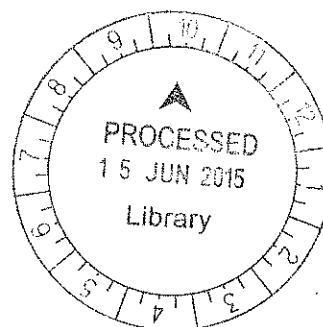


THE REMOVAL OF HEXAVALENT CHROMIUM BY A NOVEL SINGLE-STEP  
CHEMICALLY ACTIVATED BIOCARBON

DYISNI SUNDARALINGAM

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
BACHELOR OF BIOTECHNOLOGY (HONOURS)



TP  
248  
2  
014  
2015

FACULTY OF SCIENCE, TEHCNOLOGY,  
ENGINEERING AND MATHEMATICS  
INTI INTERNATIONAL UNIVERSITY  
PUTRA NILAI, MALAYSIA

## NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this dissertation completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

Moreover, I have not handed in a dissertation similar in contents elsewhere.

In case of proof that the dissertation has not been constructed in accordance with this declaration, the Faculty of Science, Technology, Engineering and Mathematics has the right to consider the research dissertation as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

DYISNI SUNDARALINGAM

Name

I13002263

I.D.Number

*Dyisni*

Signature

8/5/2015

Date

## ACKNOWLEDGEMENT

Firstly and foremostly, I would like to express my sincerest gratitude to my supervisor, Associate Professor Dr. Prof Palsan bin Abdullah for his guidance, support, and motivation to throughout my research and dissertation write-up with his knowledge, patience and wise words.

Apart from that, I would like to my hardworking and loving parents for their financial and moral support. Most of all, words can never say how much gratitude I feel for Jesh, Madhavi, Saheyli, Kanimoli and Aruna for their unsurmountable care, concern and cheers.

Last but not least, I would like to give myself a pat in the back for never giving up and for succesfully completing this dissertation with unrelenting persistence and good faith. As William Ernest Henley once wrote:

“I am the master of my fate, I am the captain of my soul.”

## ABSTRACT

Heavy metal pollution in the environment has drastically increased to alarming levels worldwide because of reckless urbanization and industrialization and is expected to be worse in the projected future. Cr (III) is naturally present and crucial in certain metabolic pathways while Cr (VI) pollution exists mainly due to manmade industries other than natural causes like sewage leachates. Even at minute levels, Cr (VI) is highly hazardous to human health due to its extreme mobility and solubility in water which exists in anion form, powerfully oxidative, and 100 to 1000 times more poisonous than Cr (III). Current heavy metal remediation methods are based on physico-chemical approaches that are very expensive, energy-demanding, produces a new type of waste (sludge), requires complicated management, and the technology is limited to developed countries. Functional groups on the biocarbon's cell wall polysaccharides, proteins and lipids can bind to metal ions. The novel biocarbon with single-step chemical activation had a maximum adsorption capacity,  $Q_{max}$  of 14.286 mg/g with an optimum pH at 2. Cr (VI) removal was 92.33% for 100 mg/L of initial Cr (VI) concentration at a contact time of 30 minutes. Optimum biocarbon dosage used was 0.125 g at 25°C r.t. and the  $pH_{pzc}$  value was 2.3. Langmuir isotherm type-1 had the best fit to the available data with a  $R^2$  of 0.994 and Langmuir affinity constant,  $b$  of 5.385 L/g.

**Keywords:** Anion, biocarbon, waste, pollution, groundwater, Cr (VI), remediation, activation, adsorption capacity, Langmuir, pH, dosage, contact time, functional groups.

## TABLE OF CONTENT

	PAGE
DECLARATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
TABLE OF CONTENT	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	x
CHAPTER	
1. INTRODUCTION	1
1.1 Background	1
1.2 Objectives	3
2. LITERATURE REVIEW	4
2.1 Chromium (VI) Properties	4
2.2 Current Commercial Techniques	4
2.2.1 Physico-Chemical Approaches	4
2.2.2 Biological Approaches	5
2.3 Effects of Variable Factors	5
2.3.1 pH	5
2.3.2 Contact Time	6
2.3.3 Biocarbon Dosage	7
2.3.4 Initial Chromium (VI) Concentration	7
2.4 Biocarbon	8
2.4.1 Sources	8
2.4.2 Characterization	8
2.4.3 Biosorption Mechanisms	9
2.4.4 Advantages	10
2.5 Sorption Isotherms	11
3. MATERIALS AND METHODS	12
3.1 Preparation of Chromium (VI) Stock Solution	12
3.2 Preparation of Other Chemical Reagents	12
3.3 Activation of Biocarbon	12
3.4 1,5-Diphenylcarbazide Method (DPC Assay)	13
3.5 Calibration Curve Preparation	13
3.6 Batch Biosorption Studies: Effect of Variable Factors on Chromium (VI) Removal	14

3.6.1	Effect of pH	14
3.6.2	Effect of Biocarbon Dosage	14
3.6.2	Effect of Contact Time	15
3.6.3	Effect of Initial Chromium (VI) Concentration	15
3.7	Characterization: Point of Zero-Charge ( $\text{pH}_{\text{pzc}}$ )	15
3.8	Determination of Chromium (VI) Removal and Uptake	16
3.9	Adsorption Isotherms	17
3.9.1	Langmuir	17
3.9.2	Freundlich	18
3.10	Statistical Analysis	18
<b>4.</b>	<b>RESULTS AND DISCUSSION</b>	<b>19</b>
4.1	Calibration Curve	19
4.2	Batch Biosorption Studies on Chromium (VI) Removal	19
4.2.1	pH	19
4.2.2	Biocarbon Dosage	20
4.2.3	Contact Time	21
4.2.4	Initial Chromium (VI) Concentration	22
4.2.4	Point of Zero-Charge	24
4.3	Adsorption Isotherm	24
4.3.1	Langmuir	24
4.3.2	Freundlich	26
4.3.3	Parameters of Langmuir and Freundlich Isotherms	26
4.4	Comparison with Other Biocarbons	27
<b>5.</b>	<b>CONCLUSIONS AND FUTURE PROSPECT</b>	<b>28</b>
5.1	Conclusions	28
5.2	Future Prospect	28
	<b>REFERENCES</b>	<b>29</b>
	<b>APPENDICES</b>	<b>32</b>

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
2.4.2	Absorption capacity of a few biocarbon (Datta et. al., 2013).	18
3.5	Known Cr (VI) standard curve preparation.	23
3.9	Different types of linearized form of Langmuir and Freundlich isotherm with graph parameters. (Adapted from Armagan & Toprak, 2013)	27
4.3.3	Isotherm parameters of linearized of Langmuir and Freundlich isotherms	35
4.4	Comparison of Cr (VI) removal parameters among a few biocarbons	36

## LIST OF FIGURES

Figure		Page
2.2.1	Effect of pH on Cr (VI) absorption (Ilyas et. al., 2014).	15
2.2.2 (a)	Effect of contact time on Cr(VI) removal by sawdust (Ilyas et. al., 2014)	16
2.2.2 (b)	Contact time effect of neem leaf powder on % absorption (Kovo et. al., 2014).	16
2.2.2 (c)	Effect of contact time on Cr(VI) removal of various biocarbons (Gupta & Mote, 2014)	16
2.4.3 (a)	Pretreatment on biomass supplies to produce biosorbents (Gautam et. al., 2014).	19
2.4.3 (b)	Cr (VI) interface with <i>Sargassum</i> biomass cell wall surface by ion exchange or complexation (Zheng et. al., 2011).	19
2.5 (a)	Langmuir isotherm where $C_e$ (mg/L) = [metal ion] in solution at equilibrium; $q_e$ (mg/g) = bound metal ions; $Q_{max}$ (mg/g) = (monolayer sorption capacity) Langmuir constant; $b$ (L/mg) = constant(binding sites' affinity) (Hubbe et. al., 2011).	20
2.5 (b)	Freundlich isotherm where $K_f$ & $n$ = Freundlich constants( $n$ = measure of the strength and nature of active sites distribution and sorption process & $K_f$ = indicates sorption capacity) (Hubbe et. al., 2011).	20
4.1	Calibration curve obtained for known Cr (VI) concentration range of 0-1 mg/L. Linear equation $y = 0.6695x$ with coefficient correlation, $R^2 = 0.9990$ . High $R^2$ value indicates high reliability and accuracy of data. Absorbance increased with increased metal ion concentrations.	29
4.2.1 (a)	Cr (VI) removal values decreased drastically from 97.83% to 26.50% at higher pH with removal being best at pH 2.	30
4.2.1 (b)	Metal uptake also reduced drastically with increased pH from 20.24mg/g to 4.83 mg/g. Uptake was best at pH 2.	30
4.2.2 (a)	Cr (VI) removal increased with higher biocarbon dosage to 100%.	31
4.2.2 (b)	Metal uptake, $q_e$ values reduced with increased dosage.	31
4.2.3 (a)	Mean removal values of contact time for each bar plotted were mean of triplicates measurements. Good removal was achieved	32



	even at 30 minutes.	
4.2.3 (b)	Mean metal uptake values for each contact time bar plotted were of triplicates.	32
4.2.4 (a)	Removal decreased from 99.24% to 87.02% with increasing initial Cr (VI) concentration used.	33
4.2.4 (b)	Uptake increased gradually with increasing initial Cr (VI) concentration with 2.08 mg/g for 10 mg/L to 17.16 mg/g at 100 mg/L.	33
4.2.5	The point of zero charge was determined by plotting the difference between final pH and initial pH against initial pH. The point where the curve crosses the x-axis is the pH-pzc; pH 2.3.	34
4.3.1 (a)	Linearized Langmuir type 1 isotherm showing monolayer absorption.	34
4.3.1 (b)	Linearized type 2 Langmuir isotherm	35
4.3.1 (c)	Linearized type 3 Langmuir isotherm	35
4.3.1 (d)	Linearized type 4 Langmuir isotherm	35
4.3.2	Linearized Freundlich isotherm	36

## LIST OF ABBREVIATIONS

Cr (III)	Trivalent chromium
Cr (VI)	Hexavalent chromium
$\text{CrO}_4^-$	Chromate
$\text{Cr}_2\text{O}_7^{2-}$	Dichromate
dedH <sub>2</sub> O	Deionised distilled water
DPC	1,5-diphenylcarbazine
HCl	Hydrochloric acid
$\text{HCrO}_4^-$	Hydrogen chromate
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
NaOH	Sodium hydroxide
NaCl	Sodium chloride
NLP	Neem leaves powder
(-NH <sub>2</sub> )	Amine functional group
(-NH <sub>3</sub> <sup>+</sup> )	Protonated amine group
pzc	Point of zero charge
r.t.	Room temperature
USEPA	United States Environment Protection Agency
WHO	World Health Organization

## 1.0 CHAPTER 1:

### INTRODUCTION

#### 1.1 BACKGROUND

Presently, drastic urbanization and industrialization has exponentially increased the level of heavy metal pollution in the environment to worrying levels and is expected to double or triple in the coming future. These metals are persistent, unmanageable and non-biodegradable. Toxic heavy metals accumulate in the waterways, seeps into the soil, taken up by plants and livestock alike and eventually end up in humans as we consume these for food. It can cause severe groundwater, soil and air pollution. Chromium is the 21<sup>st</sup> most plentiful element of this earth's outer layer with a range of oxidation states, from -4 to +6 (Were, Moturi, & Wafula, 2014). Amongst them, chromium (III) and chromium (VI) are commonly found in the environment. Chromium (III) is naturally present and crucial in certain metabolic pathways while Cr (VI) pollution exists mainly due to manmade industries other than natural causes like sewage leachates. Processing and production activities like electroplating, leather tanning, paint, dye, coal mining and petrochemical facilities cause the rise of toxic Cr (VI) pollution in the environment. The current permissible concentration of chromium standards in drinking water by the United States Environment Protection Agency (USEPA) is total Cr < 0.1 mg/L while for World Health Organization (WHO), total Cr < 2 mg/L and toxic Cr (VI) < 0.05 mg/L (U.S. Geological Survey, 2007).

Chromium (VI) is highly hazardous to human health due to its extreme mobility and solubility in water which exists in anion form; chromate ( $\text{CrO}_4^-$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), powerfully oxidative, and 100 to 1000 times more poisonous than Cr (III) even at minute levels (Gautam, Mudhoo, Lofrano, & Chattopadhyaya, 2014). It is also mutagenic due to its ability to form free radicals and carcinogenic due to its high penetration power. A study at Kenya within the tannery sector revealed it is corrosive to the skin when ingested or inhaled directly with a higher incidence of dermatological and respiratory problems with long term contact (20% and 30%) (Were et. al., 2014). Other symptoms were gastrointestinal hemorrhage, damaged

blood and lungs system, weakened immune system, tissue necrosis, hepatic and renal destruction, liver and lung cancer which eventually lead to death (Were et. al., 2014).

Current heavy metal remediation methods are based on physico-chemical approaches such as coagulation-flocculation, chemical precipitation, ion exchange resins, activated carbon adsorption, and membrane filtration (Sannasi, 2013). The downside of these methods are they are highly expensive; energy-demanding for example in tannery where copious amount of water used in areas where groundwater is scarce (Belay, 2010); produces a new type of waste (sludge) instead of eliminating waste; and requires complicated management (skilled employees); technology limited to developed countries; time-consuming, and less efficient (55–60%) compared to biocarbon usage (Belay, 2010).

This can be overcome by using waste as biocarbon where functional groups on cell wall polysaccharides, proteins and lipids bind to metal ions (Murugavelh & Vinothkumar, 2010). Functional groups like carboxyl go through ion exchange or complexation with Cr (VI) anions (Zheng et. al., 2011). As a thriving field to explore, it presents great opportunities due to the small amount of data available for full-scale practical applications on the design and adsorption unit tests (Gautam et. al., 2014). In this experiment, a novel biocarbon source was chemically activated in a single step to determine the point of zero-charge as well as effects of pH, dosage, contact time, and initial concentration to finally determine its maximum adsorptive capacity via isotherms. This innovative biotechnological approach can be applied to wastewater treatment facilities, industries using heavy metals, factory flue gases, landfills, and even in domestic household settings. Recovery of these metals are also possible with relatively simple precipitation methods that may generate a new field of economy altogether in the near future.

## 1.2 OBJECTIVES

1. To successfully prepare and activate the novel biocarbon via chemical activation.
2. To calculate, prepare and recheck chromium (VI) stock solutions and other chemical reagents.
3. To investigate the effects of contact time on chromium (VI) removal and metal uptake,  $q$  by the activated biocarbon.
4. To investigate the effect of pH on chromium (VI) removal and metal uptake,  $q$  by the activated biocarbon.
5. To investigate the effect of biocarbon dosage on chromium (VI) removal and metal uptake,  $q$ .
6. To investigate the effect of initial chromium (VI) concentration on chromium (VI) removal and metal uptake,  $q$ .
7. To determine the point of zero-charge (pzc) of the activated biocarbon.
8. To determine the maximum adsorptive capacity via Langmuir and Freundlich adsorption isotherms.

## 2.0 CHAPTER 2:

### LITERATURE REVIEW

#### 2.1 CHROMIUM (VI) PROPERTIES

The stability and reactivity of Cr (VI) relies on the pH of the environment and thus it exists in diverse forms ( $\text{Cr}_4\text{O}_{13}^{2-}$ ,  $\text{Cr}_3\text{O}_{10}^{2-}$ ,  $\text{Cr}_2\text{O}_7^{2-}$ , and  $\text{HCrO}_4^{2-}$ ) in acidic solutions from pH 1 to 6 with hydrogen chromate,  $\text{HCrO}_4^{2-}$  being the majority (Shouman, Fathy, Khedr, & Attia, 2013). Therefore, more amino groups ( $-\text{NH}_2$ ) would be in the protonated form ( $-\text{NH}_3^+$ ) which causes an electrostatic interaction between the biocarbon surface and the anionic  $\text{HCrO}_4^{2-}$  resulting in high chromium removal such as 90% in palm branches as found by Shouman, et. al. (2013). When in acidic medium, depending on the arrangement of the biocarbon's cell wall surface molecules, the palm branches' surfactant head (trimethyl ammonium group) had many positive charges (Shouman et. al., 2013).

#### 2.2 CURRENT COMMERCIAL TECHNIQUES

##### 2.2.1 Physico-Chemical Approaches

Electrochemical and chemical precipitation is useless, particularly when metal ion concentrations in solutions are minute and these methods create large amount of sludge which needs additional treatment (Gautam et. al., 2014). Presently, the most widely utilised adsorbent is activated carbon which has a good adsorption capacity for carcinogenic metals in wastewater and industrial effluent with low concentration of heavy metal ions but unfortunately, not practical to be applied in large-scale industries (Gautam et. al., 2014). Also, its high cost and loss of 10-15% during regeneration has hindered its usage by developing countries (Datta, Mishra, & Chakraborty, 2013). Despite being used widely, it still is expensive due to its processing procedure (Gautam et. al., 2014).

## **2.2.2 Biological Approaches**

The use of living and dead biomass can be used to absorb heavy metals as these bacterial and plants cells can perform biosorption mechanisms like bioaccumulation, complexation, ion exchange and others to remedy these toxic metals (Sannasi, 2013). However, living biomass may not be able to fully reiterate the absorptive capacities as obtained in the laboratory studies in real-life practical situations where many factors will influence its bioaccumulation capabilities (Sannasi, 2013). Furthermore, living cells need to be frequently recalibrated and provided with nutrition to have optimum working parameters and once they die, the cost to regrow, maintain and immobilize them to matrices to absorb toxic metals can be very pricey. On the other hand, dead biomass material seems to be more promising and workable with regeneration techniques to reduce cost and reduce waste generated (Sannasi, 2013). Dead biomass' biosorption also happens passively (requires less energy) and is primarily based on the metal ion and biocarbon's affinity between each other (Gautam et. al, 2014).

## **2.3 EFFECTS OF VARIABLE FACTORS**

### **2.3.1 pH**

At very low pH values, hydronium ions would encircle the surface of biocarbon which improve the interaction of Cr (VI) ions with the biocarbon binding sites by increasing attractive forces. As the pH increases, the total surface charge on the biocarbon becomes negative and adsorption decreases. The optimum pH for the removal of Cr (VI) was found to be at pH 2 for most adsorbents (Singha, Naiya, Bhattacharya, & Das, 2011). The pH reveals the type of contamination and thus what it causes in water. In another study, maximum absorption was also found to be between pH 2 and 3. At higher pH, biocarbon surface and Cr (VI) anions increases repulsion between them which causes a decrease in the removal of Cr (VI) ions as seen in figure 2.2.1 (Ilyas, Ahmad, Ghazi, Sohail, Arif & Khan, 2014).

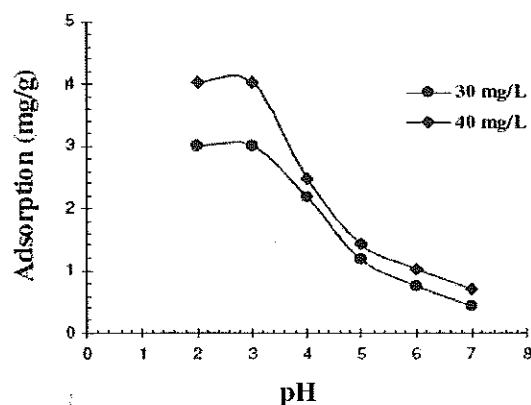
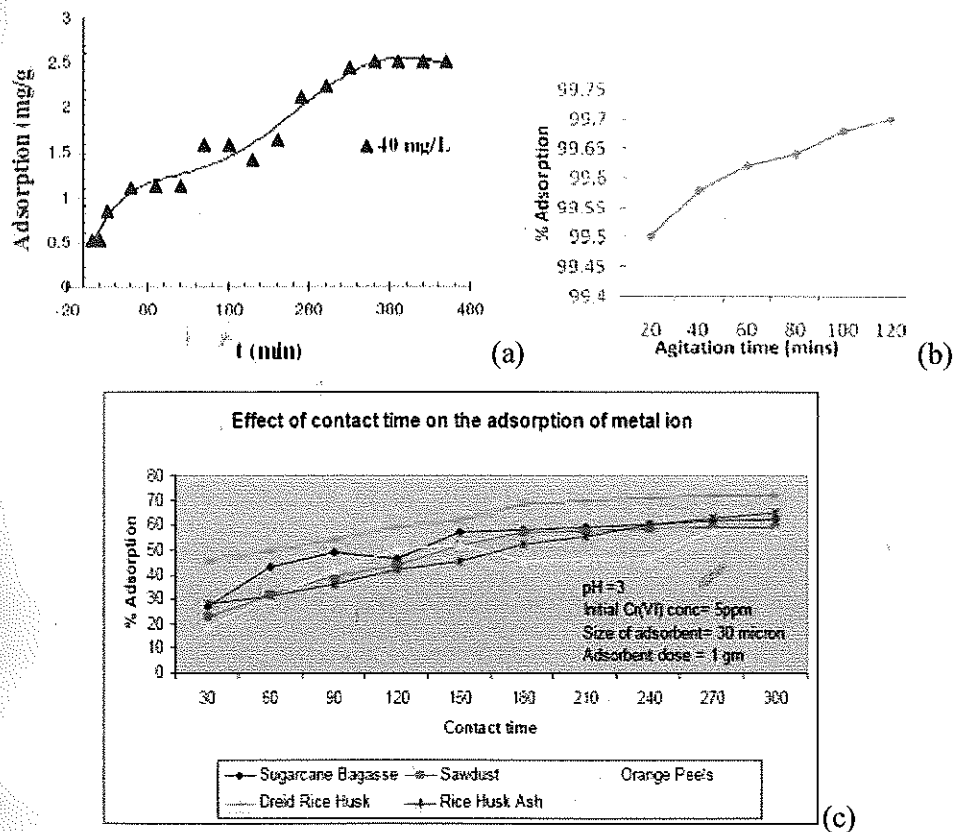


Figure 2.3.1 Effect of pH on Cr (VI) absorption (Ilyas et. al., 2014).

### 2.3.2 Contact Time

The equilibrium time for the adsorption of Cr (VI) in aqueous solutions varied for each adsorbents used from three to six hours (Singha et. al., 2011). The nature of the biocarbon and its available sites for adsorption impacts the time required to achieve equilibrium (Gupta & Mote, 2014). In one study of Cr (VI) removal using sawdust, a standard increase was observed within 30 minutes and stability was achieved within six hours with no major change viewed afterwards as seen in figure 2.2.2 (a) (Ilyas et. al., 2014). Kovo, Olu and Gwatana, 2014, had an increasing absorption percentage even after two hours but at a slower rate suggesting a constant contact time was reached as seen in figure 2.3.2.(b). Gupta & Mote, 2014, used 1 g of the biocarbon for every 20 ml of 5 mg/L Cr (VI) with varying contact times of 30 to 300 minutes with a maximal removal time of around 120 to 180 minutes as seen in figure 2.2.2 (c). At equilibrium, the active site were all saturated with metal ions and therefore shows a constant plateau-shaped graph observed at higher contact times as seen in figures 2.2.2.(a), (b) and (c).





**Figure 2.3.2** (a) Effect of contact time on Cr (VI) removal by sawdust (Ilyas et. al., 2014); (b) Contact time effect of neem leaf powder on % absorption (Kovo et. al., 2014); (c) Effect of contact time on Cr (VI) removal of various biocarbons (Gupta & Mote, 2014).

### 2.3.3 Biocarbon Dosage

The absorption percentage of Cr (VI) by neem leaves powder (NLP) increased from 99.45 to 99.70 % when more NLP was added from 200 to 1200 mg/dm<sup>3</sup> at 303 K and constant agitation for 120 minutes because more adsorption sites were available due to an abundance of functional groups (Kovo et. al., 2014). Maximum uptake was obtained at a dosage of 10 g/L for all the biocarbon sources (Singha et. al., 2011).

### 2.3.4 Initial Chromium (VI) Concentration

In Cr (VI) removal of NLP by Kovo et. al. in 2014, removal increased initially but as the initial Cr (VI) concentration was increased, removal reduced from (353.7 mg/g) 99.7% to (424.5 mg/g) 95.5% for 1.2 g of biocarbon used. This type of behavior

apparently does not usually follow literature but can occur if the initial Cr (VI) concentration required surpasses the amount of free biocarbon binding sites (Kovo et. al., 2014).

## **2.4 BIOCARBON**

### **2.4.1 Sources**

A few sources of biocarbon that have proven to be effective in removing toxic heavy metal were tea wastes (Shrestha, et. al., 2013), orange peel, neem leaves (Kovo et. al., 2014), nutshells (Altun & Pehlivan, 2012), saw dusts, rice husks, cacti barks (Fernandez-Lopez et. al., 2014), pine needles and various others; each with differing absorptive capabilities for different types of heavy metals (Datta et. al, 2013; Gautam et. al., 2014; Gupta & Mote, 2014).

### **2.4.2 Characterization**

Tea leaves' insoluble cell walls are made up largely of lignin, cellulose and hemicelluloses, condensed tannins and structural proteins. The responsible groups in lignin, tannin or other phenolic compounds are mainly carboxylate, aromatic carboxylate, phenolic hydroxyl and oxyl groups (Datta et. al., 2013). Among these materials, agricultural byproducts and biomass showed very high adsorption capacities. However, the applicability of these materials has been found to be limited due to leaching of organic substances into the solution. To overcome such problems, activation via physical or chemical treatment on solid adsorbents has been used as a technique for improving the physical and chemical properties and to increase its adsorption capacity. Without activation, the grinded tea wastes was washed with hot water (80°C) for one hour intervals until a colourless solution (normally observed within 15 washing cycles) was spectrometrically measured at room temperature to remove hydrolysable tannins and other dissolvable and coloured pigments. Then Datta et. al., 2013, dried the tea waste in oven at 105°C and used in the adsorption experiments without any further modification. However with activation, the tea waste wash was done within 5 cycles only with no remarkable colour interference at r.t. Cr (VI) adsorption was carried out in relatively cold water (25±0.3°C) and stored in a

vacuum desiccator to preserve adsorptive capability for further use. Biocarbons like tea waste (79 mg/g, 90% removal by Datta et. al., 2013; almost 100% removal of Cr (VI) of tannery contaminated water by Isah & Lawal, 2013; Nandal, Hooda & Dhanial in 2014 cited of 94-100% removal of Pb, 86% for Ni and 77% for Cd were obtained with tea waste), neem (99.7%, 125.83 mg/g; Kovo et. al., 2014), and tobacco seemed to have higher adsorption capacities (some with nearly 100% Cr (VI) removal) compared to other parts of agro-waste like nut shells, rice husk, saw dust, and clay as seen in table 2.1.2.

**Table 2.4.2** Absorption capacity of a few biocarbons (Datta et. al., 2013).

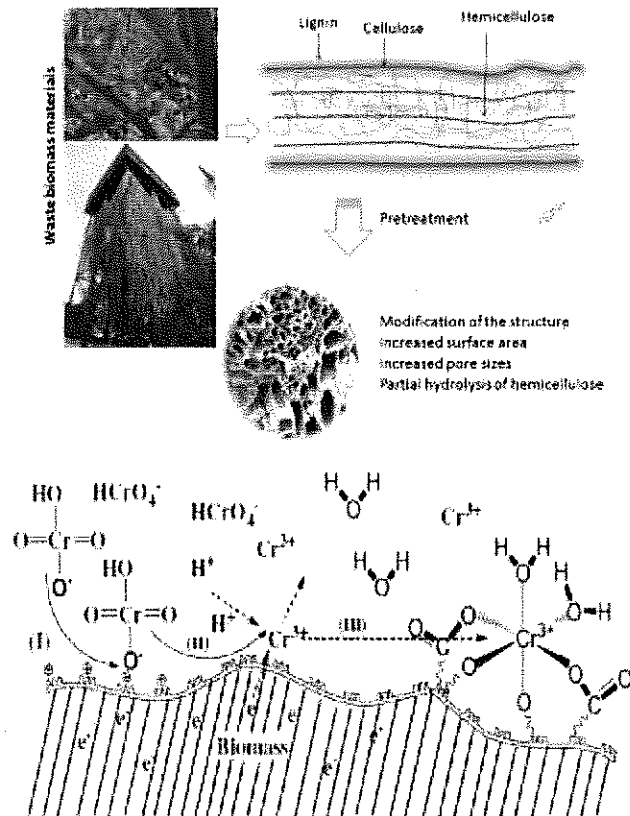
NAME OF THE ADSORBENT	ADSORPTION CAPACITY (mg/g)
Sago waste	47
Bagasse fly ash	4
Coffee residue and clay	20
Saw dust	3
Tree leaves	21
Rice husk	11
Acid washed Tea waste	79

In another study conducted by Shrestha, Kour, Homagai, Pokhrel, & Ghimire, 2013, tea leaves were found to contain hemicelluloses (11.37%), cellulose (22.33%), and lignin (28.70%) with 90% metal removal and 85 mg/g metal uptake. This was because acid treatment exposed the biocarbon to its polymeric networks, facilitating further exchange of amino (-NH) functional group with phenolic -OH and alcoholic groups available in lignin, cellulose and hemicelluloses. The N-atom of the -NH group was found to be a more effective chelating group and it has greater inclination to give away lone pair of electrons to form complexes with metal ions (Shrestha, et. al., 2013).

### 2.4.3 Biosorption Mechanisms

Biosorption process includes a combination of several mechanisms including complexation, electrostatic attraction, ion exchange, Van der Waals attraction, covalent binding, microprecipitation and adsorption (Palsan, 2013). Studies reviewed by Abdolali, Guo, Ngo, Chen, Nguyen, and Tung, 2014, revealed that Cr (VI) anions could bind to the positively charged adsorbent surface and converted it to Cr (III)

through two mechanisms: firstly, via direct reduction by touching with the biocarbon electron-donor groups; and secondly, via indirect complexation of three binding steps of Cr (VI) anions to the biocarbon's positively-charged surface; Cr (III) reduction followed by the converted Cr (III) ions release, as seen in figure 2.1.3 (a) and (b) below. The major bulk of the ion exchange capacity which reduces toxic Cr (VI) to non-toxic Cr (III), normally is because of the carboxylic acid surface-bound groups (Hubbe, Hasan & Ducoste, 2011).



**Figure 2.4.3 (a)** Pretreatment on biomass supplies to produce biocarbons (Gautam et. al., 2014); **(b)** Cr (VI) interface with *Sargassum* biomass cell wall surface by ion exchange or complexation (Zheng et. al., 2011).

#### 2.4.4 Advantages

Biocarbon is low in costs including capital and operational costs; reduces waste greatly which saves the environment; surface modifications are achievable like adding carboxyl or amine groups; have a superior surface area that allows higher absorptive capacity at smaller sizes thus saving space; regeneration and reuse of biocarbon is possible; metal recovery is possible; fuel, electricity and water energy-saving; and it is

highly efficient with up to almost 99.7% removal in various type of biocarbons (Gautam et. al., 2014).

## 2.5 SORPTION ISOTHERMS

Sorption isotherms give an idea of the maximum possible binding of the metal ion onto the surface of the given biocarbon concentration at a constant temperature (Hubbe et. al., 2011). Freundlich adsorption isotherm and Langmuir adsorption isotherm are two most commonly favoured models used (Hubbe et. al., 2011). The Freundlich model suggests multilayer sorption where there is a heterogenous distribution of active sites' energies that assumes that the stronger binding sites are bound first and that the strength reduces when more sites are bound (Hubbe et. al., 2011). The Langmuir model suggests that monolayer sorption happens where each basic site on the biocarbon surface can only adsorb one metal ion or complex and that every site is equal plus the metal ion's ability to bind is independent of whether the adjacent sites are filled or not (Hubbe et. al., 2011).

Non-linearized equation: 
$$q_e = \frac{Q_{\max} b C_e}{1 + b C_e}$$

The equation above can be linearized as follows:

$$C_e / q_e = 1 / Q_{\max} b + C_e / Q_{\max}$$

**Figure 2.5 (a) Langmuir isotherm** where  $C_e$  (mg/L) = [metal ion] in solution at equilibrium;  $q_e$  (mg/g) = bound metal ions;  $Q_{\max}$  (mg/g) = (monolayer sorption capacity) Langmuir constant;  $b$  (L/mg) = constant (binding sites' affinity) (Adapted from Hubbe et. al., 2011).

Non-linearized equation: 
$$q_e = K_f C_e^{1/n}$$

The equation above can be expressed in logarithmic terms:

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

**Figure 2.5 (b) Freundlich isotherm** where  $K_f$  &  $n$  = Freundlich constants ( $n$  = measure of the strength and nature of active sites distribution and sorption process;  $K_f$  = indicates sorption capacity) (Hubbe et. al., 2011).

### 3.0 CHAPTER 3:

## MATERIALS AND METHODS

### 3.1 PREPARATION OF CHROMIUM (VI) STOCK SOLUTION

Cr (VI) stock solution of 1000 mg/L was made by adding 99.9% potassium dichromate ( $K_2Cr_2O_7$ ; 294.19 g/mol; supplied by R & M Marketing, Essex, U.K.) of 0.565 g to 200 mL deionised distilled water (dedH<sub>2</sub>O) (Appendix C). With suitable dilutions (Appendix B), Cr (VI) concentrations of 10, 20, 40, 60, 80 and 100 mg/L were made with this stock solution. The pH of all the Cr (VI) solutions were adjusted to pH 2.0 using 3 M sulfuric acid ( $H_2SO_4$ ) and a pH meter prior to use.

### 3.2 PREPARATION OF OTHER CHEMICAL REAGENTS

Reagents like 0.25% (w.v) 1,5- diphenylcarbazide (DPC), 0.2 M  $H_2SO_4$ , 3 M  $H_2SO_4$ , 0.1 M hydrochloric acid (HCl), 1 M HCl, and 0.1 M sodium hydroxide (NaOH) were made as noted in Appendix C. Chemicals utilized were acetone (58.08 g/mol;  $\geq$  99.5% purity); DPC (242.28 g/mol;  $\geq$  99% purity); HCl (36.45 g/mol; 37% purity; 1.19 g/mL density); NaOH (40 g/mol;  $\geq$  99% purity); and  $H_2SO_4$  (98.08 g/mol; 95-98% purity; 1.84 g/mL density) supplied by R & M Marketing, Essex, U.K.

### 3.3 ACTIVATION OF BIOCARBON

The biocarbon were collected and washed with tap water to remove any soil and impurities. They were subsequently laid onto a cardboard and left to dry overnight in the incubator oven (Memmert precision incubator) at 60 °C after air-drying in the laboratory. Then, they were filled into the industrial high-speed grinder (Shanghai Jingke Scientific Instrument Co., Ltd. High-Speed Grinder) batch-wise. The grinder section was loosely-filled and ran for one to three minutes maximum to prevent overheating. The biocarbon obtained was sieved using special laboratory sieves called England Laboratory Test Sieves from Endecotts Ltd., London (Appendix B). Selected

labelled biocarbon of sieves IV and V with 125  $\mu\text{m}$  and lesser particle sizes (Appendix A) was then carbonized at a high temperature in the furnace and stored overnight in the oven in an air tight container to prevent moisture interference. Activation of the novel biocarbon labelled S2 was prepared by single-step chemical activation (Sannasi 2015, unpublished).

### 3.4 1, 5 - DIPHENYLCARBAZIDE METHOD (DPC ASSAY)

The test tube samples measured was prepared beforehand with DPC assay the day before each experiment was carried out. Each DPC assay test tube was pipetted with 4.4 mL of 0.2 M  $\text{H}_2\text{SO}_4$ , 0.1 mL of DPC and 0.5 mL of the filtered experiment sample. Initially, 1 mL of the filtered sample was first diluted in 4 mL of deionised distilled water ( $\text{dedH}_2\text{O}$ ) and vortexed for at least 15 seconds to ensure it was thoroughly mixed. It was then diluted a second time, vortexed, and 0.5 mL of this was added to the DPC assay tube. DPC was only added at the last minute as once DPC was added, the reaction was timed and left undisturbed for 15 minutes to allow the colour to develop.

Biocarbon was filtered from the test solution with Whatman No.1 (25 mm) filter paper after each experiment has finished its run time. The blank used was 0.2 M  $\text{H}_2\text{SO}_4$ . After 15 minutes, pale pinkish-purple coloured solution were formed indicating Cr (VI) concentration present in the sample and absorbance readings were taken at 540 nm via Spectronic 200 using glass cuvettes. The final total dilution factor of the sample assayed was  $250\times$  [Dilution I ( $5\times$ )  $\times$  Dilution II ( $5\times$ )  $\times$  DPC assay ( $10\times$ )]. Any measurements after 30 minutes after the reaction were unreliable due to the photo-degrading nature of the coloured DPC complex formed. Suitable dilutions are made to keep within the detection limit [Cr (VI)  $< 1$  mg/L] using  $\text{dedH}_2\text{O}$ .

### 3.5 CALIBRATION CURVE PREPARATION

Six test tubes with different known Cr (VI) concentrations of 0 mg/L, 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1.0 mg/L was prepared to plot a calibration curve as seen in Figure 4.1. The same steps were carried out as in Chapter 3.4 following the

solution volumes required in Table 3.5. A linear equation ( $y = mx + c$ ) was generated from the graph plotted and used for Cr (VI) removal analysis. The residual concentration of Cr (VI) in the solution after in contact with the activated biocarbon,  $q_e$ , of unknown Cr (VI) concentration in the flasks tested can then be determined with the linear equation obtained from the calibration curve prepared.

**Table 3.5** Known Cr (VI) standard curve preparation.

Solution	Test tube no.	Volume (mL)				
	1	2	3	4	5	6
10 mg/L Cr (VI) stock solution (mL)	0.000	0.100	0.200	0.300	0.400	0.500
0.2 M H <sub>2</sub> SO <sub>4</sub> (mL)	4.900	4.800	4.700	4.600	4.500	4.400
0.25% (w/v) DPC (mL)			0.100			
Total volume (mL)			5.000			
Final Cr (VI) concentration (mg/L)	0.000	0.200	0.400	0.600	0.800	1.000

Cr(VI) concentration range of 0 –1 mg/L.

### 3.6 BATCH BIOSORPTION STUDIES: EFFECTS OF VARIABLE FACTORS ON CHROMIUM (VI) REMOVAL

#### 3.6.1 Effect of pH

Four 50-mL test flasks with different pH of 2, 4, 6 and 8 were prepared using the pH meter by adjusting with 0.1 M HCl, 1 M HCl, 0.1 M NaOH solutions prepared (Appendix C). Experiment was run for 1 hour with 0.125 g of activated biocarbon, 100 mg/L of Cr (VI) solution, agitation speed of 250 rpm, total volume of 25 mL, r.t. of 25 °C at pH 2. DPC assay were carried out and measured with three replicates for each pH followed by Cr (VI) removal and metal uptake determination.

#### 3.6.2 Effect of Biocarbon Dosage

Three dosages of 0.125g, 0.25g and 0.5 g of activated biocarbon was weighed and added to nine 50-mL test flasks of 100 mg/L Cr (VI) solution with three replicates for



each dosage. The test flasks were run for 1 hour with agitation speed of 250 rpm, total volume of 25 mL, r.t. of 25 °C at pH 2. Then, DPC assay was carried out and recorded followed by Cr (VI) removal and metal uptake calculations.

### **3.6.3 Effect of Contact Time**

Experiment was run for two hours with interval-measurements to test for Cr (VI) removal. Six test flasks for each contact time were prepared and measured every 15, 20, 30, 60, 120 and 180 minutes via DPC assay with three replicate readings recorded. The test flasks were run with 0.125 g of activated biocarbon in each flask, 100 mg/L of initial Cr (VI) concentration, agitation speed of 250 rpm, total volume of 25 mL and r.t. of 25 °C at pH 2.

### **3.6.4 Effect of Initial Chromium (VI) Concentration**

Experiment was run in five 50-mL test flasks, each with differing initial Cr (VI) concentration of 10, 20, 40, 60, 80 and 100 mg/L, respectively. The test flasks were run for 20 minutes with 0.125 g of activated biocarbon, agitation speed of 250 rpm, total volume of 25 mL and r.t. of 25 °C at pH 2. Three replicates measurement were recorded and Cr (VI) removal and metal uptake were calculated. This readings were also used to plot and determine the adsorption isotherms in Chapter 3.9.

## **3.7 CHARACTERIZATION: POINT OF ZERO-CHARGE ( $\text{pH}_{\text{pzc}}$ )**

The pH drift method adapted from Zaini, Okayama and Machida, 2009, was used to determine the  $\text{pH}_{\text{pzc}}$  when the equilibrium pH was equal to the initial pH. Ten 100-mL flasks were utilized containing 0.1 g of activated biocarbon and 50 mL 0.1 M sodium chloride (NaCl) with varying initial pH in each of the flasks. The initial pH was adjusted using the pH meter and 0.1M HCl, 1M HCl or 0.1M NaOH prepared beforehand (Appendix C). The test flasks were allowed to equilibrate for 48 hours at r.t. and agitation speed of 100 rpm. Graph of difference between initial and final pH was plotted against initial pH. The  $x$ -intercept of the curve shows the point of zero charge of the activated biocarbon.

### 3.8 DETERMINATION OF CHROMIUM (VI) REMOVAL AND METAL UPTAKE

The two main parameters analysed were removal percentage, % and metal uptake,  $q$ . The amount of Cr (VI) uptake ( $q_e$ , mg/g) and the Cr (VI) removal (%) from aqueous solution were calculated using these following formulas (Sannasi, 2013):

- (i) Percentage of Cr (VI) removal (%)

$$= \frac{C_0 - C_e}{C_0} \times 100\%$$

$C_0$  = initial concentration (corrected with H<sub>2</sub>SO<sub>4</sub> blank) of Cr (VI) in test solution (mg/L)

$C_e$  = residual concentration of Cr (VI) in the test solution at equilibrium (mg/L)

- (ii) Metal uptake,  $q_e$  (mg/g)

$$= \frac{C_0 - C_e}{\text{Dry weight of biocarbon (g)}} \times \text{Volume of solution (0.025 L)}$$

To study whether Cr (VI) really did reduce to Cr (III), the left over filtrate with residual Cr (VI),  $C_e$  (mg/L) was oxidized as to the Cr (III) oxidation procedure (Appendix D). The remaining known volume of the flask filtrate was boiled to 100°C and then added by two drops of potassium permanganate (KMnO<sub>4</sub>). Drops of KMnO<sub>4</sub> were added drop-wise until a stable slight pink color was retained. Successively, further extra final drops of KMnO<sub>4</sub> were added to ensure that KMnO<sub>4</sub> was in excess and every Cr (VI) was converted. As usual as the DPC assay, 1-mL of the oxidized filtrate was subjected to the DPC assay to determine the amount of remaining Cr (VI) ( $C_f$ , mg/L) after oxidation.

- (iii) The amount of Cr (VI) reduced to Cr (III) [ $C_{red}$ , mg/L];

$$C_{red} = C_f - C_e$$

where,  $C_f$  = concentration of Cr (VI) in filtrate after oxidation with KMnO<sub>4</sub> (mg/L)

- (iv) The % of Cr (VI) reduced to Cr (III) [%  $C_{red}$ ], was calculated by the following:

$$\% C_{red} = \left[ \frac{C_{red}}{C_0} \right] \times 100\%$$

- (v) The actual uptake of Cr onto biocarbon ( $q_a$ , mg/g), was calculated by the following:

$$\frac{C_a}{\text{Dry weight of biocarbon (g)}} \times \text{Volume of solution (0.025 L)}$$

- (vi) The actual % of Cr adsorbed onto biocarbon (%  $C_a$ ), was calculated by the following:

$$\% C_a = \left[ \frac{C_a}{C_0} \right] \times 100\%$$

- (vii) Actual uptake of Cr onto biocarbon ( $q_a$ , mg/g):

$$\frac{C_a}{\text{Dry weight of biocarbon (g)}} \times \text{Volume of solution (0.025 L)}$$

### 3.9 ADSORPTION ISOTHERMS

The data gained for the removal of Cr (VI) using the biocarbon will be fitted to the Freundlich and Langmuir isotherms. Adsorption isotherm was used to examine the correlation between the residual concentration of Cr (VI) at equilibrium, ( $C_e$ , mg/L) and the amount of metal uptake, ( $q_e$ , mg/g) to know the adsorption efficacy (Hubbe et. al., 2011). The suitability of the Langmuir and Freundlich models will be judged by comparing the (coefficient of determination values),  $R^2$  and constants of isotherm that are known from the models in figures 2.5 (a) and (b) using the experimental data obtained.

#### 3.9.1 Langmuir Isotherm

Langmuir assumes that each metal ion is located at a single site and that the biocarbon surface has identical sites of energy (Slimani, Ouahabi, Hachoumi, Riadi, Anouzla, Haddad, Antri, & Lazar, 2014). Therefore, it forecasts a monolayer formation of metal ions on the biocarbon surface (Slimani et. al., 2014). Four types of linearized

Langmuir models were used to plot the experiment data obtained and graph plots of x and y axis parameters as in table 3.9 were plotted to determine the best isotherm type fit for the data available.

### 3.9.2 Freundlich Isotherm

This commonly applied isotherm describes the equilibrium of the biosorption process. It presumes that the adsorption process at equilibrium takes place on surfaces that are heterogeneous which therefore does not presume monolayer absorption and the adsorption capacity was connected to the Cr (VI) concentration (Shouman et. al., 2014). The graph plotted was as in table 3.9.  $R^2$ ,  $Q_{max}$  and  $b$  values were determined.

**Table 3.9** Different types of linearized form of Langmuir and Freundlich isotherm with graph parameters.

Isotherm	Equation	Linear expression	Plot	Parameters
Type 1 Langmuir		$\frac{C_e}{q_e} = \frac{1}{Q_m} C_e + \frac{1}{bQ_m}$	$\frac{C_e}{q_e}$ vs. $C_e$	$Q_m = 1/\text{slope}$ , $b = \text{slope}/\text{intercept}$
Type 2 Langmuir	$\frac{q_e = Q_m b C_e}{1 + b C_e}$	$\frac{1}{q_e} = \frac{1}{bQ_m} \left(\frac{1}{C_e}\right) + \frac{1}{Q_m}$	$\frac{1}{q_e}$ vs. $\frac{1}{C_e}$	$Q_m = 1/\text{intercept}$ , $b = \text{intercept}/\text{slope}$
Type 3 Langmuir		$q_e = Q_m - \left(\frac{1}{b}\right) \frac{q_e}{C_e}$	$q_e$ vs. $\frac{q_e}{C_e}$	$Q_m = \text{intercept}$ , $b = -1/\text{slope}$
Type 4 Langmuir		$\frac{q_e}{C_e} = bQ_m - bq_e$	$\frac{q_e}{C_e}$ vs. $q_e$	$Q_m = -$ intercept/slope, $b = -$ slope
Freundlich	$\frac{q_e = K_f C_e^{1/n}}$	$\log(q_e) = \log(K_f) + \frac{1}{n} \log(C_e)$	$\log(q_e)$ vs. $\log(C_e)$	$\log(K_f) =$ intercept, $n = 1/\text{slope}$

where  $q_e$  (mg/g) = amount of metal uptake;  $Q_m$  (mg/g) = Langmuir monolayer maximum absorption capacity;  $b$  (L/mg) = Langmuir constant-binding sites affinity for metal ions; and  $C_e$  (mg/L) = Cr(VI) residual concentration at equilibrium

(Source: Adapted from Armagan & Toprak, 2013).

### 3.10 STATISTICAL ANALYSIS

Each tests were done in triplicates and analyzed with SPSS version 20; One-way ANOVA, Duncan's test, and Fisher's LSD post hoc tests for multiple comparisons with significance level of 5%, ( $\alpha = 0.05$ ).

## 4.0 CHAPTER 4:

### RESULTS AND DISCUSSION

#### 4.1. CALIBRATION CURVE

The linear equation obtained allows estimation and extrapolation of unknown Cr (VI) concentration even at a higher concentration like 100 mg/L assuming that absorbance increases linearly with increasing metal ion concentration and does not plateau. The standard deviation of the values between absorbance of known concentration were calculated to be within 10% to reduce error and increase accuracy and precision ( $R^2$ ).

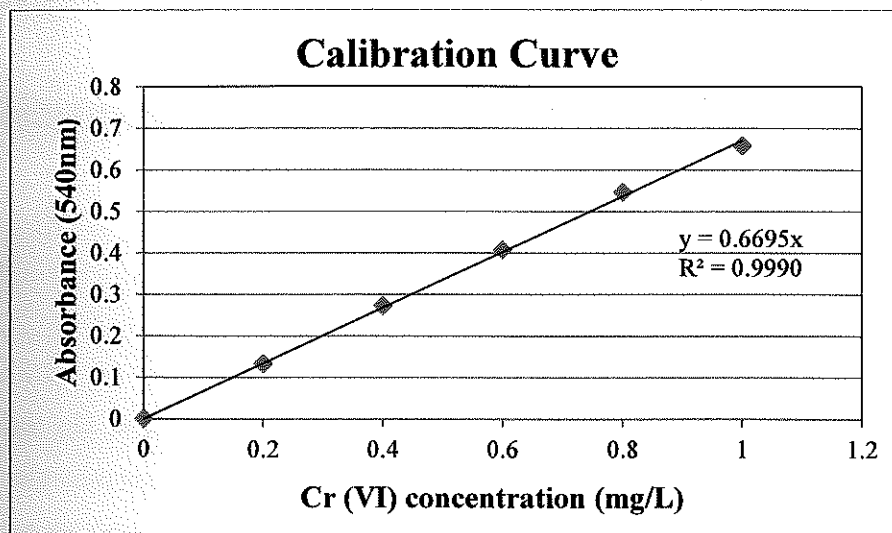


Figure 4.1 Calibration curve obtained for known Cr (VI) concentration range of 0-1 mg/L. Linear equation  $y = 0.6695x$  with coefficient correlation,  $R^2 = 0.9990$ . High  $R^2$  value indicates high reliability and accuracy of data. Absorbance increased with increased metal ion concentrations.

#### 4.2 BATCH BIOSORPTION STUDIES ON CHROMIUM (VI) REMOVAL

##### 4.2.1 Effect of pH

Cr (VI) removal and uptake was highest at pH 2 of 97.83% and 20.24 mg/g. Low pH has more hydrogen chromate anions,  $\text{HCrO}_4^-$  hydrolyzed from dichromate ions which binds better to the protonated biocarbon surface's amino and hydronium functional group, strengthening the oppositely charged metal anions's bond with the biocarbon's surface (Ilyas et. al., 2014). Biosorption was fortified at low pH also because of the protonation of the biocarbon surface allowing more binding sites to be activated in acidic aqueous systems and increases Cr (VI) removal. At  $\text{pH} > 6.5$ ,  $\text{Cr}_2\text{O}_7^{2-}$  and  $\text{CrO}_4^{2-}$  are formed which repels the surface of the biocarbon due to their double anion charge that causes Cr (VI) removal to be poor.

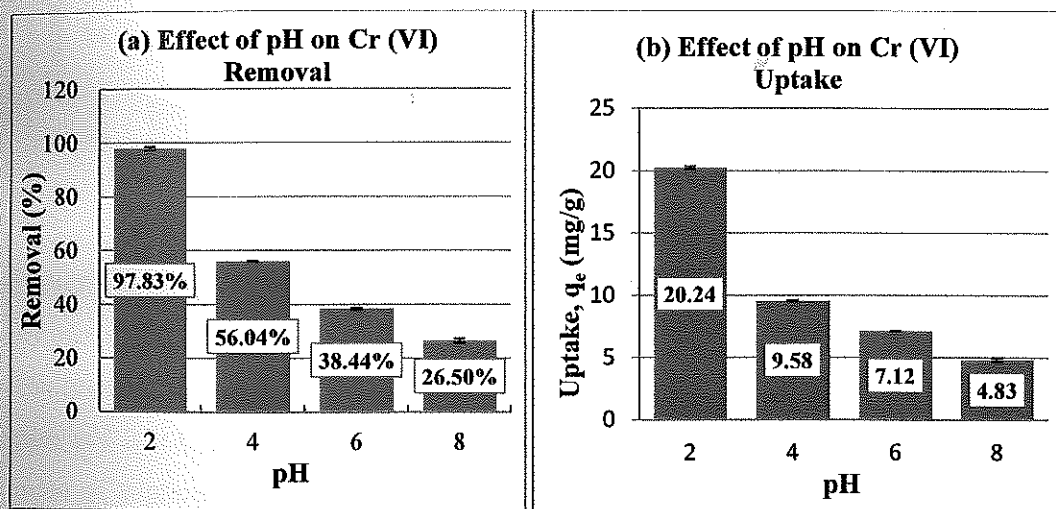


Figure 4.2.1 (a) Cr (VI) removal values decreased drastically from 97.83% to 26.50% at higher pH with removal being best at pH 2. (b) Metal uptake also reduced drastically with increased pH from 20.24 mg/g to 4.83 mg/g. Uptake was best at pH 2. [Parameters: time = 20 min; dosage = 0.125 g; 100 mg/L Cr (VI); 250 rpm; pH 2, 4, 6 and 8; r.t. Absorbance was measured at 250 $\times$  dilution. Values for each bar are mean of triplicate readings. Both removal and uptake values decreased drastically with increased pH. [n = 3  $\pm$  standard error (s.e.)]; (p < 0.05)]

#### 4.2.2 Effect of Biocarbon Dosage

The higher the amount of biocarbon particles present, the more sites are available for the metal ions to bind thus more efficient Cr (VI) removal. Removal of 100% was achieved with just a small dose. However, metal uptake decreased with increased dosage because more particles were present with many more binding sites that were