Spirulina Platensis for Decontamination of Chromium Laden Aqueous Effluent

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

By
Mahesh Kumar Gagrai
(08610709)

Department of Chemical Engineering
Indian Institute of Technology Guwahati
December 2013

Dedication

To My Parents and Family

CERTIFICATE

*This is to certify that the thesis entitled "**Spirulina Platensis for Decontamination of Chromium Laden Aqueous Effluent**", being submitted by Mr. Mahesh Kumar Gagrai to the Indian Institute of Technology Guwahati, India for the award of Doctor of Philosophy, is a record of bonafide research work carried out by him under our guidance and supervision. The work embodied in this thesis has not been submitted for any other degree or diploma. In our joint opinion, the thesis is up to the standard of fulfilling the requirements of the doctoral degree as prescribed by the regulations of this Institute.*

_____

Date: [Signature (s) of Thesis Supervisor (s)]

[Animes Kr. Golder]

[Assistant Professor]
[Department of Chemical Engineering, Indian Institute of Technology Guwahati]

_____

Date: [Signature (s) of Thesis Supervisor (s)]

[Dr. Chandan Das]

[Assistant Professor]
[Department of Chemical Engineering, Indian Institute of Technology Guwahati]

ACKNOWLEDGEMENT

I take this opportunity to express my deep sense of respect and gratitude to my thesis supervisor, Dr. Animes Kumar Golder, for providing inspiring and valuable guidance, and insight. I am also indebted to Dr. Golder for instilling in me a craving for perfection. A person with an amicable and positive disposition, he always made himself available to clarify my doubts despite his busy schedules and I consider it as a great opportunity to do my doctoral work under his guidance and to learn from his research expertise. I believe, it will always remain with me in my future life. It has really been a remarkable experience working with him.

I also wish to express my deep sense of gratefulness to my joint thesis supervisor, Dr. Chandan Das, for his valuable guidance and support. He has also provided insightful discussions on my research and laboratory facilities for the experimental work.

I express my sincere gratitude and indebtedness to the chair of the doctoral committee, Prof. Aloke Kumar Ghoshal, for his critical comments and valuable suggestions at various stages of my work. His observations and comments helped me to establish the overall direction of the research and to move forward with in-depth studies.

I would like to express my sincere thanks to Dr. Kaustubha Mohanty, for serving as a member of my doctoral committee and generously sharing knowledge on biological techniques.

Furthermore, I am very grateful to the another member of the doctoral committee, Dr. Saswati Chakraborty, Department of Civil Engineering, for providing insightful comments both on my work as well as on the thesis.

I am thankful to Central Instruments Facility for providing the analytical facilities towards this work. I am grateful to Indian Institute of Technology Guwahati, for providing the research fellowship.

I am grateful to National Chemical Laboratory, Pune, for supplying live strain of *Spirulina platensis*. I am also obliged to Mr. Ajay Kanaujia, Scientist, UP Jal Nigam, for his help during collection of industrial chrome tanning effluent.

I am thankful to my friends Mr. Amit Kumr Bharti, Indian Railways (Sectional Engineer) and Mr. Daya Sankar Sakya, UP Roadways (Assistant Engineer) for their useful support in transportation of industrial chrome tanning effluent from collection point to the Institute.

I am obliged to Mr. Harsaraj Biswanath, Mr. Prasun Bhattacharjee, Mr. Lukumoni Borah, Mr. Kaustavmoni Deka, Mr. Jayanta Kumar Mout, Mr. Dipak Kumar Barman, Mr. Balen Chandra Mahanta, Mrs. Ritumoni Kalita, Mr. Kalyan Boro, Mr. Bhagya Boro and all other staff members of Chemical Engineering Department, for their sincere and timely help at various parts of this work.

I am gratified to my colleagues, Mr. N. Vinothkumar and S. Murugavelh for encouraging and supporting me at the initial stage of this work. I have enjoyed numerous fruitful discussions with them throughout this work.

I am happy to oblige my friends, Mr. Ardhendu Sekhar Giri, Mr. Vijay Singh, Mr. Arijit Das, Mr. Sujoy Bose, Mr. Suman Saha and Mr. Raj Kumar Das, for their enriching friendship and friendly assistance in the experimental run. I am also thankful to Mr. Ch Venkatanarasimha Rao, Mr. Kuntal Maiti, Mr. Ankur Patowary Momin, Mr. Harendra Rathore and Mr. Gourav Kumar, for their support at several stages of the experiment and analysis. I also like to thank Ms. Snigdha Khuntia, for her valuable cooperation at the preliminary stage of this thesis work.

I am fortunate to come across to many funny & good friends, without whom life would be bleak. I am thankful to Ms. Chitrita Kundu, Mr. Avijit Ghosh, Mr. Leela Manohar Aeshala, Mr. Manish Kumar, Mr. Rajasekhara Reddy B., Mr. Ruhit Jyoti Konwar, Mr. Sanjib Barma, Mr. Sankar Chakma, Mr. S. Yadav and all research scholars, for their valued friendship and useful support during my stay at this Institute.

Finally, I express my humble regards and respect to my parents, my wholehearted appreciation to my brothers and my beloved wife. They have always encouraged me throughout this work with their selfless love and affection.

Indian Institute of Guwahati *Mahesh Kumar Gagrai*
Guwahati
December 2013

Vita

Mahesh Kumar Gagrai

Date of Birth: 7 September 1978
Email: mgagraihbti21@gmail.com

Education:

- Ph.D. student
  Department of Chemical Engineering
  Indian Institute of Technology Guwahati
  Guwahati, Assam, India.
  2009 -2013

- M.Tech in Chemical Engineering
  Department of Chemical Engineering
  Indian Institute of Technology Guwahati
  Guwahati, Assam, India.
  2007

- B.Tech in Biochemical Engineering
  HBTI Kanpur, India
  2002

Research Experience:

IIT Kanpur, India (April 2005-July 2005)
- Project Associate: Microbial degradation of textile dyes (DST sponsored).

IIT Kanpur, India (Aug 2004-April 2005)
- Project Assistant: Defluoridation of drinking water (UNICEF sponsored).

Industrial Experience

SM India, New Delhi, India (May 2008 December 2008)
- Chemical Engineer: Process control and documentation.

BC Exports, India (Jan 2008-May 2008)
- Chemical Engineer: ETP installation, production and quality control.

Publications

Journal Papers

M.K. Gagrai, C. Das and A.K. Golder, "Non-ideal metal binding model for Cr(III) sorption using *Spirulina platenesis* biomass: experimental and theoretical approach", ***Canadian Journal of Chemical Engineering***, 2013, 91(12), 1904-1912

M.K. Gagrai, S. Khuntia, C. Das and A.K. Golder, "Effect of background ions on reduction of Cr (VI) to Cr (III) using saline water algae", ***Research Journal of Chemistry Environment,*** 2011, 15 (2), 450-453.

M.K. Gagrai, C. Das and A.K. Golder, "Reduction of Cr(VI) ion by dead *Spirulina* biomass in aqueous solution: A kinetic reduction model", ***Chemosphere,*** 2013, 93(7), 1366-1371**.**

Ch.V. Rao, M.K. Gagrai, V.V. Goud and A.K. Golder, "Oxidative brilliant green dye degradation in Fenton process," ***International Journal of Cheminformatics and Chemical Engineering*** **(accepted).**

Manuscript communicated

M.K. Gagrai, C. Das and A.K. Golder, "Biological reduction and removal of Cr(VI) with microbial consortia collected from local water treatment plant", *International Journal of Environment Management and Technology* (Under review).

M.K. Gagrai, C. Das and A.K. Golder, "Metabolites of *Spirulina sp*. cultivated in Cr(III) precipitated industrial chrome tanning effluent", *Journal of Algological Studies*, (Under review).

Papers in Conferences

- M.K. Gagrai, C. Das and A.K. Golder, "Metabolite of *Spirulina sp.* cultivated in precipitated chrome tanning effluent (PTE)", International Conference on Algal Biorefinery: A Potential Source of Food, Feed, Biochemicals, Biofuels and

Biofertilizers (**ICAB 2013**), 10-12 January, 2013, IIT Kharagpur, India.

- M.K. Gagrai, A. P. Momin, C. Das and A.K. Golder, "Influence of phenol treatment on Cr(III) Biosorption with *Spirulina*", Indian Chemical Engineering Congress (**CHEMCON-2012**), 27-30, December, 2012 at NIT Jalandhar, India.
- M.K. Gagrai, C. Das and A.K. Golder, "Two parameter equilibrium sorption isotherm model: Influence of Temperature on Cr(III) sorption onto *Spirulina* biomass", Indian Chemical Engineering Congress (**CHEMCON-2012**), 27-30, December, 2012 at NIT Jalandhar, India.
- M.K. Gagrai, C. Das and A.K. Golder, "Cultivation of *Spirulina sp*. in chemically treated spent tanning liquor (Characterization of biomass)", accepted at 7th CUTSE Conference (**CUTSE-2012**), 6-7 November 2012, Miri, Sarawak, Malaysia.
- S. Khuntia, M.K. Gagrai, C. Das and A.K. Golder, "Effect of background ions on reduction of Cr (VI) to Cr (III) using saline water algae", Accepted at 5th International Congress of Chemistry and Environment (**ICCE-2011**), 27- 29th May, 2011, Kuching, Sarawak, MALAYSIA.
- M.K. Gagrai, C. Das and A.K. Golder, "Cr(III) biosorption by a saline microalgae *Spirulina platensis*: Studies on two parameters isotherms" Indian Chemical Engineering Congress (**CHEMCON-2011**), 27 - 29th December, 2011, M S Ramaiah Institute of Technology, Bangalore, India.
- M.K. Gagrai, C. Das and A.K. Golder, "Studies on the hazardous nature of spent bio-sludge laden with Cr(III): Toxicity characteristic leaching procedure" Indian Chemical Engineering Congress (**CHEMCON-2011**), 27 - 29th December, 2011, M S Ramaiah Institute of Technology, Bangalore, India.
- M.K. Gagrai, C. Das and A.K. Golder, "Study on the mechanism of aqueous Cr(VI) sorption by *Spirulina platensis* biomass", Microbes in Wastewater & Waste Treatment, Bioremediation and Energy Production (**MWT 2011**) 24 - 27th January, 2011, BITS Goa, India.
- M.K. Gagrai, C. Das and A.K. Golder, "Biosorption of heavy metal ions to a microalga *Spirulina Sp*." Indian Chemical Engineering Congress (**CHEMCON-2010**), 27 - 29th December, 2010, Annamalai University, Tamilnadu, India.

Spirulina platensis for decontamination of chromium laden aqueous effluent

ABSTRACT

Spirulina platensis, a blue-green microalga, grows in high saline (13 to 35 g Cl^- L^{-1}) and alkaline (pH 8 to 10.5) condition. The acid-base characteristic of a biomaterial is an important factor determining the efficiency of metal removal. The quality and quantity of acidic sites of algal species strongly depend on its cultivation conditions.

This study investigates the applicability of *Spirulina platensis* for removal of both Cr(III) and Cr(VI) in batch reactor. The acidic sites of dead *Spirulina platensis* biomass were determined from potentiometric titration by employing a simple proton binding model. The concentration of functional groups was in the order of phosphatic>> carboxyl > amine. Maximum Cr(III) sorption was noted at equilibrium pH of 6.2. A modified metal binding model was tested and Cr(III) binding constants of 80.9, 3.9×10^3 and 3.5×10^4 L mol^{-1} were found for carboxylic, phosphatic and amine groups, respectively. Cr(III) uptake model showed dual sites coverage of Cr(III) on *Spirulina* surface with predominant occupancy to phosphatic sites. The amine group however, according to metal binding model, was less significant in Cr(III) binding. The overall Cr(III) binding constant was found as 1.13×10^{10} L mol^{-1}. pH and temperature exhibited major influence on Cr(III) uptake capacity of dead biomass. [Cr(III)] < 10 mg L^{-1} showed negligible effect on *Spirulina* growth, while [Cr(III)] > 50 mg L^{-1} was resulted in decrease of cell density in Zarrouk media composition (ZMC) because of $Cr(OH)_3$ precipitates.

The active functional groups are capable to detoxify Cr(VI) to Cr(III). A 2nd order kinetic model was developed in terms of concentration of protonated acidic groups of dead *Spirulina sp.* biomass for the reduction of Cr(VI) into Cr(III). Cr(VI) reduction model was validated over a broad range of pH, temperature and anionic strength. Lower pH favoured Cr(VI) reduction reaction and the experimental results well fitted to the kinetic model. The overall rate constant (k_t) decreased logarithmically from 22.7 to 2.8 mM^{-1} s^{-1} with rise of pH from 0.5 to 6.0. Whereas, k_t, increased nearly by 23% with elevation temperature from 25 to 45°C. Higher

concentration (> 0.235 mM) of background anions (Cl^-, SO_4^{2-} and NO_3^-) was resulted in decreases in k_t values in case of dead biomass. Cr(VI) inhibition on cell growth in ZMC was almost at the same extent of Cr(III) with 12 h photo period per day. Concentration of Cr(VI) in solution was almost unaffected at pH 8.5.

Exhausted chrome tanning stream typically contains upto 15 g L^{-1} of Cl^-. The feasibility of precipitated chrome tanning effluent (PCTE) for the cultivation of *Spirulina sp*. was investigated to utilize its Cl^- resource. Hydroxide precipitation although reduced its Cr(III) loading but Cl^- concentration was unchanged. The kinetics of cell growth were recorded in ZMC with addition of Cr(III), Cr(VI) and PCTE. The inhibition on growth was in the order of (ZMC+PCTE)>> (ZMC+Cr(VI))> (ZMC+Cr(III). *Spirulina sp*. growth rate was about three fold less in PCTE+ZMC as compared to native *Spirulina* in ZMC. ZMC+ PCTE cultivated *Spirulina* was resulted in decrease of formation of carbohydrate and chlorophyll b by 58 and 70%, respectively. Whereas, chlorophyll a formation increased by 40% in comparison to ZMC cultivation. A kinetic model was tested by incorporating the rate of photosynthesis, nutrient uptake and respiration for *Spirulina* cell growth in ZMC, ZMC+Cr(III), ZMC+Cr(VI) and ZMC+PCTE. The growth parameters were found to be in the close proximity of the earliest reports with other biomaterials. The minimum amount of nutrient required to support *Spirulina* growth in ZMC+ PCTE was lower than ZMC+Cr(III) and ZMC+Cr(VI).

LIST OF CAPTIONS OF FIGURES

LIST OF CAPTIONS OF TABLES

TABLE OF CONTENTS

Chapter 1

Introduction and Literature Survey

This chapter introduces the problem, includes literature survey and outlines the research objectives.

1.1 Heavy metal pollution

The current patterns of industrial activity alter the natural biome and introduce heavy metals into the environment (Faisal and Hasnain, 2004). The increase in rate of effluent discharge into water bodies is a major environmental concern today. Most of these effluents contain toxic pollutants especially heavy metals. These contaminations exist in aqueous waste streams of many industries, such as metal plating facilities, mining operations, and tanneries. Major heavy metals present in industrial effluents are As(II), Cd(II) Cr(VI), Cr(III), Co(II), Fe(II), Hg(II), Ni(II), Pb(II), Se, V, Zn(II) etc. (Sud et al., 2008). These metals are toxic in both their chemically combined forms as well as the elemental form (Manahan, 2010). They are not

biodegradable and tend to accumulate in living organisms, causing various diseases and disorders. Accumulation of toxic metals in food structures is one of the major problems. The contaminated food can cause poisoning in humans and animals. Although some heavy metals are essential for metabolic process, above certain limit these are toxic to plant and animals. The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the recommended limits. Although, individual metals exhibit specific signs of their toxicity, following are general symptoms associated with Cd(II), Pb(II), As(II), Hg(II), Zn(II) and Cu(II) poisoning: gastrointestinal disorders, diarrhoea, stomatitis, tremor, hemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled (McCluggage, 1991). The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic.

1.1.1 Heavy metal toxicity

The term toxicity can be defined as the capacity of an element/chemical to adversely affect any activity of an organism such as its growth, lifespan, health and reproductive capacity (Luckey et al., 1975). The toxicity of heavy metals came to the highlight after the historic tragedy of ‘Minamata disease’ in 1953-60 due to mercury toxicity and ‘Itai-Itai’ disease due to cadmium toxicity after World War II in Japan (De, 1987). In general, toxic metals attack the active sites of the enzymes and inhibit the essential enzymatic activity. They are also dangerous because they tend to bio-accumulate. The major toxic effects of heavy metals are summarized in Table 1.1. Chemical speciation studies are important to assess the toxicity/bioavailability of an element/chemical in the environment. The toxicity of these compounds is dependent on their chemical forms (De, 1987). Although total concentration of

an element/chemical have been traditionally used to assess the environmental impact and health risk. It is now established that no meaningful interpretation can be made without speciation information. For example, Cr(VI) compounds are more toxic compared to Cr(III) compounds. Toxicity of arsenic to animals varies from the more toxic arsenite to the moderately toxic arsenate. Therefore chemical speciation studies are essential for heavy metals/metalloids to find out their significance on the environment and potential health risk.

Table 1.1. Major toxic effect of regulated heavy metals/regulated metalloids (De, 1987; Sud et al., 2008).

Element	Effects		Permissible limits for industrial effluent discharge Indian Standards: 2490 (1974) (in mg L^{-1})		
	Acute Exposure	Chronic Exposure	Inland surface waters	Public Sewers	Land for irrigation
Cd(II)	Necrosis, Anaemia, Emphysema	Cancer, Bronchitis, Hypertension, Cardiovascular Disease	2.0	1.0	--
Pd(II)	Anaemia, Constipation and Obstipation, Malaise, Colic, Pallor, Nephropathy	Encephalopathy, Suspected carcinogen, Kidney problem	2.0	1.0	--
As	Mild Bronchitis, Hyperpigmentation, Palmar and Plantar, Hyperkeratosis, Warts, Contact Dermatitis, Ulceration and Perforation of the nasal septum	Liver injury, Blackfoot disease	0.2	0.2	0.2
Cr(VI)	Skin disease, Dermatitis, Perforation of nasal septum	Cancer	0.1	2.0	--
Hg(II)	Pulmonary irritation, Neural damage	Haemolysis, Insomnia, Delirium	0.01	0.01	--
Zn(II)	Diarrhoea	Testicular tumour	5.0	15.0	--
Cu(II)	Metamorphosis, Necrosis	Wilson's disease	3.0	3.0	--
Se	Pallor, Coated tongue, Gastrointestinal disorders	Garlicky odour or breath, Chronic selenosis	0.05	0.05	--
Ag	Arteriosclerosis	Argyria	--	--	--
Ni(II)	Pulmonary, Cerebral edema	Thrombocytopenia, Anemia, Leucopenia, Eosinophilia, Motocytosis	3.0	3.0	--

Anthropogenic source(s) of regulated heavy metals and metalloids (†) from various industrial effluents where the concentration of these toxic chemicals found to be above the critical discharge limit have been tabulated in Table 1.2 (Dean et al., 1972; Patterson, 1978). Their removal/recovery processes from contaminated water are important for protecting the environment and health.

Table 1.2. Regulated heavy metals and/or regulated metalloids† found in major industries (Dean et al., 1972; Patterson, 1978).

Name of industries	Cd	Cr	Cu	Hg	Pb	Ni	Zn	Ag	As†	Sb†	Se†
Pulp, Paper mills, Paperboard, Building paper, Board mills		+	+	+	+	+	+				+
Organic chemicals, Petrochemicals	+	+		+	+		+		+		
Alkalis, Chlorine, Inorganic chemicals	+	+		+	+		+		+		
Fertilizers	+	+	+	+	+	+	+		+		
Petroleum refining	+	+	+		+	+	+		+		
Basic steel works, Foundries	+	+	+	+	+	+	+		+	+	
Basic nonferrous metal works, Foundries	+	+	+	+	+		+	+	+	+	
Motor vehicles, Aircraft-plating and finishing	+	+	+	+		+		+			
Glass, Cement, Asbestos products		+							+		+
Textile mill products		+									
Leather tanning		+									
Steam generation power plants		+									
Photographic				+	+			+			
Paints, Pigments, Dyes, Insecticides	+		+		+				+		+
Wood preservatives		+							+		

1.1.2 Chromium contamination

Chromium is a naturally occurring element and generally found in soil, rocks, plants, living creature and volcano (EPA, 1998). Soils typically contain 100 mg Cr kg^{-1} soil, while its concentration in surface water varies from parts per million to parts per billion range (Asano et al., 2007). Chromium has oxidation states from -2 to +6. But trivalent and hexavalent chromium commonly exist in natural water and soil system. Cr(III) and Cr(VI) are characterised by their different physiochemical behaviour, mobility and toxicity (Pettine et al., 1998b). 3 d-electrons of the trivalent chromium are in high spin state in an octahedral complex and are stable energetically. Cr(III) has highest crystal field of stabilization. It makes

Cr(III) ion a strong kinetically inert complex with high stability of hydration ion sphere and resulted in limitation of electron transfer reaction. Cr(III) is strongly hydrolysed into $Cr(OH)_3$ in the pH range of 6.5 to 10.5 (Rai et al., 1987). Cr(III) has low solubility and strong tendency to adsorb on surfaces. Cr(VI) ion exists as oxo species such as CrO_3 and CrO_4^{2-} as strongly oxidising agents. The oxy-compounds of Cr(VI) has higher affinity to adsorb on certain types of proton specific mineral surfaces (Pettine et al., 1998a). The common oxy ion species of Cr(VI) is CrO_4^{2-} pH> 7.0. At lower pH < 1, H_2CrO_4 predominates and $HCrO_4^-$ at pH range from 1 and 6. The bio-availability of Cr(VI) is much high as compared to Cr(III) and transport through the cell as analogous to sulfate ions (Bielicka et al., 2005).

Chromium is used in stainless steel and non-iron alloy production for metalplating, development of pigments, leather processing, catalysts production, surface treatments and in refractories. Leather industry is responsible for the major influx of chromium to the biosphere, scoring for 40% of the total industrial use (Table 1.3). In India, tannery industries are the major users of chromium compounds. The recommended limit for chromium concentration in water are set differently for Cr(III) (2 mg L^{-1}) and Cr(VI) (0.1 mg L^{-1}) (Chandra et al., 1997).

Table 1.3. Industrial consumption of chromium and chromium compounds (Barnhart, 1997).

Industries	World (%)	USA (%)
Wood preservation	15	52
Leather tanning	40	13
Metal electroplating	17	13
Pigments	15	12
Refractory	3	3
Other	10	7

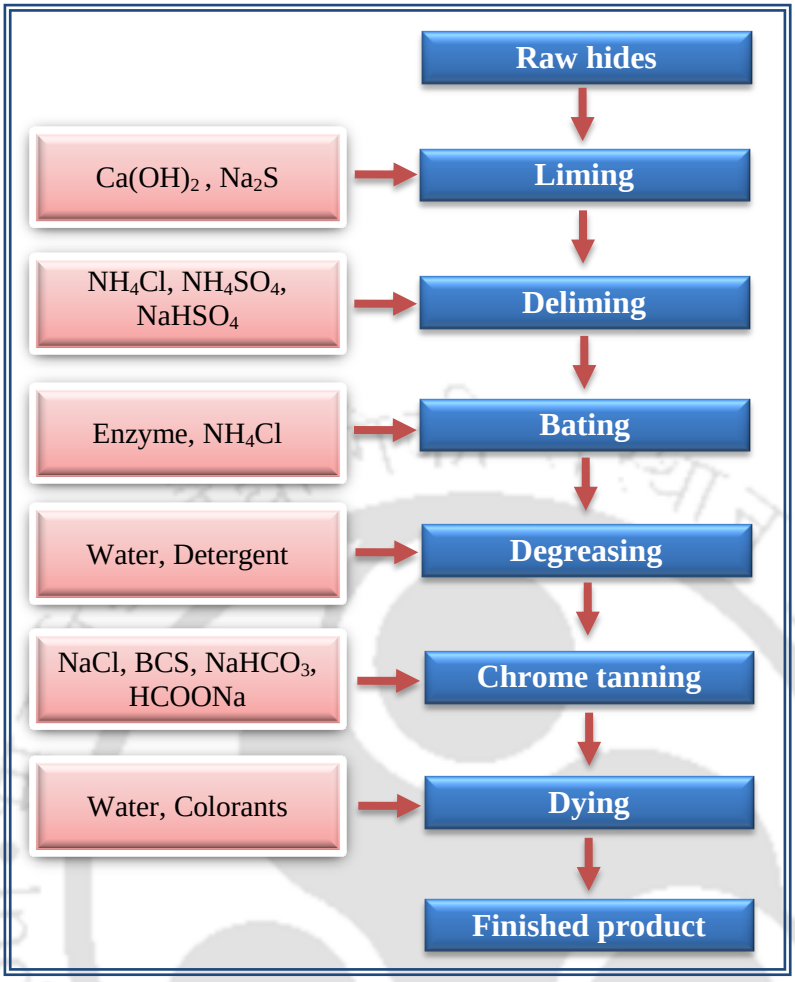

Figure 1.1. Typical wastewater generating processes in a tannery (Cassano et al., 1997; Cassano et al., 2001).

1.1.3 Chrome tanning industries: Indian scenario

According to the initial project report (2003-2004) of the Blacksmith Institute, about eighteen most polluted locations in India have been identified as places where human health and the environment are affected by heavy metals contamination. About 26.5 million people in these places are potentially affected and out of this 20 million belong to the State of West Bengal. Serious contamination of both surface and ground water has been reported in some area of Tamilnadu, as a result of uncontrolled discharge of untreated wastewater by about 80 tanneries for the last three decades (Mondal et al., 2005). The health of the rural farming

community and people working in the tanning industries has been seriously suffering from occupational diseases such as asthma, chromium ulcers and skin diseases.

In India, tanning industries is the major user of chromium compounds. Leather industry in particular, generates large quantity of polluting wastewater. Major wastewater generating processes in tannery operations is illustrated in Figure 1. Basic chrome sulphate (BCS, $Cr(OH)SO_4$) is most widely used for tanning of animal hides and skins in conventional single bath chrome tanning process. Wastewater generated contains more than one third of the initial tanning salt charged (Cassano et al., 2001). About 80% of tanneries engaged in chrome tanning process consume BCS. Consumption of BCS has increased from 25,000 to 40,000 tons per annum (Agrawal et al., 2006) during 1990s. However, presence of Cr(VI) in chrome tanning effluent is primarily resulted from Cr(III) oxidation at high pH and temperature and in exposure to UV light (Zhao et al., 2010). There are more than 2,500 tanneries all over India with processing capacity of 0.7 million tons of hide and skin annually (Ram et al., 1999; Agrawal et al., 2006). About 88% of the tanneries are in the states of Tamil Nadu, Uttar Pradesh and West Bengal. During tannage operation, the skin/hide is treated with substances like chromium salts, vegetable substances, aldehydes, oils etc to prevent putrefaction (Belay, 2010).

Table 1.4 summarizes the average pollutant loading in various operations of typical Indian tanneries and the maximum discharge limit of Central Pollution Control Board (CPCB), Govt of India.

Tanning effluent is a major source of aquatic pollution with high chemical oxygen demand (COD), biochemical oxygen demand (BOD) and chromium compounds. The concentration of

chromium (total) in chrome tanning effluent is usually in the range 1.5 to 3 g L^{-1} (Table 1.4). Cr(VI) loading is usually much smaller in concentration (1 to 5 mg L^{-1}).

Table 1.4. Physiochemical characteristics of different water quality parameters of tannery effluent (Rao et al., 2003).

Parameters	Operating unit					
	Soaking	**Beam house operation**	**Pickling and chrome tanning**	**Wet finish-rechroming and dyeing**	**Washing**	**Maximum discharge limit (CPCB)**
pH	7.5-8.5	8-12	2.2-4.0	3.5-4.5	7.0-9.0	6-9
BOD	1100-2500	2000-8000	400-800	1000-2000	1200-3000	30
COD	3000-6000	3000-15000	1000-3000	2500-7000	2500-8000	250
Sulphides		20-200			30-150	2
Total solid	35000-55000	6000-20000	30000-60000	4000-10000	15000-25000	--
Chlorides (as Cl^-)	15000-30000	3000-6000	15000-25000	500-1000	6000-9500	1000
Total Cr	--	--	1500-3000	30-60	80-20	2.0

All values except pH are expressed in mg L^{-1}

1.2 Removal of chromium compounds

1.2.1 Non-biological treatment

Various waste streams from upstream units of the leather plant, except chrome tanning, are combined and sent to the common effluent treatment plant (CETP) (Agrawal et al., 2006). The spent tanning liquors are mostly segregated for possible recovery of chromium with the aim of recycling it back to the system or producing a value-added product, or for safe disposal of chromium containing wastes. The exhausted stream coming from chrome tanning contains about 30-40% of initial tanning salt (Cassano et al., 2001; Sreeram and Ramasami, 2003). Chromium in tannery waste occurs in complex forms and their availability on the some extent depends on the technique used in tanning operation.

There are several specialized treatment technologies/processes for tannery wastewater such as chemical precipitation, coagulation/flocculation, ion exchange, membrane separation and adsorption.

Precipitation: Chemical precipitation is the most widely used process for removal of heavy metals from various industrial metal contaminated water and wastewater. Precipitation technique for heavy metals removal is typically classified as hydroxide precipitation, carbonate precipitation and sulphide precipitation. Some combinations of the three are also used for metal removal and recovery (Fabbricino et al., 2013). Precipitation normally depends on (a) the type of reagent, particle size, reaction products, volume of precipitate and settling velocity, and (b) the operating conditions such as the feeding rate of chemical, temperature, pH and stirring time. The common method of chromium removal from the spent tanning liquor is by precipitation as $Cr(OH)_3(s)$. Precipitated sludge may be either treated with sulphuric acid for chromium recovery or sent to a stabilization/conditioning process prior to final disposal for landfill purpose (Almeida, 1993; Fabbricino et al., 2013).

Even though precipitation is efficient treatment technique but treated effluent can't be directly disposed off to the environment as the concentration of heavy metal is still high (Guo et al., 2006). The process is associated with generation of large amount of sludge and disposal problem. This sludge also contains minerals and toxic metals which may leach out to ground water/receiving water bodies if treated/stabilized properly.

Coagulation and flocculation: Removal of heavy metals from wastewater by chemical coagulation/flocculation is a process compatible with other existing wastewater treatment techniques. Coagulation/flocculation usually consists of two separate process steps. The inorganic coagulant, such as ferrous or ferric chloride, aluminium sulphate or lime is added. A

synthetic organic polyelectrolyte is then mixed up to agglomerate the suspended solids into larger aggregates that can be readily removed by sedimentation and/ or filtration (Bolto and Gregory, 2007).

Cr(VI) is converted to Cr(III) by addition of reducing agent and precipitated as hydroxide by increasing the solution pH. The efficiency of the coagulation process has been studied using different salts (Orhon, 1998; Song et al., 2004). Talinli (1994) showed that coagulation with high doses of lime assisted by non-ionic polyelectrolyte at pH 11 resulted in 63% reduction of COD value from tannery effluent. Coagulation with $FeCl_3$ and lime at pH 8.8 reduces COD only by 43%. $Al_2(SO_4)_3$ is capable to reduce COD value of around 47% under the same experimental conditions. Coagulation by Al and Fe salts without lime addition is even lowers the removal efficiency (Garrote et al., 1995).

Addition of coagulating/flocculating agents for the treatment of highly concentrated effluent is generally efficient to decrease the level of pollutant. However, at lower concentration this process is rather costly. Like precipitation technique, coagulation process also generates secondary solid wastes and sometimes suffers from high sludge disposal cost (Garrote et al., 1995).

Membrane based technology: Membrane processes are long been applied in the treatment of various industrial wastewaters for separation and removal of toxic chemicals (Marcucci et al., 2002; Brans et al., 2004). Cassano et al. (2001) presented a systematic conceptual possibility of various applications of the membrane based processes, e.g., microfiltration (MF), ultra filtration (UF), nanofiltration (NF), and reverse osmosis (RO) on wastewater of different tannery operations. Application of NF to the effluent from the liming process and UF from the degreasing unit has been reported (Cassano et al., 1998; Cassano et al., 1999). The treatment

of chromium rich tanning effluents using UF and NF is mainly investigated for the recovery and reuse of tanning chemicals (Cassano et al., 1996; Padilla and Tavani, 1999). Membrane process can ably remove more than 85% proteic and colloidal material (Cortese and Drioli, 1976; Cortese and Drioli, 1978), about 97% oil (Holdich et al., 1998), 28% Cr (Fabiani et al., 1996), 55% COD and 75-80% color (Cassano et al., 2001) from industrial tanning effluents.

Although membrane technology is investigated for treating industrial effluent, however the operational and maintenance cost are much higher. Major limitations associated with membrane separation processes include (i) membrane fouling, (ii) limited life of membranes and, (iii) dissolution of the membrane material by strong oxidizing agents, solvents and by others organic compounds. Pretreatment of effluents is generally required prior to entry into the membrane module to reduce membrane fouling and flux decline.

Adsorption: Adsorption is a well-studied technique for removal of heavy metals from wastewater. There are various types of adsorbents available for treatment of tannery wastewater. Activated carbon, low cost adsorbents such as clay minerals (Ping and Guochang, 2006), soils (Tor et al., 2009), laterite (Xiaohong et al., 2009), biomass (Lokeshwari and Joshi, 2009; Sharma and Goyal, 2010), peat etc are often used for metal uptake. Activated charcoals, granular activated carbon and activated carbons prepared from low cost materials show considerable Cr removal from synthetic wastewater at acidic condition (Lyubchik et al., 2005). Faout et al. (2008) used the various types of wastewater sludges namely, primary sludge (PWS), secondary sludge (SWS), mixed sludge (MWS), physicochemical treatment sludge (CWS), and agro-processing industry sludge (AWS) as adsorbent for the removal of chromium from synthetic wastewater solution. They obtained about 40 % removal from 143 mg L^{-1} initial Cr with SWS. Sharma and Goyal (2010) employed the biomass, *Aspergillus* sp, for the treatment of tannery effluent and synthetic wastewater. They observed 94 and 64%

removal of Cr from tannery and synthetic solution, respectively, at pH 4 and with 1% biomass loading.

Removal performance of adsorption process is equilibrium limited and sometimes regeneration of adsorbents is difficult. Adsorption is also ineffective at very low concentrations of metal ions and spent adsorbent, if not regenerated, may cause a disposal problem.

Electrocoagulation: In the electrocoagulation (EC) process electrodes placed in the effluent are connected to external DC power supply. The coagulant is generated in situ by electrolytic oxidation of the anodic material. In this process, charged ionic species are removed from wastewater by allowing it to react and/or to get destabilized with ions having opposite charge and/or with floc of metallic hydroxides generated within the effluent. OH^- ions are generated at the cathode increasing solution pH. Heavy metal like Cr(III), Zn(II) etc get precipitated along with $Fe(OH)_3(s)$ and/or $Al(OH)_3(s)$ when iron and aluminium are used as electrode materials (Mollah et al., 2001). Zaroual et al. (2005) used the EC process for tannery wastewater treatment. The effects of EC time, current and cell potential on process performance are examined. An iron electrode is used as a sacrificial anode. The optimal conditions are 0.15 A cm^{-2}, 30 min and 90 min for composite and tannage effluents, respectively. The removal efficiency of chromium exceeds 99% for the both wastewaters.

Cell current density and pH are as major variables that significantly affect the removal efficiency. Removal of Cr(III) is mainly due to precipitation as $Cr(OH)_3(s)$ and co-precipitation as [Cr, Fe or Al]$(OH)_3(s)$. Aqueous solution of volume 800 ml containing 1000 mg L^{-1} Cr(III), 1000 mg L^{-1} of NaCl as supporting electrolyte having initial pH 3.4 after electrotreatment at 48.78 mA cm^{-2} current density for 40 min brings down the Cr(III)

concentration to 2 mg L^{-1} using a pair of MS electrodes (Golder et al., 2007a; Golder et al., 2007b; Golder et al., 2007c). In case of Al electrode, the time to reach the discharge limit is 60 min. Cr(III) removal is almost complete (99.9%) after 50 min of treatment in case of multiple bipolar electrodes arrangement at 1000 mA cell current against 81.5% removal for multiple monopolar electrode arrangement using MS electrodes (Golder et al., 2007c). Bipolar electrode arrangement significantly improves the removal of Cr(III) at higher pH mainly due to combined effect of precipitation and co-precipitation (Golder et al., 2007c). Current efficiency of using a pair of electrodes with respect to iron and aluminium dissolution from electrode is to be of the order of 105 and 169% (Golder et al., 2007a; Golder et al., 2007b). Current efficiency based on iron dissolution is to be about 64.5 and 91.7% for multiple bipolar and monopolar electrodes arrangement (Golder et al., 2007c). In EC process, toxic solid sludge is also generated and associated with disposal problems.

Ion-exchange: Conventional methods like precipitation, coagulation are unfavourable especially when dealing with large volumes of effluent containing heavy metal ions at low concentration. Ion-exchange is an ideal method for removal of trace amounts of heavy metals from dilute wastewaters with high quality treated water ready for reuse. Many researchers have been successfully used ion exchange resin for treatment of water from industrial processes (Korngold et al., 2003). Though it is relatively expensive as compared to the other methods, it has the ability to achieve parts per billion levels of scavenging while treating a relatively large volume. Kabir and Ogbeide (2008) studied the efficiency of commercially available resin containing polyamine functionality for the removal of chromium from tannery wastewater. The effect of other co-ions like chloride and sulphide has negligible effect on removal efficiency. With initial concentration of 10 mg L^{-1} of chromium, removal efficiency reported is 98%. The resin has high selectivity towards chromium at operated pH of 4. Sahu et

al. (2009) investigated macro-porous strongly acidic cation-exchange resin of sulphonated polystyrene group for the treatment of Cr from synthetic tannery wastewater. The resin shows selective sorption of Cr(III) in the pH range of 0.5 to 3.5 with 92% extraction efficiency from 500 mg L^{-1} of chromium solution. The major problems typically associated with ion exchange treatment include (i) metal fouling (from Fe, Mn, Cu, etc) on the ion exchange media, (ii) difficulty to find a suitable resin for treatment of wastewaters containing mixed metal ions, (iii) fouling due to oil, grease, silt, clay, colloidal silica, organic materials and microbes and, (iv) fairly high operational costs. The choice of a proper cleaning program can restore much of the lost efficiency.

Table 1.5 summarizes the most outstanding metal removal performance among the various physio-chemical treatments techniques. Ion-exchange achieved a complete removal of Cd(II), Cr(III), Cu(II), Ni(II) and Zn(II) with an initial concentration of 100 mg L^{-1}, respectively. The removal efficiency are comparable to reverse osmosis with 99% of Cd(II) rejection with an initial concentration of 200 mg L^{-1}. Precipitation is one of the most effective techniques to treat inorganic effluent with a metal concentration of higher than 1000 mg L^{-1}. Flotation method showed comparable metal removal (100%) to RO.

The main disadvantages of these processes include (i) ineffective removal of various metals from multiple metals bearing wastewater because of variation of minimum solubility of different metals with pH values, (ii) high chemical consumption and, (iii) formation of huge sludge (Almeida, 1993). A filtration step is generally necessary after precipitation/sedimentation due to relatively small particle size of the precipitates.

Table 1.5. Comparison of metal removal performance among treatment techniques (Kurniawan et al., 2006).

Treatment techniques	Metal species	Precipitant/ coagulant/ion exchanger/membrane/ collector/electrode	Optimum dose ($g\ L^{-1}$)	Initial metal concentration ($mg\ L^{-1}$)	Optimum pH	Removal efficiency/metal rejection rate (%)
Chemical Precipitation	Mn(II)	$Ca(OH)_2$	10	1085	11.0	99.3
Coagulation	Cu(II)	Poly-ferric sulfate	25	20	10-11.5	99
Flotation	Zn(II)	Surfactin-105	0.04	50	6.0	100
	Zn(II)	SDS	0.05	50	7-9	100
Ion exchange	Cr(III)	Synthetic (NAP1) zeolite	2.5	100	NA	100
	Ni(II)	--	2.5	100	NA	100
	Zn(II)	--	2.5	100	NA	100
	Cu(II)	--	2.5	100	NA	100
	Cd(II)	--	2.5	100	NA	100
	Cr(III)	IRN77	3.0	100	3-5	100
Ultra filtration	Cu(II)	YM1	--	79	8.5-9.5	100
Nano filtration	Cd(II)	Polyamide	--	200	4-11	99
Reverse osmosis	Cu(II)	ES20	--	50	7-9	100
	Ni(II)	ES20	--	50	7-9	100
Membrane electro dialysis	Cr(VI)	Iron rotary	--	130	8.5	99.6

The performance characteristics of different techniques for treatment of chrome tanning effluent are summarized in Table 1.6. The spent tanning solutions generated is normally separated for possible chromium recovery and/or treatment of tanning effluent with the aim of recycling back to the process or producing a value-added and/or for safe disposal of chromium containing waste/sludge (Agrawal et al., 2006).

Researchers have been looking for more economic and effective process to remediate heavy metal contaminated water and reduce its toxicity. Bio-techniques could remove heavy metals from aqueous streams even at very low concentration in environmental friendly and economic manners (Volesky, 1990). The major advantages of biological methods over conventional processes include low operating cost, high efficiency at low metal concentration, minimum chemical/biological sludge production, no or minimum additional nutrient requirement and possibility of metal recovery for further use (Kratochvil and Volesky, 1998).

Table 1.6. Comparative performance of various heavy metal removal/recovery techniques (Agrawal et al., 2006).

Selection guide	Treatment techniques						
	Adsorption	Electro-chemical	Ion-exchange	Membrane	Solvent extraction	Precipitation	
						Hydroxide	Sulfide
pH range	Limited tolerance	Tolerant	Limited tolerance	Limited tolerance	Some system pH tolerance	Tolerant	Limited tolerance
Metal selectivity	Moderate	Moderate	Resins can be elective	Moderate	Metal selective	Non selective	Limited selective
Influence of suspended solids	Fouled	Can be engineered to tolerate	Fouled	Fouled	Fouled	Tolerant	Tolerant
Tolerance of organic molecules	Can be poisoned	Can be accommodated	Can be poisoned	Intolerant	Intolerant	Tolerant	Tolerant
Working level for appropriate metal (mg L^{-1})	< 10	> 10	< 100	> 10	> 100	> 10	> 10

1.2.2 Biological route: An emerging effluent treatment technique

It was quite natural and also creative to think of microorganisms as potential and renewable cost effective resources for the removal of heavy metals. The biomass/biomaterials are abundant in nature and it requires little processing, prior to application for heavy metal decontamination. The board definition of the term biosorption is "processes whereby micro-organisms, alive or dead, or their derivatives, are employed for the sequestration of heavy metals from the environment" (Hawari and Mulligan, 2006).

Bio-scavenging is resulted both from chemical and physical interaction between biomass and metals. Cell wall of microorganisms, mainly consist of proteins, polysaccharides and lipids. It provides many functional groups such as carboxylate, hydroxyl, thiol, sulphonate, phosphate, amino and imidazole groups for binding of metal ions (Daughney et al., 1998).

Microbes have complex mechanisms to detoxify heavy metals through sorption and accumulation inside the cells. Microbes play a major role in the biogeochemical cycle of toxic heavy metals also in cleaning up metal-contaminated environments. There is a correlation between heavy metal tolerance and antibiotic resistance. Heavy metals are transition elements with incompletely filled d orbitals. These d orbitals of heavy-metal cations provide the ability to form complex compounds which may or may not be redox-active. Thus, heavy-metal cations play an important role as "trace elements" in many biochemical reactions. Heavy metals can be classified as essential (e.g. Co(II), Ni(II), Cu(II)) or non-essential (e.g. Cd(II), Hg(II), Pb(II)) depending on their biological role for microorganisms (Bruins et al., 2000). Essential metals have growth stimulatory effects up to a certain concentration, with inhibitory effects above this level.

Microalgae are found in aquatic habitats, freshwater, marine and moist soil. They contain chlorophyll and carry out oxygenic photosynthesis. These are eukaryotic microorganisms and carry out the process of photosynthesis. The surface of microalgae is consisting of polysaccharide and provides active sites for metal binding. The characteristic of surface depends on its cultivation conditions. These microalgae are present in variety of environment such as acidic, alkaline and saline conditions. Photosynthetic microorganisms are used for the production of primary and secondary metabolites and bio-elimination of contaminants from the wastewater streams. Table 1.7 summarise a few microalgae depending on their natural habitats.

Table 1.7. pH tolerance of various microalgae species.

Microalgae species	pH	Treatment conditions	Sorption efficiency (mg g^{-1}/%)	Source
Thalassiosira sp.		Cd(II), Cr(III), Cu(II), Pb(II), Ni(II), Zn(II), light 4 klux, photo period 16 h, temp 28ºC	Toxic effects studied	Pun et al., 1995
Cladophora glomerata		Ni(II), V, Cd(II), Pb(II), Cr(total), refinery sewage lagoon	Pb(II) 7.9×10^{-3} mg g^{-1} Cd(II) 0.1×10^{-3} mg g^{-1} Ni(II) 15.6×10^{-3} mg g^{-1} Cr(total) 1.7×10^{-3} mg g^{-1} V 37.6×10^{-3} mg g^{-1}	Chmielewská and Medved, 2001
Dunaliella sp.		Cr(VI) 300 mg L^{-1}, light 2 klux, photo period 24 h, temp 25ºC	58.3 mg g^{-1}	Donmez and Aksu, 2002
Spirulina sp.	Alkaline pH (pH>8)	Cr(VI) 300 mg L^{-1}, light 1.5 klux, photo period 24 h, temp 25ºC	99%	Pandi et al., 2009
Ulva lactuca		Pd(II) 80 mg L^{-1}, Cd(II) 100 mg L^{-1}, Hg(II) 60 mg L^{-1}, Pb(II) 80 mg L^{-1}, light 250 µmol m^{-2} s^{-1}, photo period 12 h, temp 15ºC	50 nmol g^{-1} Pd(II) 30 nmol g^{-1} Cd(II) 100 nmol g^{-1} Hg(II) 30 nmol g^{-1} Pb(II)	Turner et al., 2008
Scenedesmus vacuolatus		Fluoxetine, norfluoxetine, propranolol, lidocaine, trimipramine light 170 µE m^{-2} s^{-1}, photo period 24h, temp 25ºC	Toxic effects studied	Neuwoehner and Escher, 2011
Chlorella sp.		Cu(II) 31.5 µmol L^{-1}, Ni(II) 34 µmol L^{-1}, light 72 µE m^{-2} s^{-1}, photo period 14 h, temp 24ºC	20.31 µmol L^{-1} Cu(II) 20.51 µmol L^{-1} Ni(II)	Rai et al., 1996
Chroococcus sp., *N. calcicola*	Acidic pH (< 4)	Cr(VI) 20 mg L^{-1}, light 3 klux, photo period 24 h, temp 28ºC	21.36 mg g^{-1} *Chroococcus sp* 12.23 mg g^{-1} *N. calcicola*	Anjana et al., 2007
Gloeocapsa sp.		Pb(II) 20 mg L^{-1}, light 400 µE m^{-2} s^{-1}, photo period 24 h, temp 25ºC	232.56 mg g^{-1}	Raungsomboon et al., 2008
Oocystis sp.	Neutral pH (5-7)	Cd(II) 35 mg L^{-1}, Cu(II) 25 mg L^{-1}, light 5 klux, photo period 24 h, temp 28ºC	Cd(II) 230 mg g^{-1} *Oocystis sp* Cu(II) 75 mg g^{-1} *Oocystis sp*	Gin et al., 2002
Chlorella vulgaris		phosphorous 7.7 mg L^{-1}, light 4.1 klux, photo period 24 h, temp 20ºC	78%	Aslan, and Kapdan, 2006
Scenedesmus sp.		phosphorous 2 mg L^{-1}, nitrogen 25 mg L^{-1},	Phosphorous 99% Nitrogen 99%	Xin et al., 2010

	light 60 µE m^{-2} s^{-1}, photo period 14 h, temp 25ºC

Iaconi and co-workers (2002) studied the biodegradation performance of tannery wastewater in a sequencing batch reactor (SBR) by chemical oxidation using ozone. Ozone was supplied in a continuous flow of ozonised air. The removal performances increase up to 97, 98 and 99.9% for COD, NH_4-N and TSS, respectively. Farabegoli et al. (2004) reported the influence of Cr(VI) on denitrification bacteria in SBR. They showed that the inhibition effect is induced by chromium when the concentration reaches 120 mg L^{-1} during denitrification in aerobic condition. The inhibitory effect increases as the chromium concentration in the batch progressively increases up to the maximum value of 190 mg L^{-1}. The denitrification rates are similar without and with chromium in anoxic condition at a concentration of 150 mg L^{-1} of Cr(VI). However, chromium inhibition starts at 180 mg L^{-1}. Dermou et al. (2005) studied biological removal of Cr(VI) in trickling filter with indigenous bacteria collected from industrial sludge. They used sodium acetate as carbon source with different mode of operations to increase the efficiency of the filter. SBR operation with recirculation is a very effective operating mode as it ensures uniform wetting and distribution of precipitates all over the filter. First, the pilot-scale trickling filter is being operated as a batch reactor, consequently as a SBR with recirculation, and finally as a continuous flow reactor. The pilot-scale trickling filter consists of a plexiglas tube of 160 cm high and 9 cm i.d. The unit achieves removal rates up to 530 g Cr(VI) m^{-2} day^{-1} with hydraulic loading up to 680 m^3 m^{-2} day^{-1}. While aeration takes place naturally without the use of any external means. Ganesh et al. (2006) investigated the influence of respirometry combined with SBR for tannery wastewater treatment for the reduction of COD, TKN, NH_3. It reduces the hydraulic retention time and enhances the denitrification rate. They achieved removal efficiency of 80-82% for COD, 78-80% for TKN and 83-99% for NH_3-N. Hawari and Mulligan (2006) tested the

feasibility of anaerobic granules for the sorption of lead, copper, cadmium, and nickel from aqueous solution. Anaerobic sludge of microbial aggregates provides compact and porous structure along with excellent settling ability. They reported that binding capacity of viable biomass is higher than those for nonviable biomass. Over the pH range of 4.0 to 5.5, they didn't observe any significant effect on metal sorption. They obtained q_{max} for Pb(II), Cd(II), Cu(II), and Ni(II) ions as 255, 60, 55, and 26 mg g^{-1}, respectively, by fitting Langmuir isotherm equation. Guillen-Jimenez and co-workers (2008) isolated Yeast *Candida sp.* for sulphate ion and Cr(VI) reduction from the tannery wastewater. *Candida sp.* is capable of reducing almost 100% of 1.7 mM Cr(VI) and 84% of 3.3 mM Cr(VI) in synthetic media of wastewater. The presence of sulphate in the synthetic media at pH 6 enhances the efficiency of Cr(VI) reduction and growth rate.

Precipitated chrome tanning effluent using NaOH/$Ca(OH)_2$ etc. contains about Cr(III),10 mg L^{-1} at pH < 8.5. However, the salinity level (15 to 25 g L^{-1} Cl^-) remains unchanged. High salinity, alkaline pH and also presence restrict the growth of micro-organism.

High salinity shows up an extreme environment that only a few organisms are able to adapt and occupy. They are isolated from various saline environments such as salt lakes (e.g. Dead Sea, Great Salt Lake), solar salts and subsurface salt formation. They usually employ a continuous influx of ions like K^+ in order to balance the high salt environment outside the cell. Table 1.8 shows a few saline bacterial species having metal uptake capacity and employed for water treatment.

Table 1.8. Saline bacterial species for treatment of heavy metal laden aqueous effluent.

Bacterial species	Cl- conc. (g L^{-1})	Treatment conditions	Removal efficiency (%)	Source
Bacillus sp.	6×10^{-3}	Cr(VI) 20 mg L^{-1}, pH 7, Temp 30ºC	90%	Wang and Xiao, 1995
	10.86	Cr(VI) 300 mg L^{-1}, pH 7, temp 35 °C	98%	Dhal et al., 2010
Nestetenkonia *sp.*	52.5	Cr(VI) 20 mg L^{-1}, pH 8, temp 35ºC	84%	Amoozegar et al., 2007
Vigribacillus sp.	3.62	Cr(VI) 100 mg L^{-1}, pH 8, temp 35ºC	98%	Mishra et al., 2012
Pseudochrobactrum saccharolyticum	12	Cr(VI) 130 mg L^{-1}, pH 8.3, temp 28ºC	95%	Long et al., 2013

Salt resistance algae such as *Cystoseira barbata* (Topcuoğlu et al., 2003), *C. vermilara, C. crispus, F. spiralis, A. nodosum* (Romera et al., 2007) and *Spirogyra sp.* (Rajfur et al., 2010) could remove heavy metals from aqueous effluents at elevated salinity. However, they are not generally suitable at alkaline medium. *Spirulina platensis*, a blue-green microalga, enriched in protein content (upto 70% w/w), grow in high saline (13 to 35 g L^{-1} of Cl^-) and alkaline (pH 8 to 10.5) condition (Arunakumara et al., 2007; Michalak et al., 2007; Arunakumara et al., 2008; Finocchio et al., 2010). *Spirulina* also shows considerable removal of chromium, lead, nickel, cadmium etc from aqueous waste streams (Gong et al., 2005; Gokhale et. al., 2008; Lodi et al., 2008; Gokhale et al., 2009; Finocchio et al., 2010). *Spirulina sp.* seems to be good alternative for treating precipitated chrome tanning effluent as it has the ability to survive in such harsh conditions and effluent composition may favour its growth.

1.3 Micro-algae *Spirulina platensis*

1.3.1 Physicochemical characteristics of *Spirulina sp.*

Spirulina sp. poses a versatile metabolism as it can grow photoautotrophically, heterotrophically and mixotrophically (Chojnacka and Noworyta, 2004; Lodi et al., 2005;

Pappu et al., 2013). It is organized as cylindrical cells arranged in unbranched, helicoidal trichomes. *Spirulina* is produced in large scale to suffice the demand for the health food market (Pappu et al., 2013). It easily grows in non-sterile, highly saline and alkaline condition. They remain largely free from contamination by other microorganisms and can be cultivated in open-air cultures (Materassi et al., 1984; Wu et al., 1992; Wu et al., 1993; Cheng, 1996; Wu and Xiang, 1996; Pappu et al., 2013). Amino acid in protein provides different functional groups like -NH_2, -CONH-, -COO- etc. Polysaccharides of the algal cell wall also contain amino and carboxyl as well as phosphate groups (Daughney et al., 1998). Main components of the cell wall are teichuronic acid, teichoic acid, polysaccharides, peptyglycan and proteins (Schiewer and Wong, 2000). It contains 34-42% palmitic, 19-26% inoleic, 16-25% gamalinolenic and 3-8% oleic acid (Piorreck et al., 1984). Table 1.9 shows the major components of *Spirulina* and their variations depending on the geographical location.

Table 1.9. Percentage abundance of various components of *Spirulina* on dry weight basis (Ahsan et al., 2008).

Components	Reporting Institutes			
	FOI	SAC	IPGSR	BAU
Crude protein	65	55-70	61	60
Soluble carbohydrate	19	--	14	--
Crude lipid	4	5-7	6	7
Crude fiber	3	5-7	--	--
Ash	3	3-6	9	11
Moisture	--	4-6	6	9
Nitrogen free extract	--	15-20	4	17

FOI: French Oil Institute; SAC: Siam algae Co. Ltd, Thailand; IPGSR: Institute of Post graduate Studies and Research Laboratory, University of Malaysia; BAU: Bangladesh Agriculture University

1.3.2 Role of *Spirulina sp*. for metal detoxification

The biomass functional groups provide the active sites for metal binding. The abundance of charged sites is not only related with the microbial species but also depends on the growth conditions, nutrient availability, stress growth etc. *Spirulina* biomass in particular is very

effective for the uptake of heavy metals like Cr(III), Cd(II), Cu(II) etc when the concentration remains < 100 mg L^{-1} (Costa and Franca, 1998; Michalak et al., 2007; Arunakumara et al., 2007; Arunakumara et al., 2008; Finocchio et al., 2010).

Ayala and Bravo (1984) showed that the human and animal wastes are suitable for *Spirulina* cultivation. Richmond and Grobbelaar (1986) reported that the optimum growth of *Spirulina* in open raceway pond is at pH 11. Rose et al. (1996) cultivated *Spirulina* in tannery wastewater (combined effluent except chrome tanning) and observed inhibitory effect of ammonia. Olguín et al. (1997) utilized untreated sea water with digested pig waste for *Spirulina* biomass production. Costa and Franca (1998) showed that Cd(II) bioaccumulation increases with rise in Cd(II) concentration. They claimed that metal uptake by live *Spirulina* cells possibly does not occur only by adsorption onto the outer side of the external layer but also transports inside the cell membrane. Live *Spirulina* have greater Cd(II) uptake capacity in comparison to dead biomass. Metal uptakes are of 47.63 mg Cd(II) g^{-1} cells for living cells and 37 mg Cd(II) g^{-1} cells for inactivated cells. pH of acid mine water varies from 1 to 7, while at the end of the process it becomes alkaline. Alkalinity causes metal precipitation. The removal efficiency is about 99% for iron and between 80 and 95% for Zn(II).

Vonshak et al. (1988) studied the response of *Spirulina platensis* on salinity stress due to NaCl in the range of 0.25 to 1M. NaCl has significant effect on photosynthesis and respiration process. Photosynthesis efficiency is about 80 and 50% at 0.5 and 1.0 M NaCl and respiration rate is 140 and 200%, respectively, as compared to control *Spirulina* growth. The growth rate falls significantly with rise of salt concentration. But it enhances total sugar content for *Spirulina* cells grown under salt stress.

Costa et al. (2001) used several sources of nitrogen for the cultivation of *Spirulina* such as NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_3PO_4$ and urea at the concentration of 0.01 to 0.05 M in an aerated photo bioreactor at 30°C with illumination intensity of 1.9 klux. The use of alternate source of nitrogen can reduce the operating cost but enhances the growth. $NaNO_3$ of 0.03 M shows maximum productivity of 1.99 g L^{-1} biomass. Nayak et al. (2003) investigated the biosorption of heavy metal and toxic radionuclides by three genera of algae from different taxonomic groups. They are *Spirulina* from Cyanophyceae, *Oedogonium* from Chlorophyceae and *Catenella* from Rhodophyceae. There are different in selectivity of various algae towards different metals in sorption and desorption processes. *Spirulina* shows a maximum radionuclide accumulation in comparison to other genera at basic pH.

Rangsayatorn et al. (2004) tested Cd(II) biosorption onto immobilized *Spirulina platensis* in alginate and silica gel. Immobilization facilitates easy sorption and desorption. Removal is higher with alginate immobilized cell compared to silica gel. Slight effect of temperature for alginate cells on Cd(II) sorption is reported. However, the sorption highly depends on initial solution pH. *Spirulina sp*. immobilized in silica gel has less effect on sorption capacity with variation of pH in range of 4 to 7. But it significantly varies with temperature change. Cd(II) sorption by alginate immobilized cells is more energy-independent in comparison to silica immobilized cells. Gong et al. (2005) presented the effect of chemical treatment of *Spirulina* maxima with $CaCl_2$ for biosorption of Pb(II). $CaCl_2$ treated *Spirulina* shows higher sorption capacity. The maximum removal of lead is about 84% in intact biomass and 92% in pretreated biomass. Pb(II) sorption capacity decreases with rise in biomass dose and the optimum pH is pH 5.5. Chojnacka et al. (2005) investigated the role functional groups in Cr(III) removal using of *Spirulina sp.* However, the authors neglected the precipitation effect

of Cr(III) even at higher pH (~7). Cr(III) is usually removed by biosorption and precipitation (at pH>5) and there are possibilities of over estimations of biomass-metal binding parameters.

Heavy metal accumulation onto *Spirulina sp*. has significant effect on cell growth (Nalimova et al., 2005). Some of the heavy metals such as Cr(III), Cu(II) and Zn(II) involve in a wide range of biochemical reactions. Cu(II) concentration is lethal at 5 mg L^{-1}, whereas 4 mg L^{-1} is lethal during the linear growth phase. Zn(II) concentration of 8.8 mg L^{-1} shows lethal effect during the linear but not lag phase of growth. *S. platensis* more efficiently accumulates Cr(III) than Cr(VI) from the nutrient medium by an order of magnitude (Frontasyeva et al., 2009). No removal of Cr(VI) was observed because of the absence of its adsorption.

Hong et al. (2007) collected activated sludge from the aeration tank of Taipa Wastewater Treatment Plant in Macau and studied the effect of chloride on nutrient removal efficiency. They conducted the experiments with synthetic and real wastewater with varying Cl^- concentration from 150 to 5000 mg L^{-1}. Cl^- concentrations both in synthetic and real wastewaters don't affect removal of ammonia and nitrogen notably. However, phosphorous removal decreases form 94% at 150 mg L^{-1} Cl^- to 74% at 1500 mg L^{-1} for synthetic wastewater. In case of real wastewater, phosphorous removal decreases to 58% at 1000 mg L^{-1} Cl^-. Phosphorous removal is completely inhibited at Cl^- concentration $\geq$ 2500 mg L^{-1} in both synthetic and real wastewater.

Choudhary et al. (2007) examined the toxic impacts of Pb(II), Cu(II), and Zn(II) over a concentration range of 0.05-0.20 mg L^{-1} on *Spirulina*. Cultivation temperature and pH are kept as 30±1°C and 9 in a thermostatically controlled incubator. Light intensity of 2±0.2 klux is illuminated in culture vessels for *Spirulina* growth. Pb(II) concentration results in growth retardation for *Spirulina* and the toxicity is in order of Pb(II)>Cu(II)>Zn(II). Lodi et al. (2008)

carried out Cr(III) sorption studies with *Spirulina platensis* biomass from synthetic solution containing 25 to 200 mg L^{-1} Cr(III) at pH 6. Cr(III) uptake is about 95% with initial 100 mg L^{-1} Cr(III) and 3 g L^{-1} biomass. Cr(III) at low concentrations likely binds to sites with higher affinity. While an increase in metal concentration involves the sites with lower affinity as well. High biomass loading increases the repulsion among cells cause to higher desorption rate of ions physically bound to the sites by means of weaker bonds. Pandi et al. (2009) employed *Spirulina fusiformis* for removal of chromium from diluted retanned chrome liquor (25 to 300 mg L^{-1} Cr(VI)). It is able for removing about 93-95% chromium at 100 mg L^{-1} initial concentration. FTIR result depicts the formation of chromium complexes with carboxyl- and -NH groups. Çelekli et al. (2009) cultivated *Spirulina platensis* at pH 10 and 0.5 g L^{-1} phosphate with Schlösser's medium. The light intensity of 2 klux and agitation speed of 90 rpm achieves 3.009 g L^{-1} *Spirulina* cell density.

Finocchio et al. (2010) used methylated *Spirulina* biomass for the removal of Cr(VI) from synthetic wastewater. Methylation process modifies the surface charge for the absorptive uptake of Cr(VI) over a neutral pH range. FTIR studies shows that different free groups like carboxyl (-COOH), amines (-NHR, $-NH_2$) and ether ($-C-OCH_{3)}$ are formed due to methylation. About 80% Cr(VI) removal achieves at pH 7 to 8.

Ferreira et al. (2011) observed the second order kinetics for Ni(II), Pb(II)and Zn(II) sorption with *Spirulina platensis* biomass at pH around 5.0. They reported pH_{zpc} as 4.0. The metal removal efficiency decreases with rise in metal concentration due to both passive and active sites on the surface of biomass. There are interactions between metal and carboxylate groups during sorption. The maximum sorption (q_{max}) of 0.354, 0.495 and 0.508 mmol g^{-1} for Ni(II), Pb(II)and Zn(II), respectively, are shown.

Çelekli and Bozkurt (2011) studied the sorption of Cd(II) and Ni(II) ions onto *Spirulina* biomass. They applied both pseudo first and pseudo second order kinetics. The process follows pseudo second-order kinetics explained by intra-particle diffusion model. *Spirulina* shows maximum uptake of 73.64 mg g^{-1} for Cd(II) and 69.04 mg g^{-1} for Ni(II) at pH 5.0.

Table 1.10 summarizes the uptake capacity of different heavy metals using various *Spirulina* species. Results indicate that *Spirulina* species are effective for removing different heavy metals over a wide pH range (1.5 < pH< 7) while using dead biomass. However, high alkalinity should be maintained for *Spirulina* cultivation along with metal scavenging from the solution. Ionic strength and nutrient composition significantly affect the metabolic functionalities and biomass production.

Table 1.10. *Spirulina* species for the treatment heavy metal laden aqueous effluent.

***Spirulina* species**	**Metals**	**Experimental conditions**	**Sorption efficiency (%/mg g^{-1})**	**Source**
Platensis	Cr(III) Cd(II) Cu(II)	Cr(III) 34.66 mg L^{-1}, Cd(II) 112.4 mg L^{-1}, Cu(II) 63.5 mg L^{-1}, pH 7, temp 35°C	185 mg g^{-1} Cr(III), 196 mg g^{-1} Cd(II), 159 mg g^{-1} Cu(II),	Chojnacka et al., 2005
Maxima	Pb(II)	Pb(II) 50 mg L^{-1}, pH 5, temp 20 °C	92%	Gong et al., 2005
Platensis	Pb(II)	Pb(II) 50 mg L^{-1}, pH 7, temp 25°C	95%	Hong and Shan, 2005
Platensis	Cu(II)	Cu(II) 400 mg L^{-1}, pH 6.7, temp 30°C	91%	Solisio et al., 2006
Platensis	Cr(VI)	Cr(VI) 100 mg L^{-1}, pH 1.5, temp 25°C	86%	Gokhale et. al., 2008
Platensis	Hg(II)	Hg(II)10 mg L^{-1}, pH 6, temp 22°C	456 mg g^{-1}	Cain et al., 2008
Platensis	Cr(III)	Cr(III) 100 mg L^{-1}, pH 6, temp 20°C	95%	Lodi et al., 2008
Platensis	U	U 60 mg L^{-1}, pH 5, temp 30°C	80%	Dheetcha and Mishra, 2008
Fusiformis	Cr(VI)	Cr(VI) 300 mg L^{-1}, pH 3.2, temp 25°C	93-99%	Pandi et al., 2009
Platensis	Ni(II) Pb(II) Zn(II)	Ni(II)176 mg L^{-1}, Pb(II) 621 mg L^{-1}, Zn(II)196 mg L^{-1}, pH 5, temp 25±1ºC	20 mg g^{-1} Ni(II), 102 mg g^{-1} Pb(II), 33 mg g^{-1} Zn(II)	Ferreira et al., 2011
Platensis	Zn(II)	Zn(II) 40 mg L^{-1}, pH 8.0, temp 25°C	30.96 mg g^{-1}	Meng et al., 2012

1.4 Objectives of the dissertation

In general, precipitation/coagulation and sedimentation processes are employed in the treatment of tanning effluent for removal of heavy metals especially trivalent chromium at elevated pH (>8.5) (Esmaeili et al., 2005). Precipitation by pH adjusting is not selective and iron present in the effluent will be precipitated initially followed by other metals. Consequently, precipitation produces large quantity of toxic solid sludge which again creates a burden for its disposal (Agrawal et al., 2006). Unfortunately, the concentration of chromium is difficult to reduce < 10 mg L^{-1} by conventional precipitation technique(s) (Table 1.6). Coupling of sophisticated techniques like membrane separation and ion-exchange processes makes the process costly for further reduction of chromium concentration below the stipulated limit.

The acid-base characteristic of biomaterials is an important factor determining the efficacy of a metal removal process. There is huge diversity in characteristics properties of biomass-acidic sites and it shows significant variation with the type of biomaterials. The quality and quantity of acidic sites of algal species shows strong dependency on its cultivation conditions. It can be seen from the literature survey that *Spirulina* is an effective biosorbent in removing chromium, lead, nickel, cadmium etc from aqueous streams. However, there is a serious lacuna of investigation for the prediction of metal uptake capacity and as well as for the development of kinetics model from the acid-base characteristic of *Spirulina sp.* biomass both in dead and live conditions.

The precipitated chrome tanning effluent (PCTE) at elevated pH and salinity may be a favorable media for *Spirulina* cultivation along with residual chromium scavenging (if any).

Removal of chromium compounds from aqueous effluents using *Spirulina platensis* along with its cultivation in PCTE to use the media supplements of tanning effluent is also warranted in this study.

Therefore, the objectives of the dissertation are:

1. *Spirulina platensis* dead biomass for removal of both Cr(III) and Cr(VI) from model wastewater:

i. To determine the uptake capacity and to elucidate the mechanisms of different forms of chromium binding onto *Spirulina platensis*.

ii. To study the acid-base characteristics of *Spirulina sp*. and its role to estimate Cr(III) attachment to functional groups following a non-ideal metal sorption model.

iii. To develop a kinetic model of Cr(VI) reduction into Cr(III) in terms of active functional groups of *Spirulina sp.*

2. Cultivation of *Spirulina platensis* in precipitated industrial chrome tanning effluent:

i. To remove residual chromium with simultaneous *Spirulina* cultivation by utilizing the growth supplements present in tanning effluent.

ii. To find out the production efficiency of metabolites of *Spirulina* biomass cultivated in waste stream.

iii. To obtain the growth parameters by employing a kinetic model that counts the rate of photosynthesis, nutrient uptake and respiration.

References

Agrawal, A., Kumar, V. and Pandey, B.D., "Remediation Options for the Treatment of Electroplating and Leather Tanning Effluent Containing Chromium - A Review", *Miner. Process. Extr. Metall. Rev.*, **27**, 99-130 (2006).

Almeida, M. F. A., *JNICT Research Project*, 2nd Annual Report, College of Engineering, Port, PEAM/C/TAI/271/93 (1993).

Amoozegar, M. A., Ghasemi, A., Razavi, R. and Nabat, S., "Evaluation of Hexavalent Chromium Reduction by Chromate-Resistant Moderately Halophile, *Nesterenkonia sp*. Strain MF2", *Process Biochem*., **42**, 1475-1479 (2007).

Anjana, K., Kaushik, A., Kiran, B. and Nisha, R., "Biosorption of Cr(VI) by Immobilized Biomass of two Indigenous Strains of Cyanobacteria isolated from Metal Contaminated Soil", *J. Hazard. Mater*., **148**, 383-386 (2007).

Arunakumara, K. K. I. U., Xuecheng, Z. and Xiaojin, S., "Bioaccumulation of Pb^{2+} and Its Effects on Growth, Morphology and Pigment Contents of *Spirulina (Arthrospira) Platensis*", *J. Ocean Univ. Chin*., **7(4),** 397-403 (2008).

Arunakumara, K. K. I. U., Xuecheng, Z. and Xiaojin, S., "Growth and Pigment Biosynthesis of *Spirulina Platensis* as Affected by Pb^{2+} Concentrations", *Bangladesh J. Bot.,* **36(2)**, 177-179 (2007).

Asano, T., Burton, F. L., Leverenz, H., Tsuchihashi, R. and Tchobanoglous, G., *Water Reuse: Issues, Technologies, and Applications*, McGraw-Hill, New York (2007)

Ahsan, M., Habib, B., Parvin, M. and Hasan, M.R., "*A Review on Culture, Production and use of Spirulina as Food for Humans and Feeds for Domestic Animals and Fish*". Fisheries and Aquaculture Circular No. 1034. FAO. http://www.fao.org (2008).

Aslan, S. and Kapdan, I. K., "Batch Kinetics of Nitrogen and Phosphorus Removal from Synthetic Wastewater by Algae", *Ecol. Eng*., **28**, 64-70 (2006).

Ayala, F. J. and Bravo, R. B., "Animal Wastes Media for *Spirulina* Production", *Arch. Hydrobiol*., **36**, 349-355 (1984).

Barnhart, J., "Occurrences, Uses, and Properties of Chromium", *Regul. Toxicol. Pharm*., **26**, S3-S7 (1997).

Belay, A. A., "Impacts of Chromium from Tannery Effluent and Evaluation of Alternative Treatment Options", *J. Environ. Prot*., **1**, 53-58 (2010).

Bielicka, A. Bojanowska, I. and Wiśniewski, A., "Two Faces of Chromium - Pollutant and Bioelement", *Pol. J. Environ. Stud*., **14(1),** 5-10 (2005).

Bolto, B. and Gregory, J., "Organic Polyelectrolytes in Water Treatment", *Water Res.*, **41(11),** 2301-2324 (2007).

Brans, G., Schroen, C. G. P. H., van der Sman, R. G. M., and Boom, R. M., "Membrane Fractionation of Milk: State of the Art and Challenges", *J. Membr. Sci.*, **243(1-2),** 263-272 (2004).

Bruins, M. R. Kapil, S. and Oehme, F. W., "Microbial Resistance to Metals in the Environment", *Ecotoxicol. Environ. Saf*., **45**, 198-207 (2000).

Cain, A., Vannela, R. and Woo, L K., "Cyanobacteria as a Biosorbent for Mercuric Ion", *Bioresour. Technol*., **99(14),** 6578-6586 (2008).

Cassano, A., Drioli, E., Molinari, R. and Bertolutti, C., "Quality Improvement of Recycled Chromium in the Tanning Operation by Membrane Processes", *Desalination*, **108(1-3),** 193-203 (1996).

Cassano, A., Driolia, E. and Molinari, R., "Recovery and Reuse of Chemicals in Unhairing, Degreasing and Chromium Tanning Processes by Membranes", *Desalination*, **113(2-3),** 251-261 (1997).

Cassano, A., Drioli, E., and Molinari, R., "Integration of Ultrafiltration into Unhairing and Degreasing Operations", *J. Soc. Leather Technol. Chem*., **82(4),** 130-135 (1998).

Cassano, A., Criscuoli, A., Drioli, E., and Molinari, R., "Clean Operations in the Tanning Industry: Aqueous Degreasing Coupled to Ultrafiltration: Experimental and Theoretical Analysis", *Clean Prod. Process.*, **1**, 257-267 (1999).

Cassano, A., Molinari, R., Romano, M. and Drioli, E., "Treatment of Aqueous Effluents of the Leather Industry by Membrane Processes-A Review", *J. Membr. Sci.,* **181(1),** 111-126 (2001).

Çelekli, A. and Bozkurt H., "Bio-sorption of Cadmium and Nickel ions Using *Spirulina Platensis*: Kinetic and Equilibrium Studies", *Desalination*, **275(1-3),** 141-147 (2011).

Çelekli, A., Yavuzatmaca, M. and Bozkurt, H., "Modeling of Biomass Production by *Spirulina Platensis* as Function of Phosphate Concentrations and pH Regimes", *Bioresour. Technol.*, **100**, 3625-3629 (2009).

Chandra, P., Sinha, S. and Rai, U.N., "Bioremediation of Cr from Water and Soil by Vascular Aquatic Plants", in *ACS Symposium Series, Phytoremediation of Soil and Water Contaminants*, 664, 274-282, (edited by Kruger EL, Anderson TA and Coats JR), Washington, DC (1997).

Cheng, W., "An Overview of the Situation of Research and Production of *Spirulina*", in *International Conference on Cyanophyta Biotechnology, Spirulina Industry India* (1996) pp. 4-6.

Chmielewská, E. and Medved, J., "Bioaccumulation of Heavy Metals by Green Algae *Cladophora Glomerata* in a Refinery Sewage Lagoon", *Croat. Chem. Acta.*, **74(1)**, 135-145 (2001).

Chojnacka, K. and Noworyta, A., "Evaluation of *Spirulina sp.* Growth in Photoautotrophic, Heterotrophic and Mixotrophic Cultures", *Enzyme Microb. Technol.*, **34(5),** 461-465 (2004).

Chojnacka, K., Chojnacki, A. and Gorecka, H., "Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by Blue-Green Algae *Spirulina sp*.: Kinetics, Equilibrium and the Mechanism of the Process", *Chemosphere*, **59(1),** 75-84 (2005)

Choudhary, M., Jetley, U. K., Abash, Khan M., Zutshi, S., and Fatma, T., "Effect of Heavy Metal Stress on Proline, Malondialdehyde, and Superoxide Dismutase Activity in the *Cyanobacterium Spirulina Platensis*-S5", *Ecotoxicol. Environ. Saf*., **66(2),** 204-209 (2007).

Cortese, B. and Drioli, E., "Ultrafiltration in the Treatment of Discharges of Liming of the Tanning Industry", *Pollution*, **20(6)**, **51-54** (1978)

Cortese, B. and Drioli, E., "Ultrafiltration in the Treatment of Discharges of the Leather Industry", *Leather Skins Mat. Tanning*, **52(6)**, 511-530 (1976).

Costa, A.C. and de Franca, F. P., "Short Communication: Cadmium Uptake by *Spirulina Maxima*: Toxicity and Mechanism", *World J. Microbiol. Biotechnol*., **14**, 579-581 (1998).

Costa, J. A. V., Cozza, K. L., Oliveira, L. and Magagnin, G., "Different Nitrogen Sources and Growth Responses of *Spirulina Platensis* in Microenvironments", *World J. Microb. Biot.*, **17**, 439-442 (2001).

Daughney, C. J., Fein, J. B. and Nathan, Y., "A Comparison of the Thermodynamics of Metal Adsorption onto Two Common Bacteria", *Chem. Geol.*, **144(3-4),** 161-176 (1998).

De, A. K., *Environmental Chemistry*, Wiley Eastern, New Delhi (1987).

Dean, J. G., Bosqui, F.L. and Lanouette, K. H., "Removing Heavy Metals from Wastewater", *Environ. Sci. Technol*., **6(6),** 518-522 (1972).

Dermou, E., Velissariou, A., Xenos, D. and Vayenas, D. V., "Biological Chromium(VI) Reduction Using a Trickling Filter", *J. Hazard. Mater*., **126(1-3),** 78-85 (2005).

Dhal, B., Thatoi, H. N., Das, N.N. and Pandey, B.D., "Reduction of Hexavalent Chromium by *Bacillus sp.* isolated from Chromite Mine Soils and Characterization of Reduced Product", *J. Chem. Technol. Biotechnol*., **85**, 1471-1479 (2010).

Dheetcha, A. and Mishra, S., "Biosequestering Potential of *Spirulina Platensis* for Uranium", *Curr. Microbiol*., **57**, 508-514 (2008).

Esmaeili, A., Mesdaghinia, A. and Vazirinejad, R., "Chromium (III) Removal and Recovery from Tannery Wastewater by Precipitation Process", *Am. J. Appl. Sci.* **2(10),** 1471-1473 (2005).

Fabbricino, M., Naviglio, B., Tortora, G. and Antonio, L., "An Environmental Friendly Cycle for Cr(III) Removal and Recovery from Tannery Wastewater", *J. Environ. Manage.*, **117**, 1-6 (2013).

Fabiani, C., Ruscio, F., Spadoni, M. and Pizzichini, M., "Chromium (III) Salts Recovery Process from Tannery Wastewaters", *Desalination*, **108(1-3)**, 183-191 (1996)

Faisal, M. and Hasnain, S., "Comparative Study of Cr(VI) Reduction in Industrial Effluent by *Ochrobactrum Intermedium* and *Brevibacterium sp*.", *Biotechnol Lett*., **26**, 1623-1628 (2004).

Faout, F., Brar, S. K., Verma, M., Tyagi, R. D., Blais, J. F., and Surampalli, R. Y., "Trivalent Chromium Ion Adsorption on Various Types of Wastewater Sludge", *Pract. Period. Hazard. Toxic Radioact. Waste Manage*., **12(3),** 181-187 (2008).

Farabegoli, G., Carucci, A., Majonec, M. and Rolle, E., "Biological Treatment of Tannery Wastewater in the Presence of Chromium", *J. Environ. Manage*., **71(4)**, 345-349 (2004).

Ferreira, L.S., Rodrigues, M.S., de Carvalho, J. C. M., Lodi, A., Finocchio, E., Perego, P. and Converti, A., "Adsorption of Ni^{2+}, Zn^{2+} and Pb^{2+} onto Dry Biomass of *Arthrospira*

(Spirulina) Platensis and *Chlorella Vulgaris*. I. Single Metal Systems", *Chem. Eng. J.*, **173(2),** 326-333 (2011).

Finocchio, E., Lodi, A., Solisio, C. and Converti, A., "Chromium (VI) Removal by Methylated Biomass of *Spirulina Platensis*: The Effect of Methylation Process", *Chem. Eng. J.*, **156(2)**, 264-269 (2010).

Frontasyeva, M. F., Pavlov, S. S., Aksenova, N. G., Mosulishvili, L. M., Belokobylskii, A. I., Kirkesali, E. I., Ginturi, E. N. and Kuchava, N. E., "Chromium Interaction with Blue-Green Microalga *Spirulina Platensis*", *J. Anal. Chem.*, **64(7),** 746-749 (2009).

Ganesh, R., Balaji, G., Ramanujam, R. A., "Biodegradation of Tannery Wastewater using Sequencing Batch Reactor-Respirometric Assessment", *Bioresour. Technol.*, **97(15)**, 1815-1821 (2006).

Garrote, J. I., Bao, M., Castro, P. and Bao, M. J., "Treatment of Tannery Effluents by a Two-Step Coagulation/Flocculation Process", *Wat. Res.*, **26(11)**, 2605-2608 (1995).

Gin, K.Y.H., Tang, Y.Z. and Aziz, M.A., "Derivation and Application of a New Model for Heavy Metal Biosorption by Algae", *Water Res.*, **36**, 1313-1323 (2002).

Gokhale, S. V., Jyoti, K. K. and Lele, S. S., "Kinetic and Equilibrium Modeling of Chromium (VI) Biosorption on Fresh and Spent *Spirulina Platensis*/*Chlorella Vulgaris* Biomass", *Bioresour. Technol.*, **99(9)**, 3600-3608 (2008).

Gokhale, S. V., Jyoti, K. K. and Lele, S. S., "Modeling of Chromium (VI) Biosorption by Immobilized *Spirulina Platensis* in Packed Column", *J. Hazard. Mater.*, **170,** 735-743 (2009).

Golder, A. K., Samanta, A. N. and Ray, S., "Trivalent Chromium Removal by Electrocoagulation and Characterization of the Process Sludge", *J. Chem. Technol. Biotechnol.*, **82**, 496-503 (2007a).

Golder, A. K., Samanta, A. N. and Ray, S., "Removal of Trivalent Chromium by Electrocoagulation", *Sep. Purif. Technol*., **53**, 33-41 (2007b).

Golder, A.K., Samanta, A.N. and Ray, S., "Removal of Cr^{3+} by Electrocoagulation with Multiple Electrodes: Bipolar and Monopolar Configurations", *J. Hazard. Mater*., **141**, 653-661 (2007c).

Gong, R., Ding, Y., Liu, H., Chen, Q. and Liu, Z., "Lead biosorption and Desorption by Intact and Pretreated *Spirulina Maxima* Biomass", *Chemosphere*, **58(1),** 125-130 (2005).

Guillen-Jimenez, R. D, Morales-Barrera, L., Morales-Jimenez, J., Rodrıguez, C. H. H. and Cristiani-Urbina, E., "Modulation of Tolerance to Cr(VI) and Cr(VI) Reduction by Sulfate ion in a Candida Yeast Strain isolated from Tannery Wastewater", *J. Ind. Microbiol. Biotechnol*., **35(11),** 1277-1287 (2008).

Guo, Z., Zhang, G., Fang, J. and Dou, X., "Enhanced Chromium Recovery from Tanning Wastewater", *J. Clean. Prod*., **14(1),** 75-79 (2006).

Hawari, A.H., and Mulligan, C.N., "Heavy Metals Uptake Mechanisms in a Fixed-Bed Column by Calcium-Treated Anaerobic Biomass", *Process Biochem*., **41**, 187-198 (2006).

Holdich, R. G., Cumming, I. W. and Smith, I. D., "Crossflow Microfiltration of Oil in Water Dispersions using Surface Filtration with Imposed Fluid Rotation", *J. Membr. Sci.*, **143(1),** 263-274 (1998).

Hong, C. and Shan-shan, P., "Bioremediation Potential of *Spirulina*: Toxicity and Biosorption Studies of Lead", *J. Zhejiang Univ. Sci*., **6(3)**, 171-174 (2005).

Hong, C.C., Chan, S. K. and Shim, H., "Effect of Chloride on Biological Nutrient Removal from Wastewater", *J. Appl. Sci. Environ. Sanit*., **2 (3),** 85-92 (2007).

Iaconi, C. D, Lopez, A., Ramadori, R., Pinto, A. C. D. and Passino, R., "Combined Chemical and Biological Degradation of Tannery Wastewater by a Periodic Submerged Filter (SBBR)", *Water Res*., **36(9)**, 2205-2214 (2002).

Kabir, G. and Ogbeide, S. E. "Removal of Chromate in Trace Concentration Using Ion Exchange from Tannery Wastewater", *Int. J. Environ. Res*., **2(4),** 377-384 (2008).

Korngold, E., Belayev, N. and Aronov, L., "Removal of Chromates from Drinking Water by Anion Exchangers", *Sep. Purif. Technol*., **33(2),** 179-187 (2003).

Kratochvil, D. and Volesky, B., "Biosorption of Cu from Ferruginous Wastewater by Algal Biomass", *Wat. Res*., **32**, 2760-2768 (1998).

Kurniawan, T. A., Chan, G. Y. S., Lo, W. H. and Babel, S., "Physico-Chemical Treatment Techniques for Wastewater Laden with Heavy Metals", *Chem. Eng. J*., **118(1-2),** 83-98 (2006).

Lodi, A., Soletto, D., Solisio, C. and Converti, A., "Chromium (III) Removal by *Spirulina Platensis* Biomass", *Chem. Eng. J*., **136(2-3),** 151-155 (2008).

Lokeshwari, N. and Joshi, K., "Biosorption of Heavy Metal (Chromium) Using Biomass", *Global J. Environ. Res*., **3 (1),** 29-35 (2009).

Long, D., Tang, X., Cai, K., Chen, G., Chen, L., Duan, D., Zhu, J. and Chen, Y., "Cr(VI) Reduction by a Potent Novel Alkaliphilic Halotolerant Strain *Pseudochrobactrum Saccharolyticum* LY10", *J. Hazard. Mater*., **256-257**, 24-32 (2013).

Luckey, T. D., Venugopal, B. V. and Hutcheson, D., *Heavy Metal Toxicity, Safety and Hormology*, Academic Press, New York (1975).

Lyubchik, S. B., Perepichka, I. I., Galushko, O. L., Lyubchik, A. I., Lyggina, E. S. and Fonseca, I. M., "Optimization of the Conditions for the Cr (III) Adsorption on Activated Carbon", *Adsorption*, **11**, 581-593 (2005).

Manahan, S. E., *Fundamentals of Environmental Chemistry*, Lewis Publishers, Michigan (2010).

Marcucci, M., Ciardelli, G., Matteucci, A., Ranieri, L. and Russo, M., "Experimental Campaigns on Textile Wastewater for Reuse by Means of Different Membrane Processes", *Desalination*, **149(1-3),** 137-143 (2002).

Materassi, R., Tredici, M. and Balloni, W., "*Spirulina* Culture in Sea-water", *Appl. Microbiol. Biotechnol*., **19(6)**, 384-386, (1984).

McCluggage, D., *Heavy Metal Poisoning*, NCS Magazine, The Bird Hospital, CO, U.S.A (1991).

Meng, H., Xia, Y. and Chen, H., "Bioremediation of Surface Water Co-Contaminated with Zinc (II) and Linear Alkylbenzene Sulfonates by *Spirulina Platensis*", *Phys. Chem. Earth Part A/ B/C*, **47-48,**152-155 (2012).

Michalak, I., Zielinska, A., Chojnacka, K. and Matula, J., "Biosorption of Cr(III) by Microalgae and Macroalgae: Equilibrium of the Process", *Am. J. Agr. Biol. Sci*., **2**, 284-290 (2007).

Mishra, R. R., Dhal, B., Dutta, S. K., Dangar, T. K., Das, N. N. and Thatoi, H. N., "Optimization and Characterization of Chromium(VI) Reduction in Saline Condition by Moderately Halophilic *Vigribacillus sp*. Isolated from Mangrove Soil of Bhitarkanika, India", *J. Hazard. Mater*., **227-228(15),** 219-226 (2012).

Mollah, M. Y. A., Schennach, R., Parga, J. R. and Cocke, D. L., "Electrocoagulation (EC) - Science and Applications", *J. Hazard. Mater*., **B84**, 29-41 (2001).

Mondal, N. C., Saxena, V. K. and Singh, V. S., "Impact of Pollution due to Tanneries on Ground water Regime", *Curr. Sci.*, **88**, 1988-1994 (2005).

Nalimova, A. A., Popova, V. V., Tsoglin, L. N. and Pronina, N. A., "The Effects of Copper and Zinc on *Spirulina platensis* Growth and Heavy Metal Accumulation in its Cells", *Russ. J. Plant. Physl*., **52(2),** 229-234 (2005).

Nayak, D., Lahiri, S., Mukhopadhyay, A. and Pal, R., "Application of Tracer Packet Technique to the Study of the Bio-sorption of Heavy and Toxic Metal Radionuclide by Algae", *J. Radioanal. Nucl. Ch.*, **256**, 535-539 (2003).

Neuwoehner, J. and Escher, B.I., "The pH-dependent Toxicity of Basic Pharmaceuticals in the Green Algae *Scenedesmus Vacuolatuscan* be Explained with a Toxicokinetic Ion-Trapping Model", *Aquat. Toxicol*., **101**, 266-275 (2011).

Olguín, E. J., Galicia, S., Camacho, R., Mercado, G. and Pérez, T. J., "Production of *Spirulina sp*. in Sea Water Supplemented with anaerobic Effluents in Outdoor Raceways under Temperate Climatic Conditions", *Appl. Microbiol. Biotechnol*., **48**, 242-247 (1997).

Orhon, D., Sgzen, S., Cokgsr, E. U. and Ates, E., "The Effect of Chemical Settling on the Kinetics and Design of Activated Sludges for Tannery Wastewater", *IAWQ 19th Biennial International Conference*, Vancouver, Canada, (1998) pp. 256.

Padilla, A. P. and Tavani, E. L., "Treatment of an Industrial Effluent by Reverse Osmosis", *Desalination*, **123(1-3)**, 219-226 (1999).

Pandi, M., Shashirekha, V. and Swamy, M., "Bioabsorption of Chromium from Retan Chrome Liquor by Cyanobacteria", *Microbiol. Res*., **164(4),** 420-428 (2009)

Pappu, J. S. M., Vijayakumar, G. K. and Ramamurthy, V., "Artificial Neural Network Model for Predicting Production of *Spirulina Platensis* in Outdoor Culture", *Bioresour. Technol*., **130**, 224-230 (2013).

Patterson, J. W., *Wastewater Treatment Technology*, 3rd ed., ANN Arbor Science Publishers, Ann Arbor, Michigan (1978).

Pettine, M., Barra, I., Campanella, L. and Millero, F. J., "Effect of Metals on the Reduction of Chromium (VI) with Hydrogen Sulphide", *Water Res.*, **32(9)**, 2807-2813 (1998a).

Pettine, M., D'Ottone, L., Campanella, L., Millero, F. J., and Passino, R., "The Reduction of Chromium (VI) by Iron (II) in Aqueous Solutions", *Geochim. Cosmochim. Acta*., **62(9),** 1509-1519 (1998b).

Ping, W. and Guochang, L., "Study on Absorption of Cr(VI) in Wastewater by Keggin ion Modified Attapulgite Clay", *Non-metallic Mines*, **29**, 46-46. (2006).

Piorreck, M., Baasch, K. and Pohl, P., "Biomass Production, Total Protein, Chlorophylls, Lipids and Fatty Acids of Freshwater Green and Blue Green Algae Under Different Nitrogen Regimes", *Phytochem*., **23**, 207-216 (1984).

Pun, K. C., Cheung, R. Y. H. and Wong, M. H., "Characterization of Sewage Sludge and Toxicity Evaluation with Microalgae", Mar. Pollut. Bull., **31(4-12),** 394-401 (1995).

Rai, D., Saas, B. M. and Moore, D. A., "Chromium(III) Hydrolysis Constants and Solubility of Chromium(III) Hydroxide", *Inorg. Chem.*, **26(3),** 345-349 (1987).

Rai, L. C., Rai, P. K. and Mallick, N., "Regulation of Heavy Metal Toxicity in Acid-Tolerant *Chlorella*: Physiological and Biochemical Approaches", *Environ. Exp. Bot.*, **36(1),** 99-109 (1996).

Rajfur, M., Kłos, A. and Wacławek, M., "Sorption Properties of Algae *Spirogyra Sp.* and their Use for Determination of Heavy Metal ions Concentrations in Surface Water", *Bioelectrochem*., **80,** 81-86 (2010).

Ram, B., Bajpai, P. K. and Parwana, H. K., "Kinetics of Chrome-Tannery Effluent Treatment by the Activated-Sludge System", *Process Biochem.*, **35(3-4),** 255-265 (1999).

Rangsayatorn, N., Pokethitiyook, P., Upatham, E. S. and Lanza, G. R., "Cadmium Biosorption by Cells of *Spirulina Platensis* TISTR 8217 Immobilized in Alginate and Silica Gel", *Environ. Int.*, **30(1),** 57-63 (2004).

Rao, J. P., Chandrababu, N. K., Muralidharan, C., Nair, B.U., Rao, P. G. and Ramasami, T., "Recouping the Wastewater: A Way forward for Cleaner Leather Processing," *J. Clean. Prod.*, **11**, 591-599 (2003).

Raungsomboon, S., Chidthaisong, A., Bunnag, B., Inthorn, D. and Harvey, N. W., "Removal of lead (Pb^{2+}) by the *Cyanobacterium Gloeocapsa sp.*", *Bioresour. Technol.*, **99**, 5650-5658 (2008).

Richmond, A. and Grobbelaar, J. U., "Factors Affecting the Output Rate of *Spirulina Platensis* with Reference to Mass Cultivation", *Biomass*, **10**, 253-264 (1986).

Romera, E., González, F., Ballester, A., Blázquez, M. L. and Munoz, J. A., "Comparative Study of Biosorption of Heavy Metals Using Different Types of Algae", *Bioresour. Technol*. **98**, 3344-3353 (2007).

Rose, P. D., Maart, B. A., Dunn, K. M., "Rowswell, R. A. and Britz, P., "High rate Algal Oxidation Ponding for the Treatment of Tannery Effluents", *Wat. Sci. Technol.*, **33(7),** 219-227 (1996).

Sahu, S. K., Meshrama, P., Pandey, B. D. , Kumar, V. and Mankhand, T. R., "Removal of Chromium(III) by Cation Exchange Resin, Indion 790 for Tannery Waste Treatment", *Hydrometallurgy* , **99(3)**, 170-174 (2009).

Schiewer, S. and Wong, M. H., "Ionic Strength Effects in Biosorption of Metals by Marine Algae", *Chemosphere*, **41**, 271-282 (2000).

Sharma, I. and Goyal, D., "Adsorption kinetics: Bioremoval of Trivalent Chromium from Tannery Effluent by *Aspergillus sp*. Biomass". *Res. J. Environ. Sci.*, **4**, 1-12 (2010).

Solisio, C., Lodi A., Torre, P., Converti, A. and Borghi, M. D., "Copper Removal by Dry and Re-hydrated Biomass of *Spirulina Platensis*", *Bioresour. Technol.*, **97**, 1756-1760 (2006).

Song, Z, Williams, C. J. and Edyvean, R. G. J., "Treatment of Tannery Wastewater by Chemical Coagulation", *Desalination*, **164(3),** 249-259 (2004).

Sreeram, K. J. and Ramasami, T., "Sustaining Tanning Process through Conservation, Recovery and Better Utilization of Chromium", *Resour. Conserv. Recycl.*, **81**, 185-212 (2003).

Sud, D., Mahajan, G. and Kaur, M. P., "Agricultural Waste Materials Potential Absorbent for Sequestering Heavy Metal Ions from Aqueous Solutions", *Bioresour. Technol.*, **99**, 6017-6027 (2008).

Talinli, I., "Pretreatment of Tannery Wastewaters", *Wat. Sci. Tech.*, **29(9)**, 175-178 (1994).

Topçuoĝlu, S., Gűven, K.C., Balkis, N. and Kırbaşoĝlu, C., "Heavy Metal Monitoring of Marine Algae from the Turkish Coast of the Black Sea, 1998-2000", *Chemosphere*, **52**, 1683-1688 (2003).

Tor, A., Danaoglu, N., Arslan, G. and Cengeloglu, Y., "Removal of Fluoride from Water by Using Granular Red Mud: Batch and Column Studies", *J. Hazard. Mater.*, **164**, 271-278 (2009).

Turner, A., Pedroso, S. S. and Brown, M. T., "Influence of Salinity and Humic Substances on the Uptake of Trace Metals by The Marine Macroalga, *Ulva Lactuca*: Experimental Observations and Modelling using WHAM", *Mar. Chem.*, **110**, 176-184 (2008).

U.S. EPA., *Toxicological Review of Trivalent Chromium (CAS No. 16065-83-1)*. In Support of Summary Information on the Integrated Risk Information System (IRIS), Washington, DC, U.S. Environmental Protection Agency (1988).

Volesky, B., *Removal/Recovery of Heavy Metals by Biosorption*, CRC Press, Boca Raton, Florida (1990) pp. 7-44.

Vonshak, A., Guy, R. and Guy, M., "The Response of the Filamentous Cyanobacterium *Spirulina Platensis* to Salt Stress", *Arch. Microbiol.*, **150,** 417-420 (1988).

Wang, Y. T. and Xiao, C., "Factors Affecting Hexavalent Chromium Reduction in Pure Cultures of Bacteria", *Water Res.*, **29**, 2467-2474 (1995).

Wu, B., Tseng, C. K. and Xiang, W., "Large-scale Cultivation of *Spirulina* in Seawater Based Culture Medium", *Botanica. Marina.*, **36**, 99-102. (1993).

Wu, B., Xiang, W., He, H., "Studies on Physiological Characteristics of New Strain SCS of *Spirulina Platensis*", *Tropic. Oceanol.*, **11(1),** 1-7 (1992).

Wu, B. and Xiang, W., "Retrospect and Prospect of Seawater *Spirulina*", *Spirulina Industry,* **4**, 10-13 (1996).

Xiaohong, Y. U., Lijun, Z. H. U., Baiwei, G. U. O., and Shouyang, H. E., "A study on the Adsorption of Chromium on Laterite from Guizhou Province, China", *Chin. J. Geochem.*, **28**, 220-226 (2009).

Xin, L., Hong-ying, H., Ke, G. and Ying-xue, S., "Effects of Different Nitrogen and Phosphorus Concentrations on the Growth, Nutrient Uptake, and Lipid Accumulation of a Freshwater Microalga *Scenedesmus sp*.", *Bioresour. Technol.*, **101**, 5494-5500 (2010).

Zaroual, Z., Azzi, M., Saib, N., Karhat, Y. and Zertoubi, M., "Treatment of Tannery Effluent by an Electrocoagulation Process", *J. Am. Leather Chem. Assoc.*, **100(1),** 16-21 (2005).

Zhao, Y. G., Shen, H. Y., Pan, S. D. and Hu, M. Q., "Synthesis, Characterization and Properties of Ethylenediamine-Functionalized Fe_3O_4 Magnetic Polymers for Removal of Cr(VI) in Wastewater", *J. Hazard. Mater.*, **182**, 295-302 (2010).

Chapter 2

Materials and Methods

This chapter discusses the experimental procedures of chromium detoxification studies in from both model and industrial effluents; other experimental details and methods of analysis. Specifications of all reagents and chemicals are documented in this chapter. Any specific change or deviation from what is stated here is detailed in the respective Section(s)/Chapter(s).

2.1 Chemicals and Reagents

Chemical and reagents were procured mostly form M/s Merck, Mumbai (India), M/s Titan Biotech Ltd., Delhi, (India), M/s Loba-Chemie, Mumbai (India) and M/s Changshu-Yangyuan Chemicals (China). Deionised (DI) water (M/s Millipore USA, Model: Elix 3) was used for the preparation of reagents, standard solutions and model wastewater. pH of the solution was adjusted using 0.5N H_2SO_4 or NaOH. All the plastic wares used were made of polypropylene procured from M/s Tarson Products Pvt Ltd., Kolkata (India). Glassware with

low coefficient of thermal expansion was supplied by M/s Borosil, Mumbai (India). The details of chemicals and reagents are illustrated in Table 2.1.

Table 2.1. List of common chemicals/reagents used to prepare model effluent and reagent solutions.

Reagents/chemicals	Purity (%)	Grade	CAS	Make
1,5 Diphenyl carbazide ($C_6H_5NHNHCONHNHC_6H_5$)	98	GR	140-22-7	Merck, Mumbai (India)
Acetic acid (CH_3COOH)	99	AR	64-19-7	
Acetone $(CH_3)_2CO$)	99	AR	67-64-1	
Barium dichloride ($BaCl_2$)	99	AR	10361-37-2	
Boric acid (H_3BO_3)	99.5	AR	10043-35-3	
Copper sulphate ($CuSO_4.5H_2O$)	99	AR	7758-99-7	
Dipotassium hydrogen phosphate(K_2HPO_4)	99	AR	7758-11-4	
Ethylene diamine tetra acetic acid (EDTA) ($C_{10}H_{16}N_2O_8$)	98	AR	60-00-4	
Ferroin solution ($C_{36}H_{24}FeN_6^{2+}$)	0.1 % sol w/w	AR	14634-91-4	
Ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2$)	98.5	AR	7783-85-9	
Magnesium chloride ($MnC1_2.4H_2O$)	97	AR	7791-18-6	
Mercuric sulphate ($HgSO_4$)	98	AR	7783-35-9	
Methanol (CH_4OH)	99	AR	67-56-1	
Nitric acid (HNO_3)	70	AR	7697-37-2	
Phenol(C_6H_5OH)	99	AR	108-95-2	
Potassium bromide (KBr)	99.5	GR	03-02-7758	
Potassium chromium sulphate [$CrK(SO_4)_2.12H_2O$]	99	GR	233-401-6	
Potassium dichromate ($K_2Cr_2O_7$)	99	AR	7778-50-9	
Potassium hydrogen phthalate($C_8H_5KO_4$)	99	AR	877-24-7	
Potassium nitrate (KNO_3)	99	AR	7757-79-1	
Potassium sulphate (K_2SO_4)	99	GR	7778-80-5	
Silver sulphate (Ag_2SO_4)	99	AR	10294-26-5	
Sodium acetate (CH_3COONa)	99	AR	127-09-3	
Sodium chloride (NaCl)	99.5	GR	7647-14-5	
Sodium hydroxide (NaOH)	97	AR	131073-2	
Sodium nitrate ($NaNO_3$)	99	AR	7631-99-4	
Sucrose ($C_{12}H_{22}O_{11}$)	99	AR	57-50-1	
Sulphuric acid (H_2SO_4)	98	AR	7664-93-9	
Vitamin B_{12} ($C_{63}H_{88}CoN_{14}O_{14}P$)	97	AR	68-19-9	
Zinc sulphate ($ZnSO_4.7H_2O$)	99	AR	7446-20-0	
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	99.5	AR	10034-99-8	Titan Biotech Ltd., Delhi (India)
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	95	AR	10043-52-4	Loba-Chemie, Mumbai (India)
Molybdenum trioxide (MoO_3)	99.5	GR	5758	
Sodium hydrogen carbonate ($NaHCO_3$)	99.5	AR	144-55-8	
Ethanol (C_2H_5OH)	99.9	AR	GB678-90	Changshu-Yangyuan Chemicals (China)

2.1.1 Source of *Spirulina platensis*

Spirulina platensis (NCIM No. 5143) was procured form National Chemical Laboratory (NCL) Pune, Maharashtra (India). *Spirulina* supplied was in solid surface of agar with suitable nutrient compositions. Commercially available *Spirulina* dry biomass was obtained from M/s N. Sons Ahmedabad (India). It was screened to separate out the various sizes of biomass particles and was used without making any chemical or physical modification of it.

2.1.2 Collection of tanning effluent

Industrial tanning effluent was collected from UP Jal Nigam, Kanpur, Uttar Pradesh (India). In this unit, tanning effluent is segregated from the common effluent stream and treated in a separate treatment unit. Chrome tanning effluent was collected in air tight containers and kept refrigerated till further analysis.

2.2 Analytical Methods

2.2.1 Particle size distribution

Spirulina dead biomass was composed of different particle sizes. A precision Laser Particle Size Analyzer (LPSA) (M/s Malvern Instruments UK, Model: Master Seizer 2000) was employed to determine the size distribution of biomass particles. *Spirulina* biomass obtained was screened through four different mesh sizes (-150, +150 to -85, +85 to -40 and +40) to separate out the various ranges of biomass particles. The corresponding biomass sizes were denoted with B_1, B_2, B_3 and B_4 and, stored in suitable containers to prevent from moisture.

The method of particle size calculation was based on the analysis of 'halo' of diffracted light produced when a laser beam passed through a dispersion of particles. Larger particles diffract light at narrow angles with high intensity. Whereas small particles diffract at wider angles but with low intensity (Bohren and Huffman, 2007). The particle size is determined as a volume equivalent sphere diameter. The knowledge of optical properties i.e., refractive index and imaginary component of both the sample to be measured, along with the refractive index of the dispersant are required. The optical properties of the dispersant can be found out from published data, and many modern instruments are having in-built databases that include common dispersants. The user can either measure them or estimate them using an iterative approach based upon the goodness of fit between the model data and actual data collected for the sample. Table 2.2 shows the variation of refractive indices with type of various common materials.

Table 2.2. Refractive indices relative to water of inorganic and organic marine particles (Aas, 1996).

Material	Wavelength, nm	Refractive index
Quartz	589	1.15
Feldspar	589	1.13-1.18
Mica	589	1.15-1.25
Clay minerals	589	1.11-1.22
Marine bacteria	589	1.043-1.058
Synechococcus sp.	400-740	1.032
Synechocystis	400-720	1.047-1.059
Diatoms	589	1.060-1.067
Thalassiosira pseudona	660	1.035-1.063
Chaetoceros curvisetu	400-740	1.021
Emiliana huxley	514	1.05-1.058
Isochrysis galban	436	1.023-1.031
Pavlova sp.	660	1.067-1.075
Dunaliella salina	400-740	1.092
Plalymonas suecica	400-740	1.071
Chlorella	514	1.047-1.086

2.2.2 Total chromium determination

Total chromium concentration was analysed using Atomic Absorption Spectrophotometer (M/s Varian instrument Australia, Model: 240FS). The equipment was freshly calibrated before each test. The technique uses absorption spectrometry to assess the concentration of Cr(total) present in an aqueous solution. It requires standards with known Cr(total) content (5 to 20 mg L^{-1}) to establish the relation between the measured absorbance and the standard Cr(total) concentration. In other words, the electrons of the atoms are promoted to higher orbitals in a short period of time by absorbing a defined quantity of energy (Lajunen and Perämäki, 2004). Amount of energy is specific to electron transition for a particular element. Chromium solution was atomized with an oxy-acetylene flame at air and acetylene flow rate of 13.5 and 2.9 L min^{-1}, respectively. The radiation flux with and without chromium is measured in a photo-detector (R955 photomultiplier). The ratio of two absorbances is converted to Cr(total) concentration based on the Beer-Lambert Law (Lajunen and Perämäki, 2004). Cr(total) stock of 1000 mg L^{-1} was prepared by dissolving 5.44 g potassium chromium sulfate [$CrK(SO_4)_2.12H_2O$] salt of 99% purity in 1000 mL DI water. Cr(total) standard in the range of 5 to 20 mg L^{-1} was freshly prepared by appropriate dilution of stock solution before each experiment.

2.2.3 Measurement of hexavalent chromium

Cr(VI) concentration was determined by colorimetric method (APHA, 1998). Cr(VI) Stock solution (1000 mg L^{-1}) was prepared by adding 0.2829 g of well-dried $K_2Cr_2O_7$ in 1000 mL of DI water. 1,5 diphenylcarbazide (DPC) of 0.25 g was dissolved in 50 mL acetone to get DPC solution. Standard Cr(VI) solution of 0.2 to 1 mg L^{-1} were made and solution pH was adjusted

around to 1±0.3. 2 mL DPC solution was added to 100 mL Cr(VI) to develop a complex of purple color. The intensity of color was scanned at 540 nm using an UV-vis spectrophotometer (M/s Thermofisher Scientific India, Model: Spectrascan UV-2300). The absorbance of color corresponds to Cr(VI) concentration.

2.2.4 Determination of trivalent chromium

Concentration of Cr(III) was calculated by the difference between total chromium and Cr(VI) concentration.

2.2.5 Zeta potential measurement

Spirulina dead biomass of 0.25 g was added to 50 mL $NaNO_3$ (0.1 M) solution. pH was varied from 1.5 to 7.0. It was kept in a thermostatically controlled orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 200 rpm and 25°C to attain equilibrium. Biomass suspension was filtered through 0.45 µm polyethersulfone membrane (M/s Pall Corporation, India) to collect clear solution. A flow cell chamber of 0.7 mL capacity was used for zeta potential measurement by employing the zeta potential analyser of M/s Beckman Coulter Japan (Model: Delsa Nano C).

2.2.6 Functional group characterization

The presence of different *Spirulina* functional groups was confirmed acquiring the FTIR spectra in KBr pellet method. Biomass and KBr salt were dried in a hot air oven (M/s Sonu Instrument Manufacturing Company India, Model: MTC 1201) at 60 and 105°C, respectively. KBr to *Spirulina* biomass in the ratio of 100:1 (w/w) was grinded in a clean mortar and pestle

to homogenize. It was then transferred to pellet casting die. The pressure of 5 to 7 tons was imposed to create a thin film. Background correction was done at first with pure KBr pellet by scanning in the range from 450 to 4500 cm^{-1} with a resolution of 4. The mixed sample was scanned in the same range. The number of scans was 45 for each specimen noise reduction. A FTIR spectrophotometer of M/s Shimadzu, Japan (Model: IR affinity 1) was employed for this work.

2.2.7 Potentiometric titration

Protonated acidic groups of *Spirulina* biomass were quantified with potentiometric titration as outlined by Seki et al., 2000. Biomass suspension (5 g L^{-1}) of 50 mL containing 0.1N $NaNO_3$ at different pH was agitated in a thermostatically controlled rotary orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 200 rpm and 30ºC. Shaking was continued till it equilibrated. It was then titrated with standard solution of 0.5N HCl or NaOH. A precision potentiometer (M/s Mettler Toledo Switzerland, Model: DL60) was used for titration. The number of titratable surface protonated acidic groups was determined from the difference between the bulk proton concentration in presence and in absence of *Spirulina* biomass (Seki and Suzuki, 1998).

2.2.8 Algae growth

Spirulina cell suspension of a definite quantity (1 g L^{-1}) was collected after its cultivation and serially diluted to desired values. These diluted solutions were scanned at 540 nm to record corresponding absorbance (Sivakiran et al., 2012) using UV-vis spectrophotometer (M/s Thermofisher Scientific India, Model: Spectrascan UV-2300). After that cells were collected by filtration with 0.45µm filter and dry weight was taken, absorbance observed corresponds to

Spirulina cell concentration. Mean daily division rate '*k*' (Fogg and Thake, 1987) was calculated as in Eq. 2.1:

$$k = \frac{3.32}{t}\left(\log_{10} A_t - \log_{10} A_0\right) \tag{2.1}$$

Where, *t*=number of days since inoculation, A_0 =absorbance at *t*=0 and A_t = absorbance at *t*=*t*.

2.2.9 Pigment assay

Chlorophyll and carotenoid extraction were carried out in 95% (v/v) methanol. Algae-solvent suspension (50 mL per g algae) was agitated at 200 rpm and 25°C for 30 min followed by another 30 min sonication. It was then centrifuged at 5000 rpm to collect dissolved pigment. The supernatant was analysed spectrophotometrically by recording the absorbance at 662, 646 and 470 nm. The amount of pigments was calculated from Eqs. 2.2 to 2.4 (Rainha et al., 2011):

$$C_a = 15.65A_{662} - 7.34A_{646} \tag{2.2}$$

$$C_b = 27.05A_{646} - 11.21A_{662} \tag{2.3}$$

$$C_t = 1000A_{470} - 2.86C_a - \frac{129.2C_b}{245} \tag{2.4}$$

Where, C_a, C_b and C_t represent the concentration (g L^{-1}) of chlorophyll 'a', chlorophyll 'b' and carotenoid. A_{470}, A_{646} and A_{662} are the absorbance at 470, 646 and 662 nm, respectively.

2.2.10 Total lipid content

Ethanol was used to extract lipid from algal biomass. Harvested biomass was dried at 50°C for 24 h. It was added to solvent in the ratio of 1:10 (w/v) and kept in an orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 25°C and 200 rpm for 8 h. The mixture was then centrifuged to collect supernatant followed by an additional filtering step using 0.45 µm membrane filter to remove suspended particles, if any. The clear solution was dried in a hot air oven (M/s Sonu Instrument Manufacturing Company, India (Model: MTC 1201) at 50°C to determine lipid content (Chaiklahan et al., 2008).

2.2.11 Total sugar determination

Carbohydrate content was quantified following the procedure reported by Pawar and D'Mello, (2011). 1 mL diluted phenol in DI water (5% v/v) was mixed with an equal volume of glucose solution and 5 mL of concentrated (98% pure) H_2SO_4. Glucose concentration was varied from 0 to 10 mg L^{-1}. A colour complex is formed by the reaction between phenol and glucose in acidic solution. Absorbance was measured at 488 nm against the blank solution. Glucose concentration was calculated from the calibration curve.

2.2.12 COD measurement

Determination of chemical oxygen demand (COD) was carried out in closed reflux titrimetric method (APHA, 1998). Digestion solution was prepared by dissolving 4.9 g $K_2Cr_2O_7$ in 500 mL DI water, 167 mL concentrated (98% pure) H_2SO_4, and 33.3 g $HgSO_4$. It was then diluted to 1000 mL. Ag_2SO_4 solution was made by adding Ag_2SO_4 to concentrated H_2SO_4 (5.5 g Ag_2SO_4/kg H_2SO_4) and kept for 2 days for dissolution. Standard ferrous ammonium sulphate

(FAS) titrant of 0.10M was prepared by dissolving 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in DI water with 20 mL concentrated H_2SO_4. It was cooled to room temperature and diluted to 1000 mL. FAS was standardised prior to use with standard $K_2Cr_2O_7$ solution. 1.5 mL digestion and 3.5 mL Ag_2SO_4 solutions was added to 2.5 mL sample. It was thoroughly swirled and boiled in a digester (M/s Hach USA, Model: DRB 200) at 150°C for 2 h. The sample was cooled to room temperature and titrated against standard FAS solution using ferroin as an indicator. A blank sample was run following the same procedure. The net amount of $K_2Cr_2O_7$ consumed is equivalent to O_2 required for the oxidation of organic materials present in sample.

2.2.13 Determination of sulphate

A buffer solution was prepared by dissolving 30 g $MgCl_2.6H_2O$, 5g sodium acetate ($CH_3COONa.3H_2O$), 1 g KNO_3, and 20 mL acetic acid (CH_3COOH) (99% purity) in 500 mL DI water and made up to 1000 mL. Standard SO_4^{2-} solution (5 to 40 mg L^{-1}) was prepared with 0.02N H_2SO_4. $BaCl_2$ was used to develop turbidity. 100 mL water sample was added to 20 mL buffer solution and stirred on a magnetic stirrer (M/s Tarson India, Model: Spinnot) at room temperature. While stirring, about 1 g of $BaCl_2$ was added and turbidity was recorded spectrophotometrically at 420 nm. The corresponding concentration of SO_4^{2-} was determined from the calibration curve.

2.2.14 Chloride measurement

Chloride (Cl^-) ion concentration was determined by employing an ion-meter (M/s Jenway UK, Model: 3345). The meter was initially calibrated for 10, 100 and 1000 mg L^{-1} Cl^- at room temperature. Water sample was analysed for Cl^- content with appropriate dilution.

The details of various instruments used in different parts of this work are illustrated in Table 2.3.

Table 2.3. Instrumental details for the analysis of water quality parameters and biomass characterization.

Instrument	Model and make	Purpose	Detection/ working/performance range
Analytical balance	Model: MK-200E Make: Adair Dutt Instruments, India	Weight measurement	0.01 to 210 g Resolution: 0.01 g
Atomic absorption spectrophotometer	Model: 240FS Make: Varian, Australia	Cr(total) determination	Minimum detection: 0.006 mg L^{-1}
Centrifuge	Model: TC 8100 TD Make: Elteck, India	Sludge separation	Maximum speed: 8000 rpm
Chemisorption analyser	Model: Chemsorb 2720 Make: Micromeritics, USA	Measurement of biomass surface area	Minimum specific surface area: 0.02 $m^2 g^{-1}$
Digital lux meter	Model: LX 101 Make: LT Lutron, Taiwan	Light intensity	Lux: 0 to 50 klux
Digital reactor block	Model: DRB 200 Make: Hach USA	COD	Temperature: 37 to 165ºC
DO meter	Model: HI2400 Make: Hanna Instruments, India	Dissolved oxygen	DO: 0 to 45 mg L^{-1}
FTIR	Model: IR affinity 1 Make: Shimadzu, Japan	Biomass functional group characterisation	Frequency: 350 to 7500 cm^{-1}
Hot air oven	Model: MTC 1201 Make: Sonnu Instrument Manufacturing, India	Drying	Temperature: 25 to 250º C
Ion meter	Model:3345 Make: Jenway, UK	Chloride determination	10 to 1000 mg L^{-1}
Laser particle size analyser	Model: Master Seizer 2000 Make: Malvern Instruments, UK	Determination of biomass particle size	0.02 to 2000 µm
Magnetic stirrer	Model: Spinnot Make: Tarson India	Mixing/agitation	Stirrer speed: 100 to 1000 rpm
Micropipette	Model: T100 & T1000 Make: Tarsons products pvt ltd., India	µL range liquid dispensing for analytical work	Capacity T100:10 to 100 µL T1000:100 to 1000 µL
Microwave oven	Model: MH-2046HB Make: LG Electronics, India	Microwave assisted drying of Cr(III) loaded biomass	Frequency: 2450 MHz (fixed) Microwave: 800 W (fixed)
Millipore water purification unit	Model: Elix 3 Make: Millipore, USA	Preparation of all reagent and test solutions	TOC: <30 ppb Pyrogens (endotoxins):<0.001 EU mL^{-1} Water resistivity (@ 25 °C): >5 MΩ cm
Orbital shaker	Model: LSI-3016R Make: Lab Tech, Korea	Agitation	Maximum speed: 350 rpm Temperature: ± 0.5°C
pH meter	Model: pH 510 Make: Eutech	pH measurement	pH: 0 to 14 Resolution: 0.01 pH

Potentiometer	Instruments, Singapore Model: DL60 Make: Mettler Toledo, Switzerland	Proton adsorption capacity of biomaterial	Potential range: -2000 to +2000 mV
Scanning Electron Microscopy / Energy Dispersive X-ray analysis	Model:1430VP Make: LEO, UK	Biomass morphological and elemental analysis	Magnification: 10X to 200000X
Ultrasonic bath	Model: Lab Companion UC-02 Make: Jeiotech, Korea	Lipid analysis	Frequency (sound wave): 40 kHz (fixed) Ultrasonic power: 70 W (fixed)
UV-vis spectrophotometer	Model: Spectrascan UV-2300 Make: Thermofisher Scientific, India	Cr(VI), pigments, cell density, carbohydrate analysis	Wavelength: 190-1100 nm Resolution: 0.05 nm
Zeta-potential meter	Model: Delsa Nano C Make: Beckman Coulter, Japan	Zeta potential measurement	Zeta potential range: -200 mV to +200 mV

2.3 Experimental procedure

2.3.1 *Spirulina* cultivation

Initially, *Spirulina* was cultured in typical Zarrouk media composition (ZMC). However, the cell density was less than 25% of the reported value (Goksan et al., 2007) even after one month of cultivation. Addition of appropriate quantity of EDTA, H_3BO_3, $MnC1_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, MoO_3 and vitamin B_{12} to Zarrouk media significantly increases the cell density (Pandey et al., 2010). So, *Spirulina* was transferred to the modified Zarrouk media composition as (g L^{-1}): $NaHCO_3$: 16.8, $NaNO_3$: 2.5, NaCl: 1, K_2HPO_4: 0.5, K_2SO_4: 1, $MgSO_4 \cdot 7H_2O$: 0.2, $CaCl_2 \cdot 2H_2O$: 0.04, EDTA: 0.08, H_3BO_3: 2.86×10^{-3}, $MnC1_2.4H_2O$: 1.81×10^{-3}, $ZnSO_4.7H_2O$: 0.222×10^{-3}, $CuSO_4.5H_2O$: 0.079×10^{-3}, MoO_3: 0.015×10^{-3} and vitamin B_{12}: 1×10^{-3} (Pandey et al., 2010). Initial pH of the medium was 8.5. *Spirulina platensis* was cultivated in 250 mL flasks containing 100 mL Zarrouk media. The growth was promoted by shaking in a thermostatically controlled orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) with an agitation speed of 150 rpm and temperature 30±1 °C. The light

intensity of 4 klux was employed with the help of a cool white fluorescent lamp (M/s Crompton Greaves Ltd. India, Power: 25 W) fixed just above the reactor's top.

2.3.2 Cr(III) biosorption experiment

Dead biomass: Batch biosorption experiments were conducted in 250 mL capacity cylindrical polypropylene vessels (M/s Tarsons India, Product code: 582230) containing 200 mL Cr(III) solution. Potassium chromium sulphate ($CrK(SO_4)_2.12H_2O$) was used as source of Cr(III). The initial Cr(III) concentration was varied from 5 to 20 mg L^{-1}. pH of the solution was adjusted to the desired value in the range from pH 0.5 to 11.5 followed by addition of *Spirulina* biomass (1 to 5 g L^{-1}). The suspension was agitated in a thermostatically controlled (±0.5°C) rotary orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 200 rpm till the solution attained the equilibrium. Intermediate samples were drawn at selected time intervals and biomass was separated by centrifugation (M/s Elteck India, Model: TC 8100 TD) at 5000 rpm for about 10 min. The supernatant was analyzed for residual Cr(III) concentration and solution pH. The difference between the initial and the residual Cr(III) concentration was assumed to be adsorbed on *Spirulina* biomass surface. Initial trial test showed absence of Cr(VI) at the beginning as well as no Cr(VI) was detected at the end of the experiment. Therefore; concentration measured by atomic absorption spectrophotometer was considered to be same as Cr(III). The reproducibility of the experiments was found to be ±5%, determined from the multiple runs under the similar experimental conditions.

Live biomass: Performance of live *Spirulina* for Cr(III) removal and its toxicity, if any, was tested in ZMC. The experiment was conducted in batch mode with 5 to 100 mg L^{-1} initial Cr(III) concentration. *Spirulina* was cultivated in 250 mL conical borosilicate vessels (M/s Borosil India, Product Code: 4980021). The growth was promoted in a thermostatically

controlled orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) with an agitation speed of 150 rpm and temperature 30±1 °C. A light intensity of 4 klux was provided with photo period of 12 h per day. The volume of inoculum was 10% (v/v) of media volume of 100 mL. Samples were withdrawn at selected time intervals. It was analysed for residual Cr(III) and *Spirulina* growth.

Desorption: Cr(III) desorption test was conducted for Cr(III) recovery and to explore the scope of reuse of spent biomass. Desorption solution of 50 mL was prepared in the pH range from 0.5 to 7. pH was adjusted using 0.5 N H_2SO_4 or NaOH. Cr(III) laden biomass was added to desorption solution and swirled in a thermostatically controlled (±0.5°C) rotary orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 200 rpm and 25°C for 8 h. Biomass was separated out by centrifugation at 5000 rpm. Supernatant was analysed for Cr(III).

2.3.3 Cr(III) precipitation

Model effluent: Cr(III) ($CrK(SO_4)_2.12H_2O$) precipitation experiment was carried out with 200 mL solution containing an initial Cr(III) of 20 mg L^{-1} in the pH range from 2 to 7. The solution pH was adjusted using 0.5 N H_2SO_4 or NaOH. The agitation was performed for 30 min at 200 rpm and sludge was separated by centrifugation as stated earlier.

Industrial effluent: The raw chrome tanning effluent imparted stringent odour and was dark green in appearance. The effluent was filtered using cloth filter (commercially available nylon cloth used for apparel making) to remove suspended and visible particles. It was then subjected to precipitation for the removal of Cr(III) at different pH. pH was adjusted in the range from 5 to 9 using 0.5 N NaOH with 100 mL tanning effluent in 250 mL Erlenmeyer flask. The solution was mixed on a magnetic stirrer (M/s Tarson India, Model: Spinnot) at

200 rpm and 30°C for 10 min. It was then transferred to 100 mL measuring cylinder and left overnight (~12 h) for settling of Cr(III) precipitated sludge. The clear supernatant and sludge were collected for further studies.

2.3.4 Cr(VI) reduction and biosorption experiment

Dead biomass: The experiments were conducted in batch mode. The conical borosilicate vessels (M/s Borosil India, Product Code: 4980021) of 500 mL capacity were engaged as the reactors. Potassium dichromate ($K_2Cr_2O_7$) was used as source of Cr(VI). Cr(VI) working solution of 200 mL was freshly prepared by appropriate dilution of the stock 500 mg L^{-1}. pH was adjusted in the range from 0.5 to 7 using 0.5 N H_2SO_4 or NaOH and measured by employing a digital pH meter (M/s Eutech Instruments Singapore, Model: pH 510). Initial Cr(VI) concentration was kept between 0.96×10^{-3} and 7.69×10^{-3} M (50 to 400 mg L^{-1}). *Spirulina* biomass (1 to 4 g L^{-1}) was then added to Cr(VI) solution. The reactor was agitated in a thermostatically controlled orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 200 rpm for 24 h. Samples were taken out at chosen intervals of time and centrifuged at 5000 rpm for about 10 min to draw clear solution. The volume of sample drawn was kept under 1% of the working solution. The supernatant was analysed for total chromium, Cr(VI) concentration and pH values. The ionic strength of the solution was varied between 7.49×10^{-3} and 1.42×10^{-2} M using NaCl, Na_2SO_4 or $NaNO_3$, one at a time.

Live biomass: Cr(VI) removal performance using live *Spirulina* was conducted in 250 mL conical borosilicate vessel (M/s Borosil India, Product Code: 4980021). Suitable amount of Cr(VI) stock solution was added to have the final concentration of 5 to 100 mg L^{-1} in growth media. Cr(VI) and *Spirulina* inoculum (10% v/v) were mixed to ZMC to get the final volume of 100 mL. The suspension was kept in a thermostatically controlled orbital shaker (M/s Lab

Tech Korea, Model: LSI-3016R) at 150 rpm and 30±1 °C. The samples were withdrawn at selected time intervals and analysed for residual Cr(VI) concentration and *Spirulina* cell density.

2.3.5 Industrial chrome tanning effluent treatment

Precipitation: The experimental procedure adopted for precipitation test is outlined in Section 2.3.3.

Biomass cultivation: Partially treated chrome tanning effluent was added for chloride substitution in ZMC for the cultivation of *Spirulina sp.* ZMC typically fixes the pH at around 8.5. Media pH was adjusted in the range from 6.5 to 10.5 using 0.5 N NaOH or H_2SO_4. The fully developed inoculum of *Spirulina sp.* was used to prepare 100 mL of cultivation media at 10% dilution. Culture was shaken in an orbital shaker (M/s Lab Tech Korea, Model: LSI-3016R) at 150 rpm and 30±1 °C with a photo period of 12 h per day by employing a cool white fluorescent lamp of 4 klux intensity on the top of the reactor. The cultivation period was 30 days. The samples were withdrawn at regular intervals of time for the analysis of *Spirulina* cell density and metabolic products.

References

APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 20 ed., Washington (1998).

Aas, E., "Refractive Index of Phytoplankton Derived from its Metabolite Composition", *J. Plant. Res.*, **18(12)**, 2223-2249 (1996).

Bohren, C.F. and Huffman, D.R. *Absorption and Scattering of Light by Small Particles* Wiley-VCH Verlag GmbH, Weinheim, Germany (2007).

Chaiklahan, R., Chirasuwan, N., Loha, V. and Bunnag, B. "Lipid and Fatty acids Extraction from the Cyanobacterium *Spirulina*", *Sci. Asia.*, **34**, 299-305 (2008).

Fogg, G. E. and Thake, B., *Algae Cultures and Phytoplankton Ecology*. 3rd ed, The University of Wisconsins Press Ltd., London (1987).

Goksan, T., Zekerüyaoúlu, A. and Ülknur A.K., "The Growth of *Spirulina Platensis* in Different Culture Systems under Greenhouse Condition", *Turk. J. Biol.*, **31**, 47-52 (2007).

Lajunen, L. H. J. and Perämäki, P., *Spectrochemical Analysis by Atomic Absorption and Emission*, 2nd ed, Royal Society of Chemistry, UK (2004).

Pandey, J.P., Tiwari, A. and Mishra, R.M., "Evaluation of Biomass Production of *Spirulina Maxima* on Different Reported Media", *J. Algal Biomass. Utln.*, **1(3),** 70-81 (2010).

Pawar, H.A. and D'Mello, P.M., "Spectrophotometric Estimation of Total Polysaccharides in Cassia Tora Gum", *J. Appl. Pharma. Sci.*, **01(03)**, 93-95 (2011).

Rainha, N., Lima, E., Baptista, J. and Rodrigues, C., "Antioxidant Properties, Total Phenolic, Total Carotenoid and Chlorophyll Content of Anatomical Parts of *Hypericum Foliosum", J. Med. Plant.*, **5(10)**, 1930-1940 (2011).

Seki, H. and Suzuki, A., "Biosorption of Heavy Metal ions to Brown Algae, *Macrocystis Pyrifera, Kjellmaniella Crassiforia* and *Undaria Pinnatifida*", *J. Coll. Inter. Sci.*, **206(1),** 297-301 (1998).

Seki, H., A. Suzuki and Y. Iburi, "Biosorption of Heavy Metal ions to a Marine Microalga, *Heterosigma Akashiwo (Hada) Hada*", *J. Coll. Inter. Sci.*, **229**, 196-198 (2000).

Sivakiran, R.R., Madhu, G.M., Satyanarayana, S.V. and Bindiya, P., "Bioaccumulation of Cadmium in Blue Green Algae *Spirulina (Arthrospira) Indica*", *J. Bioremed. Biodegrad.*, **3(3),** 1-4 (2012).

Chapter 3

Bioremediation of Trivalent Chromium from Simulated Effluent

This chapter investigates on bioremediation of trivalent chromium from simulated effluent using dead and live *Spirulina platensis* biomass.

3.1 Background of Cr(III) bio-scavenging

Contamination of water by chromium and their derivatives has become a major concern today, due to its deleterious effect on public health and environment (Gupta and Ali, 2004; Gupta et al., 2010). Chromium is known as one of the most toxic heavy metals because of its carcinogenic effect and potential to modify deoxyribonucleic acid transcription process. Cr(III) may cause DNA alterations such as DNA crosslinks and single-strand (Rudolf and Cervinka, 2009). Cr(III) is also converted to more toxic Cr(VI) in an oxic environment. Chromium compounds are the major constituents in various industrial effluents like electro-plating, leather tanning, and chromate production (Dalcin et al., 2011). Conventional processes namely, chemical precipitation, ion exchange and incineration are generally

employed for chromium removal. The primary drawbacks of such processes include high treatment cost, more sludge generation and also sometimes they are not efficient at low metal concentration. Biosorption techniques are gaining interest for removal of chromium for both resource conservation and environmental point of view. Different kind of biosorbents (Gupta et al., 2012) used for chromium removal include fungi (Mukhopadhyay et al., 2011), yeast (Cui et al., 2010), bacteria (Srivastava et al., 2007), algae (Gupta et al., 2001; Gupta and Rastogi, 2009), seaweeds and their derivatives (Murphy et al., 2008), wheat straw and grass (Chojnacka, 2006), industrial waste materials (Srivastava et al., 1997; Gupta and Ali, 2004; Gupta et al., 2010; Khorramabadi et al., 2012), plant derived and microbial biomasses (Loukidou et al., 2004; Saha et al., 2013) and peats (Batista et al., 2009).

The knowledge about the acidic sites speciation i.e., the type of functional groups present on biomass surface is regarded as a crucial step for the understanding and predicting of biosorption behavior. Chemical speciation of functional groups depends upon its protonation and deprotonation capacity and their interaction with other components present in the system. pH, redox potential, ionic strength and alkalinity also have considerable impact . Cr(III) sorption capacity, mechanism of sorption, biomass agglomeration, behavior of active functional groups and metal chemistry are controlled by the pH of the medium. However, most of the studies have not considered the role of functional groups in equilibrium metal sorption modeling at different pH. Researchers have shown considerable interest to investigate the effect of pH on metal binding to microbial species. However, least information are available on trivalent metal binding constants (such as Cr(III)) to different functional groups present on *Spirulina* biomass. Although, monovalent and bivalent metal binding constants with some marine algae are available. But the effects of metal precipitation as well as the sharing of functional groups in metal removal are not well studied. Chojnacka et al.

(2005) reported the role of functional groups in Cr(III) removal using *Spirulina sp.* However, the authors neglected Cr(III) precipitation effect even at higher pH (~7). Cr(III) is usually removed both by biosorption and precipitation at pH>5 and there are possibilities of over estimations of metal binding constants.

Biosorption is a surface phenomenon inwhich biomass or biomaterial is simply used as an adsorbent (Veglio, 1997; Volesky, 2001). Whereas, bioaccumulation involves growth of biomass along with contaminant (here Cr(III)) removal/binding (Kuyucak and Volesky, 1989; Dursun et al., 2003). Cr(III) removal in live condition is associated withits partial binding on cell surface. Part of it is transferred into cell interior. The latter process involves metabolic functionalities (Chojnacka, 2003) and occurs at a quite slow rate. As a result a large installation for continuous wastewater treatment plant with high residence times is needed. The mainadvantage is that Cr(III) removal can be achieved to a very low concentration (Gadd, 1991) along with removal of other contaminants such as potential nutrients.

The objective of this chapter is to quantify the net amount of Cr(III) binded onto *Spirulina sp*. surface. The role of solution pH on mutual interactions of Cr(III) and biomass functional groups is elucidated in greater details by considering Cr(III) precipitation effect. Functional groups attached to Cr(III) as a function of solution pH is determined quantitatively using proton sorption and non-ideal metal binding models (Plette et al., 1995). The effects of process conditions on Cr(III) sorption, thermodynamic behavior and biomass surface morphology are also reported. Performance of live *Spirulina* for Cr(III) removal and its toxicity, if any, are examined in Zarrouk media composition (ZMC).

3.2 Theoretical consideration

3.2.1 Determination of acidic sites using equilibrium proton binding model

The acid-base properties i.e., the type of acidic sites, its concentration and acidic site dissociation, were evaluated by potentiometric titration (Please refer Section 2.2.7 of Chapter 2). The titration curves of biomass species and their derivatives are broad and ill defined. It reflects the diversity in biomass-acidic sites. FTIR spectra revealed that *Spirulina* biomass is consisting of a number of functional groups (Figure 3.1). Three functional groups i.e., carboxylic, phosphatic and amine were significant. These groups primarily involve in metal binding (Finocchio et al., 2010) and hence they were considered for proton binding model development. The cultivation conditions of *Spirulina* may change its surface functional groups compositions. The dissociation reactions of *Spirulina* biomass functional groups can be written as (Seki el al., 2000):

$$\equiv B_i H \overset{K_{ai}}{\leftrightarrow} \equiv B_i^- + H^+ \qquad (i=1 \text{ to } 3) \qquad (3.1)$$

Initially (time, t=0), $$\mathbf{1} \leftrightarrow \mathbf{O} + \mathbf{O} \qquad (3.2)$$

At equilibrium(time t=t), $$(1-\alpha_i) \leftrightarrow \alpha_i + H^+ \qquad (3.3)$$

Where, $\equiv B_i$ represents the acidic sites i and α_i is the corresponding degree of dissociation. Eq. 3.2 represents fractional dissociation of *Spirulina* biomass at the beginning (t=0) of the reaction. Whereas, Eq. 3.3 represents fractional dissociation at equilibrium (Seki et al., 2000).

The acid dissociation constant K_{ai} (mol L^{-1}) can be expressed as:

$$K_{ai} = \frac{\alpha_i [H^+]}{(1-\alpha_i)} \tag{3.4}$$

By rearranging, $$\alpha_i = \frac{K_{ai}}{K_{ai} + [H^+]} \tag{3.5}$$

Then, the total number of protonated acidic sites (mol g^{-1}) is:

$$N_p = \sum_{i=1}^{3} N_i (1-\alpha_i) \tag{3.6}$$

or, $$N_p = N_1 \frac{[H^+]}{K_{a1} + [H^+]} + N_2 \frac{[H^+]}{K_{a2} + [H^+]} + N_3 \frac{[H^+]}{K_{a3} + [H^+]} \tag{3.7}$$

Where, N_1, N_2 and $N_3$are the functional groups concentration (mol g^{-1}).

3.2.2 Multiple sites equilibrium Cr(III) sorption modeling

Plette et al. (1995) purposed a multicomponent competitive binding model (MCBM) based on nonideal competitive adsorption. MCBM model provides good results for the description of metal-ion-binding data. A simplified version of metal-binding model was investigated to study the equilibrium behavior of Cr(III) biosorption onto *Spirulina* biomass. Similarly, Cr(III) metal-binding reactions of type 1, 2 and 3 sites can be written as in Eq. 3.8. The distribution of these species at different pH is illustrated in Figure 3.1. $Cr(OH)^{2+}$ is about 15 to 10 % between the pH range studied with 10 mg L^{-1} Cr(III) in solution (Figure 3.1).

Whereas, $Cr(OH)_2^+$ varies between 85 to 90%. Therefore, removal of Cr(III) is primarily resulted from $Cr(OH)_2^+$ binding to *Spirulina* biomass. The symbol '*M*' in Eq. 3.8 actually represents $Cr(OH)_2^+$.

$$\equiv B_i^- + M \overset{K_{\theta i}}{\leftrightarrow} \equiv B_i\text{-}M \tag{3.8}$$

Initially (t=0), $\alpha_{i+} \quad 0 \leftrightarrow 0$ (3.9)

At equilibrium, $\alpha_i(1-\theta_i) + [M] \overset{K_{\theta i}}{\leftrightarrow} \theta_i$ (3.10)

Where, θ_i is fraction of acidic sites (α_i =deprotonated fraction) occupied by Cr(III) ions [M]. The Cr(III) binding constant, $K_{\theta i}$,(L mol^{-1}) of acidic sites is written as:

$$K_{\theta i} = \frac{\theta_i}{\alpha_i(1-\theta_i)[M]} \tag{3.11}$$

or, $$\theta_i = \frac{K_{\theta i}\alpha_i[M]}{1+K_{\theta i}\alpha_i[M]} \tag{3.12}$$

Overall binding constant is represented as:

$$K_{\theta,overall} = K_{\theta 1} \times K_{\theta 2} \times K_{\theta 3} \tag{3.13}$$

The total number of Cr(III) ions attached to *Spirulina* biomass (mol g^{-1}),

$$X_\theta = \sum_{i=1}^{3} N_i \theta_i = N_1 \frac{K_{a1} K_{\theta 1}[M]}{K_{a1} K_{\theta 1}[M\ ] + K_{a1} + [H^+]} + N_2 \frac{K_{a2} K_{\theta 2}[M]}{K_{a2} K_{\theta 2}[M\ ] + K_{a2} + [H^+]} + N_3 \frac{K_{a3} K_{\theta 3}[M]}{K_{a3} K_{\theta 3}[M\ ] + K_{a3} + [H^+]}$$

(3.14)

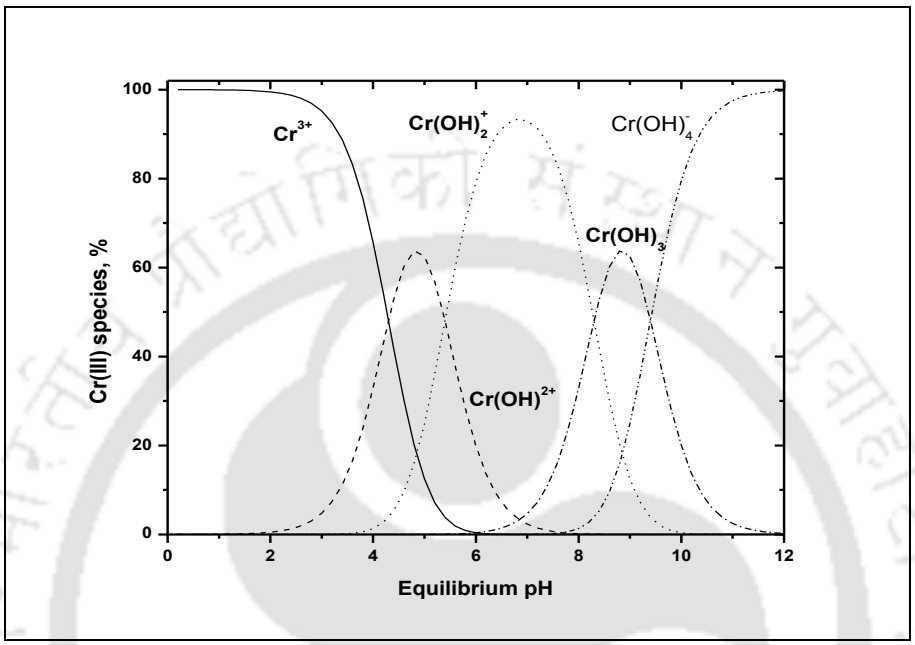

Figure 3.1 Cr(III) speciation at different pH ([Cr(III)]=1.9×10^{-4} M) (Bases and Mesmer,1976; Vilar et al., 2012).

3.3 Results and discussion

3.3.1 Studies with dead *Spirulina* biomass

3.3.1.1 Biosorbent characterizations

The interaction of Cr(III) with the functional groups was investigated to understand the mechanism of Cr(III) ion binding to available acidic sites. The broad-band at around 3434 cm^{-1} of raw *Spirulina* (Figure 3.2) indicated the presence of -OH groups of glucose and -NH groups of protein (Park et al., 2005). The absorption peaks were of-CH stretching at 2920 cm^{-1}, C=O stretching of -COOH groups at 1639 cm^{-1}, bending of amide bond of protein peptide at 1545 cm^{-1}, symmetric bending of CH_3 of acetyl moiety at 1412 cm^{-1} and -CN stretching of proteinaceous fraction at 1048 cm^{-1} (Park et al., 2005). A significant shift of spectrum peaks were observed with Cr(III) loaded biomass. The peak (-NH stretch) around 3434 cm^{-1} was shifted to 3441 cm^{-1}. While C=O stretch around 1639 cm^{-1} was shifted to 1651 cm^{-1}. The peak shifting at higher wave number with Cr(III) loaded biomass suggests chemical interaction of Cr(III) with carboxylic (-COOH) and amine (-NH) groups on the surface of *Spirulina* biomass (Park et al., 2005). The peak (-CN stretching) around 1048 cm^{-1} was disappeared probably due to Cr(III) binding. The peaks at 873 and 467 cm^{-1} may be attributed to the presence of phosphate groups on biomass surface. Cr(III) ion binding to biomass was resulted in disappearance of the peak at 873 cm^{-1}.

SEM was employed to visualize the morphological changes of *Spirulina* biomass surface before (Figure 3.3) and after Cr(III) biosorption (Figure 3.4). SEM pictures revealed high

specific surface area of raw *Spirulina* biomass due to its rough and fibrous surface nature. There was shrinkage and decrease in surface roughness attributable to Cr(III) binding and also some organic materials were dissolved during sorption experiments.

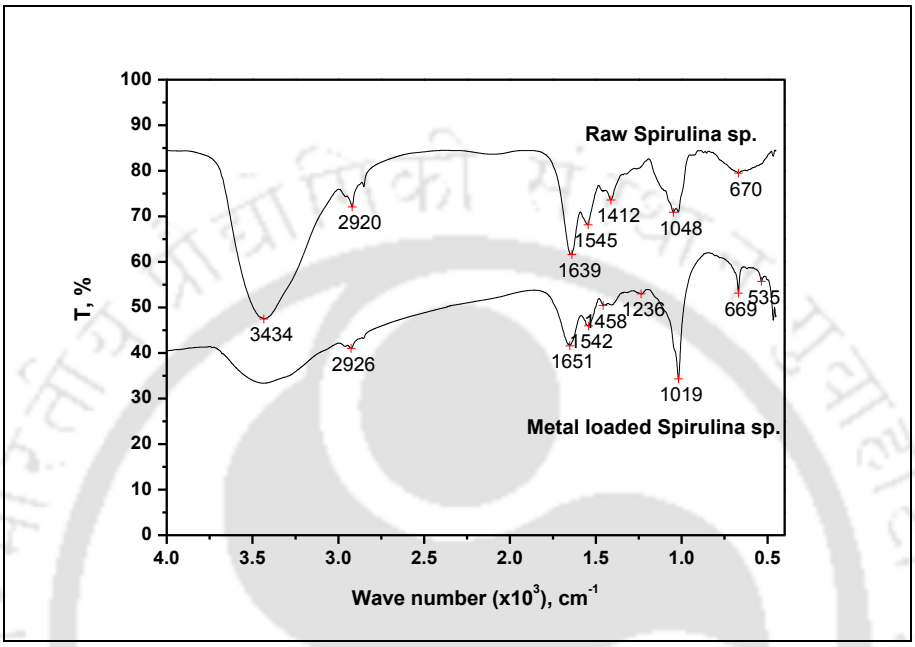

Figure 3.2 FTIR spectra of *Spirulina* biomass (stirring speed 200 rpm, pH 4, temperature 25°C and Cr(III) 10 mg L^{-1}).

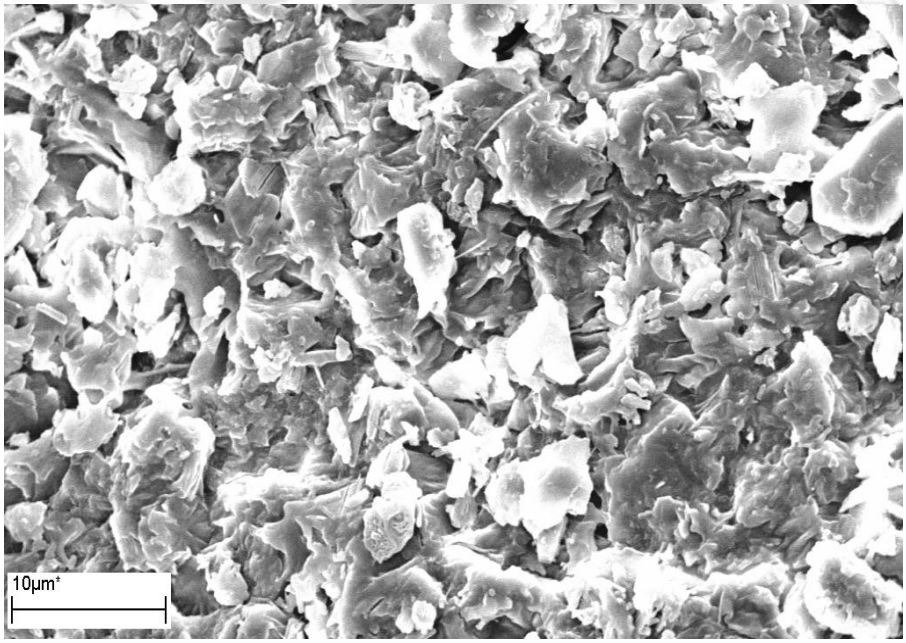

Figure 3.3 SEM images of raw biomass (biosorbent B_4).

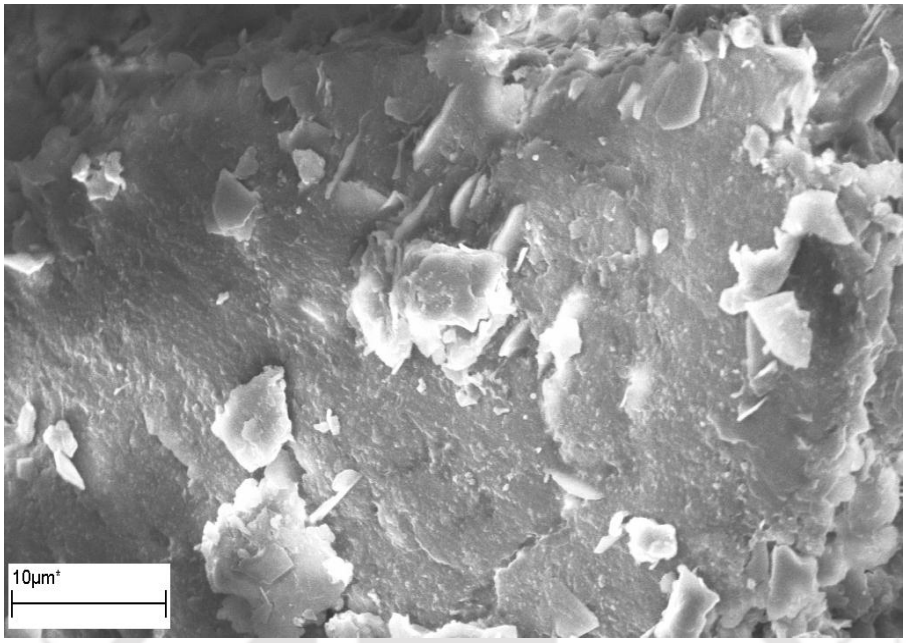

Figure 3.4 SEM image of used biomass (biosorbent B_4 2 g L^{-1}, stirring speed 200 rpm, pH 4, temperature 25°C and Cr(III) 10 mg L^{-1}).

3.3.1.2 Biomass sizes and Cr(III) uptake

Biosorbent sizes also play an important role in metal removal. *Spirulina* biomass obtained was screened through four different mesh sizes to separate out the various ranges of biomass particles as already outlined. The particle size distribution was determined using laser particle size analyser. The distribution of biomass particles and their mean diameters are shown in Figure 3.5. The distribution of both B_1 and B_2 particles were quite narrow as compared to B_3 and B_4 as wider mesh sizes was used for screening. The mean particle diameters were in the order of $B_4>B_3>B_2>B_1$ and the corresponding weight percentage were about 26.2, 14.5, 45.4 and 13.9. The effect of various sizes of biomass particles on Cr(III) uptake is illustrated in Figure 3.6. Cr(III) removal increased with decreasing biomass sizes. This was mainly attributable to higher specific surface area i.e., more active sites were available for Cr(III)

binding with lower biomass size. The removal efficiency was reduced to 58.4 from 65.3% when B_4 size was used instead of B_1. Smaller biomass particles were uniformly distributed throughout the entire solution. There was also relatively lower resistance for Cr(III) ion diffusion to biomass surface for smaller size particle. This phenomena enhanced Cr(III) sorption to biomass surface with smaller size. B_1 biomass collected after Cr(III) sorption was found to be slightly gelatinous in nature and some color pigments were also leached out to the solution. While, it was easier to separate out B_4 and the solution was almost free from the leached out biomass pigments. So, B_4 biomass was chosen for subsequent studies. The specific surface area of raw biomass (B_4) was found to be around 3.916 $m^2 g^{-1}$.

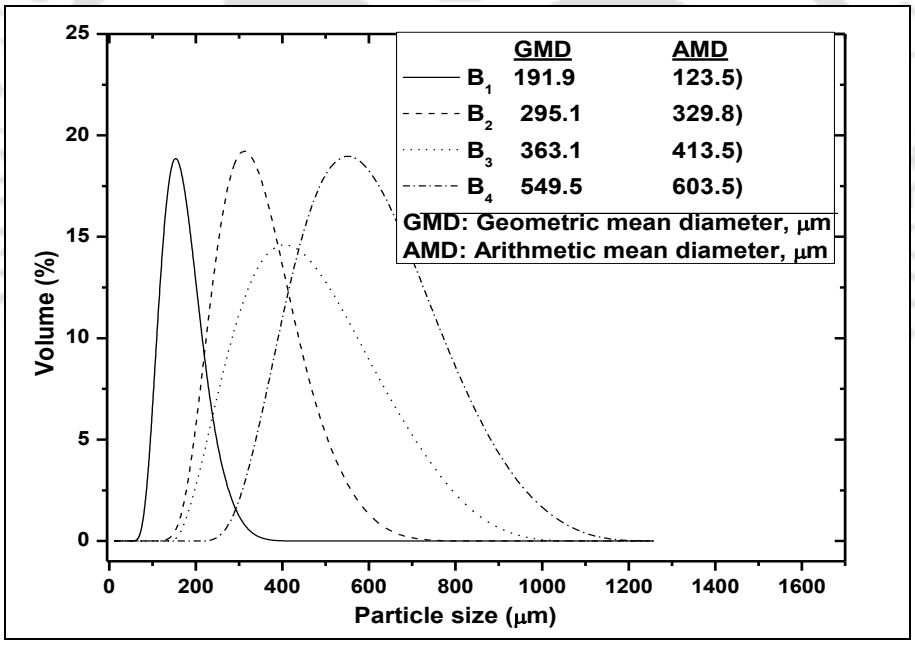

Figure 3.5 Distribution of *Spirulina* biomass particles.

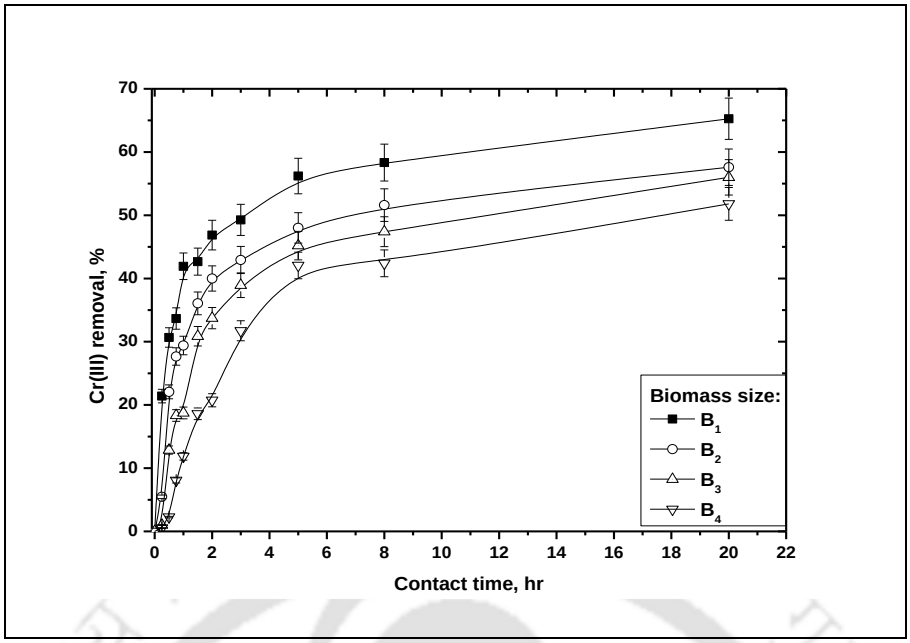

Figure 3.6 Effect of various *Spirulina* biomass sizes on Cr(III) uptake (biosorbent 2 g L^{-1}, stirring speed 200 rpm, pH 4, temperature 25°C and Cr(III) 15 mg L^{-1}).

3.3.1.3 Effect of pH on Cr(III) uptake

pH is one of the most important parameters that sets the mechanism(s) of metal biosorption from aqueous effluent. It affects the solubility of metal ions and the degree of dissociation of biomass functional groups. Cr(III) is removed by sorption onto biomass and as well as by precipitation depending on solution pH. The effect of solution pH on Cr(III) sorption was evaluated within the pH range from acidic to neutral. Figure 3.7 portrays Cr(III) sorption with equilibrium solution pH. The amount of Cr(III) sorbed was computed by subtracting Cr(III) removed by precipitation (Figure 3.8). Cr(III) precipitation test was conducted without addition of biomass under similar conditions. The variation of equilibrium pH at different initial pH for the same experiment and their effects on Cr(III) sorption, precipitation and total Cr(III) removal, can be read from Figure 3.8.

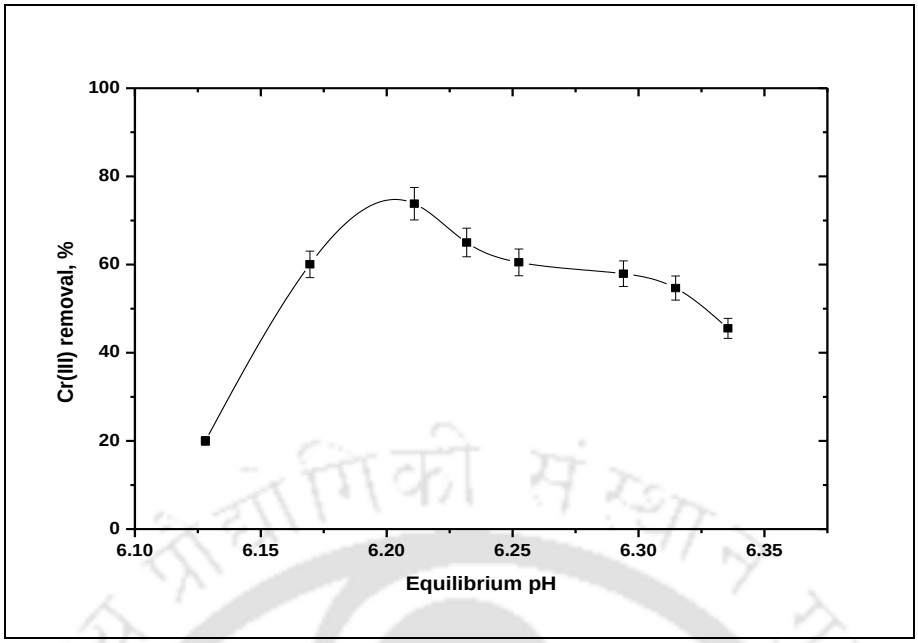

Figure 3.7 Dependency of equilibrium pH on Cr(III) biosorption: Cr(III) removal with equilibrium pH (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm, temperature 25°C and Cr(III) 10 mg L^{-1}).

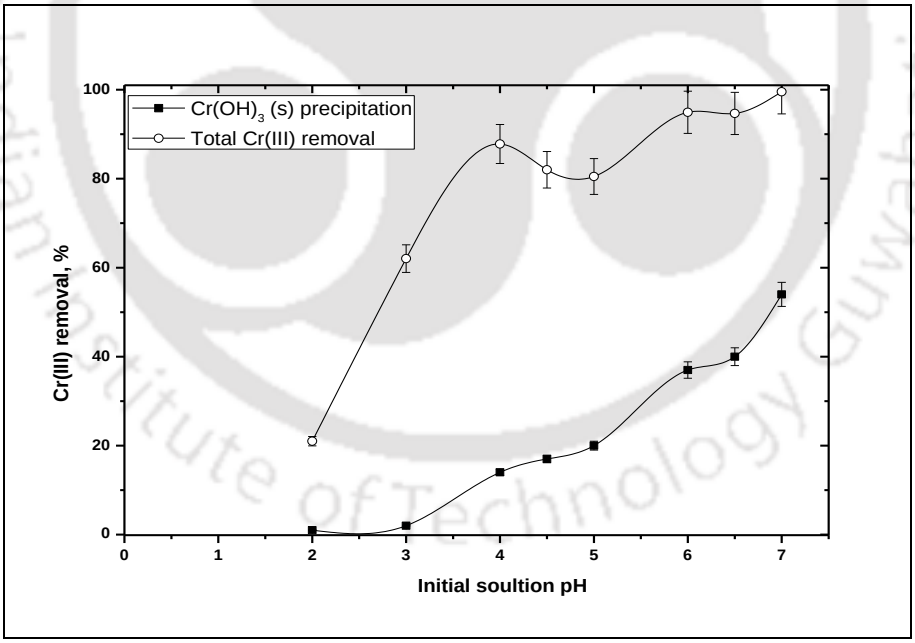

Figure 3.8 Removal of total amount of Cr(III) (precipitation + net biosorption) and Cr(III) removal by precipitation (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm , temperature 25°C and Cr(III) 10 mg L^{-1}).

Equilibrium condition was reached within 20 h of contact. Cr(III) biosorption increased with increasing the solution pH, showed a peak and then dropped. Cr(III) removal was found to be ~20% for the initial pH 2 even though zero point charge was at pH_{ZPC}=2.84 (Figure 3.9). There was negligible Cr(III) sorption at initial pH<2 due to charge repulsion between biomass particles and Cr(III) ions. Maximum Cr(III) sorption of 74% was observed at initial pH 4. The corresponding equilibrium pH was 6.2 (Figures 3.7 and 3.10). This is attributable to active participation of carboxylic and phosphatic functional groups in Cr(III) binding between initial pH 2 and 4. $Cr(OH)_3$ precipitated at higher rate at above initial of pH 5 (Esmaeili et al., 2005). Sorption of Cr(III) ions on biomass decreased at initial pH>4. The fine precipitates of $Cr(OH)_3$(s) formed probably would block the active sorption sites. It was resulted in lower sorption affinity of various hydroxylated species of Cr(III) ($Cr(OH)_2^+$, $Cr(OH)^{2+}$ etc) formed at higher pH. Hence the biosorption efficiency was reduced. *Spirulina* biomass surface contains functional groups, such as, carboxylic, phosphatic and amine (Figure 3.2). Amine group is more basic due to presence of a lone pair of electrons which would attract proton. At initial pH >5, Cr(III) also exist as $[Cr(OH)_4(H_2O)_3]^-$, $[Cr(OH)_5(H_2O)]^{2-}$ and $[Cr(OH)_6]^{3-}$, may be repelled by these functional groups. Total Cr(III) removal at higher solution pH kept on increasing as precipitation played a dominant role in Cr(III) removal. The trend of Cr(III) removal for the solution pH values between pH_{zpc} and 4, suggests that, *Spirulina* biomass sorbed more protons as compared to Cr(III) ions at pH<4 due to higher $[H^+]$. Cr(III) sorption dominated over proton sorption at initial pH 4 (Figure 3.7). The biosorption efficiency was reduced at pH>4 because of blanketing effect of $Cr(OH)_3$(s) precipitates. The stronger effect of Cr(III) precipitation with increasing pH could be seen from Figure 3.8.

The variation of equilibrium pH against its initial value without Cr(III) in solution is shown in Figure 3.10. *Spirulina* biomass showed a buffering activity around pH 6 for the initial solution

pH between 2 and 9. The rate of sorption reaction was initially faster and then gradually deceased. The increase or decrease in solution pH was because of deprotonation or protonation of the functional groups of *Spirulina* biomass.

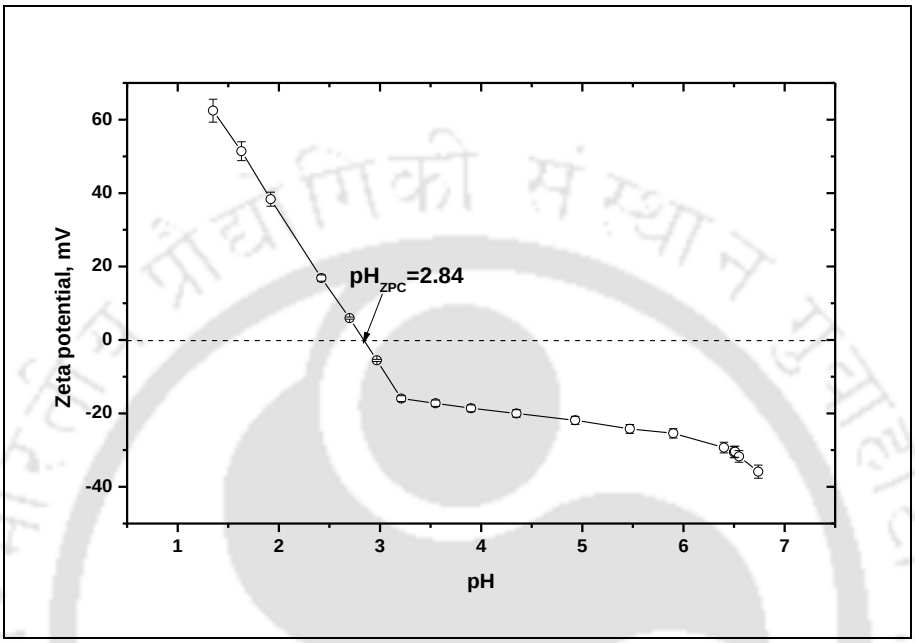

Figure 3.9 Variation of zeta potential of *Spirulina* dead biomass at temperature 25°C (biosorbent B_4 5 g $L^{-1}$and stirring speed 200 rpm).

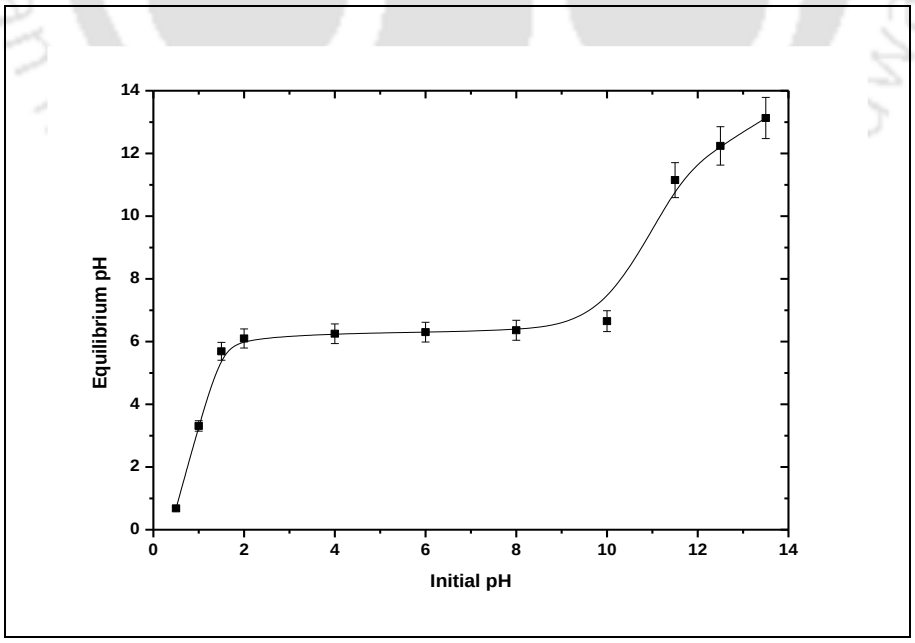

Figure 3.10 Variation of solution pH without Cr(III) in solution (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm and temperature 25°C).

3.3.1.4 Role of biomass dose on Cr(III) uptake

Biosorption efficiency significantly depends on metal ions to biomass active site ratio. The ratio of initial Cr(III) to biomass concentration in the solution could alter Cr(III) removal efficiency through combined effect of several factors, i. e., (i) availability of functional groups, (ii) driving force to overcome mass transfer resistance for Cr(III) ion transport from bulk solution to biomass surface and, (iii) effective surface area. This ratio was varied by changing Cr(III) ion and/or biomass concentration. The effect of Cr(III) to biomass ratio on removal of Cr(III) (mg) is illustrated in Figure 3.11. Removal of Cr(III) increased monotonically with increasing Cr(III) to biomass ratio i.e. at higher Cr(III) concentration with constant biomass concentration. This is attributable to higher Cr(III) uptake capacity. The biomass was further capable to remove more Cr(III) from solution. On the other hand, it was noted that with increasing Cr(III) to biomass ratio i.e. at higher biomass dose with constant Cr(III) concentration, removal of Cr(III) decreased. Higher biomass concentration reduced the effective surface area resulted from agglomeration of biomass particles. It implies that lower biomass dose enhances the process performance by increasing the uptake capacity. It was observed that, initially the biosorption rate was higher for the first 1 h because of rapid Cr(III) occupation to the functional groups and then the rate fallen down. The effects of variation of initial Cr(III) concentration and biomass dose are illustrated in Figures 3.12 and 3.13.

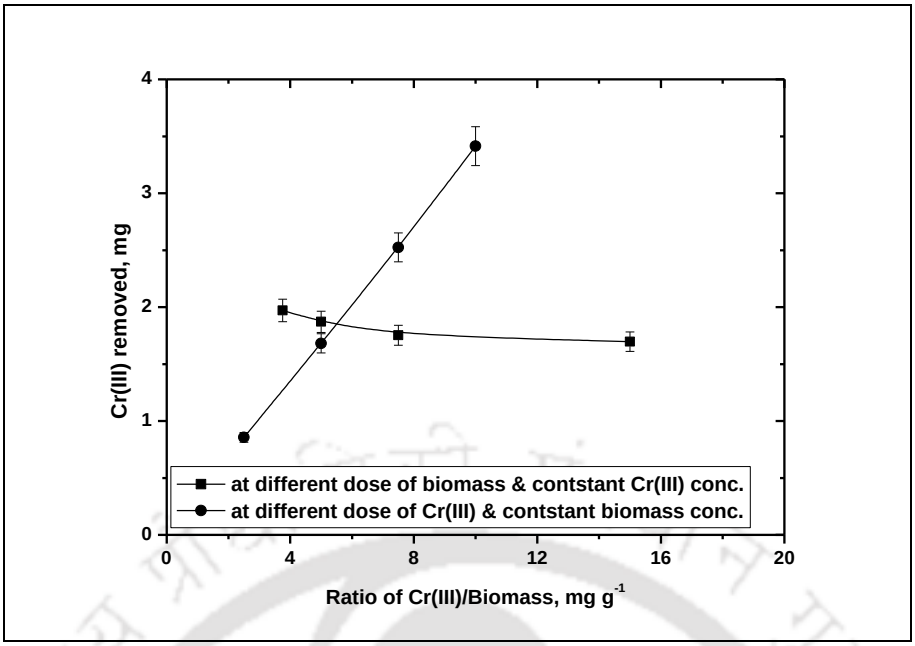

Figure 3.11 Quantity of Cr(III) removed from solution at different Cr(III) to biomass ratio (biosorbent B_4 , stirring speed 200 rpm, pH 4, and temperature 25°C).

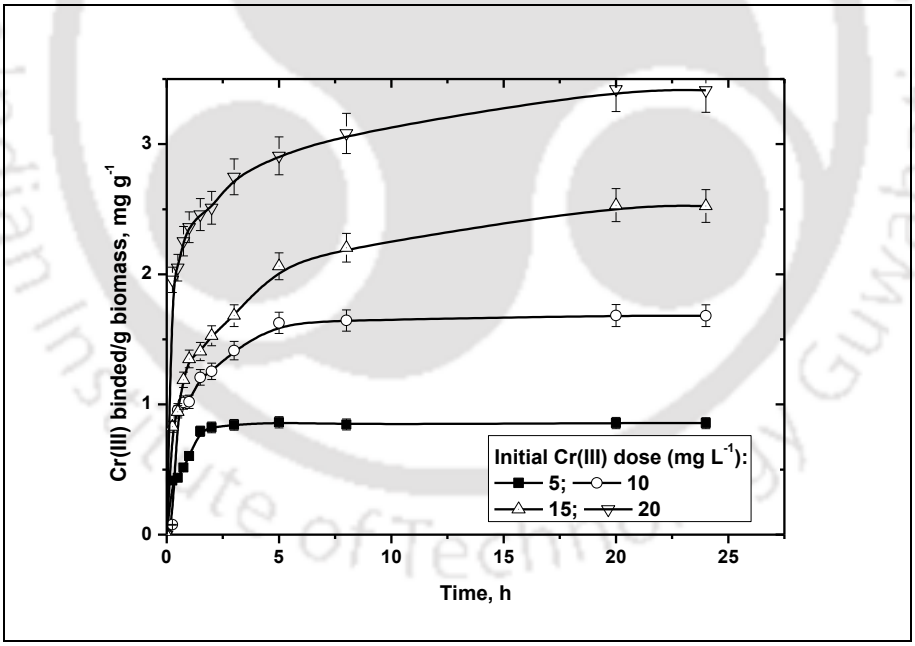

Figure 3.12 Dependency of initial Cr(III) concentration on its removal efficiency (biosorbent B_4 2 g L^{-1}, stirring speed 200 rpm, pH 4 and temperature 25°C).

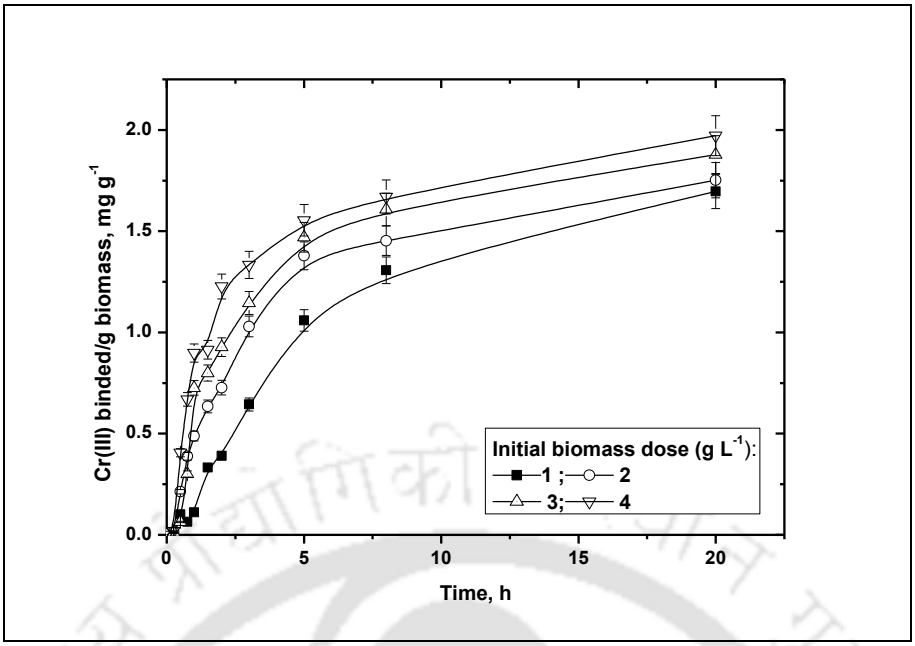

Figure 3.13 Role of biomass dose on Cr(III) removal (biosorbent B_4, stirring speed 200 rpm , pH 4, temperature 25°C and Cr(III) 15 mg L^{-1}).

3.3.1.5 Role of temperature

The effect of temperature on Cr(III) sorption onto *Spirulina* surface had been studied between 25 and 45ºC. The results indicated that Cr(III) binding efficiency decreased significantly with increasing temperature (Figure 3.14). This phenomena suggests that Cr(III) biosorption using *Spirulina* biomass was exothermic in nature. The concentration of functional groups and the boundary layer thickness were decreased at higher temperature. It was resulted as the easy escape of Cr(III) from biomass surface (Aksu and Kutsal, 1991).

The proteins and amino acids are highly sensitive to temperature and pH changes (Bendall and Swatland, 1988; Offer and Knight, 1988). Some proteins are also reported to be denaturated even at temperature >35°C. The decrease in biosorption at higher temperature may be due to partial denaturation of biomass proteins and amino acids.

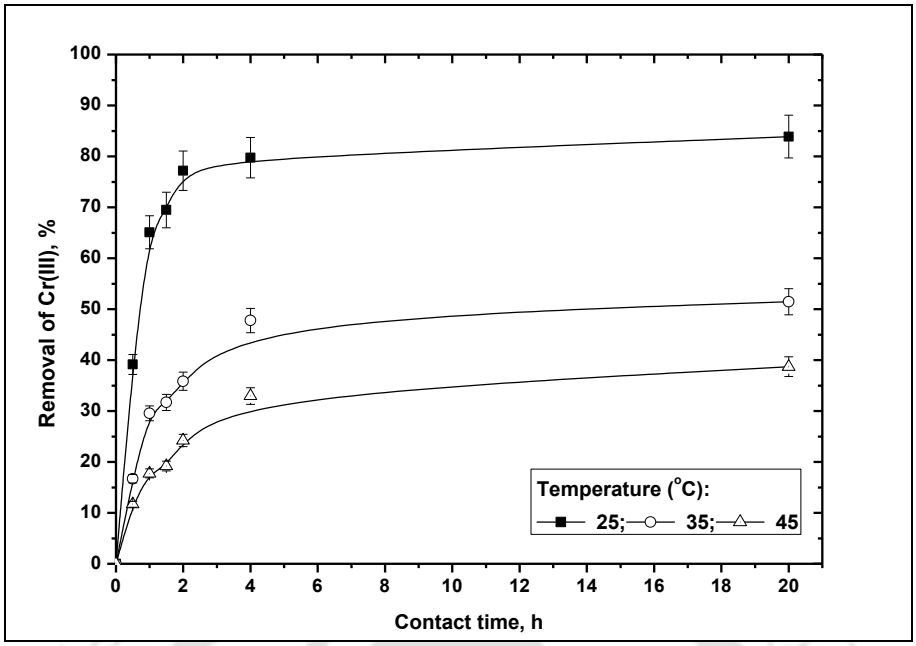

Figure 3.14 Effect of temperature on Cr(III) biosorption (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm , pH 4 and Cr(III) 20 mg L^{-1}).

3.3.1.6 Desorption studies

Desorption test was carried out in order to assess the reusability of *Spirulina* dead biomass i.e., regeneration of biosorbent for its successive use. Cr(III) loaded-biomasses was separated by centrifugation. Spent biomass containing about 1.46 mg Cr(III) per g biomass was added in 50 mL DI water maintained at different pH using 0.5N H_2SO_4. Desorption efficiencies with solution pH is illustrated in Figure 3.15. About 77% desorption i.e. Cr(III) concentration on biomass decreased from 1.46 to 0.34 mgg^{-1} biomass at pH 0.5. It decreased with rise of pH. High Cr(III) recovery suggests that Cr(III) ion was easily replaced by protons.It implies that the contribution of ion exchange plays a notable effect on desorption mechanism. Many authors also have reported complete or almost complete recovery of metals from biosorbents using mineral acid treatment (Kratochvil et al., 1998).

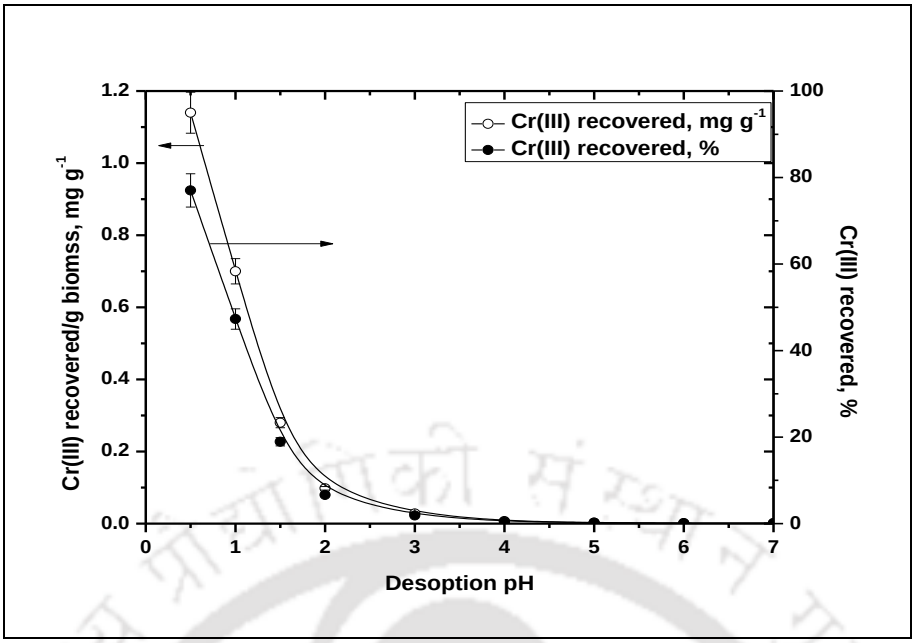

Figure 3.15 Cr(III) desorption at varying solution pH (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm , pH 4, temperature 25°C and Cr(III) 10 mg L^{-1}).

3.3.1.7 Acidic site determination

Figure 3.16 shows the protonation characteristics of acidic sites of *Spirulina* biomass obtained by potentiometric titration. The equilibrium proton binding model was employed to predict the behaviour of functional groups with varying the solution pH. The model Eq.3.7 was based on the dissociation of *Spirulina* biomass functional groups. The constants (K_{a1},K_{a2},K_{a3},N_1, N_2, and N_3) were determined by fitting the model equation with the experimental results (Figure 3.16) using least square fit method. The model showed an excellent agreement to the experiments. The best fitted constants are listed in Table 3.1. *Spirulina* biomass contains both weak acid (pK_{a1}=1.19) and base (pK_{a3}=8.07) which are corresponding to carboxylic and amine groups. The concentration (mol g^{-1}) of functional groups were in the order as phosphatic (0.28×10^{-3})>carboxyl (0.18×10^{-3})>amine (0.146×10^{-4}). The total number of acidic sites (N_p)was the combined quantity of these major functional groups based on pK_a values. The distribution of protonated acidic sites as a function of solution pH obtained from Eq.3.7 is

shown in Figure 3.17. In case of phosphatic site, it increased, showed maxima at around pH 3 and then fell down. The protonated quantity of amine group increased with rise of solution pH. It clearly indicates that the proton sorption capacity of both phosphate and carboxyl groups decreased graduallywith rise of solution pH upto pH_{zpc}=2.84. The capacity of proton sorption of carboxyl and amine groups is insignificant at pH_{zpc}. On the other way, it implies that phosphate group is neutral in nature. Similarly, the surface became more negatively charged due to decrease in protonated fraction with increase in pH values>pH_{zpc}. The buffering tendency of biomass in the initial pH from 2 to 9 (Figure 3.10) was probably due to the combined protonation and deprotonation effect of the functional groups. The concentration of the acidic sites of *Spirulina* was compared with other available data (Table 3.1).

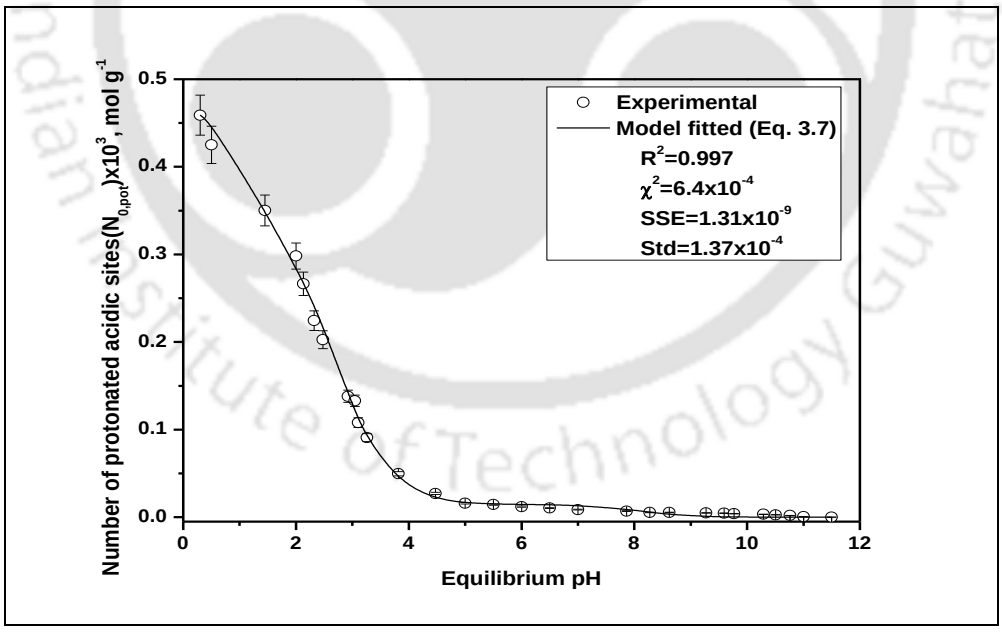

Figure 3.16 Number of protonated acidic sites of *Spirulina* biomass: experimental and model fitted (Eq. 3.7) (biosorbent B_4 5 g L^{-1}, volume 50 mL, stirring speed 200 rpm and temperature 25°C) (SSE: Error sum of squares, Std: Standard deviation, χ^2: Chi square and R^2: Coefficient of determination).

Table 3.1. Comparison of equilibrium parameters of acid dissociation of *Spirulina platensis*.

Investigation	Biomass type	Number of acidic sites (mol g^{-1})			Acid dissociation constant (mol L^{-1})		
		N_1	N_2	N_3	pk_{a1}	pk_{a2}	pk_{a3}
Present study	*Spirulina platensis*	1.8×10^{-4}	2.8×10^{-4}	1.46×10^{-5}	1.19 (Carb-oxyl)	2.87 (Pho-sphate)	8.07 (Amine)
Seki et al., 2000	*Heterosigma akashiwoHada*	1.79×10^{-3}	0.70×10^{-3}	2.79×10^{-3}	3.90	6.64	9.51
Seki et al., 1998	*R. sphaeroides*	6.43×10^{-3}	4.10×10^{-3}	3.27×10^{-3}	4.17	6.14	9.85
	A. eutrophus	1.43×10^{-3}	1.08×10^{-3}	1.92×10^{-3}	4.53	7.08	9.26
Seki and Suzuki, 1998	*Macrocystis pyrifera*	0.87×10^{-3}			3.19		
	Kjellmaniella crassiforia	1.16×10^{-3}			3.14		
	Undaria pinnatifida	1.21×10^{-3}			3.20		
Martinez et al., 2002	*B. subtilis*	0.10×10^{-3}	0.11×10^{-3}	0.08×10^{-3}	3.59	4.33	5.94
	E. coli	0.06×10^{-3}	0.15×10^{-3}	0.05×10^{-3}	3.72	4.85	6.56
Pagnaelli et al., 2000	*Arthrobacter sp.*	0.272×10^{-3}	0.138×10^{-3}		6.88	10.11	

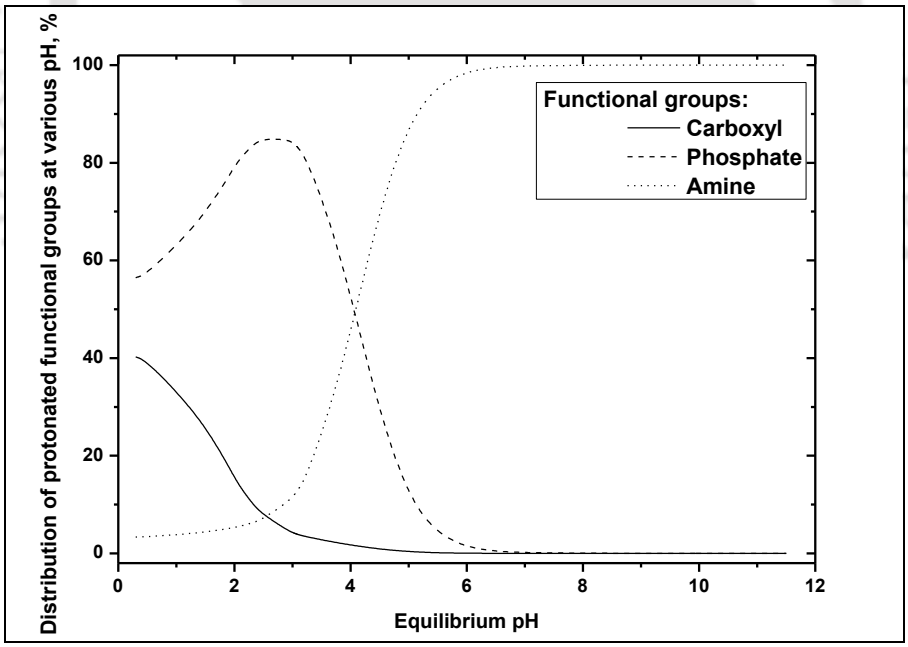

Figure 3.17 Distribution of protonated functional groups at various pH determined from Eq.3.7 (biosorbent B_4 5 g L^{-1}, volume 50 mL, stirring speed 200 rpm and temperature 25°C).

The quality and quantity of acidic sites of microbial species depend on its cultivation conditions. The acid dissociation constants of carboxylic and phosphatic sites was found be lower as compared to the other reported microbial species. These two weak acidic sites were available for Cr(III) binding at slightly acidic region (pH 4 to 6). Whereas, amine sites (pk_{a3}=8.0) could participate in Cr(III) binding at slightly alkaline region. The distribution of acidic sites of *Spirulina* surface is in accordance with this phenomenon (Figure 3.17).

3.3.1.8 Multiple sites equilibrium Cr(III) sorption modeling

MCBM model was employed to investigate Cr(III) binding to functional groups available at *Spirulina* biomass surface. The constants ($K_{\theta 1}$,$K_{\theta 2}$ and $K_{\theta 3}$,) of Eq.3.14 were found out by fitting with the experimental results of Cr(III) biosorption only (Figure 3.7) and previously calculated constants in Eq.3.7. The model showed an excellent agreement for Cr(III) biosorption (Figure 3.18). To avoid predominant Cr(III) precipitation, the model fitting was limited for the initial pH<7. The best fitted model parameters are listed in Table 3.2.

Participation of various functional groups ($N_i\theta_i$) in Cr(III) binding is obtained from Eq. 3.14. The results are shown in Figure 3.19. Cr(III) removal in the acidic regime was due to predominant involvement of carboxylic and phosphatic groups (Figure 3.17). Cr(III) binding capacity of phosphatic groups increased with rise of equilibrium solution pH and the maximum Cr(III) bindings was obtained at pH 6.2 (Figure 3.19). Chojnaka et al. (2005) reported similar results of leading contribution of carboxylic and phosphatic groups for Cr(III) biosorption using wheat straw and grass in the pH range from 2 to 5. The concentration of protonated amine site increased sharply at pH> 3 (Figure 3.17). On the other way, it indicates that the deprotonated fraction (α_i) attached to Cr(III), fell down. Its role on Cr(III) uptake was therefore negligible in equilibrium pH range studied. The range of equilibrium pH was quite

narrow. Therefore this particular experiment was conducted in quintuple to minimize the experimental error and subsequently to obtain a better model prediction.

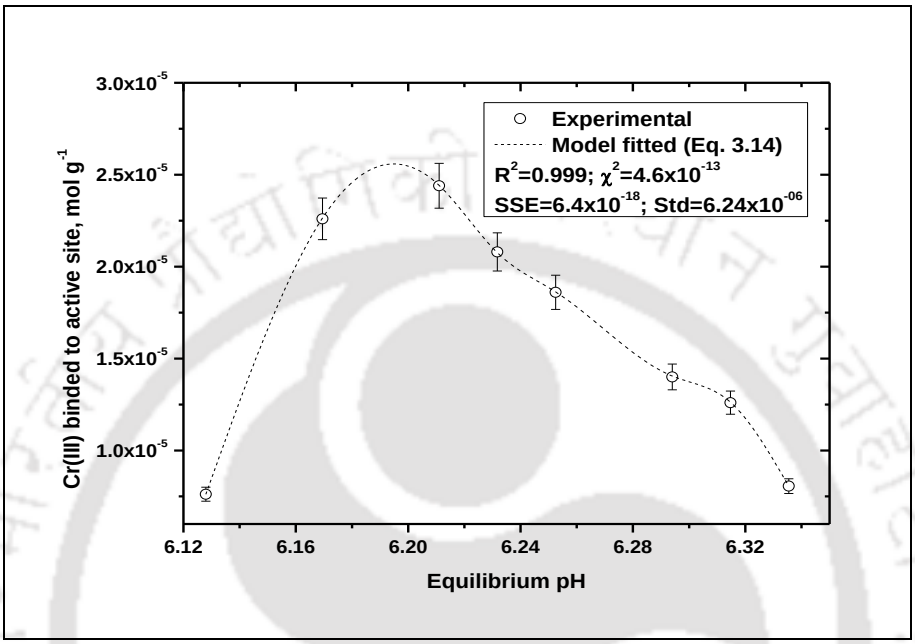

Figure 3.18 Equilibrium Cr(III) sorption: experimental and model fitted (Eq. 3.14) (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm , temperature 25°C and Cr(III) 10 mg L^{-1}) (SSE: Error sum of squares, Std: Standard deviation, χ^2 : Chi square and R^2:Coefficient of determination).

The binding constants of Cr(III) with *Spirulina* and the literature reported values for the single metal sorption study are summarized in Table 3.2. Total acidic sites (X_N) of 1.47×10^{-5} mol g^{-1} were available for Cr(III) binding at equilibrium pH of 6.2 (Eq. 3.14) and 74% Cr(III) was removed (Figure 3.4). Absorbed Cr(III) of 3.77×10^{-7}, 2.4×10^{-5} and 1.31×10^{-13} mol per g of biomass were occupied to carboxylic, phosphatic and amine sites, respectively. It indicates that Cr(III) attachments were 98.4 and 1.5% to phosphatic and carboxylic sites. The pK_θ

values determined for *Spirulina* biomass in Cr(III) sorption are in accordance with sorption-site specific removal efficiency.

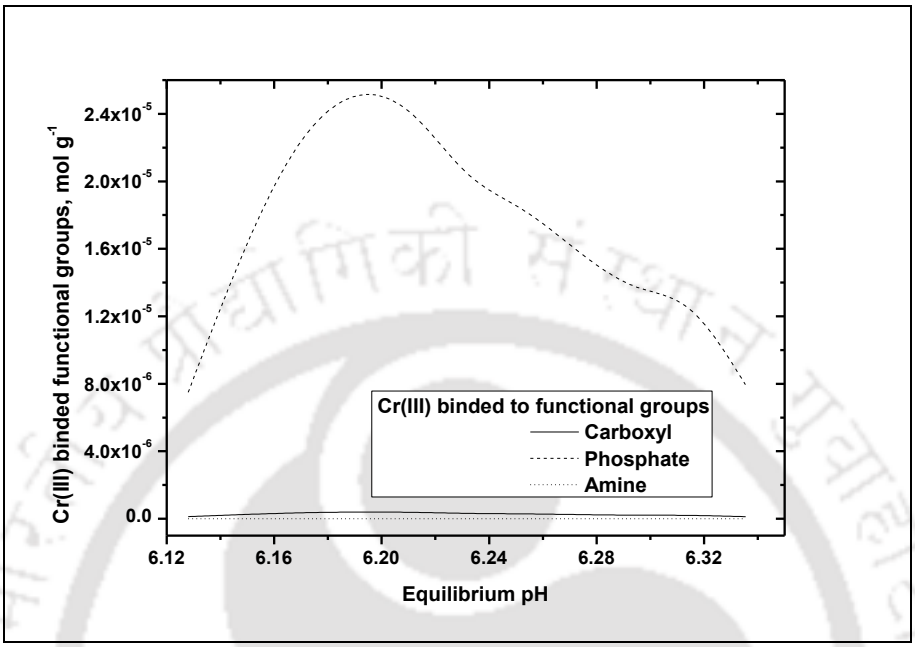

Figure 3.19 Participation of various functional groups for Cr(III) removal calculated from Eq.3.14 (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm, temperature 25°C and Cr(III) 10 mg L^{-1}).

Table 3.2. Metal binding constants of Cr(III) biosorption.

Study	***Biosorbent***	***Binding constants***			
		$K_{\theta 1}$	$K_{\theta 2}$	$K_{\theta 3}$	$K_{\theta,overall}$
Present study	*Spirulina platensis*	80.9 (L mol^{-1})	3.9×10^{3} (L mol^{-1})	3.5×10^{4} (L mol^{-1})	1.13×10^{10} (L mol^{-1})
Quaries et al., 2011	*Transferrin*		1.41×10^{10} mol^{-1}		
Liu et al., 2006	*Apoovotransferrin*		1.42×10^{10} mol^{-1}		
Sun et al., 2006	*ApoLMWCr*		1.54×10^{21} mol^{-1}		
Arakawa et al., 2000	*Calf Thymus*		3.15×10^{3} mol^{-1}		
Fukushima et al., 1995	*Humic acid*		3.4-4.9 mol^{-1}		

3.3.1.9 Thermodynamic studies

Cr(III) binding to carboxyl and amine groupswas negligible compared to phosphate group.It is already shown that maximum Cr(III)attachmentto carboxyl and amine groupswere 1.5 and 0.1%, respectively. In order to describe the thermodynamic behaviour of Cr(III) uptake onto *Spirulina* biomass, the change in free energy (ΔGº), enthalpy (ΔHº) and entropy (ΔSº) were determined in terms of the equilibrium Cr(III) constant ($K_{\theta 2}$) based on its attachment to phosphate group. The variation of $K_{\theta 2}$ with temperature is illustrated in Figure 3.20. The standard free energy change at equilibrium can be written as:

$$\Delta G^{\circ} = -RT\ln K_{\theta 2} \qquad (3.15)$$

where, *R* is gas constant (8.314 J mol^{-1} K^{-1}) and T is absolute temperature (K). The relation among ΔGº, ΔHºandΔSº is expressed as:

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} \qquad (3.16)$$

Combining Eqs. 3.15 and 3.16,

$$RT\ln K_{\theta 2} = \Delta H^{\circ} - T\Delta S^{\circ} \qquad (3.17)$$

Upon rearrangement,

$$\ln K_{\theta 2} = -\frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} \qquad (3.18)$$

The value of ΔHº and ΔSº are determined from the slope and intercept of linear plot of Eq.3.18 (Figure 3.21).

The computed values of thermodynamic parameters are shown in Table 3.3. The standard free energy change (ΔG°) was calculated as -20.48, -18.62 and -17.11kJ mol^{-1} at 25, 35 and 45ºC, respectively. The negative value of ΔG°values indicates thermodynamic feasibility and spontaneous nature of Cr(III) biosorption onto *Spirulina* biomass. The decrease in ΔG°values with increasing solution temperature suggests that the spontaneity of the sorption reaction fallen down. The enthalpy change (-6.24 kJ mol^{-1}) shows the exothermic sorption nature and

the process is energetically stable. The degree of freedom of sorbed Cr(III) ion is in line with the standard entropy change (4.78×10^{-2}kJ mol^{-1} K^{-1}).

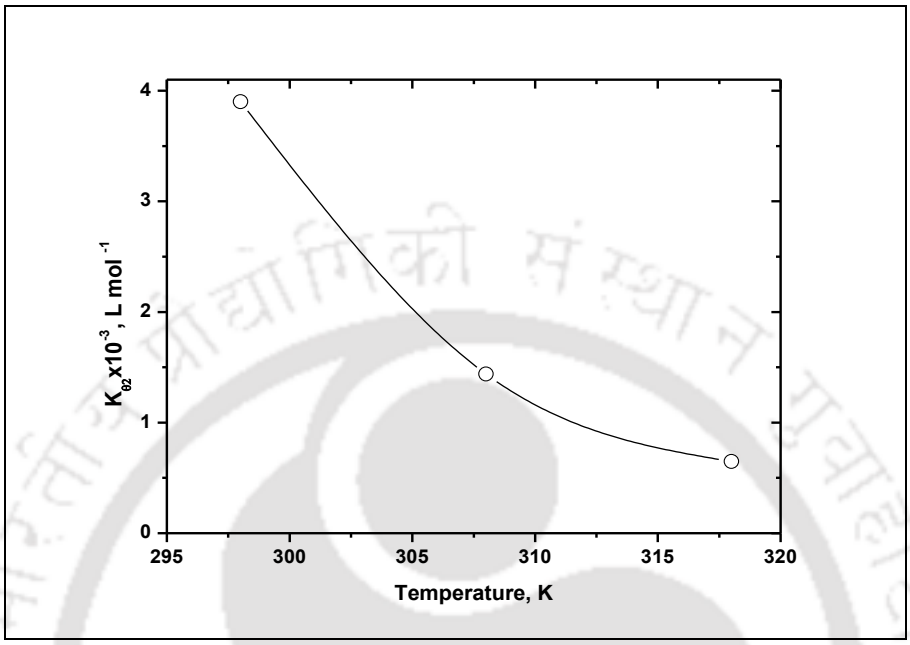

Figure 3.20 Effect of temperature on Cr(III) binding constant($K_{\theta 2}$) onto phosphate group (biosorbent B_4 5 g L^{-1}, stirring speed 200 rpm, pH 4 and Cr(III) 20 mg L^{-1}).

Table 3.3. Thermodynamic parameters for Cr(III) ion sorption on *Spirulina* biomass.

Temperature (°C)	**ΔG° (kJ mol^{-1})**	**ΔH° (kJ mol^{-1})**	**ΔS° (kJ mol^{-1} K^{-1})**	**R^2**
25	-20.48			
35	-18.62	-6.24	4.78×10^{-2}	0.999
45	-17.11			

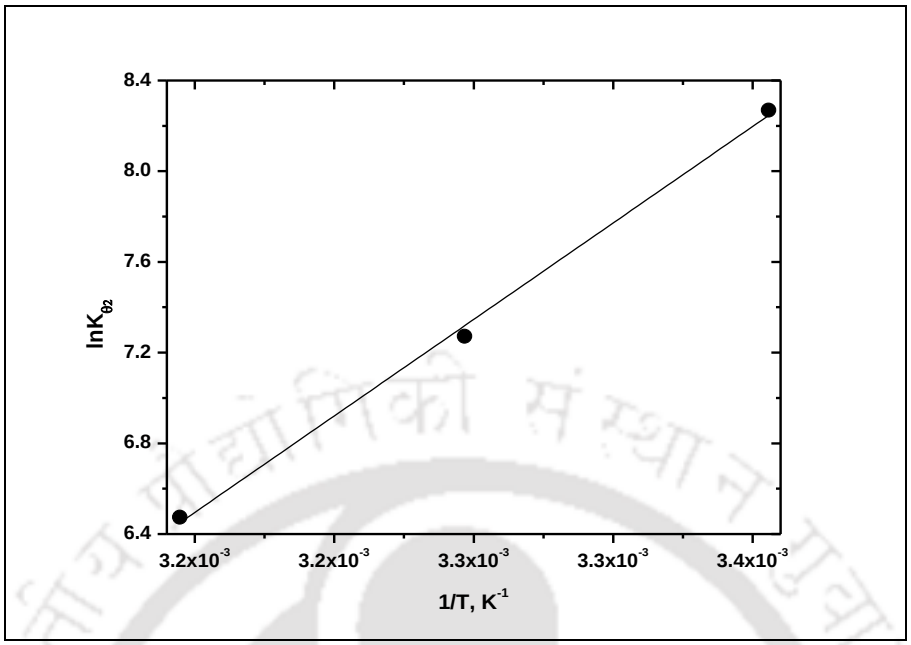

Figure 3.21 Plot of $\ln K_{\theta 2}$ against reciprocal temperature for Cr(III) binding onto phosphate group of *Spirulina* biomass.

3.3.2 Live *Spirulina*biomass and Cr(III) removal

The effect of different initial Cr(III) concentrations in nutrient medium on the growth of *Spirulina platensis* cell is shown in Table 3.4. [Cr(III)]≤10 mg L^{-1} had negligible effect on *Spirulina* growth.While [Cr(III)]>50 mg L^{-1} was resulted in decrease of cell density considerably. Complete Cr(III) removal was achieved upto 20 mg L^{-1} Cr(III). There were traces of Cr(III) in solution (0.5 to 1.5 mg L^{-1}) at higher concentration.

Literature reports that the performance of bioaccumulation of metal ions decreases notably beyond the concentration where the toxic effects begin (Wong and Tan, 1998). With an initial Cr(III) ions concentration ≤10 mg L^{-1}, no notable effect of Cr(III) was observed on cell density. This is in line with literature. The cell growth is not affected for the maximum concentration of Cr(III) ions for microalga *Scenedesmus acutus* and *Chlorella vulgaris* of 15

and 45 mg L^{-1}, respectively (Travieso et al., 1999). ZMC sets the solution pH at around 8.5. A large fraction of Cr(III) is precipitated at higher Cr(III) concentration and pH. $Cr(OH)_3(s)$ could settle on biomass particles and would hinder the nutrient uptake. This results in inhibition of *Spirulina* growth. Cr(III) solution at different concentration was precipitated at pH 8.5 prior to *Spirulina* cultivation. Sludge was separated out by centrifugation and clear solution was used for *Spirulina* cell growth. The cell density was almost same for all the cases (2.01±0.1 g L^{-1}). There are a numbers of microalgal species display the appearance of resistant cells when exposed to Cr(III) by means of mutation (Pereira et al., 2013).However, I could not find any such specific studies on *Spirulina sp*. The dynamics of cell growth and evaluation of growth parameters are provided in Chapter 5 (Section 5.3.7).

Table 3.4. Influence of [Cr(III)] on *Spirulina* growth in Zarrouk media composition with 12 h photo period per day.

$[Cr(III)]_0$, mg L^{-1}	***Spirulina* growth, g L^{-1}**	**ResidualCr(III), mg L^{-1}**
0	2.03	-
5	2.01	N.D.
10	1.94	N.D.
20	1.83	N.D.
50	1.76	0.5
100	1.58	1.5

N.D.: Not detected

3.4 Important findings

The present study reports aqueous Cr(III) uptake behavior onto *Spirulina sp.* biomass. Cr(III), a micronutrient, may be possible to supply with *Spirulina sp*. as poultry or animal food. The major findings are:

- Maximum Cr(III) sorption was noted at equilibrium pH of 6.2.

- The acid dissociation constants (pK_a) of 1.19, 2.82 and 8.07 mol L^{-1} could be assigned to carboxyl, phosphate and amine sites of *Spirulina*. The concentration of functional groups was estimated in the order of phosphatic>>carboxyl >amine.

- Cr(III) binding constants (K_θ) to surface sites were found to be 80.9, 3.9×10^3 and 3.5×10^4 (L mol^{-1}) for carboxylic, phosphate and amine sites, respectively.

- Cr(III) uptake modeling showed dual sites coverage of Cr(III) on *Spirulina* biomass surface with predominant occupancy to phosphatic sites. The amine group however, according to metal binding model, was less significant in Cr(III) binding. The overall binding constant of 1.13×10^{10} L mol^{-1} was calculated for Cr(III) sorption.

- Highest Cr(III) desorption of 77% was noted at the lowest pH of 0.5.

- In case of live *Spirulina platensis*, cell growth decreased by about maximum 22% because of predominant inhibition of Cr(III) precipitates.

Nomenclature

$\equiv B_i$	Acidic sites *i* of *Spirulina* biomass
ΔG°	Change in Gibbs free energy (kJ mol^{-1})
ΔH°	Enthalpy change (kJ mol^{-1})
$[H^+]$	Hydrogen ion concentration (mol L^{-1})
K_{ai}	Acid dissociation constant of type *i* (mol L^{-1})
$K_{\theta i}$	Metal binding constant of type *i* (L mol^{-1})
$K_{\theta,overall}$	Overall Cr(III) binding constant to acidic sites (L mol^{-1})
[M]	Cr(III) ion concentration (mol L^{-1})
N_i	Number of *i* type acidic sites (mol g^{-1})
$N_i\theta_i$	Cr(III) ion attached to *i* type acidic sites (mol g^{-1})
ΔS°	Entropy change (kJ mol^{-1} K^{-1})
X_N	Number of protonated acidic sites (mol g^{-1})
X_θ	Number of metal ions binded to *Spirulina* biomass (mol g^{-1})
R^2	Coefficient of determination

Greek symbols

α_i	Degree of dissociation of type *i* acidic sites
θ_i	Fraction of type *i* acidic sites occupied by Cr(III) ions
χ^2	Chi square

Acronyms

AAS	Atomic Absorption Spectrophotometer
AMD	Arithmetic mean diameter
FTIR	Fourier Transform Infrared Spectroscopy
GMD	Geometric mean diameter
KBr pellets	Potassium bromide pellets
MCMB	Multicomponent competitive binding model
SEM	Scanning Electron Microscopy
SSE	Sum of squared error
Std	Standard deviation
LMWCr	Low-molecular-weight chromium-binding substance

References

Aksu, Z. and Kutsal, T. A., "A Bioseparation Process for Removing Pb(II) ions from Wastewater by using *C. Vulgaris*," *J. Chem. Technol. Biotechnol.*,**52**, 108-118 (1991).

Arakawa, H., Ahmad, R., Naoui, M., and Riahi, H. A. T., "A Comparative Study of Calf Thymus DNA Binding to Cr(III) and Cr(VI) Ions: Evidence for the Guanine N-7 Chromium–Phosphate Chelate Formation",*J. Biol. Chem.*,**275**, 10150-10153 (2000).

Batista, A.P.S., Romao, L.P.C., Arguelho, M.L.P.M., Garcia, C.A.B., Alves, J.P.H., Passos E.A. and Rosa, A.H., "Biosorption of Cr (III) using in Natural and Chemically treated Tropical Peats",*J. Hazard. Mater.*, **163(2-3)**, 517-523 (2009).

Bases C.F. and Mesmer, R.E. *The Hydrolysis of Cations*, Wiley: New York, 1976

Bendall, J. R. and Swatland, H.J., "A Review of the Relation-ships of pH with Physical Aspects of Pork Quality",*Meat Sci.*,**24**, 85-126 (1988).

Chojnacka, K., *Heavy Metal Ions Removal by Microalgae Spirulina sp. in the Processes of Biosorption and Bioaccumulation*, PhD Dissertation, Wroclaw University of Technology, Poland (2003).

Chojnacka, K., "Biosorption of Cr(III) ions by Wheat Straw and Grass: A Systematic Characterization of New Biosorbents", *P. J. Environ. Stud.*, **15(6)**, 845-852 (2006).

Chojnaka, K., Chojnaki A. and Gorecka, H. "Biosorption of Cr^{3+}, Cd^{2+}, and Cu^{2+} ions by Blue-Green Algae *Spirulina sp.*: Kinetics, Equilibrium and the Mechanism of the Process",*Chemosphere*,**59**, 75-84 (2005).

Cui, L., Wu G. and Jeong, T.S., "Adsorption Performance of Nickel and Cadmium Ions onto Brewer's Yeast",*Can. J. Chem. Eng.*,**89**, 109-115 (2010)

Dalcin, M.G., Pirete, M.M., Lemos, D.A., Ribeiro, E.J., Cardoso V.L. and de Resende, M.M., "Evaluation of Hexavalent Chromium Removal in a Continuous Biological Filter with

the use of Central Composite Design (CCD)", *J. Environ. Manage.*, **92**, 1165-1173 (2011).

Das, B., Mondal, N.K., Roy, P. and Chattaraj, S., "Equilibrium, Kinetic and Thermodynamic Study on Chromium(VI) Removal from Aqueous Solution Using *Pistia Stratiotes* Biomass", *Chem Sci Trans.*, **2(1)**, 85-104 (2013).

Dursun, A. Y., Uslu, G., Tepe, O., Cuciand Y. and Ekiz, H.I., "A Comparative Investigation on the Bioaccumulation of Heavy Metal Ions by Growing *Rhizopus Arrhizus* and *Aspergillus Niger*", *Biochem. Eng. J.*, **15(2)**, 87- 92 (2003).

Esmaeili, A., Mesdaghinia A. and Vazirinejad, R., "Chromium (III) Removal and Recovery from Tannery Wastewater by Precipitation Process",*Am. J. Appl. Sci.*, **2(10)**, 1471-1473 (2005).

Finocchio, E., Lodi, A., Solisio, C. and Converti, A., "Chromium (VI) Removal by Methylated Biomass of *Spirulina Platensis*: The effect of Methylation Process",*Chem. Eng. J.*,**156**, 264-269 (2010).

Fukushima, M., Nakayasu, K., Tanaka S. and Nakamura, H., "Chromium (III) Binding Abilities of Humic Acids",*Anal. Chim. Acta.*, **317**, 195-206 (1995).

Gadd, G.M., "Heavy Metal Accumulation by Bacteria and other Microorganisms", *Experientia*, **46**, 834-840 (1991).

Gupta, V. K., Srivastava, A. K. and Jain, N., "Biosorption of Chromium (VI) from Aqueous Solutions by Green Algae *Spirogyra Species*",*Wat. Res.*,**35(17)**, 4079-4085 (2001).

Gupta, V. K. and Rastogi, A., "Biosorption of Hexavalent Chromium by Raw and Acid-Treated Green Alga *Oedogonium Hatei*from Aqueous Solutions",*J. Hazard. Mater.*,**163**, 396-402 (2009).

Gupta, V.K., Rastogi, A. and Nayak, A., "Adsorption Studies on the Removal of Hexavalent Chromium from Aqueous Solution Using A Low Cost Fertilizer Industry Waste Material", *J. Colloid. Inter. Sci.,***342**, 135-141 (2010).

Gupta, V. K. and Ali, I., "Removal of Lead and Chromium from Wastewater Using Bagasse Fly Ash-A Sugar Industry Waste",*J. Colloid Interface Sci.,***271**, 321-328 (2004).

Gupta, V. K., Carrott, P. J. M., Ribeiro, M. M. L., Carrott and Suhas, "Low-Cost Adsorbents: Growing Approach to Wastewater Treatment-A Review",*Crit. Rev. Env. Sci. Technol.,***39(10),** 783-842 (2012).

Ho, Y. S. and Ofomaja, A. E., "Kinetics and Thermodynamics of Lead Ion Sorption on Palm Kernel Fibre from Aqueous Solution",*Process Biochem.,***40 (11)**, 3455-3461 (2005).

Khorramabadi, H. S., Soltani, R. D. C., Rezaee, A., Kharee A. R. and Jafar, A. J., "Utilisation of Immobilised Activated Sludge for the Biosorption of Chromium (VI)",*Can. J. Chem. Eng.,***90(6),** 1539-1546 (2012).

Kratochvil, D., Pimentel, P. and Volesky, B., "Removal of Trivalent and Hexavalent Chromium by Seaweed Biosorbent", *Environ. Sci. Technol.,***32**, 2693-2698 (1998)

Kuyucak, N. and Volesky, B., "Accumulation of Cobalt by Marine Alga", *Biotechnol. Bioeng.*, **33(7**), 809-14 (1989).

Liu, B., Li, Y. Q., Yang, B. S. and Huang, S. P., "Synthesis, Characterization and Properties of Chromium (III) Complex $[Cr(SA)(en)_2]Cl.2H_2O$",*J. Inorg. Biochem.,***100**, 1462-1469 (2006).

Loukidou, M. X., Karapantsios, T. D., Zouboulis, A. I. and Matis, K. A., "Diffusion Kinetic Study of Chromium(VI) Biosorption by *Aeromonas Caviae*", *Ind. Eng. Chem. Res.,***43,** 1748-1755 (2004).

Martinez, R. E., Smith, D. S., Kulczycki, E.and Ferris, F. G., "Determination of Intrinsic Bacterial Surface Acidity Constants using a Donnan Shell Model and a Continuous pK_a Distribution Method",*J. Coll. Inter. Sci.*,**253**, 130-139 (2002).

Mukhopadhyay M., Noronha, S. B. and Suraishkumar, G. K., "A Review on Experimental Studies of Biosorption of Heavy Metals by *Aspergillus Niger",Can. J. Chem. Eng*.,**89,** 889-900 (2011).

Murphy, V., Hughes, H. and McLoughlin, P., "Comparative Study of Chromium Biosorption by Red, Green and Brown Seaweed Biomass",*Chemosphere*,**70(6),** 1128-34 (2008)

Offer, G. and Knight, P.,*The Structural Basis of Water Holding in Meat*, Part 1 General Principles and Water Uptake in Meat Processing', in Lawrie R A,Dev. Meat Sci 4, London, Elsevier Applied Science, (1998) pp. 63-171.

Pagnanelli, F., Papini, M. P., Toro, L., Trifoni, M. and Veglio, F., "Biosorption of Metal ions on *Anthrobacter sp.*: Biomass Characterization and Biosorption Modelling", *Environ. Sci. Technol.*,**34(13),** 2773-2778 (2000).

Park, D., Yun Y. S. and Park, J. M., "Studies on Hexavalent Chromium Biosorption by Chemically-Treated Biomass of *Ecklonia sp*.",*Chemosphere*,**60**, 1356-1364 (2005).

Plette, A. C. C., Riemsdijk, W. H. V., Benedetti M. F. and van derWal, A., "pH Dependent Charging Behavior of Isolated Cell Walls of a Gram-Positive Soil Bacterium",*J. Coll. Inter. Sci.*,**173**, 354-363 (1995).

Quaries, Jr. C. D., Marcus, R. K. and Brumaghim, J. L., "Competitive Binding of Fe^{3+}, Cr^{3+}, and Ni^{2+} to Transferrin",*J. Biol. Inorg. Chem*.,**16**, 913-921 (2011).

Rudolf, E. and Cervinka, M., "Trivalent Chromium Activates Rac-1 and Src and Induces Switch in the Cell Death Mode in Human Dermal Fibroblasts",*Toxicol. Lett.*,**188**, 236-242 (2009).

Saha, R., Saha, I., Nandi, R., Ghosh, A., Basu, A., Ghosh S. K. and Saha, B., "Application of Chattim Tree (devil tree, *Alstonia scholaris*) Saw Dust as a Biosorbent for Removal of Hexavalent Chromium from Contaminated Water", *Can. J. Chem. Eng.*,**91(5),** 814-821 (2013).

Seki, H. and Suzuki, A., "Biosorption of Heavy Metal ions to Brown Algae, *Macrocystis Pyrifera, Kjellmaniella Crassiforia*and *Undaria Pinnatifida*", *J. Coll. Inter. Sci.*,**206(1),** 297-301 (1998).

Seki, H., Suzuki A.and Mitsueda, S. I., "Biosorption of Heavy Metal ions on *Rhodobacter Sphaeroides* and *Alcaligenes Eutrophus H16*", *J. Coll. Inter. Sci.*,**197**, 185-190 (1998).

Seki, H., Suzuki, A.and Iburi, Y., "Biosorption of Heavy Metal ions to a Marine Microalga, *Heterosigma Akashiwo (Hada) Hada*",*J. Coll. Inter. Sci.*,**229**, 196-198 (2000).

Srivastava, S., Ahmad A.H. and Thakur, I. S., "Removal of Chromium and Pentachlorophenol from Tannery Effluents", *Bioresour. Technol*., **98**, 1128-1132 (2007).

Srivastava, S. K., Gupta V. K.and Mohan, D., "Removal of Lead and Chromium by Activated Slag-A Blast-Furnace Waste",*J. Environ. Eng.*,**123**, 461-468 (1997).

Sun, Y., Ramire, J. Woski, S. A. and Vincent, J. B., "The Binding of Trivalent Chromium to Low-Molecular-Weight Chromium-Binding Substance (LMWCr) and the Transfer of Chromium from Transferrin and Chromium Picolinate to LMWCr", *J. Biol. Inorg. Chem*.,**5(1),** 129-136 (2000).

Veglio, F. and Beolchini, F., "Removal of Metals by Biosorption, A Review", *Hydrometallurgy*, **44(3)**, 301-316 (1997).

Vilar, V.J.P., Valle, J.A.B., Bhatnagar, A., Santos, J.C., Guelli U. de Souza, S.M.A., Augusto U. de Souza, A., Cidália M.S. Botelho, C.M.S., Boaventura, R.A.R., "Insights Iinto Trivalent Chromium Biosorption onto Protonated Brown Algae *Pelvetiacanaliculata*:

Distribution of Chromium Ionic Species on the Binding Sites", Chem. Eng. J. 200-202, 140-148, (2012).

Volesky, B., "Detoxification of Metal Bearing Effluents, Biosorption for the Next Century", *Hydrometallurgy*, **59(2- 3)**, 203-216 (2001).

Chapter 4

Bioremediation of Hexavalent Chromium from Simulated Effluent

This chapter investigateson bioremediation of hexavalent chromium (Cr(VI)) from simulated effluent using dead and live *Spirulina platensis* biomass.

4.1 Background of Cr(VI) removal and reduction into Cr(III)

Cr(VI) and Cr(III) are characterized by their different physiochemical behaviours, mobilities and toxicities (Park et al., 2007). Cr(VI) is a known carcinogen including a number of noxious human health effects like skin rashes, upset stomachs, respiratory problems, weakened immune systems, kidney damage and genetic alteration. Bio-availability of Cr(VI) is much higher compared to Cr(III) and the transport through cell is analogous to SO_4^{2-} ions (Pettine et al., 1998). Hydrolysis of dichromate ions generates Cr(VI) oxy-anions in dilute aqueous solution. CrO_4^{2-} is predominant at pH>7. H_2CrO_4 dominates at pH <1. Whereas, $HCrO_4^-$ prevails between pH 1 and 6.

The techniques for the removal of Cr(VI) include ion-exchange (Alvarado et al., 2013), filtration (Hsu et al., 2011), reduction-precipitation (Dermou et al., 2005; Kurniawan et al., 2006), adsorption (Verma et al., 2006) and electro-coagulation (Golder et al., 2011). The most practiced method involves reduction of Cr(VI) into Cr(III) followed by $Cr(OH)_3(s)$ precipitation. Inorganic chemicals such as Fe(II) salts, H_2S and sodium thiosulfate are the commonly used reducing agents. In such treatments, solution pH is first reduced by addition of acid for proficient Cr(VI) reduction. Then pH is further increased by addition of lime/caustic soda to precipitate the hydroxides. The optimum pH for the precipitation depends on the accompanying heavy metal species in the effluent. High chemical consumption and large volume of sludge generation make such processes costly (Tels, 1987). It has motivated the environmental researchers to look for alternative treatment techniques.

Biological tool is a promising option for the detoxification of Cr(VI). A number of microbial species are found to be capable for Cr(VI) removal (Ishibashi et al., 1990; Chirwa and Wang, 1997; Park et al., 2004; Park et al., 2006; Gokhale et al., 2008; Finocchio et al., 2010).

The concentration of functional groups of biomass is found to be in the order of: phosphatic>>carboxyl>amine as stated in Chapter 3. *Spirulina* biomass carries net positive charge at pH<2.84 (Figure 3.8). Cr(VI) and Cr(III) sorption are significant at below and above the pH_{ZPC}. Indeed, it can be expected that sorbed Cr(VI) is reduced to Cr(III) by *Spirulina* dead biomass.

Gokhale et al. (2008) noted increasing Cr(VI) sorption using dry *Spirulina* biomass below (up to pH 1) as well as above (up to pH 5) the pH_{ZPC}. Maximum Cr(VI) sorption of 73.6% is reported at pH 1.5, $[Cr(VI)]_0$ 1.92×10^{-3} M (100 mg L^{-1}) and 1 g L^{-1} biomass loading. Cr(VI)

sorption shows a rapid zero-order followed by a slow non-linear sorption regime. *Spirulina platensis* immobilized in calcium alginate gives about 99 % Cr(VI) sorption at pH 1.5, $[Cr(VI)]_0$ 1.92×10^{-3} M at optimized bead size (2.6 mm), biomass loading (2.6% w/v) and calcium alginate concentration (2% w/v) (Gokhale et al., 2009). Cr(VI) is considered to be removed by sorption and Cr(VI) uptake follows the Freundlich isotherm. One year later in 2010, Finocchio et al. (2010) reported that *Spirulina platensis* cannot sorb CrO_4^{2-} near to the neutral pH. The authors used modified *Spirulina* dry biomass. Esterification of carboxyl groups (Eq. 4.1) is performed for Cr(VI) sorption at pH 7-8. Methylated biomass (2 to 4 g L^{-1}) can sorb ≥80% Cr(VI)with 0.34×10^{-3} to 0.48×10^{-3} M $[Cr(VI)]_0$ (17.68 to 25 mg L^{-1}). The Langmuir sorption isotherm shows better agreement to the experimental Cr(VI) uptake.

$$RCOOH + CH_3OH \xrightarrow{H^+} RCOOCH_3 + H_2O \qquad (4.1)$$

Cr(VI) is predominantly reduced to Cr(III) in contact with other biomass species at acidic pH<5 (Park et al., 2004; Yang and Chan, 2008; Park et al., 2008a; Park et al., 2008b; Lopez-Garcıa et al., 2012; Guo et al., 2012). Cr(VI) is irreversibly converted to Cr(III) by *Ecklonia* biomass with almost 100% Cr(VI) removal at 1≤pH≤5 (Park et al., 2004). Cr(VI) reduction shows an inverse dependency on proton concentration. XPS spectra reveal the existence of Cr(III) on *Ecklonia* surface. Raw *Sargassum sp*. seaweed can remove around 48 and 77% Cr(VI) at pH 1 and 2, respectively.

Live microalgae has been successfully used as metal detoxifying agent, because they use light as an energy source facilitating the maintenance of metabolism in the absence of organic carbon sources and electron acceptor required by bacteria or fungi. But it is not clear whether cellular uptake of toxic Cr(VI) occurs with its reduction through adsorption in/on cytoplasm

or periplasm, and/or electrons transferred outside of the cells for extra-cellular Cr(VI) reduction. The mechanism of detoxification of Cr(VI) with live microorganism is greatly dependent on its species and cultivation conditions. The solution pH also influences speciation of Cr(VI) and its removal behaviour. Microbial Cr(VI) reduction is a complex process involving multiple possible pathways. Evidence of two Cr redox intermediates i.e., Cr(IV) and Cr(V), in the microbial reduction of Cr(VI) have been reported by many authors (Suzuki et al., 1992; Myers et al., 2000; Neal et al., 2002; Kalabegishvili et al., 2003).

Generally, Cr(VI) oxy-anions are reduced to Cr(III) either by direct contact with proton donor groups or through adsorption of Cr(VI) at the active sites below the pH_{zpc}(Park et al., 2004; Park et al., 2005). Cr(III) is then desorbed due to electrostatic repulsion (Park et al., 2005). The foremost question comes in our minds that Cr(VI) removal using *Spirulina* biomass is solely by Cr(VI) sorption or, along with Cr(VI) reduction to Cr(III) at acidic pH. *Spirulina* is a cost effective biomaterial and enriched in various functional groups as iterated before, in particular, important for metal scavenging. Therefore, the role of pH on Cr(VI) removal mechanisms using *Spirulina* sp. biomass is explored in this work. The factors affecting Cr(VI) reduction efficiency is investigated. Furthermore, a kinetic model for reduction of Cr(VI) into Cr(III) by protonated functional groups is developed which is not yet reported in the literature. *Spirulina* cultivation in Zarrouk media composition (ZMC) was performed at pH 8.5 with 5 to 100 mg $L^{-1}$of Cr(VI). The performance of live *Spirulina* for Cr(VI) removal and its toxicity on cell growth, if any, was tested in ZMC.

4.2 Kinetic model development

Biomaterials are complex in nature.Itis reflected onthe extent of different Cr(VI) removal mechanisms. The acid dissociation of each mole of *Spirulina* functional group releases equal amount of H^+.Please refer to Chapter 3 (Eq. 3.1). The dissociation reaction of *Spirulina* biomass and determination of concentration of proton donor functional groups are already outlined in Chapter 3. Cr(VI) reduction by a single organic compound is typically 1st order reaction with respect to both Cr(VI) and the organic compound even with significant differences in the reactants (Elovitz and Fish, 1995; Wittbrodt and Palmer, 1995; Park et al., 2007). The reaction of Cr(VI) reduction can be expressed in terms of the biomass-organic compounds capable for the Cr(VI) conversion to Cr(III). It was considered that each protonated functional ground acts a single organic compound in Cr(VI) reduction reaction (Eq. 4.2). The equation ultimately will be empirical in nature rather than mechanistic because of poorly defined organic compound structures (Park et al., 2007).

Cr(VI) reduction reaction into Cr(III) by these proton donor groups at a definite pH, temperature and anionic strength is written as (Eq. 4.2):

$$C_{pi} + Cr(VI) \rightarrow C_{pi,ox} + Cr(III) \quad (i\text{=}1 \text{ to } n) \qquad (4.2)$$

Where, C_{pi} is the concentration of protonated fraction (M) of functional group 'i'of *Spirulina* biomass present in solution. $C_{pi,ox}$ is the amount of C_{pi} oxidized.

The rate of Cr(VI) reduction by proton donor groups at a definite pH, temperature and anionic strength, therefore iswritten as (Eq. 4.3):

$$-\frac{d[Cr(VI)]}{dt} = [Cr(VI)]\sum_{i=1}^{n} k_i[C_{pi}] \quad (4.3)$$

Where, k_i, is the constant (M^{-1} s^{-1}) of Cr(VI) reduction by the proton donor group 'i'.Eq. (4.3) shows first order dependency with respect to both [Cr(VI)] and functional groups concentration. The effect of $[H^+]$ on the degree of protonation/deprotonation is taken care by expressing the rate equation in terms of C_{pi}. Eq. 4.3 is written in terms of weighted overall rate constant (Park et al., 2007) and total protonated functional groups concentration (Eq. 4.4).

$$-\frac{d[Cr(VI)]}{dt} = k_t[C_{pt}][Cr(VI)] \quad (4.4)$$

Where, the overall rate constant,k_t, can be related as $\sum k_i f_i$. $f_i$is the fraction of protonated functional group, '*i*' and C_{pt}, is the total concentration (M) of protonated groups at time,'*t*', It can be experimentally determined by potentiometric titration as outlined in Chapter 3. C_{pt} equals to C_{p0} at $t = 0$. It is assumed that Cr(VI) reduction to Cr(III) takes place primarily by protonated groups.Then we can write on the equivalent reaction basis as in Eq. 4.5 and it takes the form of Eq. (4.6).

$$-\frac{d[Cr(VI)]}{dt} \approx -\sum\frac{d[C_{pi}]}{dt} \quad (4.5)$$

$$[Cr(VI)]_0 - [Cr(VI)] \approx C_{p0} - C_{pt} \quad (4.6)$$

By rearranging the above equation and substituting to Eq. 4.4:

$$-\frac{d[Cr(VI)]}{dt} = k_t\left(\left[C_{p0}\right] - [Cr(VI)]_0 + [Cr(VI)]\right)[Cr(VI)] \quad (4.7)$$

Eq. 4.8 is obtained by integrating Eq. 4.7 with the boundary conditions, at, $t = 0, [Cr(VI)] = [Cr(VI)]_0$ and; at, $t = t, [Cr(VI) = [Cr(VI)]$.

$$[Cr(VI)] = \frac{C_{p0} - [Cr(VI)]_0}{\exp\left\{k_t(C_{p0} - [Cr(VI)]_0)t + ln\frac{C_{p0}}{[Cr(VI)]_0}\right\} - 1} \quad (4.8)$$

The concentration of total proton donor groups initially present on biomass can be estimated from Eq. 4.9. $X$is the amount of biomass (g L^{-1}) added for the reaction.

$$N_{p0} = \frac{C_{p0}}{X} \quad \text{(mol } g^{-1}\text{)} \quad (4.9)$$

4.3 Results and discussion

4.3.1 Studies with dead *Spirulina* biomass

4.3.1.1 Biomass characterization

Cr(VI) reduction is significantly affected with variation of growth media composition even for the same type of biomaterials. The interaction between functional groups and Cr(VI) were explored by comparing the IR spectra of native and Cr(VI) treated *Spirulina* biomass at pH 0.5. The results are illustrated in Figure 4.1. The spectra of native *Spirulina* have broad-band of -OH and -NH groups of glucose and protein at around 3434 cm^{-1}(Park et al., 2005). –CH and C=O stretching of –COOH groups at 2920 and 1639 cm^{-1} can be seen from this figure. The bending of -NH groups at 1545 cm^{-1} due to amide bond of protein peptide, the symmetric bending of CH_3 of acetyl moiety at 1412 cm^{-1} and -CN stretching of proteinaceous fraction at 1048 cm^{-1} were noted (Park et al., 2005). A similar explanation to native *Spirulina* biomass is already provided in Chapter 3 (Section 3.3.1.1). The spectra of Cr(VI) treated *Spirulina* showed the peaks at 1415, 2920 and 2847 cm^{-1}corresponding to C-C, -CH and –OH, respectively. These groups were diminished with Cr(VI) contact to native biomass. -CN at 1048 cm^{-1} was shifted towards 1100 cm^{-1}. The peaks at 873 and 467 cm^{-1} may be attributed to phosphatic groups on native biomass surface. Cr(VI) reduction to Cr(III) resulted in disappearance of the peak at 873 cm^{-1}.The change of functional groups can be attributed to the interaction between Cr(VI) and *Spirulina* biomass.

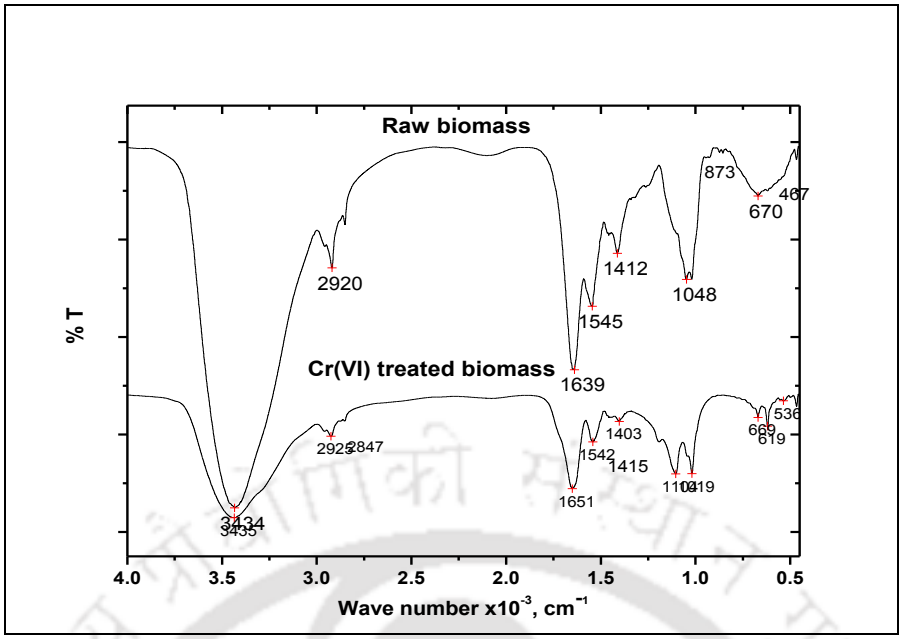

Figure 4.1 IR spectra of Cr(VI) treated and native *Spirulina* biomass. Experimental details with Cr(VI): biosorbent B_4 1 g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and Cr(VI) 3.84×10^{-3} M (100 mg L^{-1}).

4.3.1.2 Influence of pH

Unless or otherwise stated, "reduction" indicates "reduction of Cr(VI) into Cr(III)" and "removal" signifies "removal of total chromium".pH notably affects the solution chemistry of Cr(VI) and as well as the distribution of functional groups of biomass species. The influence of pH on Cr speciation in contact with *Spirulina* biomass is illustrated in Figure 4.2.The results clearly indicate that Cr(VI) reduction reaction favoured acidic pH and it fell down with pH rise. More than 90% Cr(VI) reduction to Cr(III) was achieved at pH 0.5.The electrons needed for the reduction reaction (Eqs. (4.10) to (4.12)) was provided by the biomass functional groups. At higher proton concentration (pH<2), the negative charge inside the pores of biomass got neutralized and new active positive sites were formed. It increased $HCrO_4^-$contact with biomass functional groups and the reduction rate was enhanced (Singh et al., 2005).

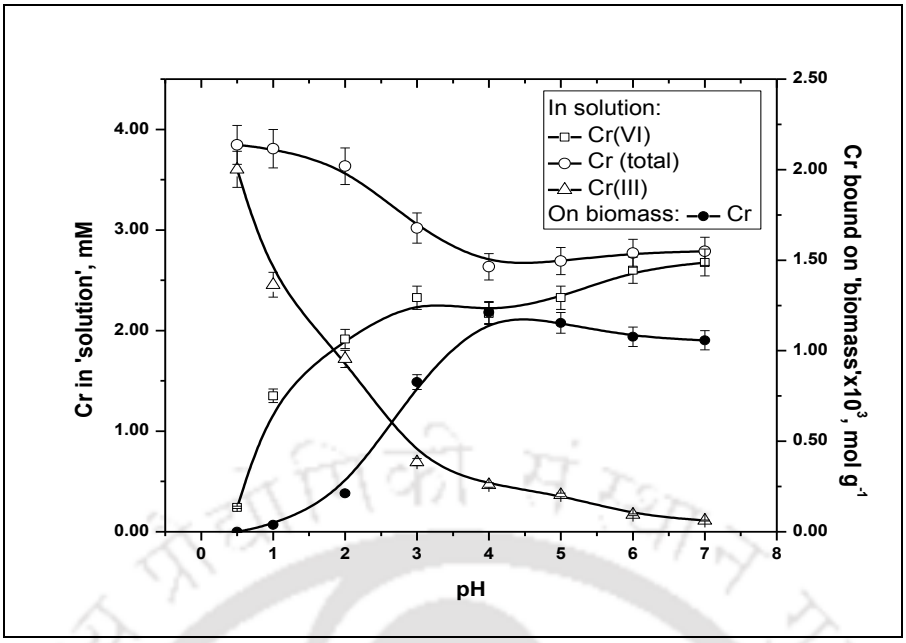

Figure4.2 Chromium speciation as a function of solution pH (contact time 24 h, biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

Cr uptake gradually increased with pH elevation till 4 and then slightly dropped ($4\leq pH\leq7$). The net biomass surface charge was negative at pH_{zpc}>2.84. The major functional groups of *Spirulina* biomass were carboxyl, phosphate and amine (Figure 4.1). These groups were primarily involved in Cr(VI) reduction preceded by Cr(VI) sorption (Park et al., 2004). The presence of Cr on biomass surface increased possibly due to attachment of Cr(VI)) with amines(pK_a=8) and, Cr(III) withcarboxyl (pK_a= 1.19) and phosphatic (pK_a=2.87) groups, respectively. It was also possible that Cr(III) repulsion to strong basic site could decrease Cr(III) transport resistance to acid sites and it might got chelated. Cr(III) occupies to carboxyl and phosphatic sites ($2\leq pH\leq7$) with predominant coverage to the later. Amine groups show lower affinity in Cr(III) binding.

Three moles of electrons are required for the reduction of 1 mol of Cr(VI) to Cr(III) (Eqs. 4.10 to 4.12). Electrons were probably supplied from biomass causing oxidation of biomass organic compounds. It was evidenced by Park et al. (2004) from the increase in the concentrations of dissolved organic compounds and inorganic carbons during reduction Cr(VI) into Cr(III) by *Ecklonia* biomass. They reported that 1 g of *Ecklonia* biomass could supply about 13.5 mmol of available electrons for Cr(VI) reduction. In the present study, *Spirulina* biomass of 1 g at pH 0.5 could reduce 3.6×10^{-3} M of Cr(VI) i.e. 93.4% reduction efficiency was achieved with 3.84×10^{-3} M $[Cr(VI)]_0$ (100 mg L^{-1}). It implies that 1 g biomass could provide about 1.08×10^{-2} M of electrons (Eqs. 4.10 to 4.12).260 g of *Spirulina* biomass is required for the reduction 1 M of Cr(VI). Around 843 g of $FeSO_4.7H_2O$ is needed for the same quantity of Cr(VI) reduction to Cr(III). It is calculated with Cr(VI) to Fe(II) stoichiometric ratio of 1:3 and 99 % purity of ferrous salt by considering 100% efficiency of Fe(II) in Cr(VI) reduction reaction.

$$CrO_4^{2-} + 8H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \qquad E^0 = 1.48 \text{ V vs. NHE} \qquad (4.10)$$

$$HCrO_4^- + 7H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \qquad E^0 = 1.35 \text{ V vs. NHE} \qquad (4.11)$$

$$H_2CrO_4 + 6H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \qquad E^0 = 1.33 \text{ V vs. NHE} \qquad (4.12)$$

The initial rate of Cr(VI) reduction increased with decrease in solution pH (Figure4.3). The effect of pH on the initial rate was almost insignificant towards the higher pH>3. Cr(VI) reduction rate fell down. k_t, and C_{p0} were estimated by least square fit method (Eq. 4.8). The best fit plots at different pH are shown in Figure4.3. The model showed excellent agreement

(R^2=0.99 for all runs) to the experimental results between the whole pH range. $N_{p0,mod}$ was determined from Eq. 4.9.Fitted parameters are summarized in Table 4.1. $N_{p0,mod}$ decreased with increase in pH. This is due to deprotonation of the functional groups. $N_{p0,mod}$ was compared with $N_{p0,pot}$ determined by potentiometric titration. We know that *Spirulina* biomass is consisting of mainly three functional groups i.e. carboxylic, amine and phosphatic and $N_{p0,pot}$ was their cumulative amount. $N_{p0,mod}$ showed very good agreement to $N_{p0,pot}$ (Table 4.1). Again it confirms that Cr(VI) reduction by protonated functional groups of *Spirulina* biomass follows a second order kinetics. The overall rate constant, k_t, was strongly dependent on proton concentration. It showed a logarithmic variation with pH ($logk_t = 0.11 - 2.018\text{pH}^{0.19}$ with R^2 =0.99 and σ =0.0066). Park et al.(2007) reported similar pH dependency on a second order rate constant with brown seaweed *Ecklonia* biomass.

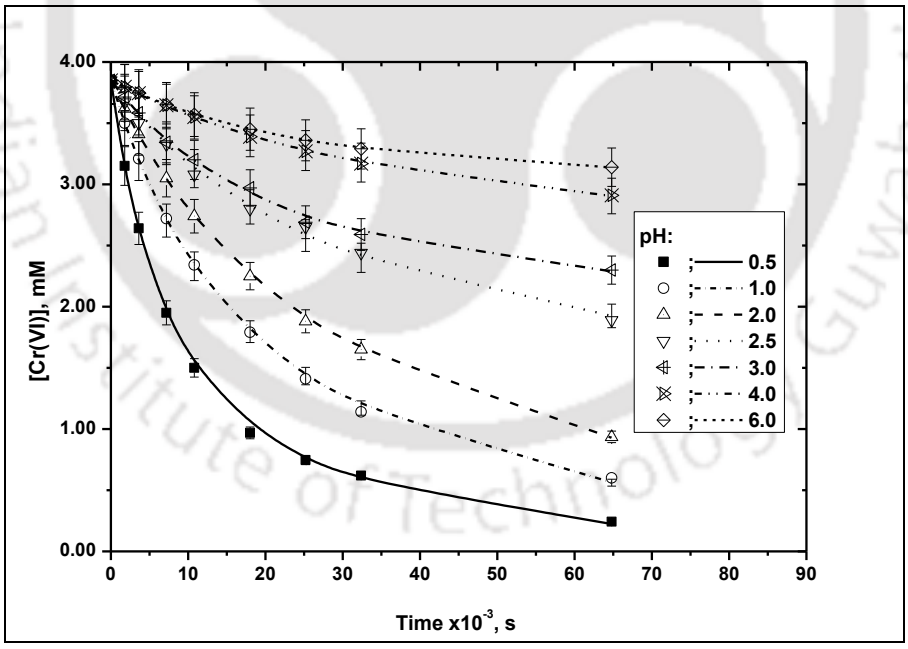

Figure 4.3 Dynamics of Cr(VI) reduction at different pH (biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})). Symbols represent experimental data. Lines are model fitted.

Table 4.1. Rate constants as a function of pH (biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

pH	$^{\dagger}N_{p0,pot}$ (mol g^{-1})	$N_{p0,mod}$ (mol g^{-1})	k_t (M^{-1} s^{-1})	R^2	var	rmsd
0.5	5.21×10^{-4}	5.31×10^{-4}	2.27×10^{-2}	0.99	9.05×10^{-20}	8.84×10^{-11}
1	4.72×10^{-4}	4.51×10^{-4}	1.10×10^{-2}	0.99	5.43×10^{-10}	6.85×10^{-6}
2	3.65×10^{-4}	3.75×10^{-4}	6.09×10^{-3}	0.99	2.14×10^{-10}	4.30×10^{-6}
2.5	3.65×10^{-4}	3.70×10^{-4}	5.20×10^{-3}	0.99	2.14×10^{-9}	1.36×10^{-5}
3	1.63×10^{-4}	1.24×10^{-4}	4.50×10^{-3}	0.99	7.52×10^{-10}	8.06×10^{-6}
4	5.04×10^{-5}	5.83×10^{-5}	3.78×10^{-3}	0.99	9.32×10^{-11}	2.83×10^{-6}
6	1.96×10^{-5}	1.56×10^{-5}	2.80×10^{-3}	0.99	1.42×10^{-11}	1.10×10^{-6}

4.3.1.3 Dependency on the variation of *Spirulina* concentration

Biomass dose can change the reduction efficiency of Cr(VI) through combined effects of two important factors i.e., concentration of proton donor groups and mass transfer resistance of Cr(VI) transport to active sites. The influence of acidic site concentration on Cr(VI) reduction kinetics was investigated with 3.84×10^{-3} M initial Cr(VI) (100 mg L^{-1}) at 25°C and pH 0.5. The results are shown in Figure 4.4. The initial fast rate period even became more faster with increase in biomass concentration and Cr(VI) concentration in solution dropped rapidly. The initial fast rate period was extended upto 1.5 h irrespective to biomass concentration as marked by the vertical dotted line in Figure 4.4. Cr(VI) reduction increased from 41 to 87% when biomass concentration was increased from 1 to 4 g L^{-1}. However, the corresponding amount of Cr(VI) reduction (mmol) per unit biomass dose decreased from 1.74 to 0.95 at pH 0.5. It indicates that the extended Cr(VI)-biomass contact increased cumulative Cr(VI) reduction efficiency at lower dose. The effect was less significant at higher dose of biomass.

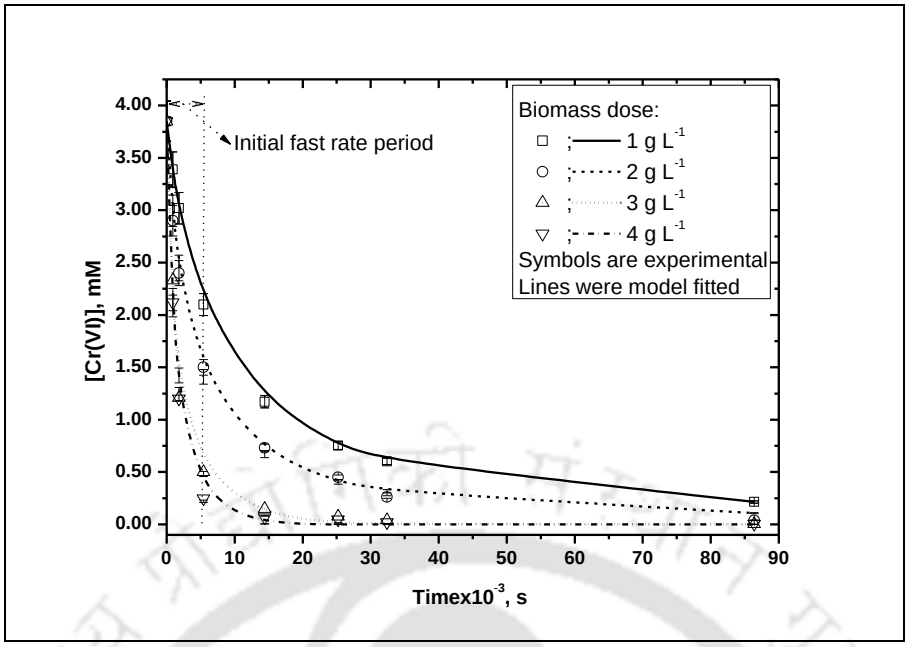

Figure4.4 Biomass dose vs. Cr(VI) reduction kinetic (biosorbent B_4, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

The model exhibited good agreement to the experimental results at different biomass concentrations (Figure4.4). The overall rate constant,k_t, was independent on biomass dose (Table 4.2). The well accordance of$N_{p0,mod}$ and $N_{p0,pot}$werealso noted. Slight fall in rate constant (max 6%) was noted at higher biomass dose. It might be resulted from the agglomeration of biomass particles.

Table 4.2. Fitted parameters with different biomass dose (biosorbent B_4, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

Biomass (g L^{-1})	$N_{p0,pot}$ (mol g^{-1})	$N_{p0,model}$ (mol g^{-1})	k_t (M^{-1} s^{-1})	R^2	var	rmsd
1	5.21×10^{-4}	5.31×10^{-4}	2.27×10^{-2}	0.99	1.05×10^{-9}	9.56×10^{-6}
2	5.20×10^{-4}	5.40×10^{-4}	2.25×10^{-2}	0.98	3.77×10^{-8}	5.94×10^{-5}
3	5.20×10^{-4}	5.30×10^{-4}	2.24×10^{-2}	0.99	1.48×10^{-8}	3.73×10^{-5}
4	5.30×10^{-4}	5.00×10^{-4}	2.23×10^{-2}	0.99	1.71×10^{-9}	1.26×10^{-5}

4.3.1.4 Effect of variation of $[Cr(VI)]_0$

The increase in Cr(VI) concentration improved Cr(VI) reduction per unit biomassas the ratio of Cr(VI) to functional group concentration increased. Significant biomass oxidation was also observed at higher Cr(VI) concentration (>7.69×10^{-3} M Cr(VI) per g of biomass).The percentage Cr(VI) reduction decreased from 99.2 to 75.7 with increase in initial Cr(VI) from 0.96×10^{-3} to 7.69×10^{-3} M at pH 0.5 (Figure4.5). The corresponding $N_{p0,pot}$ varies from 0.53 to 0.535mmol per g. Therefore, there about 0.53 to 0.535mM $N_{p0,pot}$ was added in solution with 1 g L^{-1} *Spirulina* biomass at pH 0.5. It implies that the initial concentration of Cr(VI) is almost 1.8 to 14.5 times that of the surface sites.

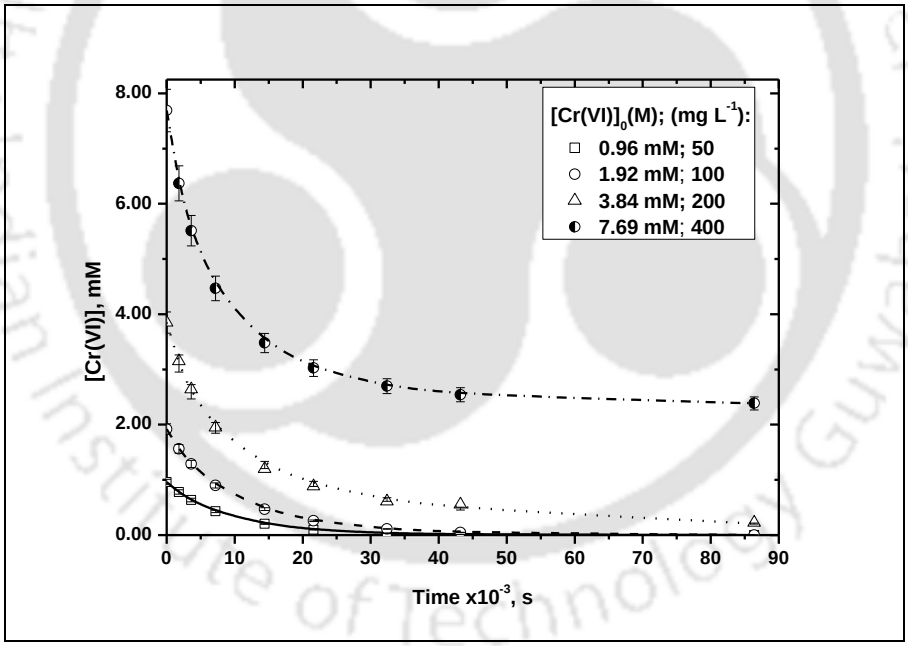

Figure 4.5 Different initial Cr(VI) concentration vs. reduction kinetic (biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5 and temperature 25°C). Symbols represent experimental data. Lines are model fitted.

Chromium on the biomass surface was mostly in trivalent form. Sorbed Cr(VI) on biomass, if any, was determined by dissolving it (digesting) in 10% (w/v) NaOH solution. Mg^{2+} in

phosphate buffer was added to suppress Cr(III) oxidation to the alkaline solution. No Cr(VI) was found by alkaline digestion. The observation is in line with the literature (Park et al., 2007). Cr(III) sorption increased with rise of Cr(VI) dose even at low pH. Functional groups were oxidized and; biomass probably got some negative sites which would bind Cr(III). Proton consumption was also increased with rise of Cr(VI) dose.It caused proton deficiency. The biomass surface became favourable towards Cr(III) binding. Cr(total) sorbed onto biomass surface showed linearity with Cr(III) concentration in solution at equilibrium (Figure 4.6). It can be expressed by the relationship as: $\mathrm{Cr_{total}}\left(\frac{\mathrm{mM}}{\mathrm{g\ biomass}}\right) = 0.333[\mathrm{Cr(III)}] - 0.0002(\mathrm{R}^2 = 0.99\ \text{and}\ \sigma = 0.0005)$.

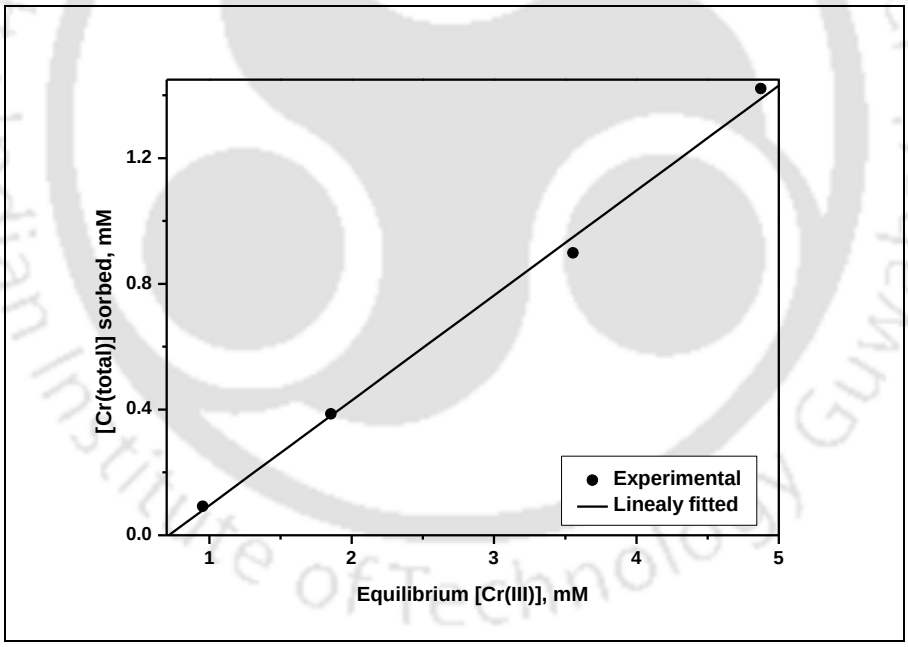

Figure 4.6 Sorption of total chromium at equilibrium Cr(III) concentration (contact time 24 h, biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5 and temperature 25°C).

The average k_t of 2.27×10^{-2} M^{-1} s^{-1}($\sigma=1\times10^{-4}$) and $N_{p0,mod}$ of 5.31×10^{-4}mol g^{-1}($\sigma=2.22\times10^{-6}$) were found with different initial Cr(VI) concentrations at pH 0.5 and 1 g L^{-1} of biomass.

R^2=0.99 was for the all runs (Table 4.3). These results are in good line with Park et al. (2007). However, they reported that the overall second order rate constant with respect to [Cr(VI)] and equivalent concentration of organic compounds, slightly fallen down to 2.40×10^{3} from 2.96×10^{3} M^{-1} s^{-1} by elevating $[Cr(VI)]_0$ from 0.962×10^{-3} to 3.864×10^{-3} M (50 to 200 mg L^{-1}).

Lopez-Garcia and co-workers (2012) showed that first order rate constant of Cr(VI) reduction by *Sargassum* biomass is greatly influenced by initial concentration of Cr(VI). There is around 235% fall in rate constant when initial Cr(VI) concentration was varied from 3.84×10^{-4} to 3.84×10^{-3} M (20 to 200 mg L^{-1}). It corroborates that Cr(VI) reduction by biomaterials are rather 2nd order dependent.

Table 4.3. Best fit parameters with different initial Cr(VI) concentrations (biosorbent B_4, 1g L^{-1}, stirring speed 200 rpm, pH 0.5 and temperature 25°C).

Cr(VI) mg L^{-1}	$N_{po,model}$ (molg^{-1})	k_t (M^{-1} s^{-1})	R^2	σ	var	rmsd
50	5.30×10^{-4}	2.27×10^{-2}	0.99	1.2×10^{-3}	2.58×10^{-20}	4.72×10^{-11}
100	5.31×10^{-4}	2.27×10^{-2}	0.99	1.2×10^{-3}	6.67×10^{-20}	7.59×10-^{11}
200	5.31×10^{-4}	2.27×10^{-2}	0.99	1.2×10^{-3}	9.05×10^{-20}	8.84×10^{-11}
400	5.35×10^{-4}	2.29×10^{-2}	0.99	1.2×10^{-3}	9.59×10^{-20}	9.10×10^{-11}

4.3.1.5 Influence of background anions

Industrial effluents generally contain large amounts of background anions along with heavy metal ions which can affect the removal of primary metal (Ting and Teo, 1994; Matheickala and Yu, 1999). The effects of concentration of different anions on the reduction efficiency of Cr(VI) were studied with Cl^-, SO_4^{2-} and NO_3^- (Figure 4.7 to 4.9). The dependency of background anions on Cr(VI) reduction efficiency was studied at pH 0.5 and 25ºC. The results show that the presence of anions supresses the reduction of Cr(VI) with *Spirulina* biomass. The suppression effect of various anions is found to be different under identical

experimental conditions. It increases with the increase in anions concentration. The different species of chromium also interact differently with these anions. $HCrO_4^-$and $CrO_4^{2-}$are the two dominating species in presence of nitrate and chloride; however these species go on decreasing in presence of sulphate. The anionic strength was varied from 5.88×10^{-4} to 5.63×10^{-3} M. The corresponding ionic strength was between 1.18×10^{-3} and1.69×10^{-2} M(Table 4.4). The presence of common anions decreased the values of the constants of the rate equation. The effect was moreat higher anions concentration. The inhibition effect of NO_3^-on Cr(VI) reduction to Cr(III) was more than that of SO_4^{2-} and Cl^-. This is possibly due to different accessibility of available protonated *Spirulina* biomass surfaces/sites by different anions.

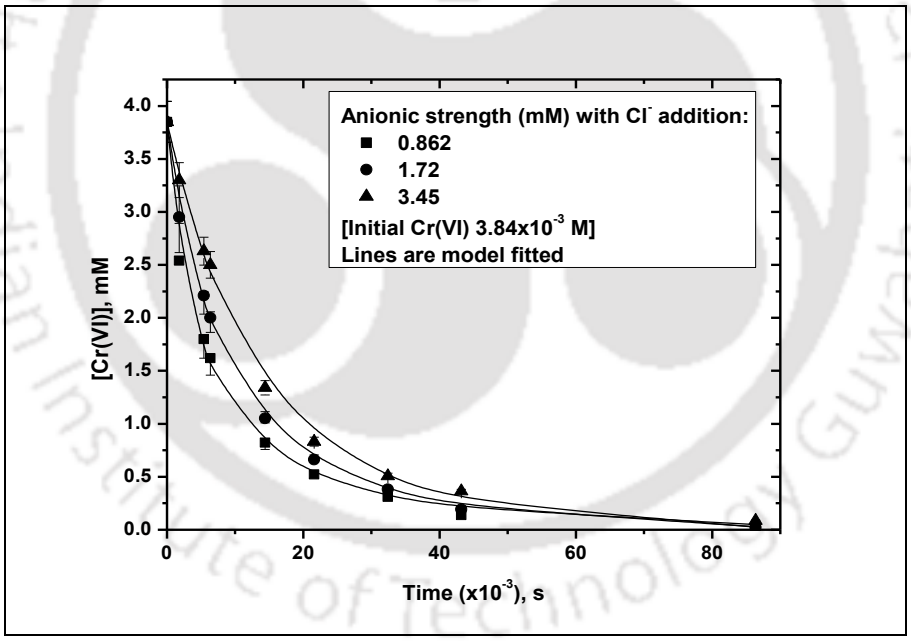

Figure 4.7 Effect of Cl^- on Cr(VI) reduction(biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

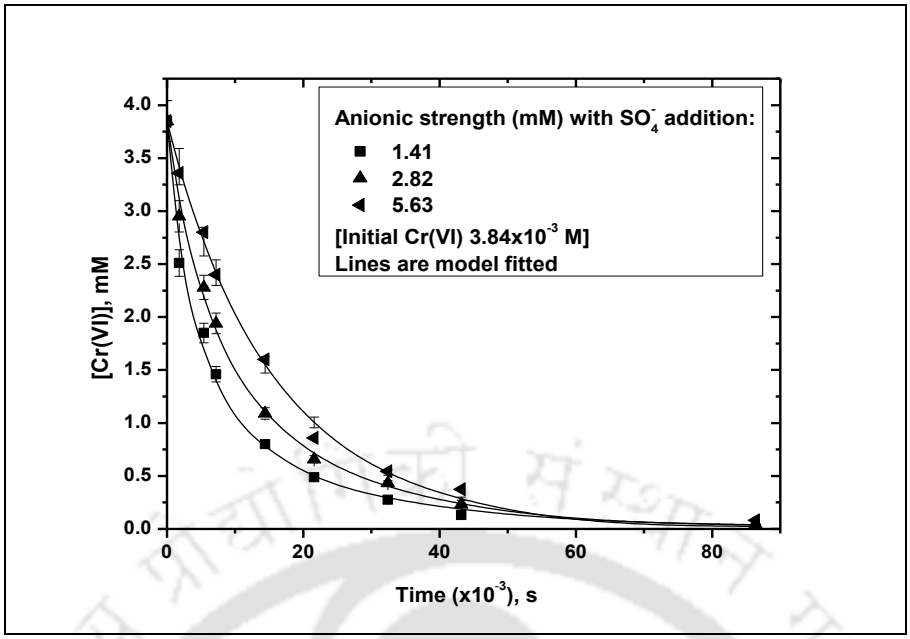

Figure 4.8 Effect of SO_4^{2-} on Cr(VI) reduction(biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

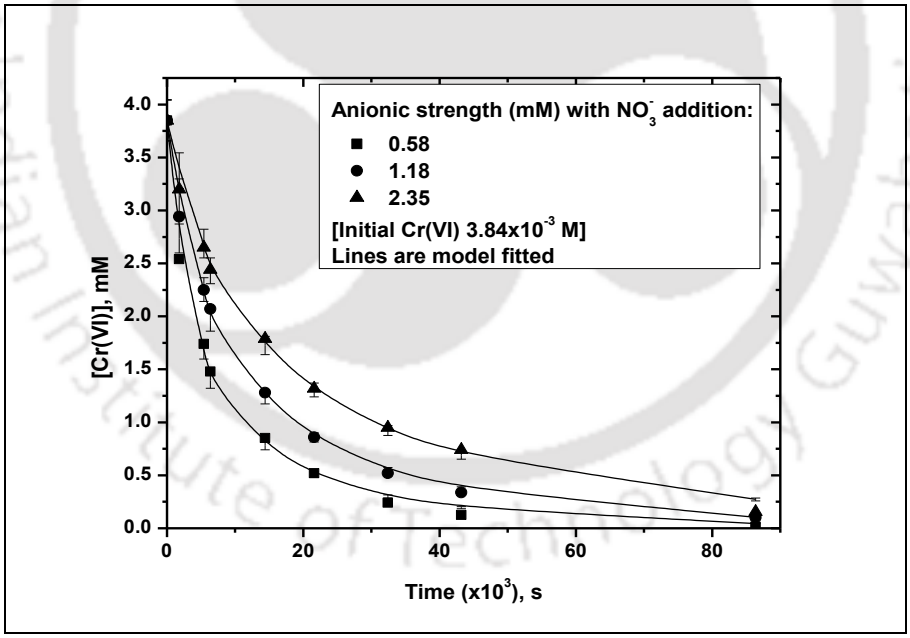

Figure 4.9 Effect of NO_3^- on Cr(VI) reduction(biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

Anions could compete to Cr(VI) sorption for the same acidic sites. The hydration energy of anions plays an important role for the sorption. Anions with lower hydration energy are easily adsorbed(Lo and Shiue, 1998;Gu et al., 2004; Han et al. 2008). SO_4^{2-} has the highest energy followed by Cl^- and then NO_3^-. While, a new species $CrO_3SO_4^{2-}$ could form in SO_4^{2-} system which is easily sorbed onto biomass (Han et al. 2008). Han et al. (2008) reported that the inhibitory effect of anions for Cr(VI) removal with *Chlorella miniata* occurs in the order of $NO_3^- > Cl^- > SO_4^{2-}$. They pointed out that $HCrO_4^-$ and CrO_4^{2-} anions are dominating in presence of NO_3^- and Cl^-, and they go on decreasing in SO_4^{2-} system. However, we couldn't find any specific relation between the effect of Cr(VI) speciation and reduction kinetics.

High concentration of background anions was resulted in fall of k_t values. It can be seen from Table 4.4 that $N_{p0,mod}$ notably decreased with rise of NO_3^- concentration as compared to SO_4^{2-} and Cl^-. The extent of protonation of functional groups dropped with NO_3^- at the same pH.

Table 4.4. Rate constants at varying anionic strength (biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5, temperature 25°C and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

Ion source	Anionic strength I, (M)	Ionic strength, (M)	$N_{p0,mod}$ (mol g^{-1})	k_t (M^{-1} s^{-1})	R^2	var	rmsd
Cl^-	8.62×10^{-4}	1.71×10^{-3}	5.31×10^{-4}	2.63×10^{-2}	0.99	1.16×10^{-8}	3.17×10^{-5}
	1.72×10^{-3}	3.42×10^{-3}	5.72×10^{-4}	2.53×10^{-2}	0.98	1.14×10^{-8}	3.14×10^{-5}
	3.45×10^{-3}	6.85×10^{-3}	5.87×10^{-4}	2.29×10^{-2}	0.98	1.69×10^{-8}	3.83×10^{-5}
SO_4^{2-}	1.41×10^{-3}	4.23×10^{-3}	5.55×10^{-4}	2.82×10^{-2}	0.98	2.12×10^{-8}	4.28×10^{-5}
	2.82×10^{-3}	8.45×10^{-3}	5.68×10^{-4}	2.37×10^{-2}	0.99	9.16×10^{-9}	2.81×10^{-5}
	5.63×10^{-3}	1.69×10^{-2}	5.71×10^{-4}	2.35×10^{-2}	0.99	1.40×10^{-8}	3.48×10^{-5}
NO_3^-	5.88×10^{-4}	1.18×10^{-3}	5.89×10^{-4}	2.80×10^{-2}	0.98	3.80×10^{-8}	5.73×10^{-5}
	1.18×10^{-3}	2.35×10^{-3}	5.14×10^{-4}	2.24×10^{-2}	0.98	1.81×10^{-8}	3.96×10^{-5}
	2.35×10^{-3}	4.71×10^{-3}	5.12×10^{-4}	2.13×10^{-2}	0.98	3.02×10^{-8}	5.11×10^{-5}

4.3.1.6 Temperature dependency

Temperature was varied from 25 to 45°C. The reduction efficiency increased with rise of temperature. Cr(VI) reduction of about 25, 35 and 55 % were observed within the first 1 h at 25, 35 and 45ºC, respectively (Figure4.10). This attributes to: (i) the reduction reaction is endothermic in nature, (ii) the mobility of Cr(VI) ions is augmented with rise of solution temperature and, (iii) higher temperature might produce a swelling effect within the internal structure of *Spirulina* biomass enabling enhanced biomass-Cr(VI) contact (Dogan and Alkan, 2003).

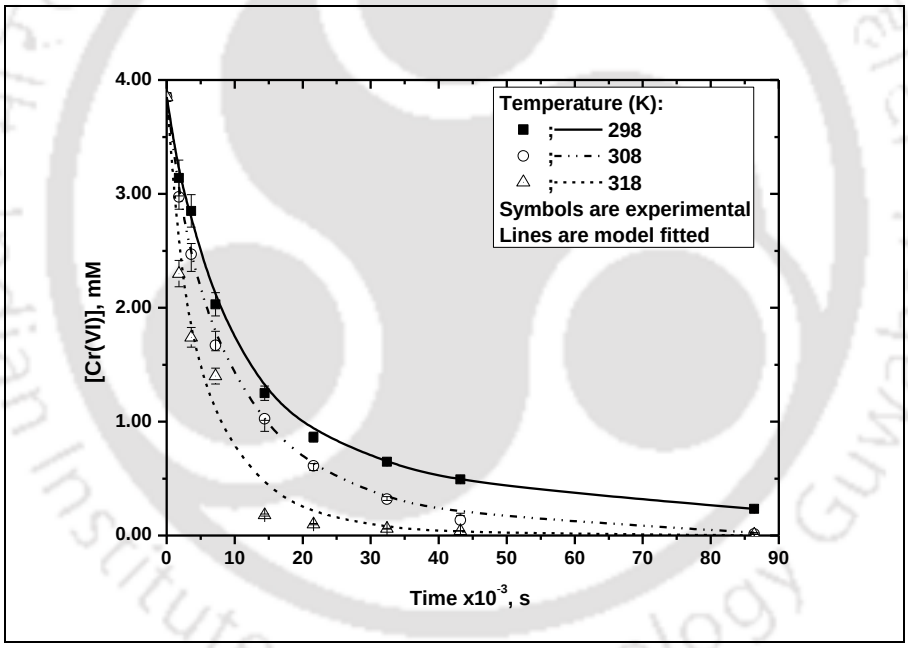

Figure 4.10 Effect of temperature on the variation of Cr(VI) reduction rate (biosorbent B_4 1 g L^{-1}, stirring speed 200 rpm, pH 0.5 and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

The variation of rate law constants with temperature is summarized in Table 4.5. At higher temperature, the concentration of protonated function groups increased slightly (max 8.5%).

Increase in $N_{p0,mod}$ at higher temperature might be resulted from activation or reopening of new sites.

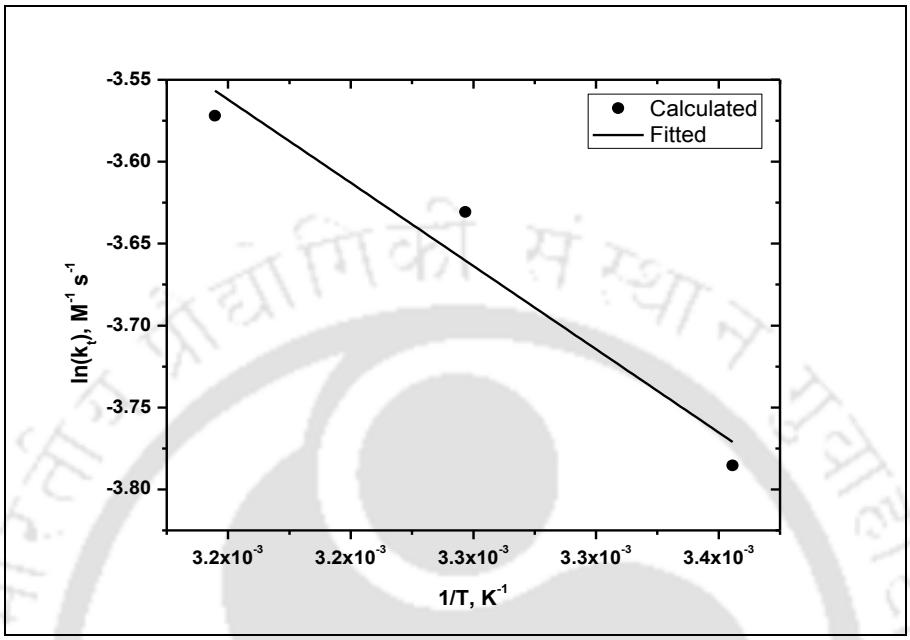

Figure 4.11 Activation energy of Cr(VI) reduction into Cr(III) using *Spirulina platensis*(biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5 and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

The overall rate constant exhibited excellent agreement to the Arrhenius equation (σ=0.002, var= 1.06×10^{-5},R^2 =0.99). The activation energy and the pre-exponential factor were found as 8.4 kJ mol^{-1} and 1.43 M^{-1} s^{-1} (Figure 4.11).The order of magnitude of the activation energy indicates that the chemical reaction control for Cr(VI) reduction is not likely, instead of that an apparent catalytic effect of biomass is more significant (Huang et al., 2011). This is in close agreement to the activation energy of 7.8±0.4 for Cr(VI) reduction using *Aspergillus niger* (Park et al., 2005).

Table 4.5. Rate constants and acidic site concentration for the reduction of Cr(VI) with *Spirulina* biomass as a function of temperature (biosorbent B_4 1g L^{-1}, stirring speed 200 rpm, pH 0.5 and initial Cr(VI) 3.84×10^{-3} M (100 mg L^{-1})).

Temperature (K)	$N_{p0,mod}$ (mol g^{-1})	k_t (M^{-1} s^{-1})	R^2	var	rmsd
298	5.31×10^{-4}	2.27×10^{-2}	0.99	1.05×10^{-9}	9.56×10^{-6}
308	5.55×10^{-4}	2.65×10^{-2}	0.98	1.53×10^{-9}	1.15×10^{-5}
318	5.77×10^{-4}	2.81×10^{-2}	0.99	3.69×10^{-8}	5.64×10^{-5}

4.3.1.7 Overall rate constant

It was observed that the rate of Cr(VI) reduction onto *Spirulina* biomass was dependent on solution chemistry. The dependency of the overall rate constant on proton concentration, anionic strength and temperature can be expressed by the following relationship (Eq.4.13). This equation can be used to calculate the rate of Cr(VI) reduction using *Spirulina* biomass over a broad range of experimental conditions.

$$\log k_t = 0.106 - 1.91pH^{0.18} + 0.0496I^{3.36\times10^{-10}} - \frac{20.8}{T} \qquad (4.13)$$

(σ=0.327, var=0.107, R^2=0.976)

k_t showed strong dependency on pH thanreaction temperature and anionic strength. The rate constants of Cr(VI) reduction have been compared with the previous studies for other biomaterials. Park et al. (2007) reported that Cr(VI) reduction rate is proportional to Cr(VI) and equivalent concentration of bio-organic compounds of *Ecklonia* biomass. The overall rate constant, k_t, varieslinearly with $[H^+]^{0.714}$at $3.5 \leq pH \leq 1.5$. An average k_t of 9.33 ± 0.65 μM^{-1} $h^{-1}$is obtained at pH 2 with 5 to 20 g L^{-1} biomass and 0.962×10^{-3} to 3.846×10^{-3}M initial concentration of Cr(VI) (50 to 200 mg L^{-1}).The model doesn'texhibit any specific relation

between the rate constant and at pH>3.5.The rate constant obtained in our study was higher in comparison to pine needle in the same pH range (Park et al., 2008b). Guo et al. (2012) observed that the rate constant with *Escherichia coli* significantly increases (176%)with rise of temperature from 20 to 50°C.Lopez-Garcıa et al. (2012)reported that first order rate constant using *Sargassum muticum* increased with increase in Cr(VI) concentration.

Biomaterial is complex in nature and the composition is affected by its origin and cultivation conditions. Functional groups provide electrons for Cr(VI) reduction and they themself get oxidized. The degree of protonation and deprotonation of biomass functional groups are primarily controlled by solution pH (Vilar et al., 2012). The overall second order rate constant in terms [Cr(VI)] and proton donor functional groups of *Spirulina* biomass were independent on [Cr(VI)] and biomass dose.

4.3.1.8 Conceptual mechanism of Cr(VI) reduction into Cr(III) using biomaterials

There are two strong possible pathways for Cr(VI) reduction into Cr(III) using non-living biomass. (i) Direct Cr(VI) reduction could take place by direct contact with the electron-donating groups of biomass. (ii) Whereas, indirect reduction involves adsorption of Cr(VI) at the positively charged sites and then reduction to Cr(III). Electrons required for this reaction is supplied by the electron donor groups which is adjacent to the adsorbed Cr(VI) on the biomass sites and Cr(III) is expelled from the surface because of charge repulsion (Park et al. 2005). H^+ can easily coordinate with carboxyl and amine groups at lower pH and abundance of these electron donor groups enhances Cr(VI) reduction in indirect route (Park et al. 2005). However, low pH favours Cr(VI) reduction in both the cases. The contribution of a specific mechanism for Cr(VI) reduction depends on pH, reaction temperature, type of biomass

material and concentration of biomass, Cr(VI) and background ions. Non-living biomass forms clusters or lumps of individual biomass particles (Section 3.3.1.2 of Chapter 3) and pores can be originated between the inter-particles void spaces. The mobility of Cr(VI) is limited in pores even with bulk agitation. Hence, it seems that the second mechanism of Cr(VI) reduction prevails inside the void spaces/pores. A diagrammatic presentation of Cr(VI) reduction mechanisms is illustrated in Figure 4.12.

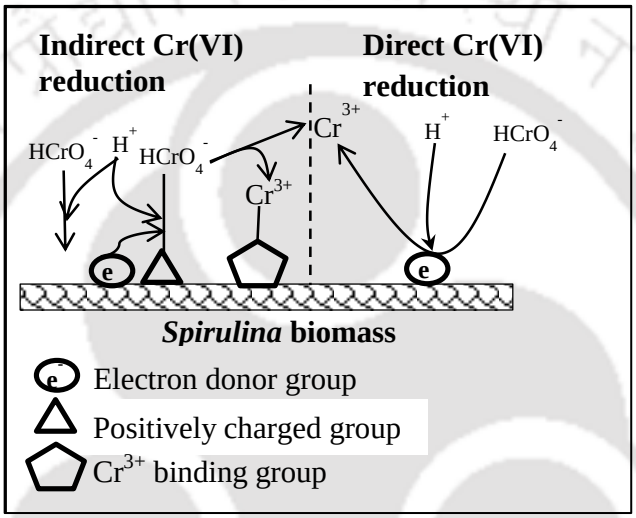

Figure 4.12. Schematic representation of reduction mechanisms of Cr(VI) using *Spirulina* biomass.

4.3.2 Performance of live *Spirulina* for Cr(VI) reduction and its toxicity on cell growth

Cr(VI) in ZMC was used for the cultivation of *Spirulina platensis* and its simultaneous removal/reduction into Cr(III). Details of cultivation conditions are outlined in Chapter 2 (Section 2.3.4). The effect of Cr(VI) concentration in the range from 5 to 100 mg $L^{-1}$was taken to test its toxicity on *Spirulina* cultivation. Reduction of Cr(VI) and *Spirulina* growth at

pH 8.5 is summarized in Table 4.6. Addition of Cr(VI) in Zarrouk media composition didn't show inhibitory effect on *Spirulina* growth and Cr(VI) removal was almost negligible. The pH_{ZPC} of *Spirulina* biomass is around 2.85 and Cr(VI) oxy anions suffer a repulsive force on and at the biomass surfaceat growth media pH(~8.5). Lower proton concentration as well as charge repulsion limit the reduction efficiency Cr(VI) to Cr(III) using live *Spirulina platensis* at higher pH. Algal tolerance to heavy metal seems to be highly dependent on the defence responses against the possible oxidative damages (Pinto et al., 2003), exudation capacity of chelating compounds and metal uptake decreases (Gaur and Rai, 2001).Various mechanisms such as exclusion of toxic heavy metals from cells by ion selective metal transporters and excretion or compartmentalization have been proposed for the minimization of heavy metal toxicity to organisms (Hu et al., 2001; Gharieb and Gadd, 2004). The dynamics of cell growth and evaluation of growth parameters with Cr(VI) in ZMC are provided in Chapter 5 (Section 5.3.7).

Table 4.6. Influence of initial [Cr(VI)] on *Spirulina* growth.

Initial Cr(VI), mg L^{-1}	Growth period 30 days, photo: dark period (h/h)=12:12, Initial inoculum 0.219 gL^{-1},agitation speed 150 rpm		
	***Spirulina* growth, g L^{-1}**	**Residual Cr(VI), mg L^{-1}**	**Cr(III)**
0	2.03	-	-
5	1.98	4.97	N.D.
10	1.92	8.87	N.D.
20	1.94	17.42	N.D.
50	1.86	46.65	N.D.
100	1.81	97.58	N.D.

N.D.: Not detectable

4.4 Important findings

Spirulina is an effective biomass for Cr(VI) reduction into Cr(III) over a wide range of pH, anionic strength and temperature. The major findings are:

- Cr(VI) reduction into Cr(III) showed highest dependency on proton concentration. Maximum 98% reduction was obtained at pH 0.5 with 0.96×10^{-3} M $[Cr(VI)]_0$.

- Higher temperature increased Cr(VI) reduction rate as well as the acidic site concentration. The activation energy of Cr(VI) reduction reaction was found as 8.4 kJ mol^{-1}.

- Slight detrimental effect of common anions on Cr(VI) reduction was noted in the order of: $NO_3^- > Cl^- > SO_4^{2-}$.

- The overall second order rate constant can be expressed in terms of pH as: $logk_t = 0.11 - 2.018pH^{0.19}$. However, no notable effect of initial Cr(VI) and biomass concentration were observed on the estimated kinetic parameters.

- The model predicted $N_{p0,mod}$ exhibited excellent agreement to that of from the potentiometric titration based on a nonideal competitive metal sorption model.

- Live *Spirulina platensis* with Cr(VI) of 5 to 100 mgL^{-1}, didn't show any toxic effect on cell growth and Cr(VI) removal was insignificance at pH 8.5.

Nomenclature

$\equiv BH$	Protonated *Spirulina* biomass active sites
C_{p0}	Total initial concentration of protonated fraction of functional groups, M
C_{pi}	Concentration of protonated fraction of functional group 'i', M
$C_{pi,ox}$	Concentration of C_{pi} oxidized during Cr(VI) reduction, M
$[Cr(VI)]_0$	Initial Cr(VI) concentration, M
C_{pt}	Total concentration of protonated fraction of functional groups at time=t, M
f_i	Fraction of protonated functional group 'i'
I	Anionic strength, M
K_{ai}	Dissociation constant of functional group 'i' of *Spirulina* biomass, M^{-1}
k_i	Second order rate constant of Cr(VI) reduction by proton donor group 'i', M^{-1} s^{-1}
k_t	Overall weighted rate constant, M^{-1} s^{-1}
NHE	Normal hydrogen electrode
N_i	Number of 'i' type functional group, mol g^{-1}
N_{p0}	Total number of protonated functional groups, mol g^{-1}
$N_{p0,mod}$	Total number of model fitted protonated acidic site, mol g^{-1}
$N_{p0,pot}$	Total number of protonated acidic site determined potentiometrically, mol g^{-1}
pH_{zpc}	pH at the point of zero charge
t	Time of Cr(VI) reduction using *Spirulina* biomass, s
X	Amount of biomass added for Cr(VI) reduction, g L^{-1}
α_i	Degree of dissociation of functional group 'i'

References

Alvarado, L., Torres, I.R. and Chen, A., "Integration of Ion Exchange and Electrodeionization as a New Approach for the Continuous Treatment of Hexavalent Chromium Wastewater", *Sep. Purif. Technol*., **105**, 55-62 (2013).

APHA, *Standard Methods for the Examination of Water and Wastewater*, twentieth ed. American Public Health Association, Washington (1998).

Chirwa, E.M.N. and Wang, Y.T., "Hexavalent Chromium Reduction by *Bacillus Sp.* in a Packed-Bed Bioreactor", *Environ. Sci. Technol*., **31**, 1446-1451 (1997).

Dermou, E., Velissariou, A., Xenos, D. andVayenas, D.V., "Biological Chromium(VI) Reduction Using a Trickling Filter", *J. Hazard. Mater*., **B126**, 78-85 (2005).

Dogan, D. and Alkan, M., "Adsorption Kinetics of Methyl Violet onto Perlite", *Chemosphere*, **50**, 517-528 (2003).

Elovitz, M.S., and Fish, W., "Redox Interactions of Cr(VI) and Substituted Phenols: Products and Mechanism", *Environ. Sci. Technol*., **29**, 1933-1943 (1995).

Finocchio, E., Lodi, A., Solisio, C. andConverti, A., "Chromium (VI) Removal by Methylated Biomass of *Spirulina Platensis*: The Effect of Methylation Process", *Chem. Eng. J*., **156**, 264-269 (2010).

Gaur, J. P., and L. C. Rai, Heavy Metal Tolerance in Algae. In: *Algal Adaptation to Environmental Stresses: Physiological, Biochemical and Molecular Mechanisms*. Rai L. C., and Gaur J. P., eds., Springer-Verlag, Berlin, (2001) pp. 363-388.

Gharieb, M. M., and Gadd, G. M. "Role of Glutathione in Detoxification of Metal (Loid)s by *Saccharomyces Cerevisiae*", *Biometals*, **17**, 183-188(2004).

Gokhale, S.V., Jyoti, K.K. andLele, S.S., "Kinetic and Equilibrium Modeling of Chromium (VI) Biosorption on Fresh and Spent *Spirulina Platensis*/*Chlorella Vulgaris* Biomass", *Bioresour. Technol*., **99**, 3600-3608 (2008).

Gokhale, S.V., Jyoti, K.K. andLele, S.S., "Modeling of Chromium (VI) Biosorption by Immobilized *SpirulinaPlatensis* in Packed Column", *J. Hazard. Mater*., **170**, 735-743 (2009).

Golder, A.K., Chanda, A.K., Samanta, A.N. and Ray, S., "Removal of Hexavalent Chromium by Electrochemical Reduction-Precipitation: Investigation of Process Performance and Reaction Stoichiometry", *Sep. Purif. Technol*., **76(3)**, 345-350 (2011).

Gu, B., Ku, Y. K. and Jardine, P. M., "Sorption and Binary Exchange of Nitrate, Sulfate, and Uranium on an Anion-Exchange Resin", *Environ. Sci. Technol.*, **38**, 3184-3188 (2004).

Guo, J., Lian, J., Xu, Z., Xi, Z., Yang, J., Jefferson, W., Liu, C., Li, Z. andYue, L., "Reduction of Cr(VI) by *Escherichia Coli BL21*in the Presence of Redox Mediators", *Bioresour. Technol*., **123**, 713-716 (2012).

Han, X., Wong, Y.S., Wong, M.H. and Tam, N.F.Y., "Effects of Anion Species and Concentration on the Removal of Cr(VI) by a Microalgal Isolate, *Chlorella Miniata*", *J. Hazard. Mater*., **158**, 615-620 (2008).

Hsu, H.T., Chen, S.S. and Chen, Y.S., "Removal of Chromium(VI) and Naphthalenesulfonate from Textile Wastewater by Photocatalysis Combining Ionic Exchange Membrane Processes", *Sep. Purif. Technol*., **80(3)**, 663-669 (2011).

Hu, S., Lau, K. W. K. and Wu, M,. "Cadmium Sequestration in *Chlamydomonas Reinhardtii*", *Plant Sci.*,**161**, 987-996(2001).

Ishibashi, Y., Cervantes, C. and Silver, S., "Chromium Reduction in *Pseudomonas Putida*", *Appl. Environ. Microbiol.*, **56**, 2268-2270 (1990).

Kalabegishvili, T.L., Tsibakhashvili, N.Y. and Holman, H. Y.N. "Electron Spin Resonance Study of Chromium(V) Formation and Decomposition by Basalt-Inhabiting Bacteria", *Environ. Sci. Technol.*, **37**, 4678-4684(2003).

Kurniawan, T., Chan, G., Lo, W. and Babel, S., "Physicochemical Treatment Techniques for Wastewater Laden with Heavy Metals", *Chem. Eng. J.*, **118**, 83-98 (2006).

Lo, S. L. and Shiue, S. F., "Recovery of Cr(VI) by Quaternary Ammonium Compounds", *Water Res.*, **32**, 174-178, (1998).

Lopez-Garcıa, M., Lodeiro, P., Herrero, R. and de Vicente, M.E.S., "Cr(VI) Removal from Synthetic and Real Wastewaters: The Use of the Invasive Biomass *Sargassum Muticum* in Batch and Column Experiments", *J. Ind. Eng.Chem.*, **18**, 1370-1376 (2012).

Matheickal, J.T. and Yu, Q., "Biosorption of Lead (II) and Copper (II) from Aqueous Solutions by Pre-Treated Biomass of Australian Marine Algae", *Bioresour. Technol.*, **69(3)**, 223-229 (1999).

Myers, C.R., Carstens, B.P., Antholine, W.E. and Myers, J.M. "Chromium(VI) Reductase Activity is Associated with the Cytoplasmic Membrane of Anaerobically Grown *Shewanella Putrefaciens* MR-1", *J. Appl. Microbiol.*, **88**, 98-106(2000).

Neal, A.L., Lowe, K., Daulton, T.L., Jones-Meehan, J. and Little, B.J. "Oxidation State of Chromium Associated with Cell Surfaces of *Shewanella Oneidensis* During Chromate Reduction", *Appl. Surf. Sci.*, **202**, 150-159(2002).

Park, D., Yun, Y.S., Ahn, C.K. and Park, J.M., "Kinetics of the Reduction of Hexavalent Chromium with the Brown Seaweed *Ecklonia* Biomass", *Chemosphere*, **66**, 939-946 (2007).

Park, D., Yun, Y.S. and Park, J.M., "Studies on Hexavalent Chromium Biosorption by Chemically-Treated Biomass of *EckloniaSp.*", *Chemosphere*, **60**, 1356-1364 (2005).

Park, D., Yun, Y.S., Jo, J. H. and Park, J. M., "Mechanism of Hexavalent Chromium Removal by Dead Fungal Biomass of *Aspergillus Niger*", *Wat.Res.*, **39**, 533-540, (2005).

Park, D., Yun, Y.S., Kim, J.Y. and Park, J. M., "How to Study Cr(VI) Biosorption: Use of Fermentation Waste for Detoxifying Cr(VI) in Aqueous Solution", *Chem. Eng. J.*, **136**, 173-179 (2008a).

Park, D., Yun, Y.S., Lee, H.W. and Park, J.M., "Advanced Kinetic Model of the Cr(VI) Removal by Biomaterials at Various pHs and Temperatures", *Bioresour. Technol*., **99**, 1141-1147 (2008b).

Park, D., Yun, Y.S. and Park, J.M., "Reduction of Hexavalent Chromium with the Brown Seaweed *Ecklonia* Biomass", *Environ. Sci.Technol*., **38**, 4860-4864 (2004).

Park, D., Yun, Y.S., Park, J.M., "Mechanisms of the Removal of Hexavalent Chromium by Biomaterials or Biomaterial-Based Activated Carbons", *J. Hazard. Mater.*, **B137**, 1254-1257 (2006).

Pinto, E., Sigaud-Kutner, T. C. S., Leita˜o, M. A. S., Oka-moto, O. K. and Morse, D. "Heavy Metal-Induced Oxidative Stress in Algae", *J. Phycol.*, **39**, 1008-1018(2003).

Tels, M., "Advances in Treating Heavy Metals Containing Wastes", *Resour. Conserv*., **14**, 71-92 (1987).

Pettine, M., Barra, I., Campanella, L. andMillero, F.J., "Effect of Metals on the Reduction of Chromium(VI) with Hydrogen Sulfide", *Wat. Res*., **32(9)**, 2807-2813 (1998).

Sevim, F. and Demir, D., "Investigation of reduction kinetics of $Cr_2O_7^{2-}$ in $FeSO_4$ solution", *Chem. Eng. J.*,**143,** 161-166 (2008).

Singh, K.K., Rastogi, R. and Hasan, S.H., "Removal of Cr(VI) from Wastewater Using Rice Bran", *J. Colloid. Inter. Sci*., **290(1)**, 61-68 (2005).

Suzuki, T., Miyata, N., Horitsu, H., Kawai, K., Takamizawa, K., Tai, Y. and Okazaki, M., "NAD(P)H-dependent Chromium(VI) Reductase of *Pseudomonas Ambigua* G-1: A Cr(V) Intermediate is Formed During the Reduction of Cr(VI) to Cr(III)", *J. Bacteriol.*,**174**, 5340-5345(1992).

Ting, Y.P. andTeo, W.K., "Uptake of Cadmium and Zinc by Yeast: Effects of Co-Metal Ion and Physical/Chemical Treatment", *Bioresour. Technol*., **50**, 113-117 (1994).

Verma, A., Chakraborty, S. andBasu, J.K., "Adsorption Study of Hexavalent Chromium Using Tamarind Hull-Based Adsorbents", *Sep. Purif. Technol*., **50(3)**, 336-341 (2006).

Vilar, V.J.P., Valle, J.A.B., Bhatnagar, A., Santos, J.C., de Souza, S.M.A., de Souza, N.A.U., Botelho, C.M.S. andBoaventura, R.A.R.,"Insights into Trivalent Chromium Biosorption onto Protonated Brown Algae *Pelvetiacanaliculata*: Distribution of Chromium Ionic Species on the Binding Sites", *Chem. Eng. J.*, **200-202**, 140-148 (2012).

Wittbrodt, P.R. and Palmer, C.D., "Reduction of Cr(VI) in the Presence of of Excess Soil Fulvic Acid", *Environ. Sci. Technol.*, **29**, 255-263, (1995).

Yang, L. and Chan, J.P., "Biosorptionof Hexavalent Chromium onto Raw and Chemically Modified *Sargassum sp.", Bioresour.Technol.*, **99(2)**, 297-307 (2008).

Chapter 5

Industrial Chrome Tanning Effluent Treatment and *Spirulina* Cultivation

This chapter studies on bioremediation of chromium from industrial chrome tanning effluent using dead and live *Spirulina platensis* biomass. A growth model is tested by incorporating the rate of photosynthesis, nutrient uptake and respiration for *Spirulina platensis* cultivation at different conditions.

5.1 Background of industrial chrome tanning effluent

Photosynthesized microalgae generate a large fraction of oxygen in the earth's atmosphere and they are ubiquitous within the biosphere. Thus, their role in the development of the earth's biosphere has unique importance. The demand of microalgae has also increased with the quest for renewable energy sources. Microalgae cultivation has attracted increasing attention for three main reasons. First, they utilize CO_2. Second, it is a potentially rich source of useful products such as proteins. Third, microalgae can be employed for the abatement of

water pollutant by removing the contaminant from waste streams. Moreover, under highly stressed growth conditions, algae can thrive and produce valuable by-products such as lipids (oils), carbohydrates, proteins and various feedstocks. Biomass can be converted into biofuels and other useful materials. Microalgal metabolites pose unprecedented chemical structures as compared to terrestrial organisms and they display interesting biological activity.

Microalgae are autotrophic in nature and able to produce their food with nutrients present in water bodies namely sea, pond, lake, etc. and eaten by aquatic creature for their survival. They are able to trap the energy of sunlight and use for the metabolic process. The solar energy is converted into biomass performing photosynthesis, photorespiration and inorganic nitrogen & sulphur assimilation. Nearly about 10^{11} and 2×10^{10} tons of carbon and nitrogen are annually fixed by photosynthesis. This involves the energy accumulation of 3×10^{10} J which is about ten times than the total energy consumed in one year worldwide (Vilchez et al., 1997).

Industrial and domestic effluents are poor in oxygen content but rich in dissolved inorganic matters such as nitrogen, phosphorous, potassium and sulphur. Arsenic, mercury, cadmium, chromium, nickel, zinc, copper etc. present in wastewater is greatly hazardous to the environment due to their toxic effects. Heavy metals progressively enter into the body through the natural food chain and eventually cause serious human health risk. Contaminants of water also cause several secondary diseases and spoil the agricultural land. In order to treat such effluents, oxygen ponds are developed and microalga like *Euglena*, *Chlamydomonas*, *Spirulina*, *Chlorella*, etc are allow to grow (Vonshak, 1997). Oxygen is released through photosynthetic activities and inorganic contaminants are degraded/removed. Different microalgae tested for the removal of heavy metals include *Chlorella Vulgaris* (Ahluwalia and

Goyal, 2007), *Chlorella miniata*(Han et al., 2006),*Spirulina platensis*(Rodrigues et al., 2012), *Spirulina maxima* (Gong et al., 2005) and *Spirogyra neglecta*(Singh et al., 2012).

The taxonomy of *Spirulina* is quite complex than the other prokaryotes, because they exhibit dissimilar behaviour of the plant form (Gupta and Changwal, 1992). *Arthrospira (Spirulina) platensis* and *A. Maxima* are filamentous cyanobacteria. The arrangement of the multicellular cylindrical trichomes is an open left hand helix along the length. It is blue green in appearance and single celled. The filament is 6 to 16 μm wide. The helix pitch and diameter range from 12 to 72 and 30 to 70 μm, respectively. However, the morphology of *Spirulina* is greatly influenced by the media composition. The recent trends in drug research suggest that microalgae species are a promising group to furnish novel biochemically active substances and essential compounds for human nutrition (Cardozo et al., 2007). *Spirulina* needs high alkaline and saline conditions for their growth and survival. Few species grow at temperature of 45 to 50°C. Generally, microbes are not able to survive in such harsh conditions. Earlier it was taken as food supplements by the people living near the lakes or ponds of Chad and Niger, Nigeria (Ciferri, 1983).

Production of metabolites with microalgae is a complex process and many factors affect the growth. The major components of *Spirulina* and their variation depending on the geographical location as listed in Table 1.9 (Chapter 1). For many years, the plant physiologist, algologist and biologist are trying for their mass culturing. Bio-elimination of contaminants long with production of primary and secondary metabolites is a prudent area of algological studies.

5.1.1 Proposed route for the cultivation of microalgae (*Spirulina*) in waste-stream

We have proposed a conceptual route for the treatment of wastewater containing inorganic pollutants which may be beneficial for the cultivation of microalgae. Wastewater coming from tannage operation, domestic activities and agricultural runoff contains a number of growth media substances. Such effluents can be utilized for cultivation of selected microalgae species (Canizares-Villanueva et al., 1995; Volkmann et al., 2008; Ratana et al., 2010). Initial high metal/inorganic toxicity of effluent can be reduced by proper pre-treatment. The raw effluent should be first sent to physical treatment unit (Figure 5.1) for removal of suspended particles, colloidal solids, coarse particles and settable particles (Metcalf and Eddy, 1991). Physical treatment processes include sedimentation (clarification), screening, aeration, filtration, flotation, skimming degasification and equalization. Chemical treatment afterwards will reduce the toxicity of pollutant towards the microalgae species. Typically chemical treatment removes organic and inorganic components of wastewater such as BOD, heavy metals, chlorine, etc (Metcalf and Eddy, 1991). Conventional chemical treatment processes include chlorination, ozonation, neutralization, coagulation etc. Polyvalent metals are commonly used as coagulating chemicals in wastewater treatment and typical coagulants would include lime (that can also be used in neutralization), certain iron containing compounds (such as ferric chloride or ferric sulphate) and alum (aluminium sulphate). Neutralization consists of the addition of acid or base to adjust pH levels back to neutrality. Since lime is a base it is sometimes used in the neutralization of acid wastes. In the present study, Cr(III) was removed from the industrial chrome tanning effluent by hydroxide precipitation (pH≥8) followed by centrifugation. It also helped to obtain a favourable pH for Cl^- substitute. Nutrients (if required) and other media substances are added to supportalgalgrowth depending on the quality of the (partially) treated effluent. A typical

application of wastewater for *Spirulina* cultivation is reported by several researchers (Canizares-Villanuevaet al., 1995; Volkmann et al., 2008; Ratana et al., 2010). Different wastewater such as pig waste (Ratana et al., 2010), swine wastewater (Canizares-Villanueva et al., 1995), starch wastewater (Phang et al., 2000) and desalinator wastewater (Volkmann et al., 2008) are utilized for *Spiulina* cultivation. About 2 to 50 % of waste effluent is added to growth media for *Spirulina* cultivation both in batch and semi batch reactors depending on the concentration of nutritional and residual undesirable components (Canizares-Villanueva et al., 1995; Olguin et al., 2001; Ratana et al., 2010).

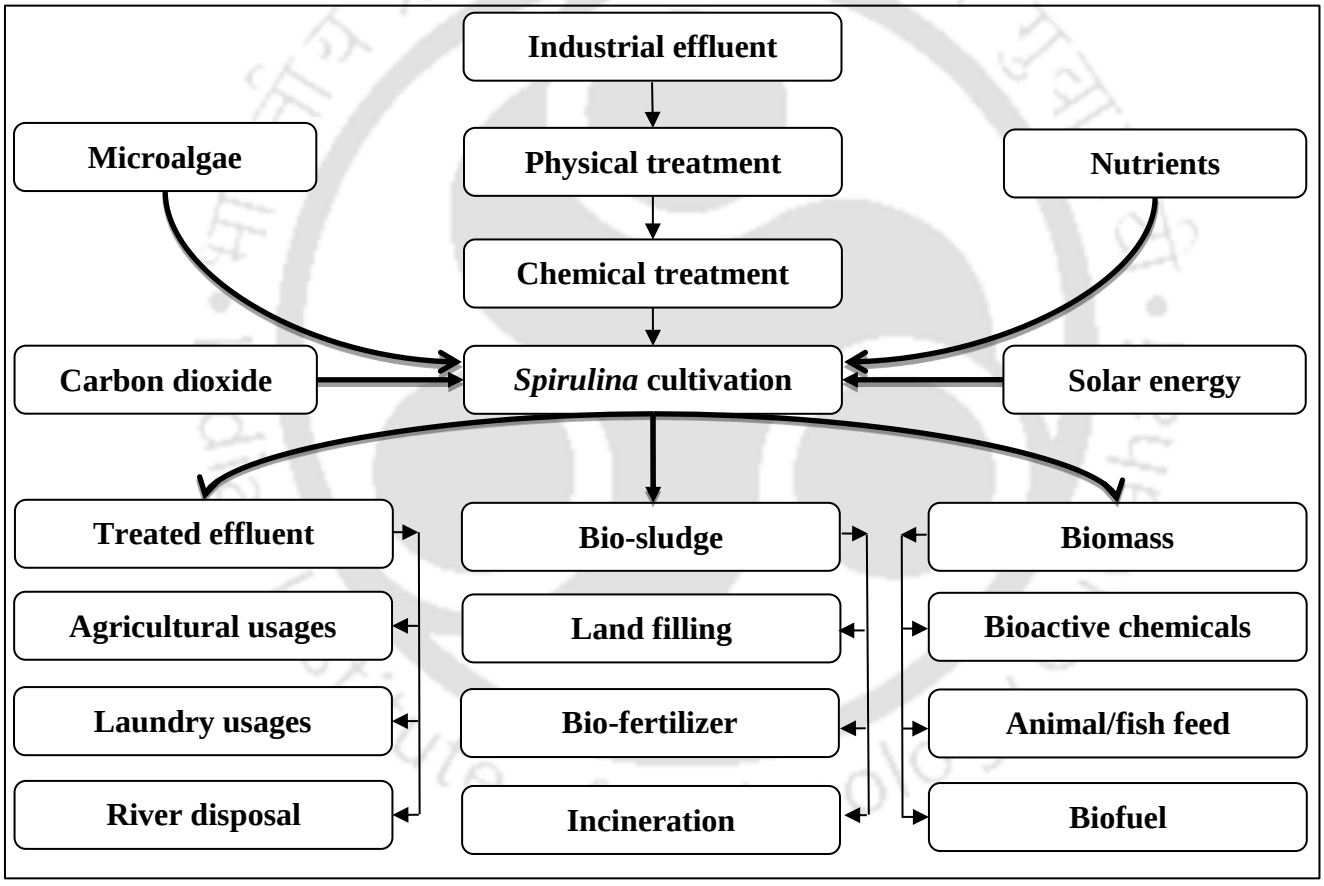

Figure 5.1 Proposed scheme for the cultivation of microalgae in waste aqueous streams containing growth media supplements.

Biomass cultivation also can be conducted in outdoors open raceway pond at ambient temperatures. Evaporation is counteracted by adding the same level of fresh water every day. Microalgae like *Spirulina sp.* can scavenge the contaminant like heavy metals present in treated effluent, if any. It can be coupled with the industrial exhaust gas stream rich in CO_2 for a favourable condition of CO_2 fixation and carbon source for microalgae. Such treatment plants can be exposed to natural light and can utilize solar energy for microalga metabolic activity. A number of designs of such photo bioreactors are available even at commercial scale (Hase et al., 2000; Kaewpintong et al., 2007).

Industrial chrome tanning process is associated with generation of an extremely contaminated effluent laden with higher concentration of Cr(III) and chloride (Cl^-). High Cl^-concentration makes the so called treatment processes uneconomical and they are not often feasible at lower Cr(III) concentration. Microbial treatment of such effluents may overcome some of the existing drawbacks. A careful selection of biological treatment of saline tanning-wastewater is needed. It is because of limited adaptation of conventional cultures at Cl^- loading>3 to 5 % w/v and ionic strength variation cause cell disruptions. *Spirulina sp*. grows in aqueous alkaline (pH 8 to 10.5) and saline (Cl^- 0.6 g L^{-1}) media. It opens up the possibility of cultivation of *Spirulina* in 'lean' chrome tanning effluent.

Therefore, the objectives of the work in this Chapter are to (i) remove bulk Cr(III) by precipitation from industrial chrome tanning effluent at an elevated pH that will be favourable towards *Spirulina* growth, (ii) supplement Cl^- for its cultivation maintaining the required salinity level, (iii) remove residual Cr(III), if any, during *Spirulina* cultivation,(vi) explore the effect of cultivation conditions on metabolic productsand, (v) obtain the kinetic parameters of

Spirulina growth incorporating the rate of photosynthesis, nutrient uptake and respiration in presence of Cr(III), Cr(VI) and PCTE in Zarrouk media composition (ZMC).

5.2 Kinetic model of micro-algal growth

5.2.1 Growth model

Substrate uptake by microalgae typically involves two kinetic steps. Firstly, substrate is adsorbed on the algal cell surfaces via equilibrium between adsorption and desorption processes. Secondly, the surface-adsorbed substrate is transported into the cells through the cell membrane. It is regulated by the feedback of intracellular substrate pool size. Algal cell growth is based on intracellular substrate uptake. Most of the previous studies have considered that the uptake rate is directly related to substrate concentration only in the second step and thus the substrate uptake is a single step kinetic process (Harison et al., 1989; Aksnes and Egge, 1991). However, the surface adsorption play an important role in nutrient uptake as it is an intermediate process taking place at the substrate-cell interface and is an equilibrium governed process. The variations in substrate concentration, temperature and pH in the substrate media can change the direction of substrate flux between the substrate and cell surface. As a result, the amount of surface-adsorbed nutrient and the transport process in the second step can be affected.

Microalgae have the ability to uncouple their growth from the uptake of nutrient and algal biomass continues to grow even for few days after nutrient exhaustion (Bernard et al., 2011). The nutrient uptake and growth is proportional to the Monod model and therefore the above behaviour cannot be explained. Moreover, microalgal growth depends on light intensity,

nutrient uptake capacity, temperature and photosynthetic rate. The Droop model was established to represent the internal quota of vitamin B_{12} for growth rate of phytoplankton and also employed for macronutrient such as nitrogen or phosphate for growth rate (Droop, 1968). Therefore, the growth rate of biomass is assumed to be related to the internal concentration of the limiting element. It was assumed that *Spirulina* growth is limited by nitrogen availability.

The surface adsorption for the nutrient uptake by several algal species is represented by the Langmuir equation with the assumption that: (i) nutrient adsorption is a single layer process, (ii) both adsorption and desorption are very rapid and the amount is fairly small, (ii) homogeneous nature of algal cell surface and, (iv) negligible effect of size of cell surface. Thus, the adsorption and desorption equations are represented as (Yao et al., 2011):

$$\rho_a = K_a S_b \left(1 - \frac{S_n}{S_{n,max}}\right) \quad (5.1)$$

$$\rho_d = K_d S_p \quad (5.2)$$

where, ρ_a and ρ_d are the rate of adsorption and desorption (mol L^{-1} h^{-1}). $K_a$andK_d are adsorption and desorption constants (h^{-1}). $S_p$is the amount of nutrient adsorbed (mol L^{-1}). It further can be expressed as: $S_p = S_n B$; where, $S_n$is the amount of surface-adsorbed nutrient per unit biomass (mol g^{-1}). $S_{n,max}$ represents the maximum amount of nutrient sorbed(mol g^{-1}). S_b indicates the concentration of nutrient in substrate (mol L^{-1}) and B is biomass concentration (g L^{-1}).

The efficiency of algal growth due to intracellular nutrient can be defined as $\emptyset_{s,i}$:

$$\emptyset_{s,i} = \left(1 - \frac{S_{n,min}}{S_n}\right) \quad (5.3)$$

where, $S_{n,min}$ is the minimum amount of nutrient required for microalgal growth (mol g^{-1}).

The efficiency of algal growth due to extracellular nutrient ($\emptyset_{s,ext}$) can be represented using the Michaelis-Menten kinetics as:

$$\emptyset_{s,ext} = \frac{S_n}{S_n + K_s} \quad (5.4)$$

The overall nutrient uptake rate is written asρ'_a:

$$\rho'_a = (\rho_a - \rho_d)\emptyset_{s,i}\emptyset_{s,ext} \quad (5.5)$$

The microalgal growth depends on nutrient uptake as well as on photon density, photosynthesis process, bio-synthesis efficiency and respiration rate. It gives a scope to couple all these processes for the calculation of specific growth rate as (Quinn et al. 2011):

$$\mu = \frac{1}{B}\frac{dB}{dt} = P_c - rR_c - \beta\rho'_a \quad (5.6)$$

where, μ, P_c, rR_c, β and ρ'_a are specific growth rate (h^{-1}), photosynthetic rate (h^{-1}), respiration rate (h^{-1}), bio-synthetic efficiency and rate of nutrient uptake (mol L^{-1} h^{-1}), respectively.

5.2.2 Photosynthetic rate

Photosynthesis involves a series of reactions including light absorption, synthesis of NADPH (nicotinamide adenine dinucleotide phosphate) and ATP (adenosine triphosphate) as energy generation with fixation of carbon in the Calvin cycle. Chlorophyll content of microalgae plays a vital role for photosynthesis, which allows energy absorption from light source. Chlorophyll molecules transfer sorbed light energy to the reaction site of the cells, where electrons are exchanged, mainly with water molecules. It is resulted in yield of oxygen gas and hydrogen ions. ATP, a nucleotide, transports energy within cells for metabolism. It is assumed that algal biomass contains about 30% carbon (w/w). Carbon specific photosynthetic rate (P_c)incorporating light intensity and light adsorption capacity, therefore, can be defined as (Quinn et al., 2011):

$$P_c = P_{max}\left(1 - \exp\left(\frac{-\alpha \emptyset_m I_{av}}{P_{max}}\right)\right) \quad (5.7)$$

where, P_{max} is calculated maximum photosynthetic rate (h^{-1}). $\alpha$is light absorption coefficient ($m^2\ g^{-1}$) and I_{av} is the average light intensity (μmol $m^{-2}\ s^{-1}$).

5.2.3 Light distribution model

Progressive algal growth increases turbidity and light penetration into nutrient media notably falls down. Moreover, the mixing affects the frequency of dark and photo periods. Light penetration is more at low algae concentration and microalgal growth rate is not influenced much due to mixing. Therefore, it is recommended to use the average light intensity for the

algal kinetics. The intensity of light decreases exponentially with light path travelled at low cell density according to the Lambert-Beer Law. The average light intensity can be defined as (Quinn et al., 2011):

$$I_{av} = I_0 exp(-\alpha BL) \quad (5.7)$$

where, I_0 is initial light intensity (μmol m^{-2} s^{-1}) and and$L$ is the light path length (m).

5.2.4 Biosynthetic efficiency

Microalgae produce protein, lipid and carbohydrate with depletion of nitrogen, phosphorus and carbonate compounds. The algal metabolism is shifted from protein synthesis to lipid and carbohydrate production depending on the extent of availability of nitrogen in media (Quinn et al. 2011). The energy necessary to breakdown the nutrient for biomass growth is accounted as biosynthesis efficiency (β) (g L^{-1}). It was calculated from the model prediction.

5.2.5 Respiration rate

The growth and photosynthesis rates typically show linear dependency on each other. The respiration and maintenance respiration rates are defined as a fraction of photosynthesis rate. The respiration rate is generally about 2% of photosynthesis rate. The model incorporates respiration losses resulted from metabolic functionalities for biosynthesis and cell maintenance. It is usually considered that the respiration rates remain same throughout day i.e. both in dark and light. It indicates that the maintenance respiration is neither stimulated

nor inhibited by growth (Geider and Osborne, 1991). However, for the present case, the maintenance respiration rate (rR_c) is like to be constant (h^{-1})as the photo period was 24 h.

5.3 Result and discussions

5.3.1 Characterization of chrome tanning effluent

The physiochemical characteristics of raw chrome tanning effluent are summarized in Table 5.1. The corresponding discharge limit provided by the Central Pollution Control Board (CPCB) of India is shown in this table. Cr(VI) concentration in raw effluent was analysed at 1:1, 1:2, 1:5 and 1:10 (v/v) dilutions and no Cr(VI) was detected. Total chromium concentration was therefore considered to be same as Cr(III). Initial dissolved oxygen (DO) was about 2.2 mg L^{-1} (Table 5.1). Low DO may destroy aquatic life. It can even kill large fish as in aquatic system if discharged without proper treatment. Some studies have suggested that DO about 4 to 5 mg L^{-1} supports diverse types of fish population (EPA, 2006). High salinity of chrome tanning effluent is also known to decrease oxygen solubility (Lawson, 2011). An aqueous stream with low DO and high amount of phosphorus and nitrogen can be utilized for microalgae cultivation. However, the toxicity of pollutant(s) present, if any, in such streams should be reduced by proper (pre)treatment.

Table 5.1. Physiochemical properties of industrial chrome tanning effluent.

Parameters	Untreated chrome tanning effluent	Discharge limit‡
pH	3.4	6-9
Cr total	5000	2.0
Cr(VI)	N.D.	0.1
Cr(III)	5000	2.0
Cl^-	14800	1.0
SO_4^{-2}	3200	2.0
COD	2500	250
DO	2.2	-
Color	Dark green	-
Odor	Stringent	-

All units are in mg L^{-1} except pH; ‡: Central Pollution Control Board regulations; N.D.: not detectable

5.3.2 Bulk Cr(III) removal by precipitation

Hydroxide precipitation at an elevated pH is the most common practice for the removal of Cr(III). The influence of pH on Cr(III) precipitation is shown in Figure 5.2. The highest Cr(III) removal of 99.99% was achieved at pH 8.4. Residual Cr(III) was 1< mg L^{-1} at this pH even with high Cl^- concentration. Cr(III) removal decreased any point below and above this value. It is in line with Cr(III) solubility at different pH. COD removal was more than 65% irrespective to the solution pH tested for $Cr(OH)_3$(s) precipitation with a bit higher removal at pH 6(Figure 5.3). Higher COD removal at this pH was because of the combined effect of surface adsorption, coagulation and sweep coagulation of gelatinous materials, if any, in sludge matrix (Golder et al. 2011).

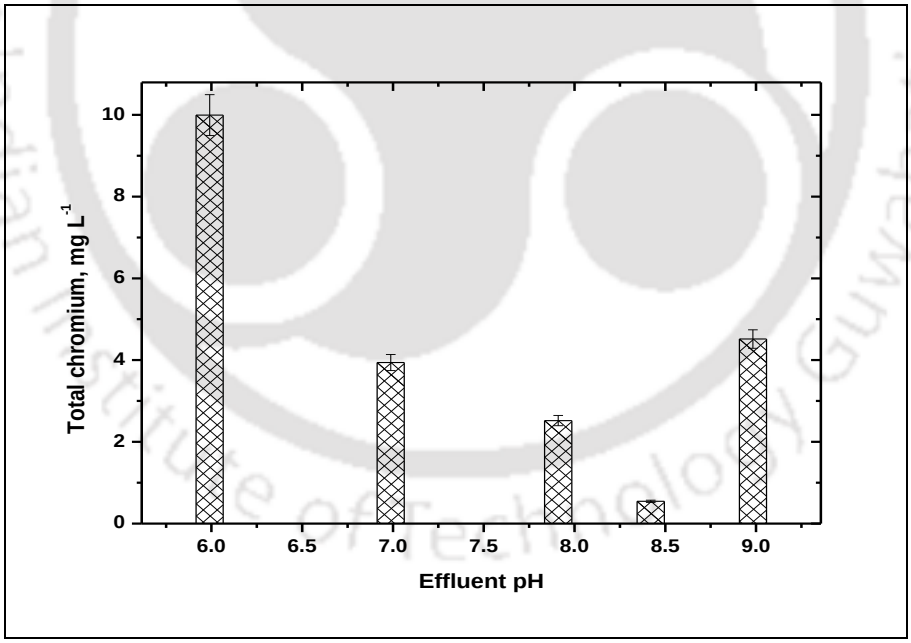

Figure 5.2 Solubility of chromium in chrome tanning effluent at different solution pH (settling time 12 h, precipitating agent 0.5 NaOH, temperature 25°C and volume 100 mL).

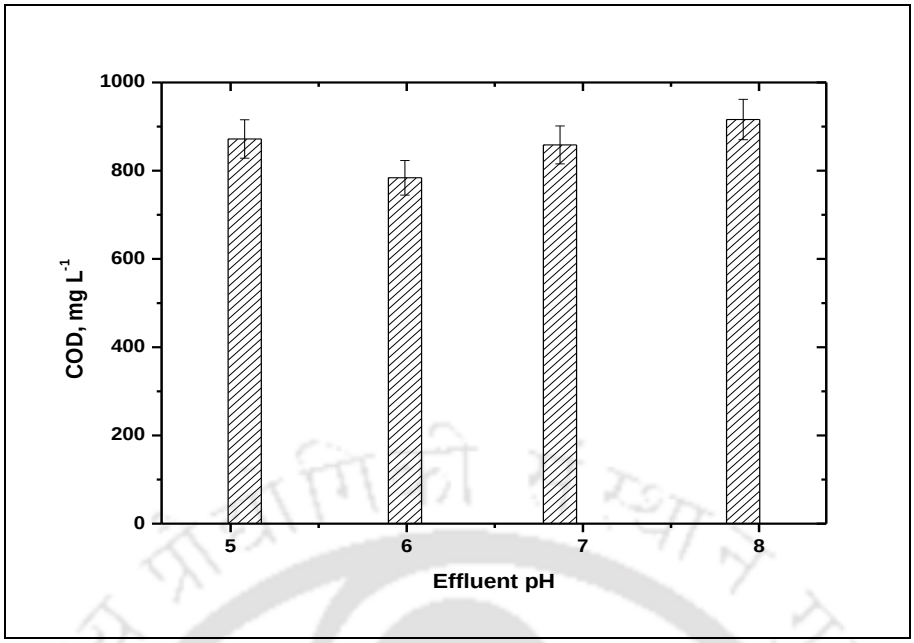

Figure 5.3 Effect of solution pH on residual COD in chrome tanning effluent maintained at different solution pH (settling time 12 h, precipitating agent 0.5 NaOH, temperature 25°C and volume 100 mL).

5.3.3 *Spirulina* cultivation in ZMC

Spirulina grown on solid media was transferred into ZMC at different solution pH. Solution pH significantly affected the microalgal cell density and metabolite production as shown in Figures 5.4 and 5.5. pH influences the solution chemistry of ZMC, CO_2 solubility as well as the sorption affinity of nutrient on algal surface. A definite quantity of inoculum (0.219 g L^{-1}) was added and the maximum growth of 2.03 g L^{-1} was obtained at pH 9.5. *Spirulina* showed notable growth at wide pH range from 7.5 to 11.5 with cell density varying from 0.25 to 0.467 g L^{-1} (Figure 5.4). The decrease in pH from ZMC value was resulted in low cell density. Slight alkaline condition favoured cell growth. However, the cell density was reduced at pH >9.5. The initial proton concentration has strong role on the net surface charge of biomass produced with the progress of growth. For the present study, it was observed that pH

increased with increase in cell density. For an example, the media pH of 9.5 was recorded at 7 days of cultivation for the initial pH of 8.5. CO_2 uptake by *Spirulina sp.*is significantly influenced by the concentration of HCO_3^-, CO_3^{2-}, CO_2 and H^+ in a carbonate system of brine water (Al-Rawajfeh, 2010). The balanced charge on *Spirulina* surface at pH 9.5 provided the most favourable condition for the growth as well as for the metabolic activities. It is necessary to mention here that pH provided in figure 5.2 to 5.7 corresponds to the initial values.

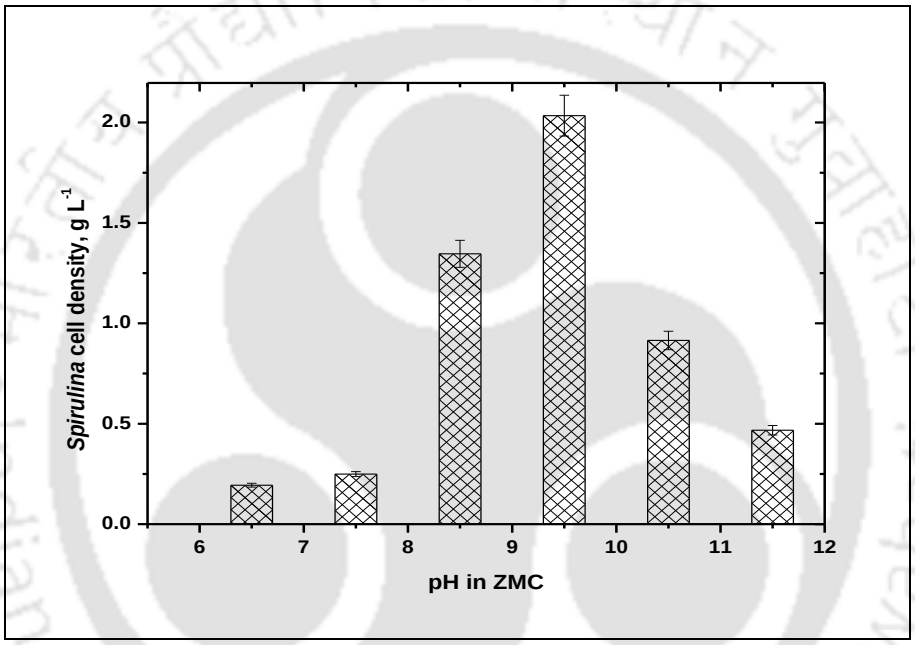

Figure 5.4 Influence of pH in ZMC on *Spirulina*cell density (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

Chlorophylls are the primary active photochemical compounds. They work as a receiver of light for driving the photosynthesis reaction. It is a critical factor especially for the faster growth of algae. So, high chlorophyll production rate is required for the rapid growth of biomass (Chauhan and Pathak, 2010). Chlorophyll a is essential in photosynthesis, while the role of chlorophyll b is seemingly unwanted. Chlorophyll b is synthesized from chlorophyll a.

The latter is reconverted to chlorophyll a and the other way around (Tanaka and Tanaka, 2011). Nevertheless, chlorophyll b is required for improving the main light-harvesting chlorophyll-binding proteins (Tanaka and Tanaka, 2011). Pigments production with the cultivation of *Spirulina sp.* at different solution pH is shown in Figure 5.5. Alkaline pH favoured high pigment formation. The maximum chlorophyll a and chlorophyll b concentration of 22.7 and 8.3 mg per g of biomass were obtained at pH 11.5. Carotene concentration reached up to about 15.7 mg g^{-1} at the same pH. The production of glucose was found to be more toward the neutral pH i.e., at 6.5. The cell growth was suppressed at this pH. Pigments are susceptible to degrade at lower pH and higher temperature (Koca et al., 2006). Higher cell growth and pigment production obtained at alkaline condition are in accordance to literature. Pigment discoloration also may occur by the conversion of chlorophylls into pheophytins by the influence of pH. In this reaction, H^+ transforms chlorophylls to pheophytins by the substitution of Mg^{2+} in the porphyrin ring (Minguez-Mosquera, 1989; Koca et al., 2006). Carbohydrate production showed the reverse dependency on pH. The highest value of 7.1 mg g^{-1} was determined at pH 6.5 (Figure 5.5).

Spirulina sp. was also cultivated in ZMC with PCTE in place of NaCl in it. Cl^- load in PCTE substituted media was varied from 0 to 11.6 g L^{-1}. The maximum growth was recorded at 1.46 g $L^{-1}Cl^-$. Then, the media pH was adjusted to different values as in Figure 5.6 with 1.46 g $L^{-1}Cl^-$. It is outlined before that without addition of PCTE, the maximum cell density was observed at pH 9.5 (Figure 5.4). Whereas, it was maximum (0.45 g L^{-1}) at pH 10.5 with PCTE supported media. Lower cell density in PCTE cultivated *Spirulina* was due to the interference of numerous substances present in tanning effluent. Glucose production was higher at pH 8.5, while pigment at pH 9.5.

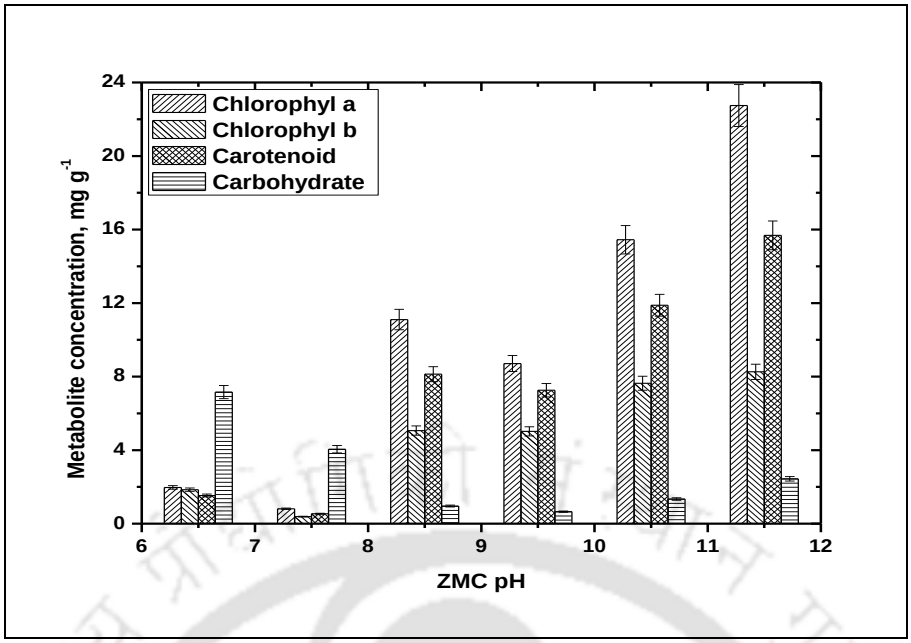

Figure 5.5 Metabolites of *Spirulina sp.* obtained at different pH maintained in ZMC (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

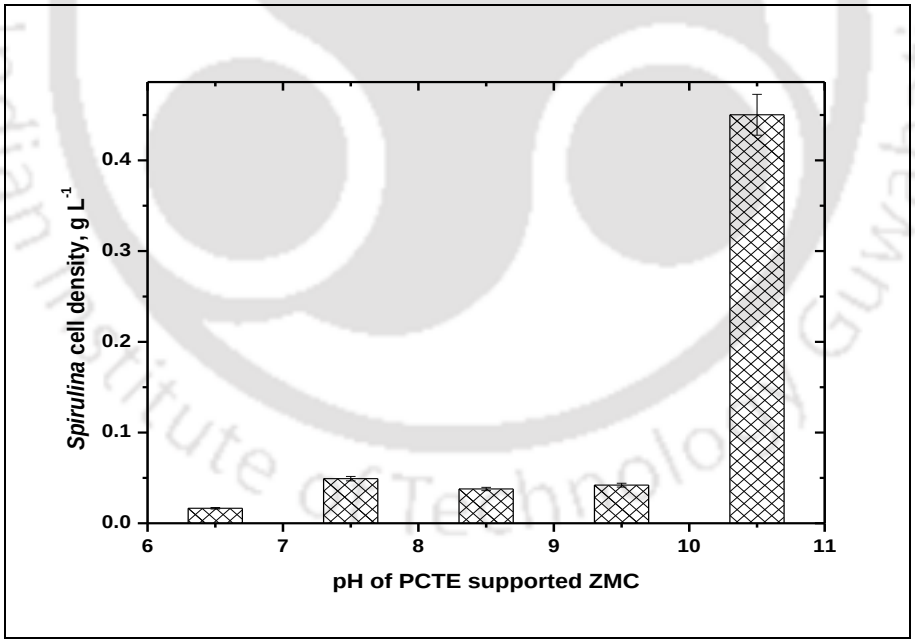

Figure 5.6 Influence of pH of PCTE supported growth media (1.46 g $L^{-1}Cl^{-}$) on *Spirulina* cell density (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

The influence of salinity on glucose production can be seen by comparing the results with and without addition of PCTE in ZMC (Figure 5.7). Glucose content decreased in PCTE. The similar effect was also observed for pigment contents. The increasing salinity (>1.46 g L^{-1} of Cl^-) inhibited the growth of biomass. Amala et al., 2013 reported maximum cell density of *Spirulina* in ZMC with 0.96 g L^{-1} of Cl^- and the growth was suppressed beyond this value. Chlorophyll a formation in PCTE substituted media was higher than ZMC. High light penetration in ZMC may reduce chlorophyll a formation (Chauhan and Pathak, 2010).

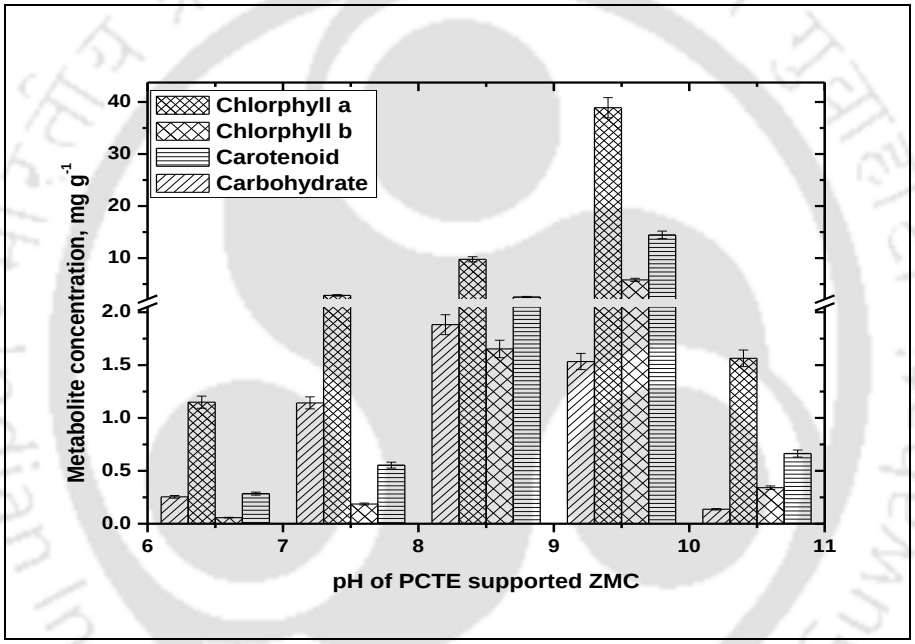

Figure 5.7 Metabolites of *Spirulina sp*. cultivated in PCTE supported media at different pH (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

A comparison of metabolites of *Spirulina* obtained in the present study and the literature reported values are summarized in Table 5.2. The results clearly indicate that the quality of *Spirulina* biomass and its metabolic products depend on their media composition and culturing conditions.

Table 5.2 Metabolic products of *Spirulina* species.

Chl a (mg g^{-1})	Chl b (mg g^{-1})	T.C. (mg g^{-1})	Lipids (%)	C.D. (g L^{-1})	Protein (%)	Carbohydrate (%)	D.T. (h)	MDD rate (day^{-1})	Source
--	--	--	--	0.99	58.3	--	51.6	--	Goksan et al., 2007
13	--	--	--	1.56	62	--	--	--	Pandey et al., 2010
--	--	--	--	3.5-3.81	--	--	--	--	Yuan et al., 2011
--	--	--	69	--	--	--	--	--	Chaiklahana et al., 2008
--	--	--	--	3.1	--	--	154	--	Celekli et al., 2009
--	--	--	--	1.5	--	--	--	--	Binaghi et al., 2003
--	--	--	4-9	--	46-63	8-14	--	--	Becker, 2007
22.6	8.2	15.6	18.33	2 .03	--	9.0	9.97	0.10	Present study in ZMC
38.7	5.7	14.4	--	0.42	--	3.7	31.47	0.03	Present study with PCTE in ZMC

Chl a: Chlorophyll a; Chl b: Chlorophyll b; T.C.: Total carotenoid; C.D.: Cell density; D.T.: Doubling time; MDD: Mean daily division

5.3.4 Influence of Cl^- and Cr(III) present in PCTE on *Spirulina* cultivation

Salt concentration in ZMC was maintained by addition of different amount of PCTE. The pictorial view of cell density of *Spirulina* obtained at one month cultivation period is shown in Figure 5.8a. The maximum growth was with 1.46 g $L^{-1}Cl^-$. Cell growth was completely inhibited with further increase in Cl^- concentration. It was quite possible that [Cl-]>1.46 gL^{-1}, caused cell osmosis which led to cell death.Figure 5.8b illustrates that [Cr(III)]<10 mg L^{-1} in PCTE had negligible effect on *Spirulina* growth. Whereas, [Cr(III)]>20 mg L^{-1} was resulted in decrease of cell density. Almost complete removal of Cr(III) was achieved upto 20 mg L^{-1}. There were traces of Cr(III) in solution (< 1.5 mg L^{-1}) at higher concentration. Above results implies that low concentration of Cr(III) present in PCTE didn't exhibit notable inhibitory effect on *Spirulina* growth.

The lipid content was found to be around 18.3 % (w/w). *Spirulina sp.*is an excellent raw material for biofuel extraction via transesterification reaction due to high lipid content. The quality and quantity of algal biomass as well as its metabolic products can be enhanced by optimizing the cultivation conditions such as salt stress, light intensity, solution pH, carbon dioxide purging rate and nutrient content.

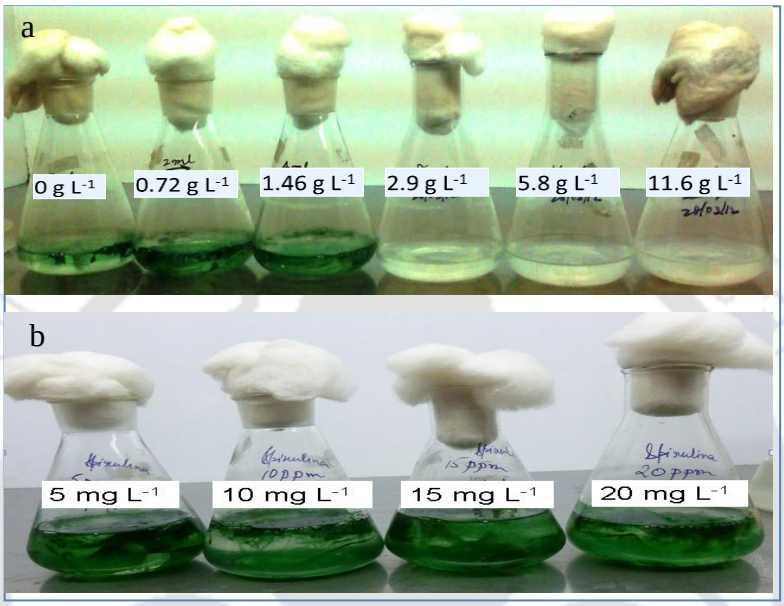

Figure 5.8 Pictorial view of *Spirulina* growth. a: Different Cl^- concentration. b: Influence of residual Cr(III) concentration on cell growth in a period of one month(inoculum 0.219 g L^{-1}, stirring speed 150 rpm, pH 8.5, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

5.3.5 FTIR spectra of *Spirulina platensis* cultivated in ZMC at different media pH

pH of ZMC was altered and the variation of surface functional groups of *Spirulina sp.* cultivated at different pH was identified by recording the FTIR spectra. The results are shown in Figure 5.9. At pH 6.5, the peaks at 3441, 2357, 1647, 1549, 1384, 1095 and 667 cm^{-1} could be attributed to –OH or –NH, P-H, C=C or C=O, N=O, -CH_3 or N=O, C-O or C-N and C-

Hstretching, respectively. The peaks at 2096, 1642, 1538 and 1386 cm^{-1} could be allocated to C=N, C=O stretch, N=O or N-H and CH_3 or N=O, respectively, at pH 8.5. It was observed that the peak around 3400 cm^{-1} was broad and can be assigned to -OH. It is necessary to mention here that the maximum cell density of *Spirulina sp.* was obtained at this pH. *Spirulina* cultivated at solution pH of 10.5 possessed the peaks at 3641, 3120, 2330, 1681, 1384, 1062 and 840 cm^{-1}. They can be assigned to –OH, C-H, P-H, C=O or C=C, S=O, C-O or N-H and C-H, respectively. The surface composition of *Spirulina* showed notable dependency on media pH.It implies the strong effect of pH on the metabolic functionalities of *Spirulina* growth.

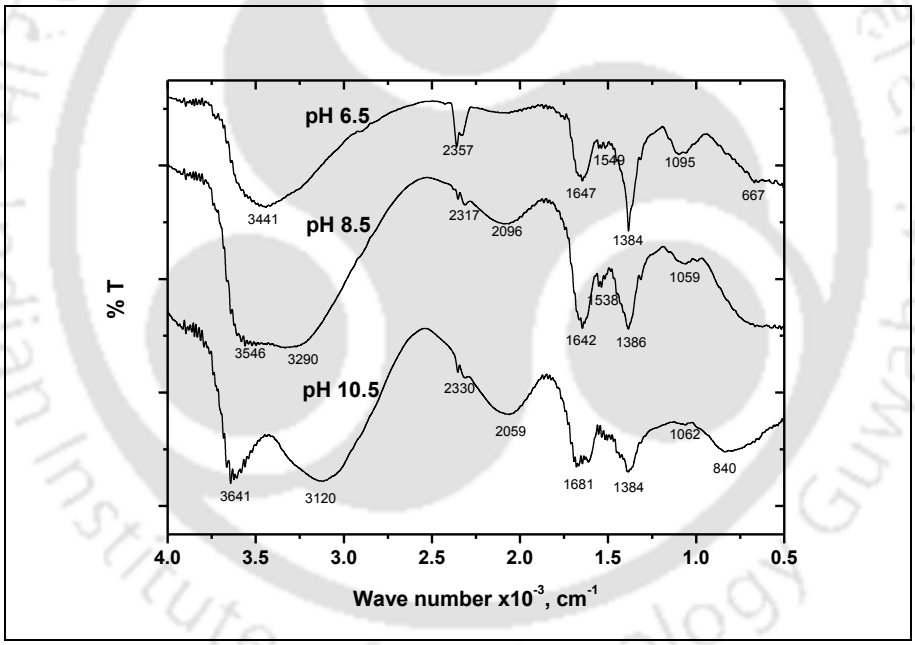

Figure 5.9 FTIR spectra of *Spirulina*biomasscultivated inZMC at different pH (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

The commercial dead *Spirulina* biomass used in Cr(III) and Cr(VI) removal studies (Chapters 3 and 4) exhibited the major peaks at 1048, 1412, 1545, 1639, 2920 and 3434 cm^{-1}

corresponding to C-N, CH_3, N-H, C=O, C-H and –OH or N-H. *Spirulina sp.* cultivated in our laboratory had shown significant resemble for the surface functional groups as expected. However, little peak shifting was noted. This may attributed to probably different cultivation conditions adopted for the cell growth.

5.3.6 FTIR spectra of *Spirulina platensis* cultivated at different Cl^- level maintained using PCTE

FTIR spectra of *Spirulina sp.* cultivated at different Cl^- concentrations are presented in Figure 5.10. The peaks appeared at 3641, 3109, 2345, 2045, 1678, 1599, 1500, 1351, 1083 and 837 cm^{-1}without Cl^-in ZMC. They can be attributed to –OH, C-H, P-H, N=C, C=O, C-O or N-H, N-O, CH_2 or CH_3, C-O and C-H, respectively. *Spirulina sp.* cultivated with Cl^- (0.72 mg L^{-1}, approximately 1% v/v dilution of PCTE) showed the peaks at 3450, 2338, 1647, 1544, 1384, 1099 and 990 cm^{-1} which may be allocated to O-H, P-H, C=O, N-H or N=O, CH_3, C-O and C-H, respectively. Similar peaks were observed with 1.46 mg $L^{-1}Cl^-$. The peaks corresponding to phosphate, C=O, O-H and CH_3 were notedfor all the cases irrespective to Cl^- concentration. However, the distinct peaks at around 3109 and 2040 cm^{-1}were observed only in case of biomass cultivated without Cl^-. The salinity levels of the nutrient media influenced the compositions of functional groups. It was also possible that the salinity dose obtained by dilution of PCTE altered the extent of availability of natural nutrient present in it.

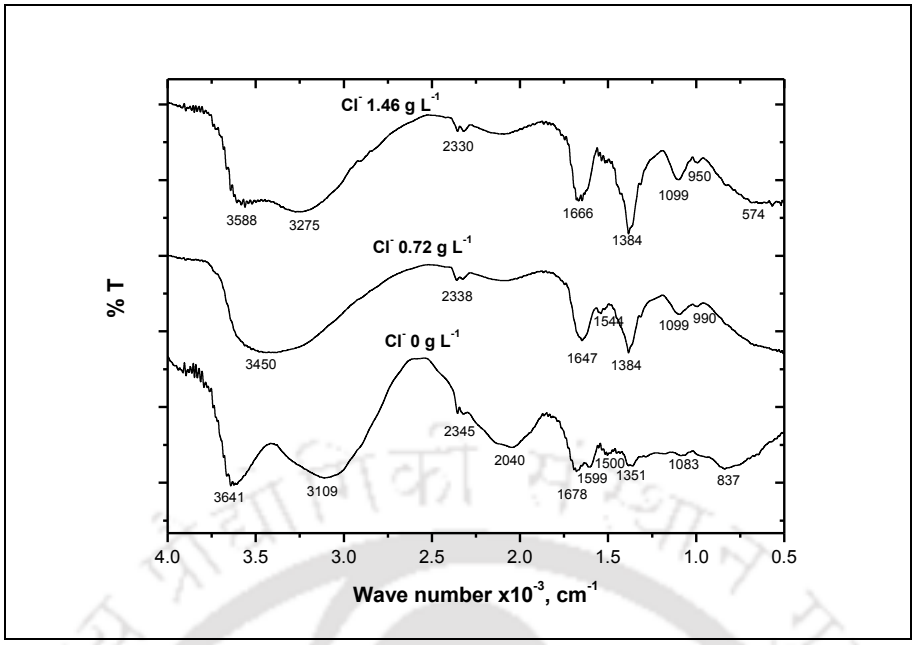

Figure 5.10 FTIR spectra of *Spirulina*biomass recorded at different Cl^- concentration maintained by addition of PCTEin ZMC (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, pH 8.5, temperature 30ºC, media volume 100 mL, light intensity 4 klux, photo period 12 h per day and cultivation period 30 days).

5.3.7 Kinetics of *Spirulina* cell growth

As shown in Figure 5.11, the cultivation conditions significantly affects cell density and growth pattern. Presence of Cr(III) and Cr(VI) and, addition of PCTE to ZMC notably decreased the growth rate of *Spirulina*. The photo period was kept 24 h i.e. the dark period was avoided to achieve maximum cell densityas well as for ease of model validation for exponential growth phase. Maximum cell density of *Spirulina platensis* was achieved at around 13th day of cultivation. PCTE in ZMC exhibited the maximum inhibition on cell growth. While, Cr(III) in ZMC showed lower growth inhibition in comparison to Cr(VI) at the same concentration.Algal tolerance to heavy metalis dependent on the defence responses against the possible oxidative harms(Pinto et al., 2003) and exudation capacity of chelating agents and likewise the nutrient uptake is reduced (Gaur and Rai, 2001).

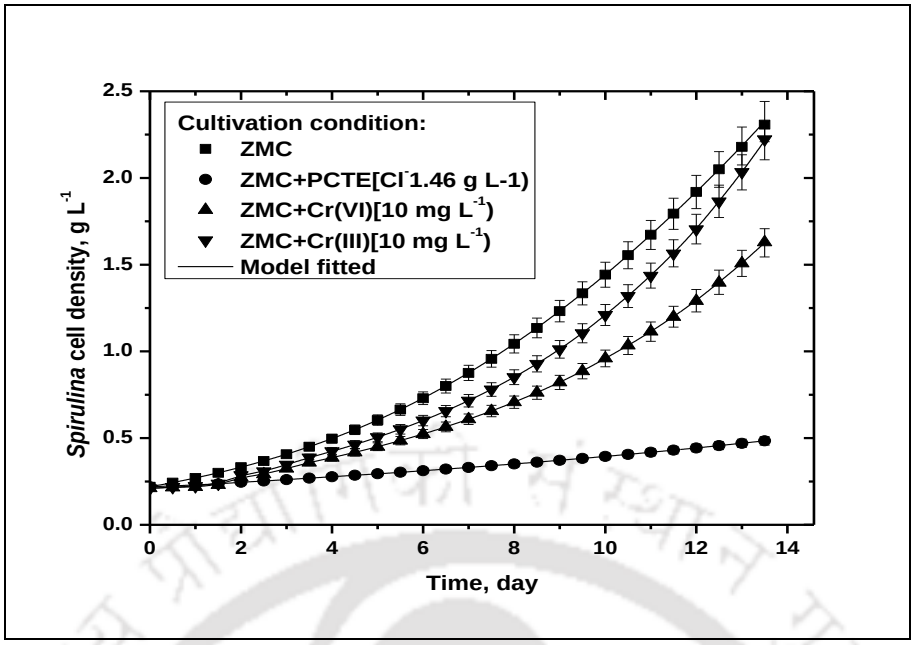

Figure 5.11 Kinetic of *Spirulina platensis* grow that different cultivation conditions and determination of kinetic constants (inoculum 0.219 g L^{-1}, stirring speed 150 rpm, pH 8.5, temperature 30ºC, media volume 100 mL, light intensity 4 klux and photo period 24 h per day).

A growth model (Eqs. 5.1 to 5.7) incorporating the rate of nutrient sorption-desorption, photosynthesis and respiration was tested for *Spirulina* cell growth in presence of Cr(III) and Cr(VI) as well as with PCTE in ZMC. The fitted parameters are shown in Table 5.3. The model showed an excellent agreement to experimentalresults and at the same time is important to mention that the number fitted parameters were ten. The value of $S_{n,min}$(mg g^{-1}) was found as 9.41(0.8 g N g^{-1} C)in ZMC for the growth of *Spirulina*. However, it increased to 41.87 (3.48 g N g^{-1} C), 42.52 (3.54 g N g^{-1} C) and 30.04 (2.50 g N g^{-1} C) with Cr(III), Cr(VI) and PCTE in ZMC, respectively.

Table 5.3. Growth parameters obtained in model validation and its comparison with literature values.

Parameters	*Spirulina platensis* (Present study)				*Nannochloro-psisoculata*	*Pseudochlo-rococcum sp.*	*S. quadricauda*
	ZMC	ZMC + Cr(III) 10 mg L^{-1}	ZMC + Cr(VI) 10 mg L^{-1}	PCTE +Cr(III) 5.4 mg L^{-1} + Cl^- 1.46 mg L^{-1}	Quinn et al. 2011	Packer et al. 2011	Yao et al. 2011
Temperature (°C)	30	30	30	30	23		
S_{nmin} (mg g^{-1})	9.41	41.87	42.52	30.04	10.0	27.8	
S_{nmax}(mg g^{-1})	412.97	372.98	427.31	399.97	150.0	93.5	
K_a(h^{-1})	0.75	0.480	0.338	0.199			1.08×10^{-3}
K_d(h^{-1})	1.5×10^{-3}	1.17×10^{-3}	5.33×10^{-4}	2.0×10^{-4}			6.3×10^{-4}
K_s (g L^{-1})	3.11	4.074	2.33	3.716	5.0×10^{-3}	1.03×10^{-5}	
α (m^{-2} g^{-1})	2.2×10^{-2}	1.0×10^{-3}	9.0×10^{-4}	4.54×10^{-3}	7.52×10^{-2}		
Φ_m (g. µmol^{-1} photons^{-1})	2.22×10^{-3}	1.0×10^{-3}	9.0×10^{-4}	2.27×10^{-3}	6.5×10^{-7}	9.84×10^{-2}	
P_{max} (h^{-1})	20.18	20.14	20.14	20.152	3.6×10^{-2}	90.1	
rR_c(h^{-1})	5.36×10^{-3}	6.85×10^{-3}	6.33×10^{-3}	1.91×10^{-3}	4.32×10^{-2}		
β (g g^{-1})	8.72	2.65	3.34	2.002	4.0(fixed)		
Variance	3.69×10^{5}	3.09×10^{5}	1.87×10^{5}	1.41×10^{4}			
std	608.60	556.47	432.71	118.89			
R^2	0.999	0.999	0.999	0.999			
T-test	0.996	0.999	0.999	0.999			
p-value	≤0.001	≤0.001	≤0.001	≤0.001			

The nutrient minimum quota for the present case is found to be lower as compared to literature data. Quinn et al. (2011) also employed a growth model for *Nannochloropsisoculata* incorporating photosynthesis, respiration, temperature, biomass growth and lipid accumulation. They observed the value of half saturation constant, K_s and α as 7.52×10^{-2} m^2 g^{-1} and 5.0×10^{-3} g L^{-1}which were lower than the present study. Yao et al. (2011) proposed a model for *S. quadricauda* growth and considered the sorption and desorption of nutrient during algae growth kinetics. But they didn't incorporate photosynthesis and respiration kinetics. They reported the sorption and desorption rate constants as 1.08×10^{-3} and 6.3×10^{-4} h^{-1}. It was about 30-81% lower for K_a and 68-138% higher for K_d in comparison to *Spirilina sp.* depending on the media composition as in Table 5.3. The values of α, Φ_m and P_{max} in present study are in close proximity with the earlier reports (Table 5.3). Both K_d and K_a(h^{-1}) showed decreasing trend with chromium and PCTE in ZMC than *Spirulina* cell growth in ZMC

without alteration of its composition. Marshall et al. (2000) proposed a photo-inhibition model for phytoplankton incorporating photo-acclimation and the aspects of nitrogen uptake and utilization. The model reflects the effects of irradiance on photosynthesis from sub-saturating to inhibitory photon flux densities, during growth on different nitrogen sources and under nutrient stress. However, their model can't explain the nature of nutrient uptake. They obtained the maximum and minimum nutrient quota as 0.17 and 0.0588 g N g^{-1} C, respectively. While, α is 0.00025 m^2 g^{-1} and P_{max} decreases with increasing in C to N ratio.

5.4 Important findings

The environment burden could be lessened by utilizing the leather industry wastewater as a source of nutrient for biomass cultivation with little processing. The study in this Chapter reports the feasibility of *Spirulina sp.* cultivation in spent tanning liquor followed by precipitation for chromium removal. The major findings are:

- The treated tanning effluent can be utilized as a source of salt and nutrient required for *Spirulina sp.* cultivation. *Spirulina sp.* biomass produced in waste tanning effluent was enriched in metabolic products.

- Cr(III) precipitation at pH >8.5 also could reduce the cost of pH adjustment to have suitable alkalinity for *Spirulina sp.* growth.

- The optimum solution pH for cell growth was found to be 10.5 with precipitated tanning effluent. Residual chromium concentration in precipitated tanning effluent didn't show inhibitory effect towards *Spirulina sp.* growth. However, salt (Cl^-) concentration above 2.96 g L^{-1} inhibited biomass growth.

- Glucose production with Zarrouk media composition was higher at solution pH 6.5. In PCTE, glucose content was higher at solution pH 8.5.

- The maximum pigments production was noted at pH 8.5 in case of both PCTE and ZMC. It can be concluded that PCTE can be used for pigment and glucose formation for the purpose of ethanol production as biofuel.

- The kinetic of *Spirulina* cell growth suggests that addition of heavy metals to ZMC interfere nutrient interaction/transport during cultivation.

- The sorption rate constant is always greater than desorption rate constant reflects positive nutrient uptake and *Spirulina* growth in all cultivation conditions. PCTE exhibited the highest growth inhibition than Cr(III) and Cr(VI) in ZMC.

Nomenclature

B	*Spirulina* cell density (g L^{-1})
I_{av}	Average light intensity (μmol m^{-2} s^{-1})
I_0	Initial light intensity (μmol m^{-2} s^{-1})
K_a	Adsorption constant (h^{-1})
K_d	Desorption constants (h^{-1})
K_s	Half saturation constant (g L^{-1})
P_c	Photosynthetic rate (carbon) (h^{-1})
P_{max}	Maximum photosynthetic rate (carbon) (h^{-1})
rR_c	Maintenance respiration rate (carbon) (h^{-1})
S_b	Bulk nutrient concentration (mol L^{-1})
S_n	Surface-adsorbed nitrate per g algae (mol g^{-1})
$S_{n,max}$	Maximum nitrate quota(maximum capacity of nitrate sorption) (mol g^{-1})
$S_{n,min}$	Minimum nitrate quota(minimum nitrate required for growth) (mol g^{-1})
α	Absorption coefficient (m^2 g^{-1})
β	Biosynthesis efficiency (g L^{-1})
$\emptyset_m$	Photon efficiency (g (μmol photons)$^{-1}$)
$\emptyset_{s,i}$	Intracellular nutrient sorption efficiency
$\emptyset_{s,ext}$	Extracellular nutrient sorption efficiency
μ	Specific growth (h^{-1})
ρ_a	Adsorption rate (mol L^{-1} h^{-1})
ρ'_a	Nutrient uptake rate (mol L^{-1} h^{-1})
ρ_d	Desorption rate (mol L^{-1} h^{-1})

References

Ahsan, M., Habib, B., Parvin, M., Huntington, T.C. and Hasan, M.R.,*A Review on Culture, Production and use of Spirulina as Food for Human and Feed for Domestic Animals and Fish*, FAO, United Nations Rome, Fisheries and Aquaculture Circular No. 1034 (2008).

Ahluwalia, S.S. and Goyal, D.,"Microbial and Plant Derived Biomass for Removal ofHeavy Metals from Wastewater", *Bioresour. Technol*., **98**, 2243-2257(2007).

Ajayan, K.V., Selvaraju, M. and Thirugnanamoorthy, K., "Enrichment of Chlorophyll and Phycobiliproteins in *Spirulina Platensis* by the use of Reflector Light and Nitrogen Sources: An in-vitro study", *Biomass Bioenerg.*, **47**, 436-441(2012).

Aksnes, D. L. andEgge, J. K., "A Theoretical Model for Nutrient Uptake in Phytoplankton", *Marine. Ecol.Prog. Ser*., **70**, 65-72 (1991).

Amala, K. and Ramanathan, N., "Chlorophyll Production from *Spirulina Platensis* (Single Cell Protein, SCP); Cultivation with Sodium Chloride in Rice Mill Waste Water", *Int. J. Chem Tech Res.,* **5(3)**, 1284-1288 (2013).

American Public Health Association (APHA), *Standard Methods for the Examination of Water and Wastewater*, 20th ed. Clesceri LS, Greenberg AE, & Eaton, AD, (Eds); American Public Health Association: Washington, DC (1998).

Becker,"Microalgae as a Source of Protein", *Biotechnol. Adv*., **25**, 207-210(2007).

Bernard O., "Hurdles and Challenges for Modelling and Control of Microalgae for CO_2Mitigation and Biofuel Production", J. Process Control, **21**, 1378-1389(2011).

Bhowmik, D., Dubey J. and Mehra, S., "Evaluating Potential of *Spirulina* as Innoculant for Pulses", *Acad. J. Plant Sci*., **3(4)**, 161-164(2010).

Binaghi, L., Borghi, A.D., Lodi, A., "Converti, A. and Borghi, M.D. Batch and Fed-batch Uptake of Carbon Dioxide by *Spirulina Platensis*", *Process Biochem.*, **38.**1341-1346 (2003).

Cafiizares-Villanueva, R.O., Dolrdnguez, A.R., Cruz M. S. and Rios-Leal, E., "Chemical Composition of Cyanobacteria Grown in Diluted Aerated Swine Wastewater", *Bioresour. Technol* .**51**, 111-116, (1995).

Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcão, V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P. and Pinto, E., "Metabolites from Algae with EconomicalImpact", *Comp. Biochem. Physiol. C*, **146**, 60-78 (2007).

Celekli, Yavuzatmaca, A.M. and Bozkurt, H.,"Modeling of Biomass Production by *SpirulinaPlatensis* as Function of Phosphate Concentrations and pH Regimes", *Bioresour. Technol.*,**100**, 3625-3629 (2009).

Chaiklahan, R., Chirasuwan, N., Loha, V. and Bunnag, B.,"Lipid and Fatty Acids Extraction from the *CyanobacteriumSpirulina*", *Sci. Asia*., **34**, 299-305 (2008).

Chauhan, U.K. and Pathak, N., "Effect of Different Conditions on the Production of Chlorophyll by *SpirulinaPlatensis*", *J. Algal Biomass Utln*., **1(4)**, 89-99 (2010).

Ciferri, O.,"*Spirulina*, the Edible Microorganism",*Microbiol. Rev.*,**47(4)**, 551-578(1983).

De Filippis, L. F., and C. K. Pallaghy, "*Heavy Metals: Sources and Biological Effects*. In: Algae and Water Pollution", Rai, L. C., et al., eds., E. SchweizerbartscheVerlagsbuch-handlung, Stuttgart(1994) pp. 31-37.

Droop, M.,"Vitamin B12 and marine ecology. IV TheKinetics of Uptake, Growth and Inhibition in MonochrysisLutheri", *J. Mar. Biol.*, **48**, 689-733(1968).

EPA,*Volunteer Estuary Monitoring Manual, A Methods Manual*, Chapter 9: Dissolved Oxygen and Biochemical Oxygen Demand, Second Edition. EPA-842-B-06-003, U.K (2006).

Gaur, J. P., and L. C. Rai, *Heavy Metal Tolerance in Algae*. In: Algal Adaptation to Environmental Stresses: Physiological, Biochemical and Molecular Mechanisms, Rai L. C., and Gaur J. P., eds., Springer-Verlag, Berlin(2001) pp. 363-388.

Geider, R.J., MacIntyre, H.L. and Kana, T.M., "A Dynamic Regulatory Model of Phytoplanktonic Acclimation to Light, Nutrientsand Temperature", *Limnol. Oceanogr*., **43**, 679-694 (1998).

Geider, R.J., Osborne, B.A., *Algal Photosynthesis: The Measurement of Gas Exchange and Related Processes*. Chapman and Hall, New York (1991) pp. 256.

Gokhale, S.V., Jyoti, K.K. and Lele, S.S., "Kinetic and Equilibrium Modeling of Chromium(VI) Biosorption on Fresh and Spent *Spirulina Platensis/Chlorella Vulgaris* Biomass", *Bioresour. Technol.*, **99**, 3600-3608 (2008).

Goksan, T., Zereriyaglu, A. and Ilknur, A.K., "The Growth of *Spirulina Platensis* in Different Culture Systems under Greenhouse Condition", *Turk. J. Biol*., **31**, 47-52 (2007).

Golder, A.K., Samanta, A.N. and Ray, S., "Removal of Chromium and Organic Pollutants from Industrial Chrome Tanning Effluent by Electrocoagulation", *Chem. Eng. Technol.*, **34 (5)**, 1-10 (2011)

Gong, R., Ding, Y., Liu, H., Chen, Q. and Liu, Z., "Lead Biosorption and Besorption by Intact and Pretreated*SpirulinaMaxima*Biomass", *Chemosphere*, **58(1)**, 125-30(2005).

Gupta, R. S. and Changwall, M. L., *Biotechnology of Mass Production of Spirulina and Arthrospira in Fresh Water*. C. V. and N. JeejiBai (Eds.) *Spirulina*, ETTA Nat. Symp. MCRC, Madras-India (1992).

Han, X., Wong, Y. S. and Tam, N.F.Y., "Surface Complexation Mechanism and Modeling in Cr(III) Biosorption by a Microalgal Isolate, *Chlorella miniata*", *J. Colloid Interface Sci.*, **303**, 365-371(2006).

Harrison, P. J., Parslow, J. S. and Conway, H. L., "Determination of Nutrient Uptake Kinetic Parameters: A Comparison of Methods", *Marine Ecol. Progr. Ser*., **52**, 301-312 (1989).

Hase, R., Oikawa, H., Sasao, C., Morita, M. and Watanabe, Y.,"Photosynthetic Production of MicroalgalBiomass in a Raceway System UnderGreenhouse Conditions in Sendai City", *J. Biosci. Bioeng.*, **89**, 157-163 (2000).

Kaewpintong, K., Shotipruk, A., Powtongsook S. and Pavasant, P., "Photoautotrophic High-Density Cultivation of Vegetative Vells of *Haematococcus Pluvialis* in Airlift Bioreactor", *Bioresour. Technol*., **98**, 288-295 (2007).

Koca, N., Karadeniz, F. and Burdurlu, H.S., "Effect of pH on Chlorophyll Degradation and Colour Loss in Blanched Green Peas", *Food Chem.*, **100**, 609-615 (2006).

Marshall, H. L., Geider, R. J. and Flynn, K. J., "A Mechanistic Model of Photoinhibition", *New Phytologist.*, **145(2)**, 347-359(2000).

Minguez-Mosquera, M. I., Garrido-Fernandez, J., and Gandul-Rojas, B., "Pigment Changes in Olives during Fermentation and Brine Storage", *J. Agric. Food Chem.*, **37**, 8-11 (1989).

Olguõn, E.J., Galicia S., Angulo-Guerrero O. and Hernandez, E., "The Effect of Low Light Flux and Nitrogen Deficiency on the Chemical Composition of *Spirulina Sp. (Arthrospira*) Grown on Digested Pig Waste", *Bioresour.Technol*. **77**, 19-24, (2001).

Packer, A., Li, Y., Andersen, T., Hu, Q., Kuang, Y. and Sommerfeld, M., "Growth and Neutral Lipid Synthesis in Green Microalgae: A Mathematical Model", *Bioresour. Technol*., **102** 111-117 (2011).

Pandey, J.P., Tiwari, A. and Mishra, R.M., "Evaluation of Biomass Production of *Spirulina Maxima* on Different Reported Media",*J. Algal Biomass. Utln.*, **1(3),** 70-81 (2010).

Pawar, H.A. and D'Mello, P.M.,"Spectrophotometric Estimation of Total Polysaccharides in Cassia ToraGum", *J. Appl. Pharma. Sci*., **01(03),**93-95 (2011).

Pereira, M., Bartolomé, M.C. and Sánchez-Fortún S., "Bioadsorption and Bioaccumulation Of Chromium Trivalent in Cr(III)-Tolerant Microalgae: A Mechanisms for Chromium Resistance", *Chemosphere*, **93**, 1057-1063, (2013).

Phang, S.M., Miah, M.S., Yeoh, B. G. and Hasim M.A., "*Spirulina* Cultivation in Digested Sago Starch Factory Wastewater", *J. Appl. Phycol*., **12**, 395-400, (2000).

Pinto, E., Sigaud-Kutner, T. C. S., Leita˜o, M. A. S., Oka-moto, O. K. and Morse, D., "Heavy Metal-Induced Oxidative Stress in Algae", *J.Phycol.*, **39**, 1008-1018 (2003).

Rainha, N., Lima, E., Baptista, J. and Rodrigues, C., "Antioxidant Properties, Total Phenolic, Total Carotenoid and Chlorophyll Content of Anatomical Parts of *HypericumFoliosum*", *J. med. Plant.*, **5(10),**1930-1940 (2011).

Ratana C., Chirasuwan, N., Siangdung W., Paithoonrangsarid, K. and Bunnag B., "Cultivation of Spirulinaplatensis Using Pig Wastewater in a Semi-Continuous Process*", J. Microbiol. Biotechnol.*, **20(3**), 609-614, (2010).

Rodrigues, M.S., Ferreira, L.S., de Carvalho, J.C.M., Lodi, A., Finocchio, E. and Converti, A.,"Metal Biosorption onto Dry Biomass of *Arthrospira(Spirulina)Platensis* and *Chlorella Vulgaris*: Multi-Metal Systems", *J. Hazard. Mater.*, **217-218**, 246-255 (2012).

Singh, A., Kumar, D. and Gaur, J.P.,"Continuous Metal Removal from Solution and Industrial Effluents Using *Spirogyra*Biomass-Packed Column Reactor", *Water Res.*,**46**, 779-788 (2012).

Tanaka, R. and Tanaka, A., "Chlorophyll Cycle Regulates the Construction and Destruction of the Light-harvesting Complexes", *Biochim. Biophys. Acta.*, **1807**, 968-976 (2011).

Vilchez, C., Garhayo, I., Lobato, M.V. and Vega, J.M., "Microalgae-Mediated Chemicals Production and Wastes Removal", *Enzyme Microb. Technol.*, **20,** 562-572 (1997).

Volkmann, H., Imianovsky, U., Oliveira, J. L.B. and Sant'Anna, E. S., "Cultivation of *Arthrospira (Spirulina) Platensisin* Desalinator Wastewater and Salinated Synthetic Medium: Protein Content and Amino-Acid Profile", *B. J. Microbiol*. **39**, 98-101, (2008).

Vonshak, A.,*SpirulinaPlatensis* (*Arthospira*): *Physiology, Cell-biology and Biotechnology*. Taylor and Francis publication, New York (1997).

Yao, B., Xi, B., Hu, C., Huo, S., Su, J. and Liu, H.,"A Model and Experimental Study of Phosphate Uptake Kinetics in Algae: Considering Surface Adsorption and P-stress", *J. Environ. Sci*., **23(2)**, 189-198 (2011).

Chapter 6

Conclusions and Recommendations for Future Work

In this chapter the salient accomplishments and major conclusions of the entire work are summarized. The directions for the further extension of the present work are alsoprovided.

6.1 Conclusions

The treatment of trivalent and hexavalent chromium both in synthetic and industrial effluents by dead and live *Spirulina platensis* were performed. The conclusions are outlined as follow:

☑ *Spirulina sp*. could effectively uptake Cr(III) from an aqueous solution. Cr(III) sorption showed the highest dependency on solution pH with maximum binding at pH 6.2. Cr(III) binding was exothermic in nature.The dissociation constants of 1.19, 2.87 and 8.07 correspond to carboxylic, phosphatic and amine groups, respectively.Cr(III) binding constants of 80.9, 3.9×10^{3} and 3.5×10^{4} L mol^{-1}were determined for carboxylic, phosphatic and amine groups, respectively. Cr(III) coverage on *Spirulina* surface was dual sites in

nature with major attachment to phosphatic sites. However, according to metal binding model, the amine group was less significant for Cr(III) removal. Cr(III) desorption was more towards the acidic pH with 77% recovery at the lowest pH of 0.5 tested. In case of live biomass, Cr(III) removal was almost complete upto an initial Cr(III) of 20 mg L^{-1}. $Cr(OH)_3$ flocs appeared at higher concentration (>10 mg L^{-1}) reduced the cell growth.

☑ Cr(VI) reduction into Cr(III)preferably took place at acidic pH because of more Cr(VI) attachment to proton donor groups and high proton concentration. The highest Cr(VI) reduction of 98% was obtained at pH 0.5. The kinetic model of Cr(VI) reduction developed in terms of functional groups and initial Cr(VI) concentrations well fitted to the experimental results upto pH 6, temperature from 25 to 45°C and anionic strength from 0.588 to 5.63 mM for different biomass doses and initial Cr(VI) concentration. The model estimated concentration of protonated functional groups exhibited excellent agreement to that of from the potentiometric titration. The kinetic parameters were independent on initial Cr(VI) concentration and biomass dose. Higher temperature augmentedthe rate of Cr(VI) reduction as well as the acidic site concentration. Slight detrimental effect of common anions (NO_3^-, Cl^-, SO_4^{2}) was noted on Cr(VI) reduction.The rate constant was expressed in terms of pH, temperature and anionic strength to calculate its value for future application. Cr(VI) exhibited similar inhibition on *Spirulina* cell growth as with Cr(III) at the same concentration.

☑ Precipitated chrome tanning effluentin ZMC (ZMC+PCTE) showed reasonable *Spirulina* cell growth. However, the cell density was much lower in comparison to growth in ZMC, ZMC+Cr(III) and ZMC+Cr(VI). ZMC+Cr(III) displayed the lowest inhibition on growth followed by ZMC+Cr(VI). ZMC+PCTE cultivated biomass produced more chlorophyll a

in ZMC+PCTE than ZMC. While, carbohydrate and chlorophyll b production were reduced in ZMC+PCTE. No major difference for the functional groups of *Spirulina sp.* cultivated in ZMC+PCTE was noted compared to biomass obtained in other experimental conditions. The growth model showed excellent agreement to the experimental data irrespective to cultivation the conditions.

6.2 Recommendations on future directions

- The present work can be extended for the treatment of effluents containing multiple heavy metals in industrial sources along with removal of inorganic pollutants.

- Future work also may include studies on theoretical metal uptake with algal growth to predict the quality of supernatant and to establish the scale up laws.

- Algal resource is one of the potential feedstocks for the third generation biofuel and biomass production is considered as a promising renewable energy source for the future. *Spirulina* cultivation in PCTE further can be investigated for its application in biofuel production. Biofuel production from *Spirulina sp.* cultivation in waste industrial and domestic effluents is also a prudent area of future research.

- This study can be extended further towards the design of an efficient photo-bioreactor for higher rate of biomass production.The genetic modification of algal strain may be taken into account to enhance the productivity of a specific product.

Publications

Journal Publications

M.K. Gagrai, C. Das and A.K. Golder, "Non-ideal metal binding model for Cr(III) sorption using *Spirulina platenesis* biomass: experimental and theoretical approach", ***Canadian Journal of Chemical Engineering***, 2013, 91(12), 1904-1912.

M.K. Gagrai, S. Khuntia, C. Das and A.K. Golder, "Effect of background ions on reduction of Cr (VI) to Cr (III) using saline water algae", ***Research Journal of Chemistry Environment***, 2011, 15 (2), 450-453.

M.K. Gagrai, C. Das and A.K. Golder, "Reduction of Cr(VI) ion by dead *Spirulina* biomass in aqueous solution: A kinetic reduction model", ***Chemosphere,*** 2013, 93(7), 1366-1371**.**

M.K. Gagrai, C. Das and A.K. Golder, "Biological reduction and removal of Cr(VI) with microbial consortia collected from local water treatment plant", ***International Journal of Environment Management and Technology*** (**Under review**).

M.K. Gagrai, C. Das and A.K. Golder, "Metabolites of *Spirulina* sp. cultivated in Cr(III) precipitated industrial chrome tanning effluent", **Journal of Algological Studies, (Under review)**.

International Conferences

M.K. Gagrai, C. Das and A.K. Golder, "Precipitated chrome tanning effluent: an alternate chloride source for *Spirulina s*p. Cultivation", Accepted at 1st International Conference On "Bioenergy, Environment & Sustainable Technologies–**BEST-2013**" 27-30 January 2013, Arunai Engineering College (AEC), Tiruvannamalai, India.

M.K. Gagrai, C. Das and A.K. Golder, "Metabolite of *Spirulina sp.* cultivated in precipitated chrome tanning effluent (PTE)", International Conference on Algal Biorefinery: A

Potential Source of Food, Feed, Biochemicals, Biofuels and Biofertilizers (**ICAB 2013**), 10-12 January, 2013, IIT Kharagpur, India.

M.K. Gagrai, A. P. Momin, C. Das and A.K. Golder, "Influence of phenol treatment on Cr(III) Biosorption with *Spirulina*", Indian Chemical Engineering Congress (**CHEMCON-2012**), 27-30, 2012 at NIT Jalandhar, India.

M.K. Gagrai, C. Das and A.K. Golder, "Two parameter equilibrium sorption isotherm model: Influence of Temperature on Cr(III) sorption onto *Spirulina* biomass", Indian Chemical Engineering Congress (**CHEMCON-2012**), 27-30, 2012 at NIT Jalandhar, India.

M.K. Gagrai, C. Das and A.K. Golder, "Cultivation of *Spirulina sp*. in chemically treated spent tanning liquor (Characterization of biomass)", accepted at 7th CUTSE Conference (**CUTSE-2012**), 6-7 November 2012, Miri, Sarawak, Malaysia.

M.K. Gagrai, C. Das and A.K. Golder, "Biological reduction and removal of Cr(VI) with microbial consortia collected from local water treatment plant", Accepted at IWA Regional Conference on Wastewater Purification & Reuse (**WWPR 2012**), 28-30th March, 2012, Heraklion, Crete, Greece.

S. Khuntia, **M.K. Gagrai**, C. Das and A.K. Golder, "Effect of background ions on reduction of Cr (VI) to Cr (III) using saline water algae", Accepted at 5th International Congress of Chemistry and Environment **(ICCE-2011)**, 27- 29th May, 2011, Kuching, Sarawak, MALAYSIA.

M.K. Gagrai, C. Das and A.K. Golder, "Cr(III) biosorption by a saline microalgae *Spirulina platensis*: Studies on two parameters isotherms" Indian Chemical Engineering Congress (**CHEMCON-2011**), 27 - 29th December, 2011, M S Ramaiah Institute of Technology, Bangalore, India.

M.K. Gagrai, C. Das and A.K. Golder, "Studies on the hazardous nature of spent bio-sludge

laden with Cr(III): Toxicity characteristic leaching procedure" Indian Chemical Engineering Congress (**CHEMCON-2011**), 27 - 29th December, 2011, M S Ramaiah Institute of Technology, Bangalore, India.

M.K. Gagrai, C. Das and A.K. Golder, "Study on the mechanism of aqueous Cr(VI) sorption by *Spirulina platensis* biomass", Microbes in Wastewater & Waste Treatment, Bioremediation and Energy Production (**MWT 2011**) 24 - 27th January, 2011, BITS Goa, India.

M.K. Gagrai, C. Das and A.K. Golder, "Biosorption of heavy metal ions to a microalga *Spirulina Sp*." Indian Chemical Engineering Congress (**CHEMCON-2010**), 27 - 29th December, 2010, Annamalai University, Tamilnadu, India.

Other Publications

Ch.V. Rao, **M.K. Gagrai**, V.V. Goud and A.K. Golder, "Oxidative brilliant green dye degradation in Fenton process," ***International Journal of Cheminformatics and Chemical Engineering*** (accepted).

A. P. Momin, **M.K. Gagrai**, C. Das and A.K. Golder, "Cr(VI) reduction to Cr(III) in aqueous solution by photo-generated Fe(II)," International Conference on Advances in Chemical Engineering **(ICACE 2013)**, 05-06th April, 2013, NIT Raipur, India.

Ch.V. Rao, **M.K. Gagrai**, V.V. Goud and A.K. Golder, "Oxidative brilliant green dye degradation in Fenton process," International Conference on Advances in Chemical Engineering **(ICACE 2013)**, 05-06th April, 2013, NIT Raipur, India.