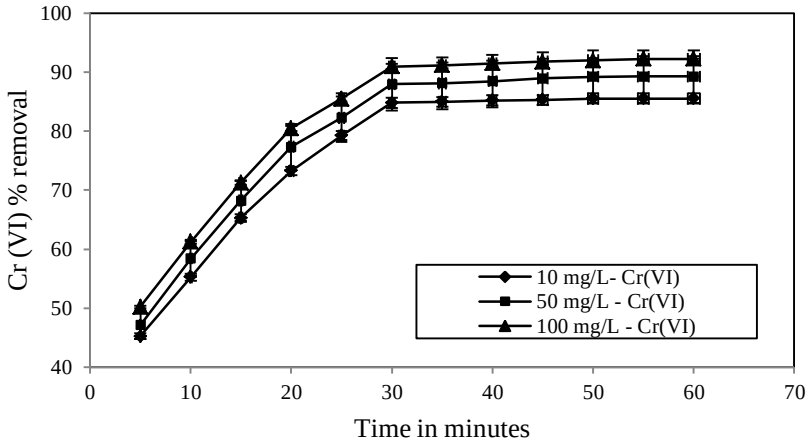


Fig. 5.92. Effect of contact time on the adsorption of chromium (VI) with initial concentration of 10 mg/L, 50 mg/L and 100 mg/L.

5.4.2.5. Adsorption kinetics

The prediction of adsorption rate gives important information for designing batch adsorption systems. The experimental data are applied to pseudo-first-order, pseudo-second-order and Intraparticle diffusion models to clarify the adsorption kinetics of chromium (VI) onto activated carbon. Adsorption of chromium (VI) ions is rapid for the first 30 minutes and its rate slowed down as it approaches towards equilibrium (Chen *et al*., 2011).

5.4.2.5.1. Pseudo-first-order or Lagergren's rate equation

Pseudo-first-order has a rate depends on the concentration of only one reactant. The rate constant K_1 for adsorption of chromium (VI) is studied by Lagergren rate equation (Lagergren, 1898; Yu *et al*., 2003; Pokhrel and Viraraghavan, 2007) for initial chromium (VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L.

$$\log (q_e - q_t) = \text{Log } q_e - \frac{K_1}{2.303} \text{ x } t$$

where q_e and q_t (mg/g) are the amounts of chromium(VI) ions adsorbed at equilibrium (mg/g) and at time '*t*' (min), respectively, and k_1 is the rate constant of the equation (min^{-1}). The adsorption rate constants (k_1) was calculated from the slope of the linear plot of log (q_e - q_t) vs. *t*, which is presented in Fig. 5.93.

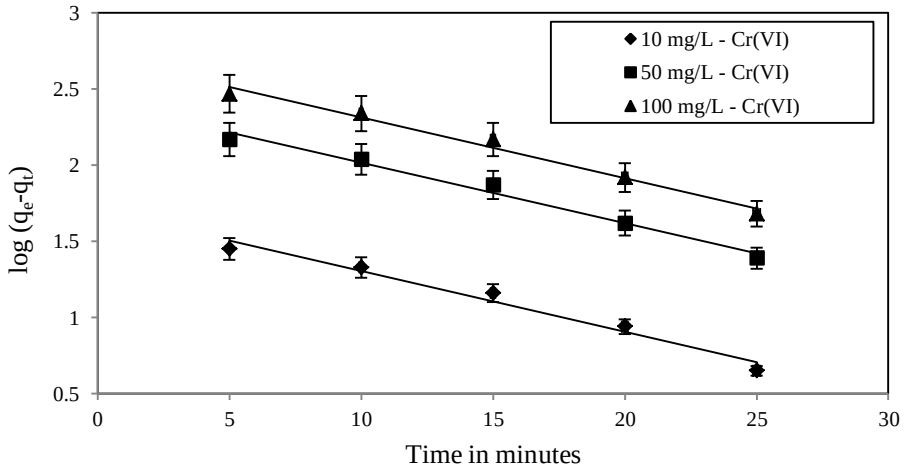


Fig. 5.93. Adsorption kinetics of time vs. log (q_e-q_t) with initial chromium (VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L.

The plots of log (q_e - q_t) vs. t for the pseudo-first-order model was almost linear, indicates the validity of Lagergren rate equation of first order kinetics (Fig. 5.93). The adsorption rate constant (k_1), calculated from the slope of the above plot is presented in Table 5.21.

5.4.2.5.2. Pseudo-second-order rate equation

Pseudo-second-order depends on the concentrations of one second-order reactant, or two first-order reactants. Experimental data were also tested by the pseudo-second-order kinetic model which is given in the following form (Ho and McKay, 1999; Brum *et al*., 2010):

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} X\ t$$

where k_2 (g/mg min) is the rate constant of the second-order equation for adsorption of initial chromium(VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L. q_t (mg/g) is the amount of adsorption time t (min) and q_e is the amount of adsorption equilibrium (mg/g).

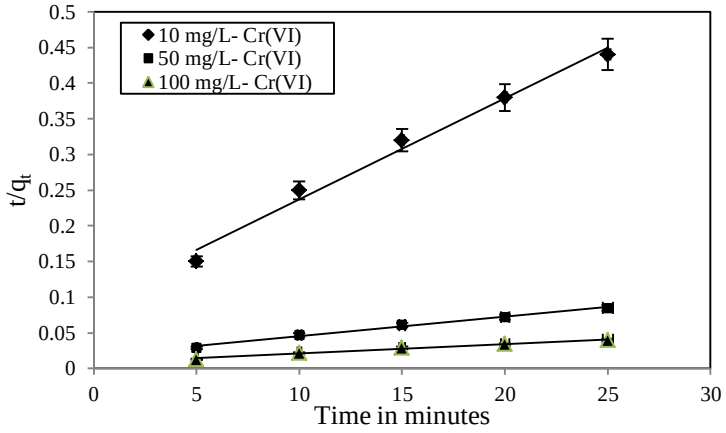


Fig. 5.94. Adsorption kinetics of time vs. t/q_t with initial chromium(VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L.

The rate constant K_2 and the R^2 values are given in Table 5.21. It is clear from these results that the R^2 values are very high in range of 0.98-0.99 for the adsorption of the chromium (VI). Fig. 5.94 shows the linear plot of t/q_t vs. t for the pseudo-second-order model is more likely to predict kinetic behavior of adsorption with chemical adsorption being the rate-controlling step.

Table 5.21. Kinetic parameters from pseudo-first-order and pseudo-second-order for chromium (VI) ions adsorption onto activated carbon at different initial concentration.

	Pseudo-first-order				Pseudo-second-order				
	Slope	Intercept	K_1 (min^{-1})	R^2	Slope	Intercept	q_0 (mg/g)	K_2 (g/mg min)	R^2
Initial Cr(VI) concentration (mg/L)									
10	-0.244	0.420	0.5619	0.983	0.264	0.0311	3.787	2.2421	0.986
50	-0.248	0.601	0.5711	0.984	0.072	0.0321	13.88	0.1610	0.987
100	-0.261	0.670	0.6010	0.986	0.026	0.0331	38.46	0.0204	0.991

5.4.2.5.3. Intraparticle diffusion rate constant (Weber-Morris equation)

During the batch mode of operation, there was a possibility of transport of adsorbate species into the pores of adsorbent, which is often the rate controlling step (Weber and Morris, 1963).The rate constants of intraparticle diffusion K_p at different initial chromium (VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L were determined using the following equation.

$$q_t = K_p t^{0.5} + C$$

Where C is the intercept and K_p is the intraparticle diffusion rate constant (mgg^{-1} $min^{-0.5}$) calculated from the slopes of respective plot q vs. $t^{0.5}$ and presented in Fig. 5.95 The rate constant K_p the R^2 values are given in Table 5.22. The intercept of the plot reflects the boundary layer effect. The larger intercept the greater the contribution of the surface adsorption in the rate controlling step. If the regression of q_t vs. $t^{0.5}$ is linear and passes through the origin, the intraparticle diffusion is the sole rate limiting step. However, the linear plots at each initial concentration of chromium (VI) did not pass through the origin. This indicates that the intraparticle diffusion was not only the rate controlling step. This indicates that the mechanism of chromium (VI) adsorption by activated carbon is a complex and both, the surface adsorption as well as intraparticle diffusion contribute to the rate determining step.

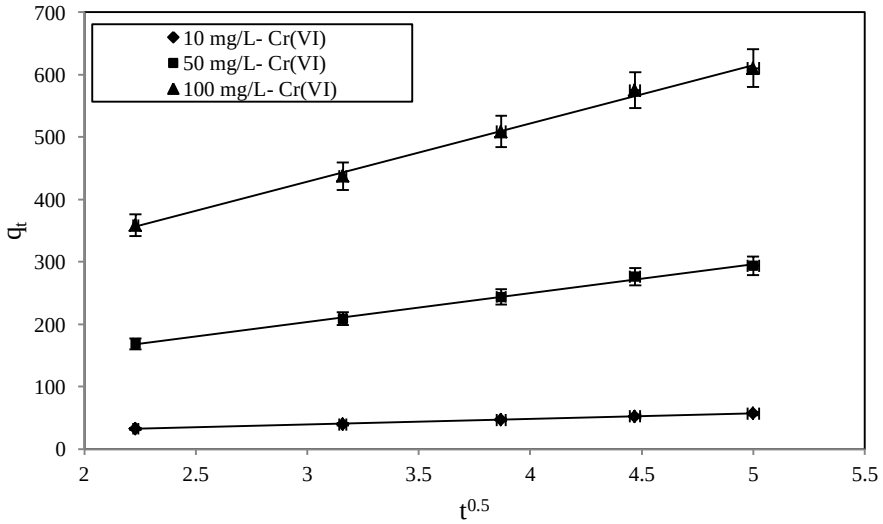


Fig.5.95. Adsorption kinetics of time vs. $t^{0.5}$ with initial chromium (VI) concentration of 10 mg/L, 50 mg/L and 100 mg/L.

Table 5.22. Intraparticle diffusion rate constants obtained from Weber- Morris equation for different initial chromium (VI) concentration.

Intraparticle diffusion rate constant				
	Slope	Intercept	K_p (mgg^{-1} $min^{-0.5}$)	R^2
Initial Cr(VI) concentration (mg/L)				
10	0.1110	-1.32510	0.1110	0.996
50	0.0314	-1.63212	0.0314	0.994
100	0.0206	-1.56063	0.0206	0.997

5.4.2.6. Effect of initial concentration on chromium (VI) removal

The adsorption of chromium (VI) onto activated carbon is studied by varying chromium (VI) concentration using optimum adsorbent dose (0.35g/50 mL) at ambient temperature (25 ± 2°C) and contact time of 60 min. The initial chromium (VI) concentration is increased from 10 mg/L to 100 mg/L and the corresponding removal gradually increases from 77.22 % to 92.24% for chromium (VI) ions. It is clear from the figure that more than 80 % adsorption of chromium (VI) took place in first 30 min and equilibrium is established after 30 min. At higher concentrations, metals need to diffuse to the adsorbent surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of chromium (VI) ion on the outer surface. From the above data it is clear that the removal method can be implemented to remove of chromium (VI) from water present in any concentration.

5.4.2.7. Adsorption isotherms

Adsorption is a well-known equilibrium separation process for water treatment. Adsorption isotherms are the equilibrium relationships between the concentrations of adsorbed metal ions and solid adsorbent at a given temperature. Adsorption isotherms have been successfully described by the well accepted adsorption isotherm models of Langmuir, Freundlich and Dubinin–Radushkevich. The study of isotherm was carried out by varying initial chromium (VI) concentration from 10 to 100 mg/L at ambient temperature (25 ± 2°C).

5.4.2.7.1. Langmuir adsorption isotherm

The adsorption data are fitted to linearly transformed Langmuir isotherm. The Langmuir model assumes that a monolayer adsorption on a homogenous surface with finite number of identical sites is given by the following equation (Chen *et al*., 2011; Langmuir, 1916).

$$\frac{1}{q_e} = \frac{1}{q_0 b C_e} + \frac{1}{q_0}$$

where q_0 is the maximum amount of the chromium (VI) ion per weight of activated carbon to form a complete monolayer on the surface (adsorption capacity) (mg/g), C_e denotes equilibrium adsorbate concentration in solution (mg/L), q_e is the amount adsorbed per unit mass of adsorbent (mg/g), and b is the binding energy constant (L/mg). From this data it can be concluded that the maximum adsorption corresponds to a saturated monolayer of adsorbate molecules on adsorbent surface. The comparison of adsorption capacity of activated carbon derived from *Eichhornia crassipes* root biomass for chromium (VI) ions with that of different adsorbent in literature is presented in Table 5.24.

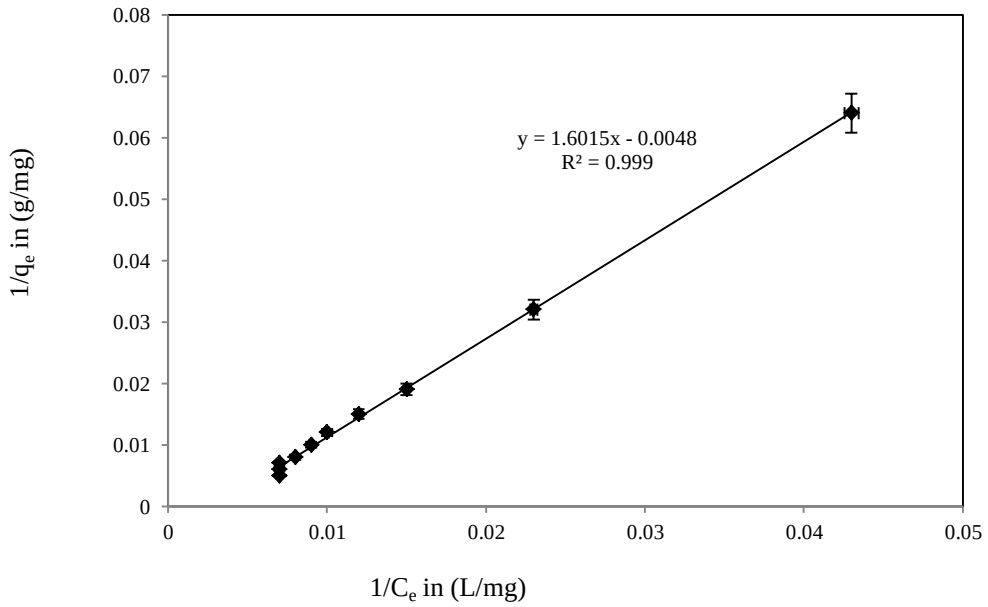


Fig.5.96. Langmuir adsorption isotherm, 1/Ce vs.1/qe.

Table 5.23. Langmuir, Freundlich and Dubinin–Radushkevich isotherm constants on the adsorptionof chromium (VI) ions from aqueous solution onto activated carbon at ambient temperature (25 ± 2°C).

	Langmuir isotherm			Freundlich isotherm			Dubinin–Radushkevich isotherm			
	b (L/mg)	q_o (mg/g)	R^2	K_f (mg/g)	*1/n*	R^2	*K* (mol^2 kJ^{-2})	q_m (g/g)	*E* (kJ mol^{-1})	R^2

Chromium (VI)										
	0.021	36.34	0.999	3.304	0.624	0.96	16.64 x 10^{-4}	0.008 3	17.36	0.9 7

In order to predict the adsorption efficiency of the adsorption process, the dimensionless equilibrium parameter is determined by using the following equation (Hall *et al*., 1966).

$$R_L = \frac{1}{1+bC_i}$$

where C_i is the initial chromium (VI) concentration (mgL^{-1}). Value of R_L indicates the shape of isotherm to be either unfavorable ($R_L > 1$) or linear ($R_L = 1$) or favorable ($0 < R_L < 1$) or irreversible ($R_L = 0$). The R_L value for initial chromium (VI) concentration of 10, 50 and 100 mgL^{-1} is found to be 0.826, 0.487 and 0.322, respectively. The values indicated a favorable isotherm shape ($0 < R_L < 1$) for adsorption of chromium (VI) on surface of activated carbon. The linear plot of $1/C_e$ versus $1/q_e$ indicates the applicability of Langmuir adsorption isotherm presented in Fig. 5.96. The values of Langmuir parameter, q_0, b and R^2 for chromium (VI) are presented in Table 5.23.

5.4.2.7.2. Freundlich adsorption isotherm

The Freundlich isotherm is an empirical equation which assumes in multilayer adsorption on heterogeneous surface of solid. The linear form of Freundlich isotherm is represented in the following equation (Freundlich, 1926).

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$

Freundlich isotherm constants n and K_f were calculated from the slope and intercept of the plot $\log q_e$ vs. $\log C_e$ shown in Fig. 5.97. The values of Freundlich parameter, K_f, $1/n$, and R^2 for chromium (VI) are presented in Table 5.23. The intensity of adsorption is an indicative of bond energies between metal ion and adsorbent, which indicate the possibility of slight chemisorptions rather than physisorption. The values of n (intensity of adsorption) between 1 and 10 (i.e., $1/n$ less than 1) represents a favorable adsorption. For the present study, the value of n also presented the same trend representing a beneficial adsorption.

Table 5.24. Comparison of adsorption capacity of activated carbon prepared from *E. crassipes* root biomass for chromium (VI) with that of different adsorbents.

Adsorbent	Type of water	pH	Cr(VI) mgg^{-1}	Reference
Saw dust activated carbon	Aqueous solution	2.0	65.8	Karthikeyan et al., 2005
Rice husk-based active carbon	Aqueous solution	2.0	0.49	Guo et al., 2003
Raw rice bran	Aqueous solution	5.0	0.07	Oliveira et al., 2005
Coconut husk fibers	Aqueous solution	2.0	29.0	Babel and Kurniawan, 2004
Hazelnut shell	Aqueous solution	1.0	170	Kobya, 2004
Saw dust	Waste waters	2.0	39.7	Sharma and Forster, 1994
Maple saw dust	Aqueous solution	6.0	5.1	Yu et al., 2003
Agricultural waste	Aqueous solution	2.0	22.29	Mohan et al., 2005
Distillery sludge	Waste water	3.0	5.7	Selvaraj et al., 2003
Cow dung carbon	Aqueous solution	4.5	3.5	Das et al, 2000
Coniferous leaves	Aqueous solution	3.0	6.3	Ayoama et al., 1999
Pine needles	Industrial water	2.0	21.5	Dakiky et al., 2002
Activated carbon derived from *E. crassipes* root biomass	Aqueous solution	4.5	36.34	Present study

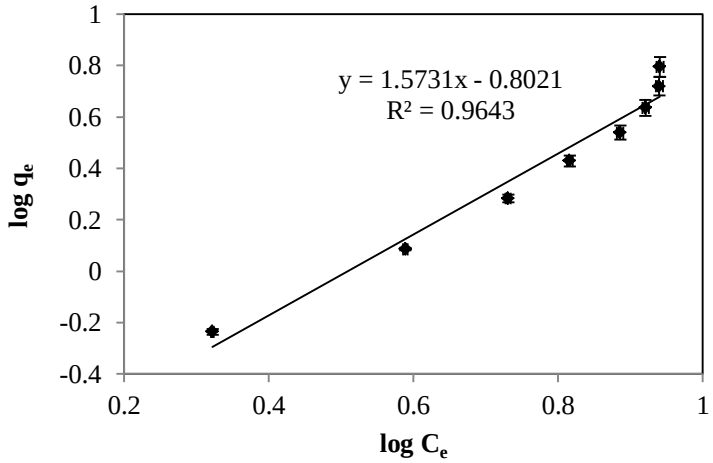


Fig.5.97. Freundlich adsorption isotherm, log q_e vs. log C_e.

5.4.2.7.3. Dubinin–Radushkevich isotherm

In order to understand the adsorption type, equilibrium data are tested with Dubinin–Radushkevich isotherm. This model envisages about the heterogeneity of the surface energies and is represented in the following linear equation (Namasivayam and Sureshkumar, 2008; Pokhrel and Viraraghavan, 2007; Chen *et al*., 2011).

$$\ln q_e = \ln q_m - K\varepsilon^2$$

where ε is Polanyi potential, and is equal to $\varepsilon = RT \ln (1 + \frac{1}{C_e})$, q_e is the amount of chromium (VI) adsorbed per unit mass of adsorbent (m mol/g), q_m is the theoretical adsorption capacity, C_e is the equilibrium concentration of chromium (VI) (m mol/L), K is the constant related to adsorption energy, R is the universal gas constant and T is the temperature in Kelvin. The isotherm constants K and q_m were calculated from the slope and intercept of the plot ln q_e versus ε^2 shown in Fig. 5.98. The values of Dubinin–Radushkevich parameter, K, q_m, and R^2 for chromium (VI) are presented in Table 5.23. The mean free energy of adsorption (E) was calculated from the constant K using the relation.

$$E = (-2K)^{-1/2}$$

It is defined as the free energy change when 1 mole of ion is transferred to the surface of the solid from infinity in solution. The value of E chromium (VI) is presented in Table 6.4. The value of E is very useful in predicting the type of sorption and if the value is less than 8 kJmol^{-1}, the adsorption process is of physical nature. If the magnitude of E is between 8 and 16 kJ mol^{-1}, then the adsorption is due to exchange of ions. The value in the present study was found to be little greater than16 kJmol^{-1}. This is due to chemical processes accompanying the ion exchange process.

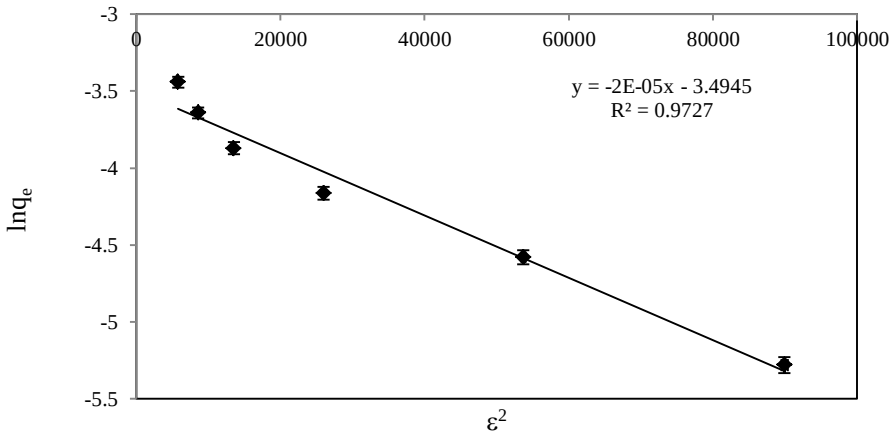


Fig.5.98. Dubinin–Radushkevich adsorption isotherm, ln q_e vs. ε^2, chromium (VI).

5.4.2.8. Effect of temperature on chromium (VI) removal

The effect of temperature on the adsorption of chromium(VI) with initial concentration 10mg/L, 50 mg/L and 100 mg/L is studied using optimum adsorbent dose (0.35 g /50 mL) and results are presented in Fig. 5.99. The percentage removal of chromium (VI) with initial concentration 10 mg/L, increased from 77.22% to 85.56%, the initial concentration 50 mg/L, increased from 78.36% to 88.32% and the initial concentration 100mg/L, increased from 79.24% to 92.24 % for 25-55 º C temperature. It can be clearly seen from the figure that, increase in temperature the percentage removal increased slowly and reached almost 92%. The increase in adsorption capacity with the increase in temperature indicates that the adsorption process is endothermic in nature.

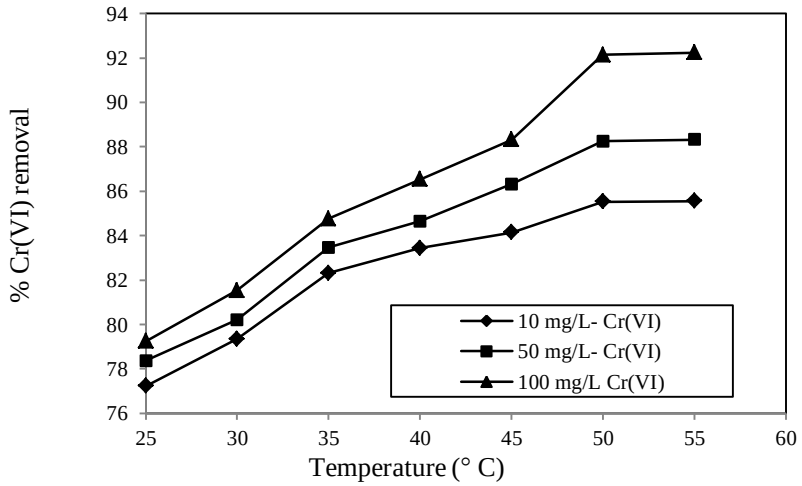


Fig. 5.99. Effect of temperature on the adsorption of chromium (VI) with initial concentration of 10 mg/L, 50 mg/L and 100 mg/L.

5.4.2.9. Thermodynamic parameters

In order to determine the thermodynamic parameters, experiments are carried out at different temperature. The change in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) of adsorption are calculated using the following equation (Chen *et al*., 2011).

$$\log K_c = \frac{\Delta S}{2.303R} + \frac{\Delta H}{2.303RT}$$

$$\Delta G = \Delta H - T\Delta S$$

where ΔS and ΔH are changes in entropy and enthalpy of adsorption, respectively. A plot of log K_c vs. 1000/T for initial chromium(VI)concentration of 10 mg/L, 50 mg/L and 100 mg/L is presented in Fig. 5.100 and the plot was found to be linear.

The K_c value is calculated using the following equation.

$$K_c = \frac{C_1}{C_2}$$

Where T is the temperature (K); R is the gas constant (8.314 J/ mol K), K_c is the equilibrium constant obtained from Langmuir isotherm. C_1 is the amount of chromium (VI) adsorbed per unit mass of activated carbon (mg/L) and C_2 is the concentration of chromium (VI) in aqueous phase (mg/L). The values of ΔH and ΔS are evaluated from the slope and intercept of Van't Hoff plots and represented in Table 5.25. Negative value of ΔG at each temperature indicates the feasibility and spontaneity of ongoing adsorption. The positive value of entropy (ΔS) indicates the increase in randomness of the ongoing process. A decrease in values of ΔG with the increase in temperature suggests more adsorption of chromium (VI) at higher temperature. The positive value of ΔH indicates the adsorption process is endothermic nature and the positive values of ΔS suggest increased randomness at the solids/solution interface during the adsorption of metal ions onto adsorbent.

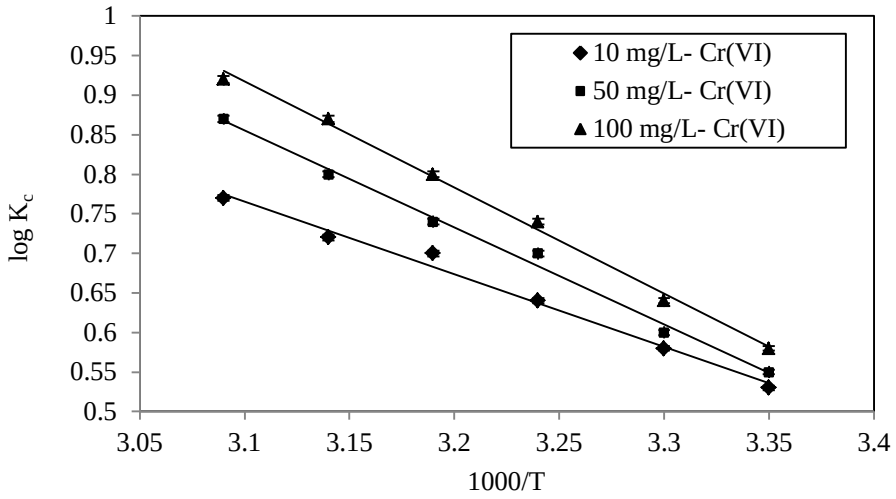


Fig. 5.100. Van't Hoff's plots, log K_c vs. 1000/T, chromium (VI).

Table 5.25. Thermodynamic parameters using chromium (VI) solution of 10 mg/L, 50 mg/L, and 100 mg/L.

	ΔH (KJ mol^{-1})	ΔS (KJ/(K mol))	ΔG (KJ mol^{-1})							R^2
			25 º C	30 º C	35 º C	40 ºC	45 ºC	50 ºC	55º C	
Initial Cr(VI) concentration (mg/L)										
10	12.501	0.04555	-0.389	-0.617	-0.845	-1.073	-1.300	-1.528	-1.756	0.989
50	12.302	0.04515	-0.475	-0.701	-0.926	-1.152	-1.378	-1.604	-1.829	0.985
100	11.901	0.04484	-0.788	-1.012	-1.237	-1.461	-1.685	-1.909	-2.133	0.987

5.4.2.10. Regeneration and reuse studies

For the sustainability of adsorption process, the adsorbents should have good desorption and reuse potential. Studies are carried out to evaluate the reuse potential of activated carbon as an adsorbent for chromium (VI). Desorption of adsorbed chromium (VI) onto activated carbon is studied by using different strength of H_2SO_4 as shown in Fig. 5.101 5N H_2SO_4 is better desorption of chromium (VI) than other. Repeated acid treatment has reduced the weight of

adsorbent considerably. As shown in Fig. 5.102 (a), there was approximately 11.23% weight loss in the first acid wash and a total of 39.22% of the weight was lost in the second wash with 5N H_2SO_4. However, there was no significant weight loss after the second wash. This shows that, in the first two cycles, almost all acid soluble material from the adsorbent is washed away and the left over material is acid resistant. The weight loss of the adsorbent may also due to corrosive nature of H_2SO_4 which may be corroding some amount of adsorbent. The percentage desorption of chromium (VI) was 72.23 %, 85.33% and 94.55 % in first, second and third cycles of operation as shown in Fig. 5.102(b). The lower desorption in the first cycle may be the irreversible adsorption of chromium (VI) to some of the functional groups (Vinodhini and Das, 2009; Sengupta and Clifford, 1986). As the acid washing proceeded, these material must have washed away (evident from weight loss) and chromium (VI) adsorption in the remaining functional groups might be reversible.

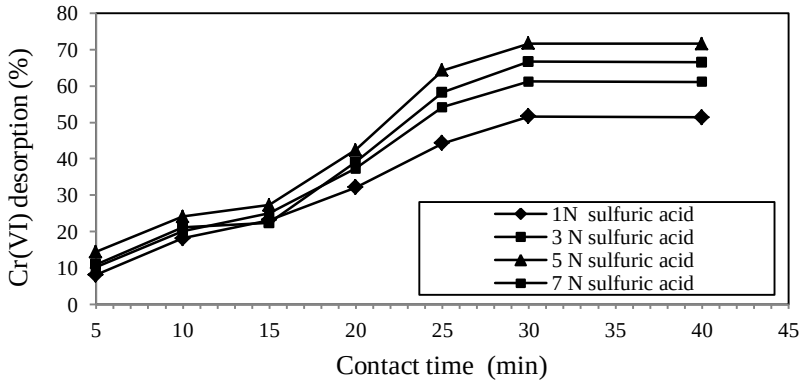
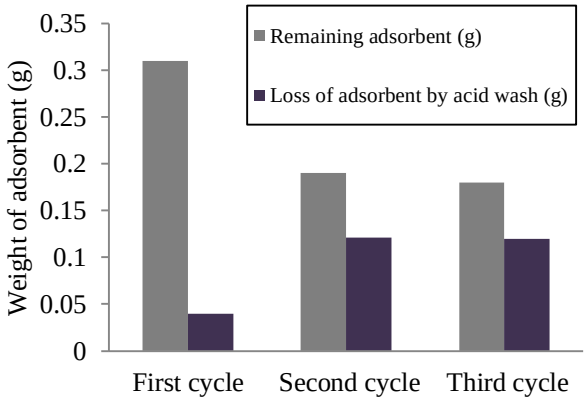


Fig. 5.101. Percentage of chromium (VI) desorption using different strength of H_2SO_4 .



(a)

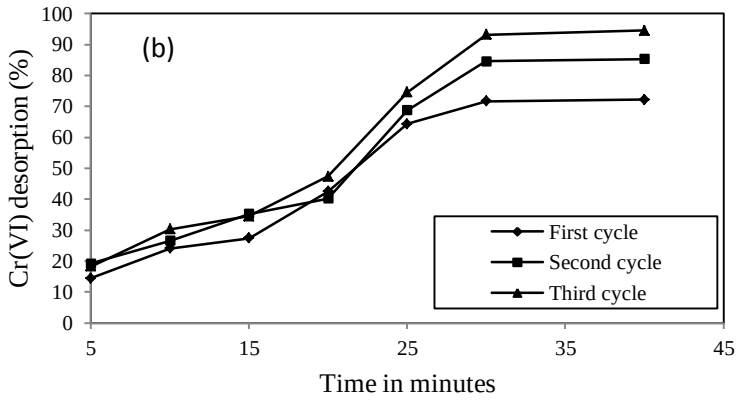


Fig. 5.102. (a) Weight losses due to acid (5N H_2SO_4) treatment in different cycles; and (b) chromium (VI) desorption from activated carbon at different cycle.

5.4.2.11. Column study for chromium (VI) removal

The breakthrough curve is a plot of the concentration measured at a fixed point in the column, usually at or near to the outlet, versus time. In a multicomponent system, the accuracy of the adsorption equilibrium model will be of great significance in the simulation of breakthrough curves when adsorption competition between the adsorbate is strong. Adsorption isotherms are used for some primary studies and obtaining the operational parameters before running more costly experiments. Hence, the practical applicability of the product for column operations was always studied to obtain some parameters necessary for better approach in water treatment method (Lizama Allende *et al*., 2012; Ahamad and Jawed, 2012). The breakthrough curves were obtained by plotting C/C_o versus the volume of 10 mg/L chromium (VI) solution, in order to determine both the volume and capacity of saturation. Fig. 5.103 shows the breakthrough curves for the column studies of chromium (VI). It can be observed that the breakthrough volumes are 35 mL. The saturation volumes (V_x) were found to be 80 mL, in 60 min (t_x). The maximum capacity of removal of chromium (VI) ions in column is given by Equation (Gupta *et al*., 1997).

$$Q = \frac{C_0 \text{ X V}}{m_s} \int_{t=0}^{t=x} (1 - \frac{C}{C_0})\, dt$$

where Q is the maximum adsorption capacity (mg g^{-1}); C_0 and C are the initial concentration of the solution and the concentration of chromium in a determined volume (mg L^{-1}), respectively; m_s is the mass of the adsorbent (g); V is the flow rate (L min^{-1}) and the time is given in minutes.

The values of Q are 0.8 mg/g for chromium (VI). Operational column parameters are helpful in designing a fixed adsorbent for chromium (VI) removal from aqueous solution.

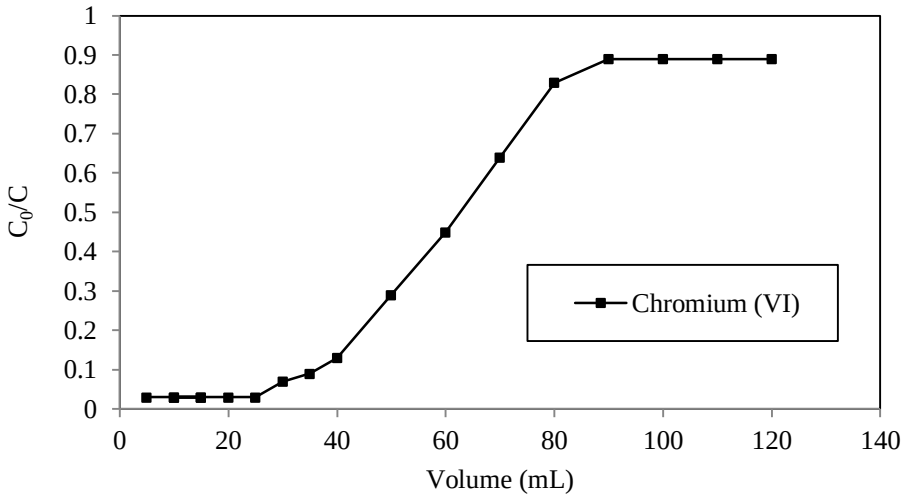


Fig. 5.103. Break through curve for the adsorption of Cr (VI) on activated carbon (initial concentration Cr (VI): 10 mg/L; flow rate: 1.2 mL/min; pH 4.5 and room temperature: 25 ± 2 °C).

The length of unused bed (LUB) is calculated using Eq.

$$LUB = L \text{ x } (1\text{-}\frac{V_b}{V^*})$$

where L is the height of the bed (cm), V_b is the breakthrough volume (mL), and V^* is the stoichiometric volume (mL), which corresponds to less than half the saturation volume (V_x). The values of LUB are 0.129 cm.

Chapter-6

6. Summary and Conclusion

Environmental pollution is increasing ceaselessly on account of an unabated increment in population, industrialization, urbanization, anthropogenic activities and natural sources. The metal/metalloids pollution is of great concerns, as these hazardous pollutants are accumulated in living organisms are responsible for many metabolic and physiological disorders. Advancement in science and technologies parallel to industrial revolution has opened new vistas to exploit the inherent traits of natural resources including green plants and microorganisms to overcome the damage to the environment by pollutants.

The Phytoremediation and bioremediation techniques are relatively new methods for abetment of hazardous ions from water, waste water and effluents. Many research organization and academic institution are actively involved in this field. However a huge scope is there to explore for future development. In this thesis, four problems have been addressed: 1. Phytoremediation of arsenic (III) and chromium (VI) by *Eichhornia crassipes* plant. 2. Bioremediation of arsenic (III) and chromium (VI) by living cells of *B. cereus* biomass. 3. Prediction of arsenic (III) and chromium (VI) removal by biosorption process using living cells of *B. cereus* biomass with multilayer feed forward artificial neural network (ANN). 4. Removal of chromium (VI) from aqueous solution using activated carbon derived from *Eichhornia crassipes* root biomass.

Eichhornia crassipes used in 'Eco-technology' for phytoextraction and phytofiltration are the best-developed subsets for removal of toxic metal from environment. Nutrient culture is an efficient method for screening heavy metal ions tolerant for free floating plants of *Eichhornia crassipes* in hydroponic culture. The mechanism of uptake, translocation and detoxification of arsenic (III) and chromium (VI) ions are well understood in plant root cells. The accumulation,

relative growth and bio-concentration factor of plant on treatment with different concentrations of arsenic(III) and chromium(VI) solution significantly increased ($P<0.05$) with the passage of time. Plants treated with 0.100 mg/L arsenic (III) accumulated the highest concentration of metal in roots (7.20 mg kg^{-1}, dry weight) and shoots (32.1 mg kg^{-1}, dry weight); while those treated with 4.0 mg/L of chromium (VI) accumulated the highest concentration of metal in roots (1320 mg/kg, dry weight) and shoots (260 mg/kg, dry weight) after 15 days. The metal uptake was ascertained by adopting various characterization methods like AAS, SEM-EDX, XRD and FTIR of the biomass before and after treatment. FTIR spectra confirmed the interaction of arsenic (III) ions with the hydroxyl, amide, thiol and amino groups present on the *Eichhornia crassipes* biomass. Similarly the FTIR spectra confirmed the interaction of chromium (VI) ions with the hydroxyl, carboxyl and amino groups present on the plant biomass. XRD pattern of arsenic ions loaded plant materials shows the presence of phases of $AlAsO_4$, As_2O_3, and $As(OH)_3$ which indicates that arsenite ion are converted into the above species and finally get adsorbed over the surface of plant materials. XRD pattern of chromium ions loaded plant materials indicates that the Cr (VI) converted into $K_3Er\ (CrO_4)_3$, some of the species are even converted into Al_8Cr_4 Dy and also remain as $K_2Cr_2\ O_7$ and finally get adsorbed over the surface of plant materials. The high removal efficiency and more accumulation capacity of arsenic and chromium ions make *Eichhornia crassipes* an excellent choice for phytoremediation processes.

Microwave extraction is becoming the choice for the extraction of solid matrices for organic analyte analysis by HPLC-ICP-MS techniques. Therefore, the method adopted in this study was tested on shoot biomass of *Eichhornia crassipes* containing 32.1 ± 0.05 $mgkg^{-1}$ arsenic (III) ions. Extraction of arsenic from plant materials was conducted using three extractant solutions: (i) Extracted by 10% (v/v) tetramethylammonium hydroxide (TMAH) with yield of 95.14%, (ii) Extracted by double deionized water with yield of 87.24% and (iii) Extracted by a modified protein extracting solution with yield of 88.92%. *Eichhornia crassipes* consisted only inorganic arsenic species as it is indicated from the results of these experiments. Arsenic (III) are present in maximum quantity, arsenic (V) in minimum quantity and the organic arsenic like monomethylarsonic (MMA) and dimethylarsinic acid (DMA) are absent. Extraction of chromium ions was conducted by same procedure from plant materials using three extractant solutions: (i) Extracted by 0.02 M ethylenediaminetetraacetic acid (EDTA), with yield of 97.24%, (ii) Extracted by double deionized water with yield of 72.21% and (iii) Extracted by

HCl solution with yield of 87%. The method developed in this study was tested on shoot biomass of *E. crassipes* containing 260 ± 0.05 $mgkg^{-1}$ chromium (VI) ions. The results in this experiments clearly indicate that chromium species adsorbed in the *Eichhornia crassipes* consisted only Cr^{+3} and Cr^{+6} but chromium (VI) are present in maximum quantity as compare to chromium (III) ions.

The biosorption of arsenic (III) and chromium (VI) from water is studied by living cells of *Bacillus cereus* biomass as bioremediation. Dependence of biosorption was studied with variation of various operating parameters, pH of solution, biomass dosage, contact time, initial concentration and temperature to achieve the optimum condition. The maximum biosorption capacity of living cells of *B. cereus* for arsenic (III) and chromium (VI) was found to be 32.42 mg/g and 39.06 mg/g at pH 7.5, at optimum conditions of contact time of 30 min, biomass dosage of 6 g/L, and temperature of 30 ± 2°C. Biosorption data of arsenic (III) chromium (VI) are fitted to linearly transformed Langmuir isotherm and pseudo-second-order model with R^2 (correlation coefficient) > 0.99. Thermodynamic parameters reveal the endothermic, spontaneous, and feasible nature of sorption process of arsenic (III) chromium (VI) onto *Bacillus cereus* biomass. The arsenic (III) and chromium (VI) ions are desorbed from *B. cereus* using both 1M HCl and 1M HNO_3. *B. cereus* biomass is characterized, using SEM-EDX, AFM and FTIR. The SEM-EDX studies reveal the morphological changes with respect to shape and size of the bacteria after sorption of arsenic (III) and chromium (VI) ions with *Bacillus cereus* cells. AFM figures reveals after the arsenic (III) and chromium (VI) ions exposure and the ultra-structures mostly disconnected with the cells adhering to each other randomly. It can be clearly observed that the biomass shape has changed into a spindle-like structure after arsenic (III) and chromium (VI) sorption. FTIR spectra may be attributed to the interaction of arsenic (III) ions with the hydroxyl, amide and amino groups present on the *Bacillus cereus* biomass. FTIR spectra may be attributed to the interaction of chromium (VI) ions with the hydroxyl, amide and amino groups present on the *Bacillus cereus* biomass.

The present work demonstrates successful removal of As (III) and Cr(VI) ions from the aqueous solutions using *Bacillus cereus* biomass with maximum removal efficiency 86.14% for As(III) and 89.24% for Cr(VI) initial concentration 1 mg/L.

Artificial neural network (ANN) techniques can enhance predicting capability of the model when mathematical or statistical methods are difficult to formulate and fails to predict with

desired accuracy. In the present work, estimation of sorption efficiency using mathematical and analytical tools is involved because the physical phenomenon for removal of arsenic (III) and chromium (VI) by living cells of *Bacillus cereus* is complex one. Therefore, artificial neural network (ANN) has been attempted in this work for prediction purpose because ANN has the capacity to map inputs and outputs efficiently in complex situations. A three layer back propagation algorithm of neural net-work is adopted to predict the response of the process. A simple back propagation network with momentum is proved meaningful supplement for the conventional and complicated mathematical models for the prediction of sorption efficiency in bioremediation process. The biosorption data of both metal ions collected from laboratory scale experimental set up is used to train a back propagation (BP) learning algorithm having 4-7-1 architecture. The model uses tangent sigmoid transfer function at input to hidden layer whereas a linear transfer function is used at output layer. The data is divided into training (75%) and testing (25%) sets. The network is found to be working satisfactorily as seven neurons are selected in the hidden layer when mean square error starts decreasing. Learning and momentum parameters are set at 0.25 and 0.20 respectively during the training phase both arsenic (III) and chromium (VI). The network is trained till minimum root mean square error is observed. A root mean square error of 0.77 is observed at epoch number 23, 644, 85 for arsenic (III) and 0.68 is observed at epoch number 23, 835, 76 for chromium (VI). The network is found to be working satisfactorily as absolute relative percentage error of 0.749 for arsenic (III) and 0.567 for chromium (VI) during training phase**.** Comparison between the model results and experimental data gives a high degree of correlation (R^2 = 0.986 for arsenic (III) and R^2 = 0.984 for chromium (VI)) indicating that the model is able to predict the sorption efficiency with reasonable accuracy.

The removal of chromium (VI) from aqueous solutions by activated carbon prepared from the *Eichhornia crassipes* root biomass. The operating parameters, pH of solution, biomass dosage, contact time, and temperature, have tremendous effect on the adsorption efficiency of chromium (VI). The adsorption capacity of activated carbon derived from *Eichhornia crassipes* root biomass was found to be 36.34 mg/g for chromium (VI), at pH 4.5, contact time of 30 min and temperature of 25 ± 2°C. The mean free energy values evaluated from the D-R model indicated that the adsorption of chromium (VI) onto activated carbon is due to by chemical ion-exchange. The kinetic data signified that the adsorption of chromium (VI) ions onto activated carbon

followed both the pseudo-first-order and pseudo-second-order kinetic model. The thermodynamic calculations showed the feasibility, endothermic and spontaneous nature of the adsorption of chromium (VI) onto activated carbon at 25-55 °C. Regeneration studies were carried out to evaluate the reuse potential of the adsorbent. The maximum removal capacity in column studies was found to be 0.8 mg/g for chromium (VI). The FTIR spectroscopic analysis confirmed that the interaction of chromium (VI) with the hydroxyl, carboxyl and amino groups present on the surface of the activated carbon. Activated carbon derived from *Eichhornia crassipes* root biomass is low-cost biomass with considerable high adsorption capacity.

From the above studies it is concluded that *Eichhornia crassipes* plant can be used for effective phytoremediation of arsenic (III) and chromium (VI). The removal of arsenic is effective but further improvement is required but for chromium (VI) it can be used effectively from effluents. *Bacillus cereus* can be used effectively for the removal of arsenic (III) and chromium (VI) by bioremediation techniques. Both the process represents a cost-effective, efficient and easy to use plant and microorganism based technology for the removal of metals from the water environment and has great potential for future applications. For the practical applicability of this technique further experiments are ongoing.

Chapter-7

7. SCOPE FOR FUTURE WORK

Human impacts leading to large scale degradation of the environment have aroused global concern on environmental issues in the recent years. Based on the findings of the present investigation, the following future scope of studies will be undertaken to carry forward the research further.

1. Microwave assisted extraction studies for arsenic, chromium, mercury, and cadmium analysis by water hyacinths with ICP-AES method which will ensure the recovery of the metal ions.
2. To study the bioaccumulation kinetics and toxic effects of Cr, As and Hg on *Monochoria hastata*.
3. Sequential eluent injection technique as a new approach for the on-line enrichment and speciation of Cr(III), Cr(VI) and As(III), As(V) species on a single column with FAAS detection.
4. Extracellular reduction of arsenate by cytochromes MtrC and OmcA of *Shewanella oneidensis* MR-1.

5. Biosorption of chromium (VI), arsenic (V) and mercury (II) by living cells of *Bacillus licheniformis* biomass.

6. Adsorption of Cr (VI) and As (III) on ureolytic mixed culture from biocatalytic calcification reactor.

7. Chromium (VI) and arsenic (III) reduction and phenol degradation in aqueous mixed culture of living cells of *Bacillus cereus* and *Bacillus licheniformis* biomass.

8. To use the natural surfactant as a feed for the microorganism which will generate the reluctant which can be used to reduce the toxicity of the metal/mettaloid?

9. To apply the mathematical model to predict of accumulation and extraction of metals/metalloids by the treatments of wastewater.

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