PHYCOREMEDIATION OF HEAVY METALS AND RESOURCE RECOVERY OF RARE EARTH ELEMENTS USING GREEN ALGAE FROM EUTROPHIC FRESHWATER BODIES

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ABSTRACT

Title: Phycoremediation of Heavy Metals and Resource Recovery of Rare Earth Elements Using Green Algae from Eutrophic Freshwater Bodies

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Biosorption is an emerging and or complementary technology to the conventional treatment methods for industrial wastewater. Biosorption involves the use of dead biological material to sequester inorganics and or organic pollutants. Micro algae have a diversity of algal species found mostly in freshwater bodies of which only a few were investigated for their biosorption potential. This study focussed on the utilisation of green algal sorbents for the recovery of rare earth elements (REE) and removal of heavy metals. The algal samples were collected from Hartbeespoort dam in South Africa and isolated using streak plating method. The pure algal species were identified using molecular methods and found to have 95-98% identities to *Chloroidium saccharophilum, Desmodesmus multivariabilis, Scenedesmus acuminutus* and *Stichococcus bacillaris. Chlorella vulgaris and Chlamydomonas reinhardtii* were purchased for comparison purposes.

The species were cultured in the laboratory under controlled conditions and dried to obtain dead algal cells for biosorption studies. Adsorption and desorption experiments were carried out to determine the sorption capacity and possible recovery in single metallic studies of lanthanum (La), thallium (Tl) and cadmium (Cd). Kinetic studies were also investigated to determine the potential rate controlling steps that are useful when designing a full scale biosorption process. Surface characterisation of algae was carried out to determine the possible mechanisms involved in biosorption processes. Multi-metallic studies were investigated to better understand the influence of competing ions in the actual environment.

In single metallic studies involving the removal and resource recovery of La, *Desmodesmus multivariabilis* was found to be the most efficient with both a high sorption capacity (q_{max})

and affinity (b) of 100 mg/g and 4.55 L/g respectively. In studies for Tl sorption, *Chlamydomonas reinhardtii* and *Chloroidium saccharophilum* had the highest q_{max} of 1000mg/g and b of 1.667 L/g. For the case of cadmium adsorption, *Chloroidium saccharophilum* had the highest q_{max} of 128.21mg/g but *Desmodesmus multivariabilis* showed a higher b of 1.490 L/g. The study aimed at selecting species with both a high uptake and recovery rate which meant those with a both a high q_{max} and high b. The most common functional groups found in all the tested algae included carboxyl, hydroxyl, phenols and amines which suggests the possible mechanisms involved being ion exchange, coordination and or complexation in addition to physical adsorption.

Adsorption using ternary system of metals showed a lower q_{max} compared to single metallic studies due to competition for binding sites. Generally the Competitive Extended Langmuir Model (ELM) performed better than Combined Langmuir and Freundlich Model (CLFM). The affinity (K_L) was highest for Tl and La at 0.244 and 0.403 L/g respectively in the ELM. The higher the K_L value, the greater the affinity of the metal for the biosorbent and possibility of metal recovery. In addition, Tl and La had a higher atomic weight than Cd which increases the atomic energy to bind on the adsorbent. Environmental factors like pH, initial metal concentration, ion exchange influenced the adsorption in the ternary system.

In the actual wastewater samples, all the available metal concentrations were generally reduced by tested algae. Cobalt and thallium were completely removed by *C. vulgaris* and *D. multivariabilis*. Light metals were found in significant quantities but were generally reduced. Chromium was also found in significant quantities which was most reduced by *S. acuminutus* and *D. multivariabilis*. Generally the best adsorbents for treatment of actual wastewater were *C. vulgaris* and *D. multivariabilis*. The algal species tested in all experiments presented great potential for use in wastewater containing heavy metals and recovery of REE. The experiments were done in batch reactors as further research is needed to find cheaper immobilizing agents before use in continuous reactors. Immobising agents improve the mechanic strength of micro algae for re-use in multiple cycles. An evaluation of cost implications would be an added advantage to reflect the realistic approach in industry through a pilot scale plant.

DECLARATION

I **Zainab Shariff Birungi**, declare that the thesis which I hereby submit for a Doctor of Philosophy in Chemical Technology degree at the University of Pretoria is my own work and has not been previously submitted by me for any degree at this or other institutions.

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Zainab Shariff Birungi Date

DEDICATION

I dedicate this thesis to my father and mother for their endless struggle to educate me. May Allah continually bless you.

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“Thank you Allah”

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NOMENCLATURE

q	metal uptake (mg/g)
v	volume of metal solution (liters)
c_i	initial metal concentration (mg/L)
c_f	initial metal concentration (mg/L)
m	amount of biomass (g).
q_e	adsorbed metal at equilibrium (mg/g)
$q_{\max}$	maximum amount of metal sorbed (mg/g)
b	constant related to the energy of sorption
C_e	equilibrium metal ion concentration (mg/L)
k	biosorption equilibrium constant capacity
n	constant indicative of biosorption intensity
X_m	maximum biosorption capacity (mmol/g)
β	activity coefficient related to mean biosorption energy (mol^{2}g^{-1}kJ^{-1})
T	temperature (k)
R	gas constant (8.314j/mol k)
R_L	separation factor
b_L	constant related to energy of adsorption from Langmuir model (L/g)
q_t	amount of adsorbed metal on the algal biomass at time (t) in (mg/g)
α	initial sorption rate (mgg^{-1}min^{-1})
β	desorption constant (gmg^{-1}) during any one experiment.
q_n	amount of Tl remaining in algae at time t_n (mg/g)
q_{n+1}	amount of Tl at time $\boldsymbol{t_n}$ minus the rate of desorption multiplied by the incremental time step (mg/g)
ΔG	Gibbs free energy(kJ/mol)
ΔH	enthalpy (kJ/mol)
ΔS	entropy (kJmol^{-1}k^{-1})
T	temperature in kelvin. K_C
R	are equilibrium constants

$q_{e(i)}$ equilibrium metal uptake for I metals (mg/g)

$C_{e.i}$ equilibrium concentration (mg/L)

$q_{m.i}$ maximum adsorption capacity (mg/g)

$K_{L..i}$ Langmuir isotherm parameters (L/g)

$b_{LF.i}$ affinity constant for adsorption (L/mg), n is the index of heterogeneity

Y_i biosorption preference (%)

Y_t total biosorption efficiency (%)

θ_i relative coverage (%)

C_i initial concentration (mg/L)

C_s concentration of metal sorbed(mg/L)

$C_{s.m}$ moles of metal biosorbed

$C_{s.m.t}$ total moles of metal biosorbed.

A_o initial surface area (m^2/g)

C_o initial metal concentration (mg/L)

R_c reduction capacity (mg.g)/ (L.m^2).

K_i intraparticle diffusion rate constant (mgg-^{1}hr$^{0.5}$) and C is the intercept

c_t concentration at time t (mg/L),

B_L surface mass transfer co- efficient (ms^{-1}),

S_s surface area of the adsorbent (m^2/g)

K constant from multiplication of Langmuir constants of q_{max} and K_L

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The negative impacts of toxic metals on the environment were observed from the aftermath of the beginning of industrial revolution until recent times. The ability to use metals of different advanced properties has been part of the history of human civilisation and indeed this has been so important in marking the milestones of human evolution, e.g., the Iron Age, followed by the bronze age, and much later, related to the utilisation of uranium, the nuclear age. As such, metals still serve as the driving factor of human progress. Thus it is no surprise that the mining and processing of the metals with its resultant pollution have been on the increase through human evolution. Unlike other types of pollutants of organic and inorganic salts, metallic pollutants tend to be intractable from the environment since metals cannot be degraded–they can only be transformed from one oxidation state to another. Therefore, pollution by toxic metallic elements tends to cause irreversible health effects and easily magnify in the higher trophic levels of the food chain (Goyal, et al., 2001; Das, et al., 2008; Gupta and Rastogi, 2008a; Chojnacka, 2010).

Heavy metal is a generic term for metals with an atomic weight higher than 40.04 are considered the most toxic including some metalloids and transitional metals (Ming-Ho, 2005). The United States Environmental Protection Agency (U.S.EPA) has listed priority metallic pollutants such as mercury, lead, cadmium, thallium, zinc, copper, nickel, beryllium, antimony. The list also includes transitional metals such as chromium and metalloids such as arsenic and selenium. Even metals such as iron, zinc, molybdenum and magnesium which are essential nutritional supplements to plants and animals can become toxic to the organisms when discharged in excess.

Unlike toxic heavy metals which are relatively abundant, some metals are scarce and unevenly distributed in the earth crust. Rare Earth Elements (REE) are ranked by as one of the most critical elements with many emerging technologies but with a diminishing supply (Eg European Commission, 2010). REE are used in many applications such as green energy technologies, nuclear oil, automobile industry, electronics industry etc. Recycling using environmentally friendly technologies act as an efficient and complementary to primary mining for sustainability of critical resources (Grosse, 2010; Binnemans, et al., 2013).

1.1.1 Environmental Metal Pollution

Toxic metals are directly or indirectly released into the environment mainly through anthropogenic related activities. Some of the most common sources of inorganic pollution are electroplating, mining, battery manufacturing, tanning, chemical manufacturing, coal combustion (Volesky, 1990a; Aksu and Açikel, 2000; Jianlong, 2002; Wang and Chen, 2009). Conventional treatment technologies such as sand filtration, GAC adsorption, precipitation-sedimentation, flotation, ion-exchange, and electrochemical deposition systems have been used for decades for the removal of toxic heavy metals Cd, Pb, Fe, Mn, Cr, and As from wastewater (Wang, et al., 2004; Aziz, et al., 2008). These technologies seem to operate relatively well for effluents containing higher metal concentrations ≥ 100 mg/L. The common failure of adsorption processes is the lack of selectivity for the target metals. Presence of other minerals such as Na, Ca, K, and Mg can inhibit the adsorption processes due to competition for adsorption sites (Ngwenya, 2007). Another limitation is the high cost of operation due to high energy requirement especially for small-scale industries with lower metal concentration (Stirk and Van Staden, 2000; Ahalya, et al., 2003). The emerging nanotechnology for treatment of inorganic pollutants provides great potential due to the high sorption efficiencies but is limited by potential environmental risk and the extremely high cost of most nano-materials (Masciangioli and Zhang, 2003; Qu, et al., 2013).

1.1.2 Potential of Biosorption

The use of biological material is an emerging and environmentally friendly technology with great prospects to effectively clean up toxic metals at low concentrations and possible recovery for re-use in industry (Gadd and White, 1993; Mack, et al., 2007; Volesky, 2007). Various living and dead organisms have been investigated for metal removal, e.g. bacteria, fungi, algae, yeast, industrial and agricultural waste. The living biomass accumulates high levels of metals but possible recovery without cell disruption seems almost impossible. Biosorption is a term often used to refer to the treatment of wastewater containing heavy metals using dead biomass. The use of dead biomass has attracted more attention as it offers the possibility of regeneration and reuse of the biosorbent (Mehta and Gaur, 2005; Chojnacka, 2010). This is mainly attributed to the active functional groups of the cell wall which provide binding sites, e.g. carboxyl, phosphate, amines, hydroxyl, phenols among others (Davis, et al., 2003; Rezaee, et al., 2006; Wang and Chen, 2009).

Recently, the feasibility of using algae as a biosorbent has been investigated. The investigations have focussed more attention on macro-algae especially brown algae (sea-weed) from saline marine environments. The brown algae are relatively abundant and are shown to effectively remove metallic species from water. However, the application of brown algae at a very large scale can only be practical in the vicinity of the sea coastline. Micro algae on the other hand proliferate everywhere in both fresh water and marine environments including inland water bodies. In spite of their local availability, only a small percentage of the fresh water algae species that has been investigated thus far has been demonstrated to be capable of adsorbing a wide range of metallic species from water (Davis, et al., 2003; Mehta and Gaur, 2005; Romera, et al., 2006; Lesmana, et al., 2009).

In addition, most biosorption studies have researched on single metal pollutant systems (Ho, et al., 1995; Volesky and Holan, 1995; Veglio and Beolchini, 1997; Akhtar, et al., 2008). Scenarios representing realistic multi-metal pollutant systems commonly encountered in the environment are limited. The use of single metal pollutant systems provides fundamental information on the adsorption potential of the various biosorbents but requires to be complimented with multi metallic studies to reflect the actual environment. Actual industrial wastewater contains different metals in varying concentrations which compete for the binding sites depending on the environmental conditions such as pH, temperature and ionic strength.

1.2 RESEARCH HYPOTHESIS

The conventional technologies used in the treatment of industrial wastewater are inefficient and costly for large volumes of water containing lower metal concentrations. The use of relatively cheap, readily available and abundant biological materials offers a better alternative for low metal concentrations. Micro-organisms like bacteria, algae, fungi are known to efficiently remove and recover metals at varying levels due to differences in binding sites specific at species level. Algae generally have higher sorption efficiency as compared to other micro-organisms and other biosorbents. Green algae have a wide variety of species in the fresh water bodies which have a great potential but not explored for biosorption. The green algae will be tested based on the following research hypothesis:

- Green algae have a higher sorption capacity than many tested algae and non- algal sorbents.
- The environmental factors like pH, temperature, competing ions and metal concentration influence the sorption efficiency.
- The functional groups on the cell surface of dead algae are responsible for effective adsorption and desorption of metals
- The algal cell wall remains active after recovery for regeneration and re-use of the biomass
- The uptake of metals in biosorbents decrease with increasing time until equilibrium is attained.

1.3 RESEARCH AIM

This study generally aimed at investigating the biosorption potential of micro algae from freshwater habitat for the removal of industrial heavy metals and resource recovery of rare earths metals for re-use in industry.

1.3.1 Specific Objectives and Tasks

The specific objectives of the project guided the formulation of tasks needed to complete the study. The specific objectives were:

i. To source and characterise laboratory culturable micro-algae from the local water bodies.
ii. To determine the adsorption and desorption potential of algal species using single metallic system of toxic and rare earth metals.
iii. To investigate the effect of pH, ionic strength, initial metal concentration, temperature on the sorption of metals.
iv. To determine the sorption and desorption rate to evaluate metal uptake and recovery efficiency.
v. To characterise algal cell wall functional groups and their interaction with metallic groups.
vi. To determine biosorption capacity of algae in multi-metal pollutant systems.

1.4 METHODOLOGY

The freshwater algae were collected from Hartbeespoort Dam which is a heavily eutrophied water body situated in the North-West, South Africa. Culturable species collected from the dam were enriched in sterile Bold Basal Medium (BBM) and further purified from single colony streaks on agar plates before species identification. The metals used in this study were based on selection of each metal as a representative of a certain class of metallic waste pollutants e.g. frequently encountered metals (cadmium), extremely toxic heavy metal but usually excluded (thallium) and rare earth metals on demand (lanthanum). The studies were carried out in batch reactors using algal species at varying initial concentration, pH, temperature, contact time and ionic strength. Lanthanum, thallium and cadmium were selected for monometallic and multi-metallic studies. Equilibrium experiments were carried out in a period of 24 hours to determine the sorption capacity and affinity of metals to the biosorbents using linearized empirical models of Langmuir, Freundlich, D-R and Temkin.

In kinetic studies, experiments were carried out for a period of 6-12 hours and data analysed using Lagergren first order model, Pseudo- second order models and Elovich model. The modified Pseudo- second order was used in desorption kinetic studies. The efficiency of metal removal and re-usability of algae was carried in 3 cycles of adsorption/desorption.

The effect of multiple metals on the sorption capacity using a ternary system under varying environmental conditions was carried out. MATLAB (version 4) and AQUASIM (version 2) were used in the analysis of multi-metallic equilibrium and kinetic data respectively. Based on the metals selected, the multi metallic studies were limited to a ternary system. Actual industrial waste samples from industry were also collected to check for commercial feasibility in treatment of multiple metals under different environmental conditions.

1.5 SIGNIFICANCE OF THE STUDY

For many decades, toxicity of metals has been recognised due to documented evidence in plants, animals and or humans. The various metal compounds are usually released into the environment directly or indirectly with the main culprit being man due to the various industrial activities. Most traditional methods currently used are limited due to the high cost of operation especially for lower concentration of metals. Biosorption is a proposed alternative technology with relatively abundant waste biomass expected to reduce the cost of industrial wastewater treatment. The main challenge is to select the most efficient biosorbents from the relatively abundant biomass. Various micro-organisms like algae, fungi and bacteria were used in biosorption with better adsorption capacities found mainly in algae giving it a competitive advantage. Unfortunately, most research efforts on biosorption was carried out on macro algae especially brown algae from marine environment. Micro-algae have a wide distribution of species found mostly in freshwater habitats but only a few have been investigated for their biosorption potential. The most common groups of micro algae include chlorophyceae (green algae), cyanophyceae (blue-green algae), bacillariophyceae (including the diatoms) and chrysophyceae (including golden algae). In this study, the focus was on the use of unwanted microbial algae from eutrophic fresh water sources for the recovery of rare earth metals and removal of toxic heavy metals. The results from this study provide information on biosorbent preference of metals, removal and recovery efficiencies, possible mechanisms involved in biosorption and reaction kinetics. The study also provides fundamental knowledge required like effect of environmental factors for selection of biosorbents before a full scale industrial biosorption process is applied. In combination with findings from other studies, the tested algal species can be immobilised for use in industrial application in the treatment of toxic metals and resource recovery of rare and precious metals.

1.6 THESIS LAYOUT

The thesis is divided into 8 chapters. The first chapter introduces the background, research hypothesis, research objectives, significance of the study general methodology and thesis lay out. Chapter 2 covers literature review related to the study. Chapter 3 provides the specific materials and methods used in this study. Chapters 4, 5 and 6 provide detailed information on the findings using different algal species as biosorbents for removal of selected metals (lanthanum, thallium and cadmium). In these chapters, equilibrium and kinetic models were used for single metallic systems. In addition, the factors influencing biosorption and surface characterisation of the algal cell wall were investigated. Chapter 7 provides information on multi-metallic studies of a ternary system using better algal sorbents. Competitive equilibrium and kinetic models for multi-metals were used for comparion of experimental and predicted data. The biosorbents were also tested using actual wastewater from industry. Finally, chapter 8 provides a general summary, conclusions and recommendations with further insights for future research. Appendices showing supplementary information are provided at the end of this thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

The discovery of heavy metals span from the ancient times of pre-Romans to mid- nineteenth century. The toxic effects of the metal elements were known after discovery and in some instances before the elements were identified. Mercury and lead were the most frequently used in ancient times, e.g. Romans knew the toxic effects of mercury and used slaves and convicts for mining mercuric sulphide (cinnabar) (Berman, 1980) which reduced their life expectancy to 6 months. Mercury was also used in many ointments to change skin colour and in the treatment of syphilis (Nriagu, 1983). Romans used lead for weather proofing of buildings, ducts for water and making cooking utensils (Nriagu, 1983). Lead was also used to improve taste and kill bacteria in wines (Berman, 1980). It is actually believed that lead poisoning of the Roman leader then contributed to the collapse of the Roman Empire (Gilfillan, 1965; Patterson, et al., 1987).

Other examples include the use of arsenic by Greeks, Romans and Arabs as a Therapeutical and poison (Partington, 1970). In 1815, arsenic poisoning was found in German houses due to the wall paper which contained green copper arsenite pigments (Craig, 1980; Thayer and Brinckman, 1982). The people of Minamata and Niigata in Japan were poisoned through eating fish which is their staple food due to accumulation of inorganic mercury contained in aquarium sediments (Jensen and Jernelöv, 1969).

Cadmium was first identified by F. Stronmeyer in 1817 when he observed a yellow colour of calamine, $ZnCO_3$ which he separated from the less soluble $CdCO_3$ (Partington, 1970). Cadmium production peaked after the First World War, when it was used for electroplating due to its resistance to corrosion (Fritz Ullmann, et al., 1985). Cadmium induced emphysema was first discovered in 1948 due to pulmonary inhalation of cadmium as fumes or dust (Friberg, et al., 1974b).

Thallium was first observed by Sir William Crookes in 1832-1919. The element was known to have caused many accidental, therapeutical and occupational poisoning since its discovery

(Mulkey and Oehme, 1993). For example, thallium was taken orally in the past to cause fall out of body hair and also used for killing rodents but discontinued due to extreme toxicity to human beings (Léonard and Gerber, 1997; Xiao, et al., 2012).

Criticism about the use of toxic metals shunned there acceptance over the years. In the mid-19th to 20th century, the use of heavy metals exacerbated due to industrialisation which is a key driver to civilization and economic development (Nixson, 1991; Hewitt and Wield, 1992). The most common sources of toxic metal effluents currently encountered come from industries such as leather tanning, petroleum industry, battery and chemical manufacturing, electroplating, smelting, coal combustion etc. (Volesky and Holan, 1995; Wang and Chen, 2009). The acceptable levels of metals in the environment are currently regulated by international standards such as Environmental Protection Agency (EPA), which sets the maximum contaminant level (MCL), e.g. Cd and Tl have a MCL in drinking water at 0.005 mg/L and 2 µg/L respectively.

2.2 HEAVY METALS IN THE ENVIRONMENT

Metallic species are non-biodegradable and can only be removed physically or chemically from contaminated wastewater (Tien, 2002; Hashim and Chu, 2004; Ahluwalia and Goyal, 2007). Metal pollution of water sources is mainly from point source pollution especially from industrial activities. Heavy metals are classified according to their chemistry coordination, a factor determined by the element’s electron environment (Beveridge and Doyle, 1989; Collins and Stotzky, 1989). In Class A (hard acids) are biologically essential, e.g. Na^+, K^+, Mg^{2+}, Ca^{2+}; Class B (soft acids) are mostly non- essential and very toxic, e.g. Cd^{2+}, Hg^{2+}, Cu^{2+}, Pd^{2+}; whereas border line ions like Fe^{2+}, Fe^{3+}, and Mn^{2+} have biological importance but can also be toxic. Hughes and Poole, 1991 provided an insight on the factors influencing individual metallic elements which included:

(a) the charge to radius ratio :(high charge density metal ions retain a potent ligand affinity, whilst the low charge-density alkali metals do not bind well),

(b) the number of electrons : ions with multiple electron shells e.g. Cd (4d 10), are more easily polarized and form stable complexes with soft, biochemically active. Hard cations are not easily polarized and are readily displaced from their binding site by competing soft cations,

(c) Zn, Cu and Cd exhibit partly filled orbitals and bind ligands more strongly than the s-block elements. Since biochemical activity is ion sensitive, the intrusion of a foreign metal with a different electron environment will prohibit many biological processes.

Therefore, in this study, the heavy metals of interest are cadmium and thallium which are further elaborated regarding there characteristic, potential sources, uses and effects on the environment.

2.2.1 Thallium

Thallium naturally occurs in 2 stable isotopes of ^{203}Tl and ^{205}Tl with relative abundance of 29.5 and 70.5% respectively (Sahl, et al., 1978). In nature, Tl minerals are rare but widely distributed in very low concentrations with 0.49 ppm in continental crust and 0.013 ppm in oceanic crust (Del Valls, et al., 1999). Natural sources of Tl from the earth crust are found in potassium minerals, e.g. Pyrite (FeS_2) and Sphalerite (ZnS) (Smith and Carson, 1977; Schoer, 1984). The major mining sources of Tl are usually from sulphide ores e.g. galena, chalcopyrite, sphalerite, pyrite (Zitko, 1975a).

Earlier studies suggested Tl (I) forms soluble compounds with, e.g. sulphates, acetate, carbonates and is more abundant than Tl (III) (Goldschmidt, 1954; Galván-Arzate and Santamaría, 1998). Tl is generally considered as a mobile element in aqueous environments usually emitted as a by-product of industrial processes like refining (iron, zinc, cadmium), cement industry and coal powered plants (Zitko, 1975b; Vink, 1993; Xiao, et al., 2000). In the past, Tl was used as a poison for rodents and insecticides, treatment of ring worms, tuberculosis, malaria and veneral disease but was discontinued due to toxicity (Nriagu, 1998; Kazantzis, 2000). Recently, Tl is used in industries for the manufacture of scintillation counters, low temperature thermometers, electronic devices, optical systems, special glass, oxidation of hydro carbons and olefins, dyes, mineralogical separation, pigments, imitation jewelry and fireworks among others (Nriagu, 1998; Ramsden, 2002). Various Tl compounds are also used as catalysts in the oxidation, reduction, aromatization, esterification and polymerisation of organic molecules (Nriagu, 1998).

Thallium is a highly toxic element usually considered worse or comparable to mercury, cadmium and lead (Peter and Viraraghavan, 2005). Despite the high risk of Tl toxicity, there is limited information on the distribution and dispersion of this metal in the environment which makes it of particular scientific interest and concern (Xiao, et al., 2004; Peter and Viraraghavan, 2005). Thallium from anthropogenic sources is more easily extracted than from natural sources because the former is adsorbed on the surface whereas the latter is incorporated in solid structure (Lehn and Schoer, 1987; Sager, 1992). Thallium has a low boiling point and easily volatilises as an impurity from coal combustion, smelting operations, cement industry, steel industry, sulphuric acid production, auto-emissions and refining processes (Jacobson, et al., 2005; Peter and Viraraghavan, 2005; Vaněk, et al., 2011).

Thallium easily replaces potassium in the biological functioning of the cell membrane due to similar ionic charge and radii (Aoyama, et al., 1988; Mulkey and Oehme, 1993). Thallium poisoning in humans mainly occurs through ingestion of contaminated food, inhalation or and through the skin. Depending on the type of exposure, dosage and age, it may be acute, sub-acute or chronic (Mulkey and Oehme, 1993). The negative effects of exposure include encephalopathy, tachycardia, mild gastro intestinal disturbances, degenerative changes in kidney, liver, heart, alteration of nervous system and eventually death (Cavanagh, et al., 1974; Nriagu, 1998; Urík, et al., 2010). Previous research on removal technologies has focussed on using pre-concentration of Tl by chelating resins (Jacobson, et al., 2005); oxidative precipitation of thallichydroxide, reductive cementation of thallium; chemical precipitation by hydroxide compounds, solvent extraction using tributyl phosphate (TBP), trioctyl phosphine oxide (TOPO) (Şenol and Ulusoy, 2010); extraction by complex forming re-agents (Zhang, et al., 2003). Adsorption of Tl using natural and chemically precipitated manganese dioxide (MnO_2) has been also widely applied (Bidoglio, et al., 1993). MnO_2 was found to be a better adsorbent than organic matter, iron oxide, clay minerals (Twidwell and Williams-Beam, 2002).

U.S.EPA specified best demonstrated available technology (BDAT) as oxidation followed by precipitation (Rosengrant and Craig, 1990). The same agency established a maximum contaminant level of Tl in wastewater at 140 µg/L and drinking water at 2 µg/L. There was minimal success in the above technologies in reducing Tl to $\leq$ 140 µg/L and the methods were associated with high cost for wastewater treatment.

2.2.2 Cadmium

Cadmium is a silvery white lustrous but tarnishable heavy metal with a relatively high vapour pressure. The metal has eight stable isotopes of which ^{112}Cd and ^{114}Cd are the most abundant at 24.13% and 28.73% respectively (Thornton, 1986). Cadmium is ubiquitous in its distribution throughout the world and found in natural deposits as ores containing other elements (Cotton and Wilkinson, 1977; Volesky and Holan, 1995). The aqueous chemistry of

Cd is mainly in the form of Cd^{2+}, $CdCO_3$, and $Cd(OH)_2$ (Faust and Aly, 1998). The metal forms soluble compounds with fluoride, chloride, bromide, iodide, nitrate and sulphate and insoluble with sulphides, carbonate oxide and hydroxide (Förstner, 1980b).

Cadmium is one of the metals with a very high demand in industrial applications. It is used in processes such as electroplating of steel, iron, copper etc., manufacture of electrode batteries, and as an additive in the making of pigments. Cadmium is also found in phosphate fertilizers, stabilizers, alloys, barrier for atomic fission control, ceramics, plastics, paints, coated fabric, printing inks rubber, glass enamel (Förstner, 1980b; Nriagu, 1980; Balkaya and Cesur, 2008). Cadmium is usually released as a by-product in wastewater causing pollution even at very low concentration along the food chain (Forstner and Wittmann, 1979; Moore and Ramamoorthy, 1984). The maximum contaminant level under the World Health Organisation for cadmium in drinking water and waste water is 0.005 mg/L and 0.1 mg/L respectively (Faust and Aly, 1998).

The longevity of Cd accumulation with a half-life of 15-1100 years creates public health concern and continuous effort to detoxify contaminated water bodies (Johannesson, 2002; Madyiwa, 2006). Acute and long-term exposure of cadmium causes negative health effects to human beings. The toxic effects are measured by the dose –response relationship, where the response is a sign of an adverse effect (Fergusson, 1990). It is estimated that 3-10% of Cd is ingested from the gastrointestinal system whereas 50% of inhaled Cd is absorbed and transported through the blood to different organs in the body (Sahmoun, et al., 2005). Several literature sources have provided commentary on the carcinogenesis of cadmium (Waalkes, et al., 1992; Waalkes, 2003; Huff, et al., 2007). The most severe form of cadmium toxicity is "itai- itai", a disease which results in multiple fractures with skeletal deformation (Murata, et al., 1970; Friberg, et al., 1974a; Kasuya, et al., 1992; Yasuda, et al., 1995).

Acute poisoning usually occurs in the lungs and gastro intestinal tract causing health effects such as bronchitis, pneumonitis, toxemia in the liver, vertigo, diarrhoea and abdominal pain (Friberg, et al., 1974a; MacFarland, 1979; Tsuchiya, 1981). A dosage of CdO fumes inhaled at 8 mgm^{-3} can be fatal (Fleischer, et al., 1974) and a dosage of > 350-500 mg is lethal (Lauwerys, et al., 1979). Chronic effects of Cd poisoning usually accumulates in the lungs, kidney and bones. Long term exposure distends air sacs reducing lung capacity; kidney damage produces proteinuria, amio aciduria, phosphaturia, glucosuria, and kidney stones. Other health risks of cadmium include hypertension, testicular atrophy, kidney failure, cancer, mucous membrane destruction, anemia, osteomalicai in bones, reproductive toxicity (Fleischer, et al., 1974; Leyva-Ramos, et al., 1997; Sarkar, 2002; Godt, et al., 2006).

Some of the conventional treatment methods used for removal of cadmium include; a 3 step process of precipitation (ppt) using nitric acid; alkali ppt by NaOH and a polishing step of sulphide ppt by Na_2S (Lin, et al., 2005). Also different studies have been carried out using zinc powder (Ku, et al., 2002; Younesi, et al., 2006). Various membrane processes have been utilised to remove cadmium like hollow fibre supported liquid membrane and emulsion liquid membrane (Mortaheb, et al., 2009). Ion exchange using resins have been investigated widely for Cd removal by different authors including among others, (Chen, et al., 1998; Malla, et al., 2002; Pehlivan and Altun, 2006).

2.3 RARE EARTH ELEMENTS IN THE ENVIRONMENT

Rare Earth Elements (REE) is a group of metals constituting 17 elements of which 15 are lanthanides and the other 2 are scandium and yttrium, as shown in Table 2.1. REE have similar chemical properties with their electronic configuration an inner shell of electrons on the 4fn orbital shielded by the outer shell composed of electrons on the 5s2, 5p6, 5d1–10, and 6s2 orbital. The difference in lanthanides is the electrostatic effect associated with increase in nuclear charge and correlated to the contraction responsible for the weak differences in chemical properties (Martins and Isolani, 2005). This explains the difficulty in separating the elements with several steps involved like solvent extraction and or ion exchange resins both very costly (Palmieri, et al., 2000; Palmieri, et al., 2002).

REE are more abundant than many other better known metals and usually occur as trace constituents of major rock forming minerals (Haskin and Paster, 1979). The three major minerals currently exploited commercially for REE include bastnasite, monazite and xenotime with a few produced from mineral sands and gangue (Bulatovic, 2010). In recent years, significant amounts of these metals are released into the environment due to increased exploitation (Volokh, et al., 1990; Shan, et al., 2002). REE are widely used in the renewable energy technologies, permanent magnets, catalysts, agriculture, forestry and aquaculture (Andres, et al., 2003; Meyer and Bras, 2011). Little attention has been paid to industrial wastewater and residue containing REE. The waste may contain low concentrations but the residues are enormous making the total REE locked in also very high (Binnemans, et al., 2013).

Table 2.1: Rare Earth Elements and Atomic Mass Composition

Rare earth elements	Chemical symbol	Atomic mass (g/mol)
Lanthanum	La	138.905
Cerium	Ce	140.116
Praseodymium	Pr	104.907
Neodymium	Nd	144.242
Promethium	Pm	145.00
Samarium	Sm	150.36
Europium	Eu	151.964
Gadolinium	Gd	157.25
Terbium	Tb	158.925
Dysprosium	Dy	162.500
Holmium	Ho	164.930
Erbium	Er	167.259
Thulium	Tm	168.934
Ytterbium	Yb	173.04
Lutetium	Lu	174.967
Scandium	Sc	44.955
Yttrium	Y	88.905

Recovery of REE from wastewater and mine tailings remains largely unexplored. Some of the industrial products which can be recycled are permanent magnets, nickel metal hydride batteries and lamp phosphors. From literature less than 1% of REE were recycled in 2011 through direct recycling of preconsumer scrap, urban mining of end-life consumer goods and landfill mining of pre and post consumer waste streams (Binnemans, et al., 2013). In addition little attention has focused on the use of biosorption of rare earth elements (Korenevsky, et al., 1999; Kazy, et al., 2006; Tsuruta, 2007). Lanthanum is one of the most abundant and commonly used REE in industrial processes which was selected a representative element.

2.3.1 Lanthanum

This element is found in minerals such as cerite, monazite, allanite, bastnasite. The main ores in which La occurs are monazite and bastnasite at 25% and 38%, respectively (Vijayaraghavan, et al., 2009; Awwad, et al., 2010). Lanthanum is used in various industries such as use in the zeolite catalysts in petroleum refining for stabilising of zeolite at high temperatures; used in the motion picture industry for making carbonic lamps; used in the optical industry for making specialised lenses; making of special ceramics and catalysts among others (Sert, et al., 2008b; Awwad, et al., 2010).

China has the highest production of lanthanum at 90% but the country possesses not more than 40% proven reserves (Binnemans, et al., 2013). Due its increasing demand, China reduced its export quota of REE from 50145 tonnes in 2009 to 31130 tonnes by 2012 (Binnemans, et al., 2013). According to the short-term criticality matrix of the U.S. Department of Energy (DOE), the most critical REEs are neodymium, europium, terbium, dysprosium, yttrium and Indium. Lanthanum, cerium and tellurium rank high in the level of importance and are near to critical depletion, Figure 2.1.

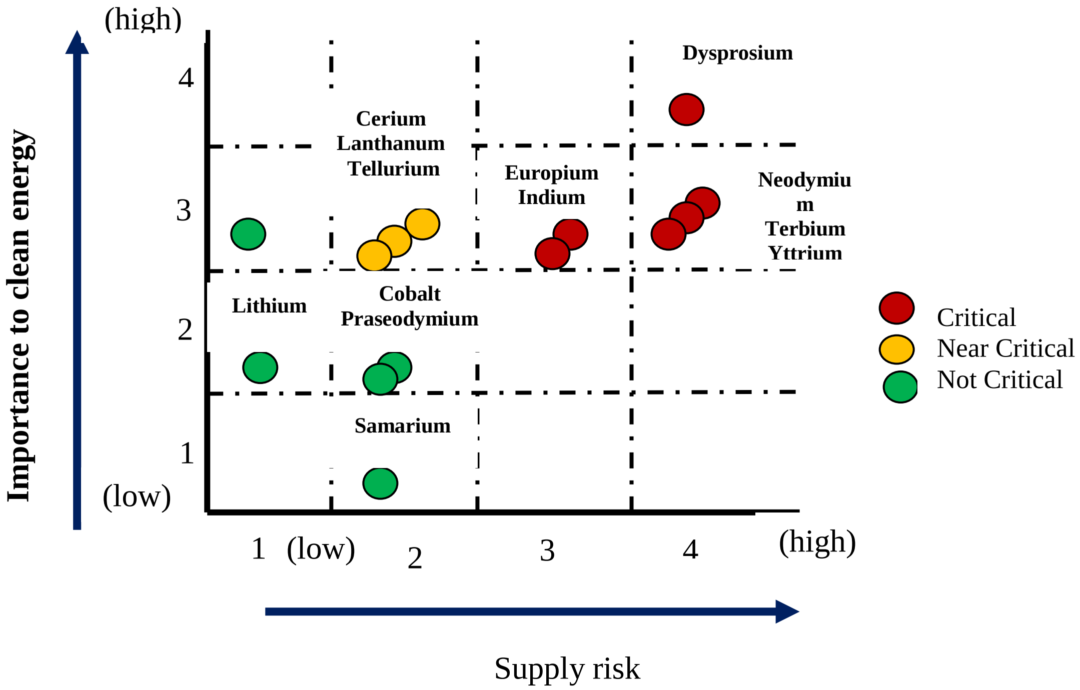


Figure 2.1: Adapted from the US Department of Energy, 2010 Supply Risk of REE (0-5 Years).

2.4 REMEDIATION AND TREATMENT OPTIONS

2.4.1 Conventional Treatment Methods

Conventional treatment technologies such as membrane filtration, GAC adsorption, precipitation-sedimentation, flotation, ion-exchange, and electrochemical deposition systems have been used for decades for the removal of toxic heavy metals Cd, Pb, Fe, Mn, Cr, and As from water (Veglio, et al., 2003; Wang, et al., 2004; Aziz, et al., 2008). Chemical precipitation (ppt) is one of the most commonly used processes for treatment of heavy metals. The process involves the use of a precipitant, e.g. lime which reacts with the metal ions to form an insoluble ppt. This technology treats effluent containing heavy metal concentrations higher than 1000mg/L (Ku and Jung, 2001; Kurniawan, et al., 2006).

In the ppt processes, balancing the pH to optimize the ppt efficiency can be problematic. For example, the pH and redox for precipitation of metals such as Pb, Al, Fe are outside the range in natural water environment. Moreover, due to overlaps in the precipitation ranges, the final sludge tends to have a mixture of metals and is therefore difficult to process further for the specific target metals (Mirbagheri and Hosseini, 2005; Mirbagheri and Hosseini, 2005; Aziz, et al., 2008). Other limitations include large chemical requirement, excessive sludge production, slow metal precipitation, poor settling (Wang, et al., 2004; Wingenfelder, et al., 2005).

Coagulation and Flocculation is another conventional method for treatment of wastewater containing heavy metals. Coagulation involves the use of coagulants, e.g aluminium, ferrous sulphate and ferric chloride to destabilise colloids. Flocculation then follows using flocculants like polyaluminium chloride (PAC), polyferic sulphate (PFS) to increase the size of unstable particles into large agglomerates (Semerjian and Ayoub, 2003). This method is not effective as it may require other techniques to complement it in the treatment of heavy metals (Chang and Wang, 2007; Fu and Wang, 2011). Other limitations include high operation cost due to chemical consumption, increased volume of generated sludge (Ayoub, et al., 2001; Fu and Wang, 2011).

Membrane filtration is a selective separation technology which allows some substances to penetrate while retaining larger partices depending on the size of the membrane. The three main membrane filtration processes include ultra filtration, nano filtration and reverse osmosis (Kurniawan, et al., 2006). Ultra filtration has a pore size of 5-20nm which enables the ions to pass through the membrane while retaining large size particles (Sablani, et al., 2001). Reverse osmosis uses pressure which allows water to pass through a semi permeable membrane while retaining heavy metals. Nanofiltration is an intermediate process between ultra-filtration and reverse osmosis (Eriksson, 1988). The main limitation for this method is the high cost especially in reverse osmosis which requires power consumption for pumping pressure, membrane fouling and process complexity.

The Flotation method uses bubble attachment in the treatment of heavy metals. The main flotation methods include dissolved air flotation, ion flotation and precipitation flotation (Wang, et al., 2004). When the particles get attached, they are separated from the suspension by bubble rise. This technique has high metal selectivity, high removal efficiencies, low detention period and production of more concentrated sludge. The limitation lies in the initial capital and operation cost (Fu and Wang, 2011).

Electrochemical techniques use electric potential in which ionised metallic species are plated out on the cathode surface, e.g. electrodialysis, membrane electrolysis and electro chemical precipitation (Van der Bruggen and Vandecasteele, 2002; Chen, 2004). This process requires fewer chemicals, produces less sludge but the high initial capital cost and expensive electricity supply renders its effectiveness.

Ion exchange is a reversible process involving the interchange of ions between the solid and liquid phase. The method is common and successfully used in the industry for the removal of heavy metals from effluent. Commonly used matrices for ion-exchange are synthetic/organic ion-exchange resins and zeolites which remove metal ions from the solution and release other ions of like charge in a chemically equivalent amount (Rengaraj, et al., 2001). This technology is associated with high removal efficiencies, and fast kinetics (Kang, et al., 2004). The disadvantage of this method is that it cannot handle concentrated metal solution as the matrix gets easily fouled by organics and other solids in the wastewater. Moreover, ion-exchange is non-selective and is highly sensitive to the pH of the solution. Other limitations include the high initial capital cost and ion exchange resins regeneration when exhausted can cause secondary pollution (Ahmed, et al., 1998).

2.4.2 Adsorption Treatment Methods

Adsorption processes are emerging technologies whereby a substance is transferred from a liquid phase to the surface of a solid by physical/chemical interactions and offer possible regeneration for re-use (Kurniawan and Babel, 2003). Recent adsorption technologies which act as possible alternatives and/or complementary to conventional technologies include Granulated activated carbon (GAC), nanotechnology and biological adsorbent systems.

Granulated activated carbon

GAC is the most common commonly used adsorbent due to its large surface area and high adsorption capacity attaining high metal removal efficiency. Modified activated carbon is generated from agricultural and industrial by-products such as coconut shell, rice husks, nut shell, saw dust and peat which act as an alternative to commercial activated carbon (Tam and Antal, 1999; Kadirvelu, et al., 2000; Amuda, et al., 2007). However, the cost of production and regeneration of spent carbon is still high and has limited its use in wastewater treatment (Babel and Kurniawan, 2004; Fu and Wang, 2011).

Nanotechnology

This technology has unique physical and chemical properties due to the crystal shapes and lattice orders of particles with a size less than 100nm. These methods offer significant improvement in performance for the treatment of heavy metals (Khajeh, et al., 2013; Qu, et al., 2013). These nano-materials have extremely high specific surface area, short intra particle distance, high chemical activity and adsorption capacity (Kalfa, et al., 2009). Examples of nano-materials tested at laboratory scale for treatment of heavy metals include;

Carbon-based nano tubes with fast kinetics and high specificity for metals (Rao, et al., 2007); metal based nano tubes (iron oxides, titanium dioxide) which are effective for metal removal and relatively cheaper than activated carbon (Sharma, et al., 2009); Nano fibre membranes from polymers and ceramics may be used for pre-treatment prior to ultra-filtration (Ramakrishna, et al., 2006); Magnetic nano tubes which can be produced in large quantities with simplicity for metal removal/recovery (Hu, et al., 2005); Nano clays which have an extensive surface area with peculiar charge characteristics, mechanical stability, high cation exchange capacity and cheaper than activated carbon (Unuabonah and Taubert, 2014).

Nanotechnology offers great promise in waste water treatment containing inorganic pollutants but faces some challenges of cost effectiveness and potential environmental and human risk (Qu, et al., 2013). The current cost of most nano materials is significantly high and owing to the small size of these particles may facilitate transport of toxic materials in the environment causing harm to cellular materials (Gorman, 2002).

Biological Adsorbent Systems

This technology uses materials of biological origin either dead or live for the treatment of wastewater containing heavy metals. The technology can be categorised under biosorption and bioaccumulation. Biosorption and bioaccumulation are always interchangeably misunderstood with the later also considered to be under the former. Biosorption is a passive and metabolically independent process that utilises non-living biological material for the removal of metals. Bioaccumulation is an active and metabolically controlled process that requires the use of living organisms to remove metals. Bioaccumulation is generally a slower process involving transport of ions across cell membranes towards the cytoplasm whereas in biosorption metal ions are quickly adsorbed on the cell surface (Modak and Natarajan, 1995; Davis, et al., 2003; Godlewska-Żyłkiewicz, 2006). Living microorganisms provide a large surface area to volume ratio but the method requires increased biological oxygen and chemical oxygen demand due to the nutrient uptake (Mohamad, et al., 2012; Monteiro, et al., 2012).

In order to deal with the pollution problem, most researchers focussed their attention onto bioaccumulation and concentration within living organisms for a long period. Some scientists used micro-organisms as a means of monitoring environmental pollution from metal bearing effluents (Hart, et al., 1979; Gipps and Coller, 1982; Costa and Leite, 1990a). The use of dead biomass for removal of toxic metals, a process referred to as biosorption was pioneered by the Volesky group from McGill University in 1981 (Tsezos and Volesky, 1981). Some earlier reviews reported that activated sludge efficiently removed radioactive metals, e.g. plutonium-239 from contaminated domestic waste water in 1949 (Ruchhoft, 1949). The first patent for biosorption apparatus was registered by Ames Crosta Mills and Company Ltd and J. R. Sanderson in 1973. The most attractive features of this technology is the high adsorption capacity, fast kinetics, metal selectivity, environmentally friendly and low initial capital and operation cost due to the relatively abundant and cheap biomass (Kotrba, 2011).

The main challenge of this technology is the selection of the most efficient biomass from the relatively abundant and available materials for use in industrial wastewater treatment. This challenge is overcome by the continuous search for biomass with better adsorption capacity and possible re-use and regeneration of biomass. This technology seems to provide greater promise in out competing conventional methods, GAC and nano technology for the treatment of toxic metals especially on issues of cost effectiveness and eco-friendliness. The biosorption technology is further elaborated below.

2.5 BIOSORPTION FOR INDUSTRIAL WASTEWATER TREATMENT

2.5.1 Biosorption Technology

Sorption is a physical-chemical process by which one substance becomes attached to another and is grouped into adsorption and absorption. In adsorption, the process occurs at the surface of another molecule while absorption involves incorporation of a substance into another of a different state (Borda and Sparks, 2008; Gadd, 2009; Gaur, et al., 2014). Biosorption can be used to describe a system where a sorbate (e.g. an atom or molecule) interacts with a biosorbent (e.g. solid surface of biological material) resulting in accumulation at the sorbate-sorbent interface. Most research currently categorises biosorption under adsorption (Michalak, et al., 2013). Biosorption involves passive and metabolically independent process using microbial organisms (Volesky, 1990b; Malik, 2004).

Some of the advantages of using non- living biomass over living micro-organisms are outlined as given by (Modak and Natarajan, 1995):

- The process is not governed by the physiological constraints of living microbial cells
- Non-living biomass behave like ion exchangers with the process being rapid between minutes and a few hours. Metal loading can also be high leading to efficient uptake
- Growth is independent as there is no need for costly nutrient requirements for growth of cells in feed solution
- The non-living cells can be operated under a wide range of conditions like pH, metal concentration, temperature unlike living cells which may require conducive environment for the growth of cells
- Possibility of recovery of valuable metals unlike living cells in which the metals may be bound intracellularly

Therefore, biosorption is a promising technology which can act as an alternative to conventional methods of industrial wastewater treatment especially at low concentrations. Some biological materials have binding capacities that are comparable with the commercial synthetic cation exchange resins (Forster and Wase, 1997). The biosorption efficiency depends on a number of factors including the capacity, affinity and specificity of the biosorbent and their physical and chemical conditions in the effluent (Singh, et al., 2006).

Conventional technologies are associated with high cost for processing of metal concentrations, lack of specificity, production of large volumes of sludge and low performance at low metal concentrations (Kotrba, 2011; Gaur, et al., 2014). The advantages of biosorption over conventional methods include low cost; high efficiency, minimisation of chemical and or biological sludge; no additional nutrient requirements; easy regeneration of biosorbent; and possibility of metal recovery (Volesky, 2001; Ahalya, et al., 2003). Figure 2.2 gives an indication on the steps involved in biosorption processes.

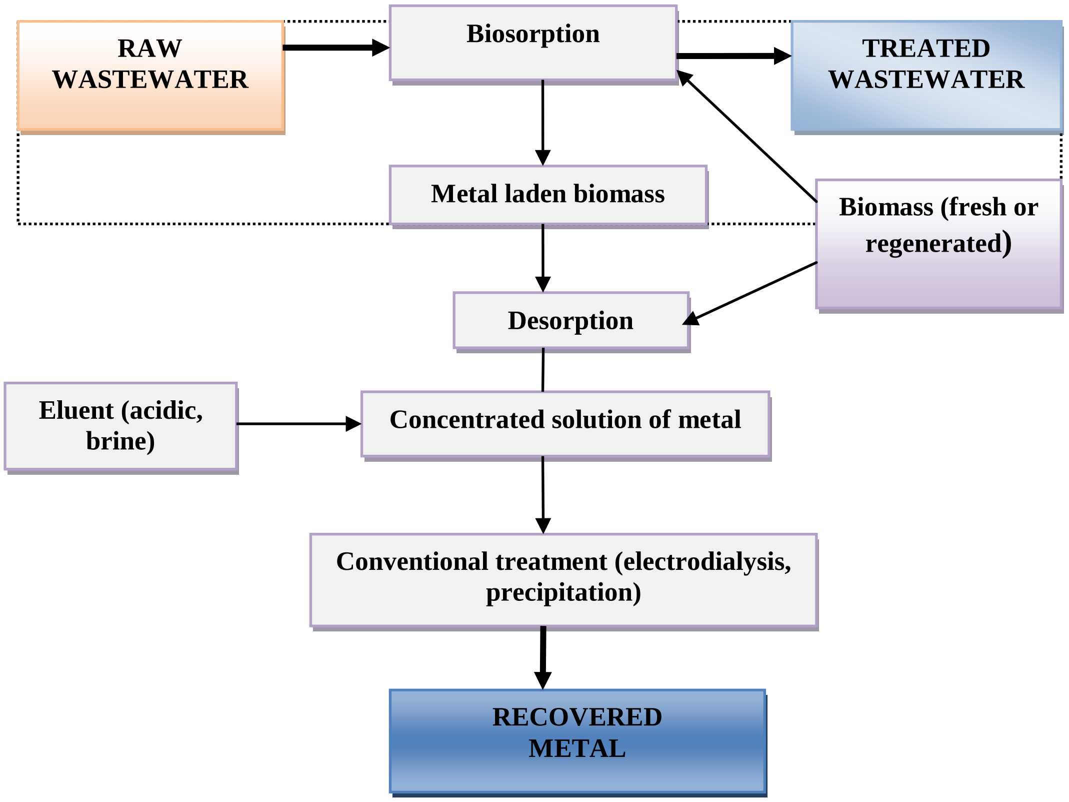


Figure 2.2: Systematically steps involved in biosorption adapted from Chojnacka, 2010

2.5.2 Biosorbents for Metal Removal and Recovery

Types of Biosorbents

The first major challenge in the biosorption field is to screen and select the most promising biomass with high binding capacity for metals and possibility of re-use (Kratochvil and Volesky, 1998). These biosorbents can be classified into low cost or natural sorbents which are usually collected from the environment, e.g., sea weed, and agricultural waste by products where there is some production cost involved. Some biosorbents belonging to the second group, such as yeast cells, can be cultured specifically for biosorption processes (Wang and Chen, 2006). This group of sorbents should have as high growth rate, high sorption capacity and re-usability (Volesky and Holan, 1995; Park, et al., 2010a). A broad range of biomass types have been tested including agricultural wastes such cane molasses, maize stocks, wood chips, grass, and maize tassels. The sorption of metals onto these biomaterials is attributed to the constituents of the cell wall which are mainly composed of carbohydrates, proteins and phenolic compounds (Choi and Yun, 2006).

Biosorption capacities of various biomass types have been compared in many review papers (Volesky and Holan, 1995; Gupta, et al., 2002; Vijayaraghavan and Yun, 2008a). Among the biosorbents tested, micro algae have proven to be better sorbents in certain cases mainly due to the constituents of their cell wall with a higher uptake capacity for metals compared to fungi, bacteria and yeast (Gong, et al., 2005; Mehta and Gaur, 2005; Tüzün, et al., 2005). In addition, algae has some advantages over other micro-organisms as they use light as an energy source in absence of an organic carbonic source, a strict requirement for bacteria and fungi (Dönmez and Aksu, 2002); have a low nutrient requirement and generally do not produce toxic substances (Das, et al., 2008).

The sorption capacity of algae varies from species to species even among strains of single species for any metal. Binding of metal ions on the algal surface depends on different conditions like ionic charge of metal ion, algal species and chemical composition of the metal ion solution (Das, et al., 2008). In general, algal biomass have a higher affinity for metal ions with greater electro negativity and smaller ionic radii (Mehta, et al., 2000). A variety of microalgae exist but only a few have been investigated for their sorption capacity. In addition, most of the studies have focused much attention on brown algae in the past 2 decades (Leusch and Volesky, 1995; Matheickal, et al., 1999; Figueira, et al., 2000; Davis, et al., 2003). (Romera, et al., 2006) also reviewed 214 references using 37 different algae of which 20 where brown algae (sea- weed), 9 red algae and only 8 green algae (micro algae). In addition, from the studies 57% were single metallic studies from pre-treated biomass, 33% natural biomass and only 10% using multi metallic systems.

Structure and Classification of Algal Sorbents

All biological material have the capacity to remove inorganic and organic pollutants but the difference lies in the cell wall composition and structure which determines the efficiency of a biomass (Gadd, 2009). Algae are eukaryotic organisms that contain chlorophyll and carry out exogenic photosynthesis. There are several characteristics for classifying algae including among others the nature of chlorophyll, cell wall structure, carbon reserves and the type of motility (Wang and Chen, 2009). Algal is usually broadly identified under two main groups viz a viz macro and micro algae. Macro algae are usually sea weeds or multicellular plants growing in salt or freshwater (McHugh, 2003). Based on their pigmentation, macro algae are further subdivided into brown sea weed, red sea weed and green sea weed. Micro algae are microscopic organisms found both in fresh water and marine environments. The three most abundant micro algae are diatoms, green algae and golden algae (Madigan, et al., 1997; Carlsson and Bowles, 2007).

Most algae contain chlorophyll and are thus green in colour but a few have specific carotenoids that sometimes mask the green colour. The green algae are one of the most diverse groups and have different forms which are unicellular, colonial, filamentous, membranous or tubular (Prescott, et al., 2002; Kotrba, 2011). Green algae mainly constitute cellulose with some modifications through addition of other polysaccharides and glycoproteins (Madigan, et al., 2000; Romera, et al., 2006).

The algal cell wall is thin rigid cellwall (3-5 nm wide) composed of a complex multi layered microfibrillar framework interspersed with an amorphous embedding material. The cell wall has different functional groups responsible for biosorption such as carboxyl, hydroxyl, amino, phoshoryl, sulphydryl. These functional groups possess negative charge which attracts the cationic form of most metals (Romero-González, et al., 2001; Skowronski, et al., 2001). The importance of any group for biosorption of a certain pollutant by a certain biomass depends on factors like the number of reactive sites in the biosorbent, accessibility of the sites, chemical state of the sites, affinity between the sites and the binding strength (Vieira and Volesky, 2010).

2.5.3 Factors Affecting Biosorption

Industrial wastewater contains varying external environment factors which influence biosorption. It is therefore, important to understand the effects of these factors on the adsorption capacity. There are a number of factors including among others, solution pH, temperature, ionic strength, initial pollutant concentration, biosorbents dosage, biosorbents size, agitation speed, co-existence of other pollutants. The major factors affecting biosorption as researched by some authors are reviewed.

Effect of pH

pH is a major influencing factor in biosorption as it determines the speciation and solubility of toxic metal ions and electrical charge of biomaterials (Xu, et al., 2006; Ofomaja and Ho, 2007; Vijayaraghavan and Yun, 2008a). pH significantly affects the dissociation site on the surface of the biomass, competition with co- existing ions and the solution chemistry of heavy metals, e.g hydrolysis, complexation, precipitation, redox reaction, speciation (Komy, et al., 2006; Guo, et al., 2008). At low pH values, the hydronium concentration makes competition among protons more favourable than metals in solution by the biomass active sites (Naja, et al., 2010). Acidic groups at low pH are protonated according to their pKa values. High pH causes deprotonation of metal ions binding sites exposed by cellular surfaces. Binding sites are available to metal ions only in the deprotonated state (Ofomaja and Ho, 2007). Every metal has its own favourable pH range in a certain industrial waste. The results from different authors found varying optimal pH ranges for adsorbents which are attributed to the other factors like initial concentration, temperature, and biosorbent dose (Naja, et al., 2005; Xu, et al., 2006; Navarro, et al., 2008).

Effect of initial concentration

Initial concentration provides a driving force by overcoming all mass transfer resistance of heavy metals between aqueous and solid phases (Ahmaruzzaman, 2011). The removal efficiency depends on the accessibility of surface function groups to bind metal ions (Arief, et al., 2008). Generally an increase in metal concentration improves adsorption capacity until all the available sites on surface functional groups become saturated. Several studies were carried to determine the effect of initial heavy metal concentration on metal uptake by biosorbents to mention but a few (Taty-Costodes, et al., 2003; Malkoc and Nuhoglu, 2007; Ghodbane, et al., 2008).

Effect of Ionic strength

The industrial effluent contains different cations and anions such as Na^+, Mg^{2+}, Ca^{2+} and Cl^-, NO_3^-, SO_4^{2-} respectively. The presence of these counter ions influences the binding of metallic ions on biosorbents affecting the uptake rate (Benaissa and Benguella, 2004; Diniz and Volesky, 2005b). Adsorption is affected by an increase in ionic strength due to competition between light and heavy metals for the binding sites. The effect is attributed to the changes in metal activity or in the properties of electric double layer (Ahmaruzzaman, 2011). Metal uptake decreases with increasing ionic strength as a result of electrostatic attraction and change in metal activity (Gonzalez-Chavez, et al., 2002; Deng, et al., 2007).

The ionic strength influences the strength of the electrostatic charges associated with adsorption radius in inner-sphere or outer-sphere complexes. Particularly, outer-sphere complexes are intrinsically more sensitive to variation in ionic strength than inner-sphere complexes, as the availability of free sorbent binding sites tends to decrease with increasing electrolyte concentration (Jonas and Norden, 1974). In the outer-sphere complexes, uptake of ionic species is mediated by the bipolar hydronium ions that form the interface layer between the charged surface (sorbent) and candidate ionic species in solution. Increasing ionic strength in the solution reduces the accessibility of sites by ionic species in solution due to the changing polarity of the surface layer (Donath, et al., 1997).

Effect of Temperature

Temperature affects biosorption to a certain degree depending on the structure and surface functional groups. Some studies showed an increase in temperature to optimum increases the surface area thus improving the sorption efficiency (Saleem, et al., 2007; Sulaymon, et al., 2013). At extremely higher temperatures, the metals may escape to the bulk phase (Kula, et al., 2008). Gibbs free energy, enthalpy and entropy are usually determined as endothermic or exothermic based on the spontaneity of the chemical reaction in the biosorption process (Uslu and Tanyol, 2006; Memon, et al., 2007; Padmavathy, 2008). A negative value of Gibbs free energy indicates feasibility and spontaneous nature of biosorption process (Bishnoi, et al., 2007; Ngah and Hanafiah, 2008; Sarı and Tuzen, 2008a). Positive values are endothermic and could be explained by an increase in the dehydration of adsorbate ions with a relatively high energy of solvation (Shen and Duvnjak, 2004; Şeker, et al., 2008).

2.5.4 Mechanisms of Biosorption

There are several mechanisms in the biosorption processes which depend on the type of species and the metal to be sorbed, the binding strength, number of binding sites, functional groups, availability and accessibility of sites (Volesky and Holan, 1995; Han, et al., 2006; Gaur, et al., 2014). Due to complexity of solution chemistry, surface composition and structure, some properties operate concurrently to varying degrees. Binding mechanisms may be either physical through London- van der waals forces, electrostatic or chemical by covalent bonding (Volesky, 2001; Naja and Volesky, 2011).

Biosorption mechanisms are classified into metabolic dependent and non- metabolic dependent according to their cell functionality (Veglio and Beolchini, 1997; Gaur, et al., 2014). The metabolic dependent mechanisms occur in living organisms and involve transport across the cell membrane and precipitation (Brierley, 1990; Costa and Leite, 1990b). The non-metabolic mechanisms which occur in biosorption processes include chemisorption by ion exchange, coordination and or chelation, complexation, physical adsorption, micro precipitation among others (Muraleedharan and Venkobachar, 1990; Holan, et al., 1993;

Wang and Chen, 2006). Figure 2.3 descibes the possible mechanisms of biosorption on the cell surface of organisms

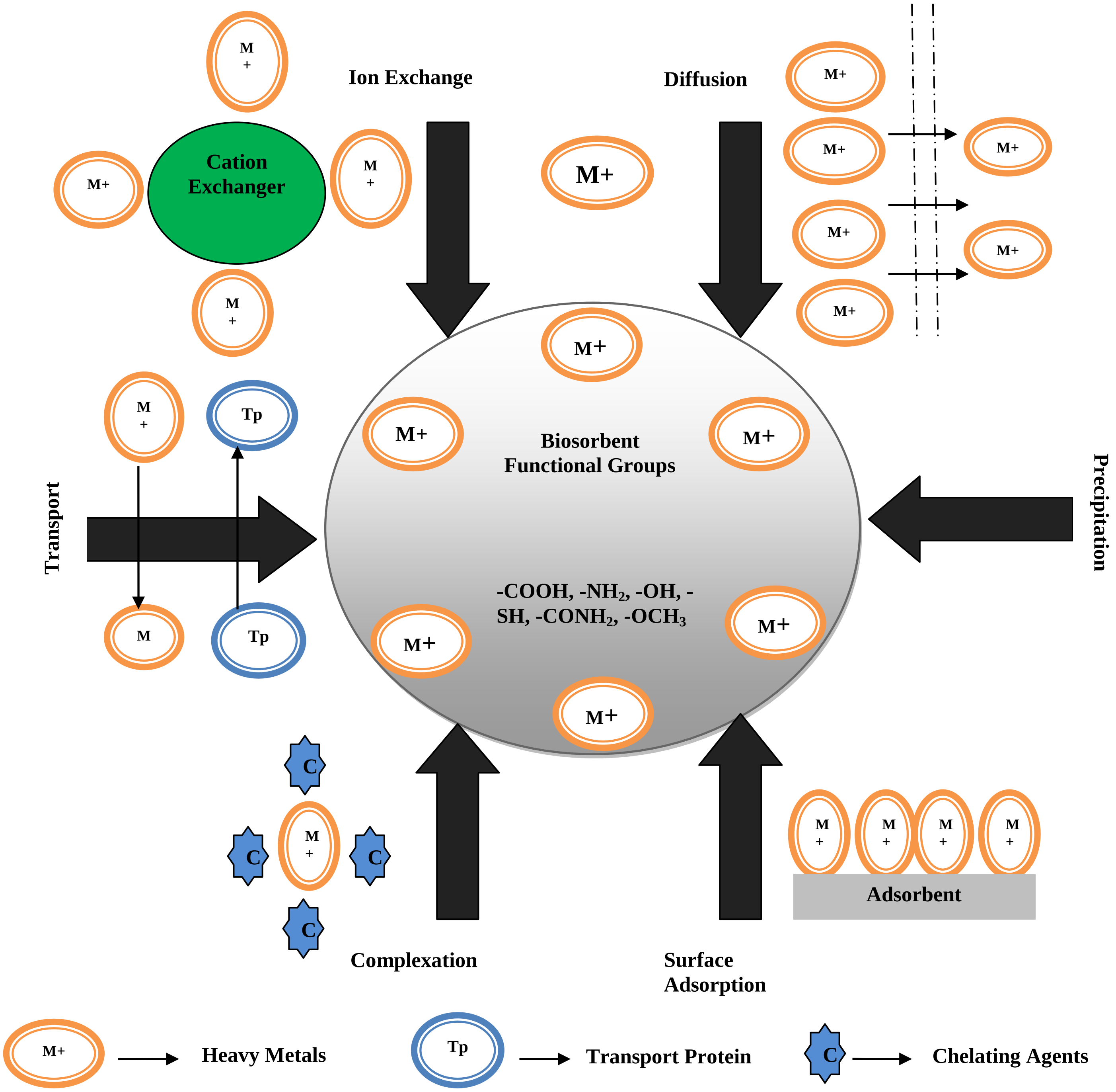


Figure 2.3: Mechanisms of biosorption based on cellular metabolism and location of biosorption adapted from Gaur, et al., 2014

Some researchers have reported that ion exchange is the principal mechanism in biosorption (Allen and Brown, 1995; Delgado, et al., 1998; Arief, et al., 2008). Ion exchange is a reversible chemical where an ion within a solution is replaced by a similarly charged ion attached onto an immobile solid particle (Han, et al., 2006). Other researchers suggest an interaction of ion exchange and formation of complexes between metal cations and ligands as well as precipitation of surface of cell wall (Crist, et al., 1994; Schiewer and Volesky, 2000). The rate of ion exchange depends on the diffusion of ions from the bulk of the liquid to the external surface of the exchanger particle; intra particle diffusion of ions through the solid to the binding sites; exchange of the ions; diffusion of the released ions to the surface of the solid; and diffusion of the released ions from the surface to the liquid bulk (Treybal Robert, 1981).

In complexation, species form 2 or more ligand with the metal in the central position. In some cases, the metals may form coordinating bonds when the central metal atom in the complexation bounds to immediate neighbours through covalent bonds as a result of metal ion occupying an electron pair. Chelation involves an atom in a molecule attached to the central metal atom or multiple donor atoms attached to the metal atom (Kotrba, 2011). Classification of metals using the hard soft acid base principle (HSAB) is important in understanding biosorption mechanism. Type A (hard acids) have a preference to oxygen containing ligands, while type B (soft acids) bind to S and N^- containing ligands. The principle is more descriptive than explanatory with bonds formed between hard and soft ligands forming covalent bonds (Gadd, 1992; Gadd, 2009). Although several mechanisms have been proposed, the actual mechanisms involved in metal biosorption are not fully understood (Wang and Chen, 2006).

Analytical techniques can be used to understand possible mechanisms as they complement each other in providing insight of biosorption processes (Park, et al., 2010b; Michalak, et al., 2013). These include among others, atomic absorption spectrophotometry (AAS), ion selective electrodes (ISE), UV–Vis spectrophotometry, potentiometric titration, scanning or transmission electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/TEM–EDX), infrared spectroscopy or Fourier-transform infrared spectroscopy (IR or FTIR), X-ray absorption spectroscopy (XAS), X-ray diffraction (XRD) analysis, electron spin resonance spectroscopy (ESR), nuclear magnetic resonance (NMR), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC), Figure 2.4 (Fomina and Gadd, 2002; Ngwenya, 2007; Michalak, et al., 2013).

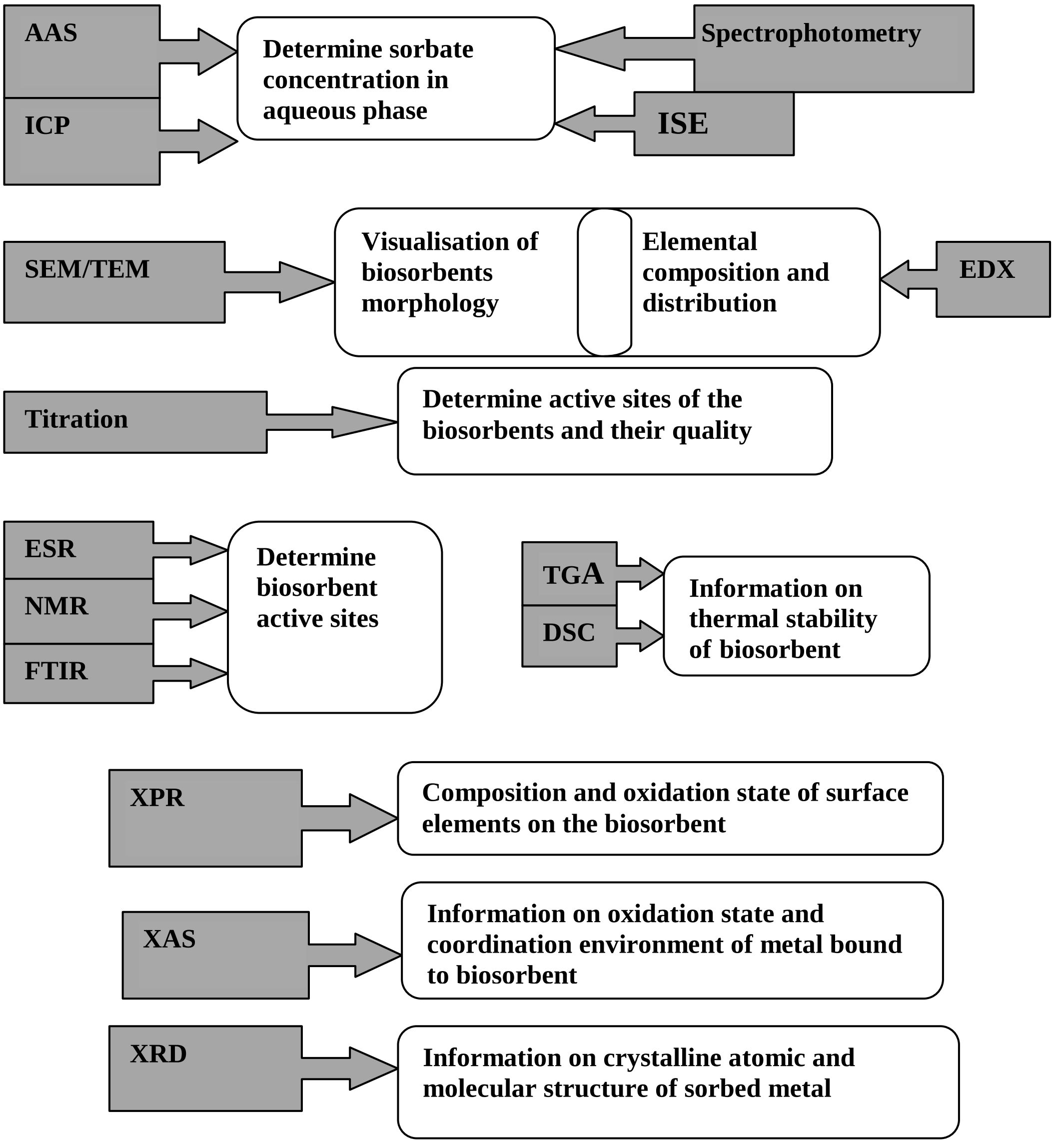


Figure 2.4: A brief description of analytical techniques in biosorption research adapted from Fomina and Gadd, 2014

2.6 MODELLING BIOSORPTION EXPERIMENTS

2.6.1 Equilibrium Models for Monometallic Systems

The biosorption isotherms are used for basic evaluation of sorption systems under optimal environmental conditions. Any comparison done at two different sorption systems can only be done at the same initial concentration for screening of sorbents with highest sorption capacity. These experiments are usually carried in batch reactors as an initial step before application into the dynamic continuous systems (Gadd, 2009). Equilibrium isotherms models are classified into empirical and mechanistic equations. In the mechanistic models, mechanisms for biosorption are explained and can predict the experimental behaviour (Pagnanelli, et al., 2002; Volesky, 2003). The empirical models assist in the evaluation of biosorption performance such as maximum sorption capacity and affinity of the sorbents for the metallic ions. The weakness with these models is they do not take into consideration of external environments associated with the actual effluents but are capable of describing adsorption isotherms (Volesky and Holan, 1995; Kratochvil and Volesky, 2000; Palmieri and Garcia, 2001; Vijayaraghavan and Yun, 2008b). Several empirical models have been used in single solute system (Freundlich, 1906; Langmuir, 1916; Brunauer, et al., 1938; Temkin and Pyzhev, 1940; Vijayaraghavan, et al., 2006). Table 2.2 summarises the most common empirical models with the linearised equation used.

The Langmuir and Freundlich are the most widely used and accepted simplistic mathematical models in literature as they usually fit the experimental done relatively well (Volesky and Holan, 1995; Arıca, et al., 2001; Mehta and Gaur, 2005). These isotherm models reflect metal binding as a function of equilibrium concentration of a known metal in solution. A Langmuir model assumes monolayer adsorption solutes onto surface containing a finite number of identical sites with homogenous distribution of adsorption energy and no transmigration of adsorbed ions. Freundlich model assumes the energy distribution is heterogeneous with binding sites having a higher affinity for the metal first occupied (Selatnia, et al., 2004; Göksungur, et al., 2005; Stumm and Morgan,).

Other common isotherm models used include Temkin model whose consideration is given to the interaction between adsorbate and adsorbent; Dubinin Radushkevich (D-R) model which expresses the adsorption mechanism with a Gaussian energy distribution on a heterogeneous surface, Table 2.2 (Gadd, 2009; Pagnanelli, 2011).

Table 2.2: Most Common Equilibrium Models for Single Metallic Studies

Isotherm type	**Linearised Equation**	**References**
Langmuir	$\boldsymbol{C_e}/\boldsymbol{q_e} = \boldsymbol{C_e}/\boldsymbol{q}_{\max} + 1/\boldsymbol{bq}_{\max}$	(Langmuir, 1916)
Freundlich	$\log\ \boldsymbol{q_e} = \log\ \boldsymbol{k} + 1/\boldsymbol{n} \log\ \boldsymbol{C_e}$	(Freundlich, 1906)
Dubinin-Radushkevich (D-R)	$\ln\ q_e = \ln\ X_m + \beta\varepsilon^2$	(Dubinin, 1960)
Temkin	$q\ = B\ \ln A_T + B\ \ln C_e$	(Temkin and Pyzhev, 1940)

2.6.2 Kinetic Models for Biosorption

Kinetic models are a fundamental process in biosorption studies as they provide useful information for the design and optimisation of operation processes. These models provide information on the rate controlling steps such as mass transport, chemical reaction processes and also determine the size of the contact equipment. However, biosorption kinetic data is still insufficient from published literature. In addition, evaluation of equilibrium sorption performance needs to be supplemented by process oriented studies of its kinetics. Numerous studies have been conducted but are required due to different operating procedures for each study (Wang and Chen, 2009; Ahmaruzzaman, 2011).

Biosorption is usually a very fast process taking place within minutes to a few hours and considering mass transfer and adsorption processes. The kinetic results can be affected by the particle size, initial concentration, mass transfer coefficients, solute diffusivity, mixing, and maximum sorption capacity of biosorbent. Kinetics studies provide the important information about the possible mechanism of biosorption that involves the diffusion (bulk, external, and intraparticle) and chemical reactions (Volesky, 2003; Michalak, et al., 2013). The rate determining step in most cases has been established to be either external film diffusion or intraparticle diffusion (Pagnanelli, 2011).

The assumption is that sorbate transport ensues in the following 4 steps. The first step involves the external diffusion from the bulk solution to the external surface surrounding the biosorbent, the second step involves transport of the solute across the boundary layer to surface of biosorbent through external diffusion, and the third step involves internal diffusion of compounds in the pores to the internal active sites of the sorbent. Finally, in the fourth stage of kinetics the metals are removed by the active sites via complexation, adsorption or precipitation (Selatnia, et al., 2004).

Different kinetic models have been suggested to describe the capacity reaction order of the adsorption system based on solution chemistry each with its own limitations, e.g. First and Pseudo-second order models, Weber and Moris, Elovich model and intra-particle diffusion. If the aim of modelling is simply having a mathematical equation resembling experimental data, empirical models neglecting the mass transfer effects can be adopted. Otherwise, if kinetic modelling aims to interpret experimental data and predict the effect of influencing factors, phenomenological models should be developed including the description of those mass transport steps limiting the global rate of sorption (Ho, et al., 1996; Mehta, et al., 2002).

Largergren's first order and Ho's Pseudo-second order models are the most commonly used in biosorption processes. The Pseudo- first order model assumes that the rate of occupation of binding sites is proportional to the number of unoccupied sites onto the biosorbent. In most cases, the first order equation does not fit well with kinetic data through the entire biosorption period as it is usually applicable for the first 20-30 minutes of the sorption process. The pseudo second order equation fits well in most cases through the entire adsorption mechanism as the rate controlling step with regression coefficients higher than 0.98 (Ho, et al., 1996; Febrianto, et al., 2009). Table 2.3 shows the most commonly used kinetic models in biosorption studies.

Table 2.3: Commonly used Kinetic Models in Biosorption

Model	**Equation**	**References**
First order	$\log(q_e - q_t) = \log q_e - {}^{k_1}\!/_{2.303}\, t$	(Lagergren, 1898)
Pseudo- second order	$t/q_t = 1/k_2 q_e^2 + 1/q_e\, t$	(Ho and McKay, 1999)
Elovich	$q_t = \beta \ln(\alpha\beta) + \beta \ln t$	(Roginsky and Zeldovich, 1934)

2.7 Modelling Multi Metallic Experiments

The actual waste water from industrial effluents contains various organic and inorganic pollutants. In most biosorption studies, most studies were undertaken using single metallic system due to the simplicity in evaluating and understanding their models (Romera, et al., 2006; Volesky, 2007). Recently, attention has shifted to multi-metallic systems as it reflects the real waste water with varying environmental conditions, e.g. pH, temperature, ionic strength and initial concentration. In studies involving multi metallic experiments, more research was done on binary systems (Chong and Volesky, 1996; Figueira, et al., 1997; Li, et al., 2004), with less on ternary systems (Chong and Volesky, 1996; Sağ, et al., 2001; Mohapatra and Gupta, 2005).

For systems with a mixture of more than one metal, the mathematical model must be modified to take into account the competition of metals for the binding sites. Low, et al., 1995 described the amount of adsorbed ion to be dependent on completion between cations, ionic size, stability of bonds between metal ions and adsorbent. A series of equilibrium biosorption models which incorporate the metal concentration, pH and ionic strength have been presented by Schiewer and Volesky, 1997. Various empirical multi metallic models have been developed including among others, extended Langmuir, modified Langmuir, Redlich–Peterson, Sheindorf–Rebuhn–Sheintuch (SRS), extended Freundlich and combined Langmuir and Freundlinch models. Table 2.4 shows the most commonly used multi metallic models with their limitation. The MATLAB software is a very useful tool used in analysis of multi metallic systems with different ions competing for the same sorption site.

Table 2.4: Commonly used Multi- Metallic Models in Biosorption

Model	**Equations**	**Limitations**	**References**
Extended Langmuir	$q_{e(i)} = \dfrac{q_{m.i} K_{L..i}\left(C_{e.i}\right)}{1+\sum_{j=1}^{N} K_{L.j}\left(C_{e.j}\right)}$	Mechanisms are not well represented	(Fritz and Schluender, 1974)
Combined Langmuir and Freundlich	$q_e = \dfrac{q_{LF.i} b_{LF.i} C_{e.i}^{1/n_{LF,i}}}{1+\sum_{a=1}^{N} b_{LF,a} C_{e.a}^{1/n_{LF.a}}}$	Requires additional parameters	(Sips, 1948)
Surface Complexation model	$q \approx f\left(C_e\right)$,e.g. following Langmuir	Equilibrium constants have to be determined for different binding sites	(Davis and Leckie, 1978; Yiacoumi and Tien, 1995)

2.8 Recovery of Rare Earths and Target Metals

The traditionally used technologies for the recovery of rare earths and or other target heavy metals include electro deposition, solvent extraction, ion exchange, membrane separation and adsorption among others (Barnes and Edwards, 1982; Mooiman, et al., 1984; Liu, et al., 2000). Due to the soaring demand of limited resources, there is an increase in price of rare earths, precious metals and other commonly used heavy metals which necessitates efficient recovery. Adsorption has garnered more attention due to its potential recovery using relatively low cost and efficient techniques (Wang and Chen, 2009; Das, 2010).

The entire biosorption process consists of adsorption-desorption cycle. The efficiency of biosorption exploitation depends on the level of regeneration of biosorbents for re-use in a number of cycles (Bishnoi and Garima, 2005). Most authors as observed by Vijayaraghavan and Yun, 2008a) have extensively done research on the biosorption capacities of different biomaterials with limited information on recovery of metals and re-use of the biosorbents. The eluents must meet the following requirements; non-damaging to the biomass, less costly, environmental friendly and effective (Vijayaraghavan and Yun, 2008a; Lesmana, et al., 2009). More research is required to focus on the regeneration ability of the biosorbents for future biosorption application (Wang and Chen, 2009).

Various chemical re-agents have been utilised in the recovery of metals especially dilute acids, e.g. nitric acid (HNO_3), sulphuric acid (H_2SO_4) and hydrochloric acid (HCl). In studies using HCl, the eluent was found to have a high recovery efficiency but the desorption capacity decreased probably due to hydrolysis of polysaccharides on the biomass surface. $CaCl_2$ and NH_4Cl and EDTA were also found to have high desorption capabilities although not acids (Mehta and Gaur, 2005). The efficiency of the eluent can be expressed as a solid/ liquid ratio (S/L) with the solid representing the metal laden biomass and the liquid as the eluent. High values of S/L are preferred (Volesky, 1987; Kuyucak and Volesky, 1989).

2.9 Summary

Biosorption is an emerging and developing technology with great potential in the treatment of industrial wastewater water using readily available and inexpensive biosorbents. A variety of biosorbents exist including fungi, bacteria, algae, agricultural waste among others of which very few have been investigated, for example literature on algae tends to suggest limited work done on micro algae despite the diversity of species. Algae usually exist as a nuisance in most water bodies causing eutrophication and yet these organisms could be utilised in the removal/recovery of toxic metals from industrial wastewater. An important criterion to be considered in biosorption is the ability of biosorbents to recover target metals, and re-usability of the biomass. It can be noted that biosorption studies need to shift from only single metallic studies but in addition tackle real industrial effluent containing different metals, salts and varying external environments. A challenge noteworthy is that biosorption is a complex system with uncertain known mechanisms which are dependent on physical, chemical and biological factors. There is therefore need for further investigation of biosorbents for improved adsorption and desorption efficiency, selectivity for target metals, understanding biosorption mechanisms, performance of models and applicability to actual industrial wastewater. Hence this study contributes to the knowledge gap in biosorption using microbial algae in the removal/recovery of metals in industrial wastewater.

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

3.1.1 Materials

The algal samples were collected using plankton nets (30 and 120 µm) into 5 collection vials (2000ml) containing Bold Basal Medium (BBM). Some samples were preserved in formalin.

3.1.2 Algal Samples from Freshwater Sources

The planktonic algae were collected from a fresh water dam in Hartbeespoort dam in North West province in South Africa. 30 litres of algal samples were scooped using a bucket from different sampling site and concentrated using planktonic nets of 30 and 120 µm. The collected samples were put into vials containing sterile Bold Basal Medium (BBM). The BBM was prepared by firstly making up stock solutions separately using distilled water. The volumes of appropriate stock solutions were based on preparing 1L of BBM media added in numerical order to a conical graduated flask containing 900mL distilled water, Table 3.1. The media was stirred for 30 min to ensure thorough mixing and the pH adjusted to 6.7. The BBM was then autoclaved and ready for use.

Some samples were concentrated to 20 ml, preserved in 10% buffered formalin (10 ml conc. formalin; 4.0 g NaH_2PO_4, H_2O; 6.50 g Na_2HPO_4) and volume made to 100 ml with distilled water. The samples were then transported to the laboratory and preserved at a temperature of 4°c. The samples stored in formalin were identified under the light microscope while still fresh to check for basic features of algae. The concentrated sample was withdrawn with a pipette and mounted on a glass slide. The sample was then covered with a cover slip and pressed to prevent evaporation and observed under the microscope

3.2 ISOLATION AND IDENTIFICATION OF ALGAL SAMPLES

3.2.1 Materials

Selected algal cultures for transfer, sterilized test tubes for inoculation, sterile pipettes, ethanol, bunsen burner, igniters, chlorinated wiping cloth and loop for streaking.

Table 3.1: Bold Basal Media Formulation for Sample Collection

Chemical name	**Formula**	**Weight (g)**	**Distilled** H_2O **Water (mL)**
Group A (10ml each to 1 litre distilled H_2O)			
di-potassium hydrogen orthophosphate	K_2HPO_4	1.875	250
Potassium di-hydrogen Orthophosphate	KH_2PO_4	4.375	250
Magnesium sulphate	$MgSO_4.7H_2O$	1.875	250
Sodium Nitrate	$NaNO_3$	6.250	250
Calcium chloride	$CaCl_2.2H_2O$	0.625	250
Sodium Chloride	NaCl	0.625	250
Group B (1ml each to 1 litre distilled H_2O)			
EDTA tetrasodium salt +	EDTA - Na_4	5.000	100
Potassium hydroxide	KOH	3.100	
Ferrous sulphate +	$FeSO_47H_2O$	0.498	100
Sulphuric acid conc	H_2SO_4	0.1mL	
Boric acid*	H_3BO_3	1.142	100
Group C (0.1ml each to 1 litre distilled H_2O)			
Zinc sulphate	$ZnSO_4.7H_2O$	0.353	25
Manganese chloride	$MnCl_2.4H_2O$	0.058	25
Cupric sulphate	$CuSO_4.5H_2O$	0.063	25
Cobaltous nitrate	$Co(NO_3)_2.6H_2O$	0.020	25
Sodium molybdite	$Na_2MoO_4.2H_2O$	0.048	25

*Needs heating to ~50-60°C to dissolve.

3.2.2 Centrifuge and Streak Plating of Algal Samples

A volume of 12 ml micro algal sample was taken from the enrichment culture and put into four centrifuge tubes. The tubes were centrifuged at 6000 rpm for 5 minutes. After removing the supernatant, the cells were suspended in fresh sterile water in each tube using vortex mixer (rotated at 1000 rpm up to homogeneous suspension). Centrifugation and washing was repeated six times to expel most of the micro-organisms presented in algal sample. The samples were then isolated using streak plating in axenic conditions on agar plates and left to grow for seven days under required light conditions (Osram L 36W/77 Floura) and temperature range of 20-23°C. Repeated streaking was done to obtain single colonies free from contamination.

The agar plates were prepared by adding 9 g of bacteriological agar to 1L of AF-6 media adapted from (Andersen, et al., 2005). The lower concentration of agar results in the formation of a semi-solid surface which acts as a more suitable substrate for algal growth. The AF-6 media was prepared by firstly preparing stock solution of trace elements and vitamins, Table 3.2. For vitamins, primary stocks of B-12 (11.4mg/10ml) and biotin (10.4mg/100ml) was prepared initially. The biotin mixture was adjusted to a pH of 10 to dissolve. All stocks were adjusted to pH 5 and made up to final volume and stored at 4°C. The vitamin stock was then prepared by adding: 1ml of biotin stock, 0.1ml B-12 primary stock, and 20mg thiamine HCl in 100ml water and brought to a final volume of 1L, 0, 5ml.L^{-1} of this solution was added to all media formulated. AF-6 media was then prepared by dissolving MES into 950ml of distilled water followed by the other compounds individually dissolved, the final volume was then adjusted to 1L and the pH adjusted to 6.6 and autoclaved.

3.2.3 Molecular Identification of Algal Species

DNA extraction from the algal samples was performed with the CTAB method of (Murray and Thompson, 1980). The Internal transcribed spacer (ITS) and 18S ribosomal RNA gene (rRNA) were used in this study. ITS1 and ITS2 primers were used to amplify the ITS region in the forward and reverse directions, respectively. To amplify the 18S rRNA gene, the specific forward primer and the conserved reverse primer were used. Polymerase chain reaction (PCR) amplification was carried out in a DNA Thermal Cycler (Gene Amp PCR System 2400 - Perkin Elmer). The conditions for the ITS1/ITS2 primer pair were 5 min at 95°C, 35 cycles of 1 min at 90°C, 2 min at 50°C, 1 min at 72°C and a final elongation step of 10 min at 72°C. All PCR amplifications were performed in 50 µl master mix solution containing 10 mM Tris HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgSO4, 200 µM dNTP, 0.4 µM of each primer, 1.0 u Taq polymerase (Promega) and 10 ng of total DNA. Phylogenetic analysis of sequences was checked for similarity using a basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/). The samples identified from BLAST search were used in taxonomic classification of algae.

Table 3.2: AF-6 Media preparation for algal culture

Chemical compound	**Unit**	**Quantity Used**
Trace metal stock solution		
$FeCl_3.6H_2O$	$g.L^{-1}$	0.98
$ZnSO_4.7H_2O$	$g.L^{-1}$	0.11
$CoCl_2.6H_2O$	$g.L^{-1}$	0.02
$MnCl_2.4H_2O$	$g.L^{-1}$	0.18
$Na_2MoO_4.2H_2O$	$g.L^{-1}$	0.01
$Na_2EDTA.2H_2O$	$g.L^{-1}$	5
Vitamin stock		
Thiamine-HCl	mg/L	200
Biotin	mg/L	1
B-12	mg/L	1
AF-6 media		
MES	$g.L^{-1}$	0.4
Fe-citrate	$g.L^{-1}$	0.002
Citric Acid	$g.L^{-1}$	0.002
$NaNO_3$	$g.L^{-1}$	0.14
NH_4NO_3	$g.L^{-1}$	0.022
$MgSO_4.7H_2O$	$g.L^{-1}$	0.03
KH_2PO_4	$g.L^{-1}$	0.01
K_2HPO_4	$g.L^{-1}$	0.005
$CaCl_2.2H_2O$	$g.L^{-1}$	0.01
Trace Metal Solution	ml	1
Vitamin (From ASW)	ml	1

3.2.4 Pure Algal Cultures

The pure colonies were picked up by loop and allowed to grow in tubes and vials containing AF-6 media as starter cultures and stock cultures. Starter cultures are usually considered as back up and were maintained under illumination in static flasks. The stock cultures were further inoculated into 1000ml and 2000ml volumetric flasks to increase the volume of cultured sample and sub- cultured twice a month, Figure 3.1. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* algal species were purchased from the Culture Collection of Algae and Protozoa (CCAP) for comparison purposes and were cultured in the modified recipe of Bold Basal Medium with 3fold Nitrogen and Vitamins; (3N-BBM+ V) formulated as shown in Table 3.3.

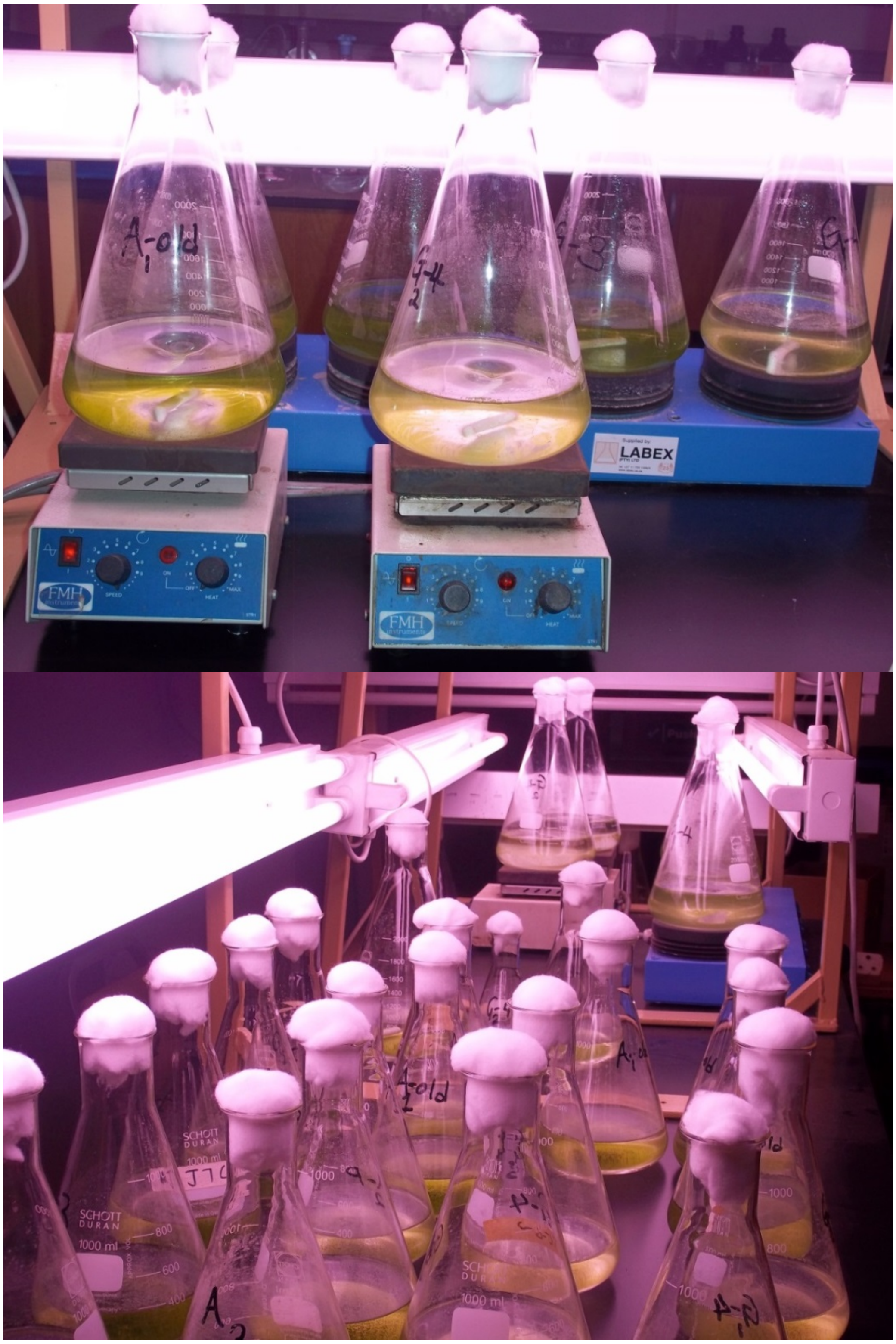


Figure 3.1: Algae cultures in 1000ml and 2000ml volumetric flasks using algal light

Table 3.3: 3N-BBM+V Modified Media from CCAP

Stock solutions in g / 1000 ml water		for 1 litre final medium, add
Chemical compound	**Units (g)**	**Unit (ml)**
	3N-BBM+V media	
$NaNO_3$	25	30
$CaCl_2.2H_2O$	2.5	10
$MgSO_4.7H_2O$	7.5	10
$K_2HPO_4.3H_2O$	7.5	10
KH_2PO_4	17.5	10
NaCl	2.5	10
Trace element solution	see below	6
Vitamin B_1	see below	1
Vitamin B_{12}	see below	1
	Vitamin B_1	
Thiaminhydrochloride	0.12	Add to 100ml of distilled water
	Vitamin B_{12}	
Cyanocobalamin	0.1	Add to 100ml of distilled water
	Trace element solution	
Na_2EDTA	0.75	Add mixture to 1000 ml of distilled water
$FeCl_3.6H_2O$	0.097	
$MnCl_2.4H_2O$	0.041	
$ZnCl_2$	0.005	
$CoCl_2.6H_2O$	0.002	
$Na_2MoO_4.2H_2O$	0.004	

3.3 ADSORPTION AND DESORPTION EXPERIMENTS

3.3.1 Materials

Algal sieve (mesh size of 0.3 mm), Erlenmeyer flasks (250ml), volumetric flasks (100ml and 10ml), magnetic stirrers, pipette tips, pH meter, timer, centrifuge tubes, buffers (HCl and NaOH), HNO_3 acid.

3.3.2 Chemical Re- Agents

All chemical re-agents used were of analytical grade. Lanthanum, thallium and cadmium standard stock solutions prepared using nitric acid were purchased from Laboratory Consumables and Suppliers in South Africa. The standard solution of sodium nitrate, magnesium nitrate, potassium nitrate and calcium nitrate were purchased from Merck in

South Africa. All glassware were soaked in 30% nitric acid and rinsed three times in distilled water before use in experiments.

3.3.3 Biosorbents

The harvested biomass for the different algal species was dried at 50°c in an oven for 24 hours, crashed and sieved using a mesh size of 0.3mm to maintain uniform size and increase the surface area. The samples were then stored in air dried containers, sealed and ready for biosorption experiments.

3.3.4 Equilibrium and Adsorption Kinetics in Batch Reactors

A standard stock solution of 1000 mg/L of La^{3+}, Cd^{2+} and Tl^{+} was used to prepare initial metal concentrations. The metal concentrations used were in the range of 15-150 mg/L for La and Cd, and 15-500 mg/L for Tl. The metals were measured into 100 ml Erlenmeyer volumetric flasks and transferred to 250 ml conical flasks. The pH was adjusted to optimum in the range of 5.5-6 and the biomass kept constant at 0.05g for all the tested metals. Preliminary kinetic experiments were carried out to determine the time required for adsorption to reach equilibrium which was found to be less than 4 hours. In all the algal biomass tested, the adsorption experiments were carried out for 6 hours, time more than required for equilibrium.

Equilibrium experiments were carried out concurrently with kinetic experiments after determining the time required for adsorption. The samples were stirred on a magnetic stirrer at a constant speed of 350 rpm and room temperature. Samples were withdrawn at pre-determined time intervals for kinetic experiments and after 6 hours for equilibrium experiments. The experiments were carried out in duplicates and a control set up for all the studies tested. pH was kept constant by adjusting hourly using 0.1M NaOH and 0.1M HCl. The samples were stirred on a magnetic stirrer at a constant speed of 350 rpm and room temperature. The effect of pH, contact time, temperature on adsorption was also tested. Samples were withdrawn at pre-determined time intervals for kinetic experiments and after 6 hours for equilibrium experiments. The samples were centrifuged for 10 minutes at 6000 rpm and the filtrate analysed using the Inductively Coupled Plasma (ICP, Spectro Arcos FHS12, Boschstroisse, Germany).

3.3.5 Desorption Kinetics and Re-Usability of Algae

The algae was tested for their re-usability for the removal and recovery of tested metals in three cycles. The metal laden biomass was rinsed twice in deionised water to remove residual solution and weighed. The main eluent used for recovery of metals in most experiments was 0.1 M HNO_3. Other eluents of sulphuric acid (H_2SO_4), Ethylenediaminetetraacetic acid (EDTA), hydrochloric acid (HCl), were also tested on recovery of thallium. Low concentrations were used for preparation of all the mineral acids. The weighed biomass was added to 50ml of 0.1M of eluent (HNO_3) and stirred on a magnetic stirrer for a period of 4-6 hours. The experiment was carried out in duplicates. The sample was drawn at the end of the experiment, centrifuged and the supernatant analysed for metal analysis. The same procedure was repeated for the other 2 cycles using the same biomass to check for re-usability. In desorption kinetics, samples were taken at predetermined time intervals, centrifuged and the filtrate analysed using ICP.

3.4 MULTI-METALLICS IN BATCH REACTORS

3.4.1 Adsorption Equilibrium and Kinetics for Ternary System

For preparation of a mixture of multi metallic, initial concentration of 25-150 mg/L were used for Cd and La, and 150-800 mg/L for Tl. The varying concentrations were measured into 100 ml volumetric flask for each metal, and then mixed to make a volume of 300ml for the 3 metals. The mixture was then divided into 2 volumes of 150 ml each into Erlenmeyer flasks to make duplicates. The pH was adjusted to optimum at 6 before the start of the experiment and a constant biomass of 0.08g was used. The experimental sets were stirred on the magnetic stirrer at room temperature and a constant speed of 350 rpm. Equilibrium and kinetic studies were carried out con-currently. The first sample was taken as soon as the biomass was added into the metal concentration at time (t_0). The pH was adjusted occasionally using 0.1M NaOH and 0.1M HCl. The preceding samples were taken at pre-determined time interval between 5-1440 minutes. The samples were centrifuged for 10 minutes at 6000 rpm and the supernatant analysed using ICP.

3.4.2 Effect of pH on Ternary System

A mixture of 3 metals was used to check the effect of pH on the ternary system at initial concentration of 150, 150 and 250 mg/L for Cd, la and Tl respectively. The initial concentration was kept constant for all the pH values in a range of 2-8. The biomass was also kept constant at 0.05g and the experiment was run for 4 hours.

3.4.3 Effect of Ionic Strength on Ternary System

Standard stock solutions of Na^+, K^+, Mg^{2+}, Ca^{2+} were purchased from Merck, South Africa. All experiments were carried out at a constant temperature and biomass. To test for effect of ionic strength, initially experiments were carried out using Na+ at low and high molar concentration without adjusting the pH as it would bias the final results. NaOH used for adjusting pH contains Na+ which would increase the molar concentration of Na+ in solution. 250mg/L of Tl, 100mg/L of Cd and La were measured using100ml volumetric flasks and then volumes of 50ml each were used for each experiment. A mixture of the 3 metals was poured into 250ml Erlenmeyer flask. Initial concentrations of 0.65 and 4.35M for Na^+, K^+. Ca^{2+} and Mg^{2+} were measured using 50ml volumetric flasks and poured into the mixture of prepared heavy metals. All experiments were carried out in duplicates. A biomass of 0.05g was added to each flask and stirred on the magnetic stirrer at a constant speed. A sample was withdrawn from each flask, centrifuged and the filtrate analysed using ICP.

3.4.4 Recovery of Metals from a Ternary System

Adsorption samples from equilibrium studies of ternary system were used in the desorption experiments. The metal loaded samples were rinsed twice in double distilled water and weighed. 0.1M nitric acid was used as the eluent and the experiments were carried out in duplicates. The biomass was added and mixture was stirred in the magnetic stirrer for 6 hrs. A sample was withdrawn, centrifuged and the filtrate analysed using ICP.

3.4.5 Comparison of Mixed Algal Sorbents

Different algal species were mixed (combo) to test for improved sorption efficiency at optimal pH and constant biomass in all experiments. The mixture was composed of 4 sets which included *Desmodesmus multivariabilis*+ *Chlamydomonas reinhardtii*; *Desmodesmus multivariabilis*+ *Chlorella vulgaris*; *Desmodesmus multivariabilis*+ *Chlorodium saccharophilum*. In addition all the four species were combined and the biomass used for each species was weighed at 0.03g. Initial concentration of 250mg/L of Tl, 100mg/L Cd and La were used in these experiments. The combo was added to the metallic solution and the samples stirred on a magnetic stirrer for 6 hours.

3.4.6 Biosorption using Actual Industrial Samples

Industrial wastewater samples were collected from 3 industries in South Africa, namely Dilakong chrome mine, Afrisam cement industry and Manganese metal company. The industrial samples were initially analysed using ICP to determine the metal types and concentration available before biosorption experiments. 100ml of waste samples were then contacted with 0.1g of algal biomass in 250ml of Erlenmeyer flasks and stirred on the magnetic stirrer at 240 rpm for 24 hours. The samples were filtered and the filtrate analysed using ICP.

3.5 CHARACTERISATION OF THE ALGAL BIOSORBENTS

3.5.1 Scanning Electron Microscope

The morphology of the biosorbents before and after biosorption was identified using a Scanning Electron Microscope (SEM, JOEL JSM 5800LV, Tokyo, Japan). The wet algal samples were prepared using traditional chemical methods before embedding for SEM (Glauert and Reid, 1975). The samples were first rinsed three times in 0.075 M phosphate buffer for 10 minutes. The buffer was used to protect the biological sample from becoming acidic. The clean cells were then fixed in 0.5% aqueous osmium tetroxide for 2 hours and rinsed three times in distilled water. Each sample was then dehydrated in ethanol (30%, 50%, 70%, 90%, and 100%) for 10 minutes. The samples were dried at critical point from liquid carbon dioxide, mount on the stub and sputtered with gold ready for identification. After sample preparation, SEM was used to obtain images at different magnification before and after adsorption.

3.5.2 Fourier Transform Infrared Spectrum

The functional groups on the algal cell wall were identified using the Fourier Transform Infrared spectrum, (FTIR, Perkin Elmer 100). The instrument was set up and scanned within a range of 4000-625 cm^{-1}. The sample holder (diamond stage) was cleaned with acetone and a background spectrum collected to check the response of the system. The algal samples before and after adsorption were each placed on the diamond stage and the pressure arm lowered on the samples. The stage was cleaned again before mounting another sample to avoid contamination.

The total pressure applied was monitored to obtain the spectrum. Data was processed with different peaks attained to represent the functional groups.

3.5.3 Brunauer, Emmett and Teller Method

The algal cell surface was characterised using the Brunauer, Emmett and Teller (BET-AccupyII 1340 gas).The BET method was based on the adsorption of gas on the surface at a given pressure. The weighed sample was first degassed at 50°C for 24 hours using the sample degas system (vac prep 061). The density of the algal sample was also obtained. The sample surface area was then analysed using liquid nitrogen in a pycnometer.

3.6 DATA ANALYSIS

3.6.1 Equilibrium Modelling

Empirical models of Langmuir, Freundlich and Dubinin-Radushkevich (D-R) models were used to quantitatively describe sorption potential of algae using linear regression. The Langmuir model assumes attachment of only one layer of molecules to the surface, has specific number of sites where solute molecules can be adsorbed and the surface of the sorbent is strongly attracted to the adsorbate. In this model, sorption capacity ($q_{\max}$) and the affinity (b) were determined. The Freundlich model on the other hand, assumes a heterogeneous adsorption surface with stronger binding sites occupied first with decreasing bind strength as the metal ion concentration in solution increases. The D-R model is temperature dependent; therefore the experiments were carried out at 23°c (296.15k). The following equations (Eq.) were used to quantitatively describe the adsorption data; the amount of metal bound by the biosorbents at any contact time (t) was calculated from data and is given by;

$$q = \frac{v(c_i - c_f)}{m} \tag{3.1}$$

Where q is metal uptake (mg/g); v is volume of metal solution (liters); c_i is initial metal concentration (mg/L); c_f is final metal concentration (mg/L); m is amount of biomass (g).

The (Langmuir, 1916) is expressed by the linearised equation;

$$C_e/q_e = C_e/q_{\max} + 1/bq_{\max} \tag{3.2}$$

where q_e is the adsorbed metal at equilibrium (mg/g), $q_{\max}$ is the maximum amount of metal sorbed (mg/g), b is a constant related to the energy of sorption and C_e is the equilibrium metal ion concentration (mg/L).

The (Freundlich, 1906) is represented by the linearised equation as follows;

$$\log q_e = \log k + 1/n \log C_e \tag{3.3}$$

where k is biosorption equilibrium constant, representative of the sorption capacity and n is a constant indicative of biosorption intensity.

The D-R model, (Dubinin, 1960) was plotted from the linearized equation of $\ln q_e$ vs. ε^2 with the intercept (X_m) and slope (β) determined from Eq. (3.4), Fig, 1c. The Polanyi potential (ε) was calculated from equation (3.5).

$$\ln q_e = \ln X_m + \beta\varepsilon^2 \tag{3.4}$$

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \tag{3.5}$$

$$E = \frac{1}{\sqrt{2.\beta}} \tag{3.6}$$

where X_m is the maximum biosorption capacity (mmol/g); β is the activity coefficient related to mean biosorption energy (mol^{2}g^{-1}kJ^{-1}); T is temperature (k); R is gas constant (8.314jmol^{-1} k^{-1})

The essential characteristics of Langmuir were expressed using a dimensionless constant separation factor, R_L (Weber and Chakravorti, 1974).

$$R_L = \frac{1}{1 + (1 + b_L C_o)} \tag{3.7}$$

where R_L is the separation factor; b_L constant related to energy of adsorption from Langmuir model (L/g); C_o is the highest initial concentration (mg/L)

The adsorption affinity of the metal using biosorbents can be expressed using the distribution co- efficient, K_d as indicated below.

$$K_d = \frac{(C_o - C_f)}{C_f} \times \frac{V}{M} \tag{3.8}$$

where C_o is the initial concentration, C_f is final concentrations (mg/L); V is the volume of solution (ml); M is the weight of algae (g).

3.6.2 Kinetic Modelling of Single Metallic Systems

Kinetic models of first-order of Lagergren, the pseudo second-order and Elovich equation were used to analyse the rate of metal sorption uptake. The first-order rate expression of (Lagergren, 1898) is given by the linearised equation as follows;

$$\log(q_e - q_t) = \log q_e - {k_1}/{2.303}\, t \qquad (3.9)$$

Pseudo second order equation of (Ho and McKay, 1999) is given by the following linearised form;

$$t/q_t = 1/k_2 q_e^2 + 1/q_e\, t \qquad (3.10)$$

where, q_t is the amount of adsorbed metal on the algal biomass at time (t) in (mg/g); q_e is the equilibrium sorption uptake (mg/g); k_1 the rate constant of Lagergren first-order adsorption (min-^{1}) and k_2 is the rate constant of second-order adsorption.

The linearised model of Elovich (Roginsky and Zeldovich, 1934) is given by equation Eq. 3.9 from which the slope and intercept were determined for β and α respectively.

$$q_t = \beta \ln(\alpha\beta) + \beta \ln t \qquad (3.11)$$

where q_t is the amount of metal sorbed at a time t, α is the initial sorption rate (mgg^{-1}min^{-1}) and β is the desorption constant (gmg^{-1}) during any one experiment.

Desorption kinetics for recovery of metals were modelled using the modified equations of first-order and the pseudo second order equations (Njikam and Schiewer, 2012). The modified first order rate equation assumes the rate of desorption is proportional to the Lanthanum filled sites and is given by differential Eq;

$$dq/dt = -k_{1,des} q\,, \qquad (3.12)$$

The modified 2nd order rate equation assumes the rate of desorption is proportional to the square of Lanthanum filled sites and is given by differential Eq.;

$$dq/dt = -k_{2,des} q^2 \qquad (3.13)$$

where k_1 is the 1^{st} order desorption rate constant; q is the remaining amount of metal bound to the biomass at time t ; k_2 is 2^{nd} order desorption rate constant. The differential equations 3.11 and 3.12 were integrated using the following Eq.;

$$q_{n+1} = q_n - \left({}^{dq}\!/_{dt}\right)_n \left(t_{n+1} - t_n\right) \tag{3.14}$$

where q_n is the amount of Tl remaining in algae at time t_n and q_{n+1} is the amount of Tl at time t_n minus the rate of desorption multiplied by the incremental time step, k_1 the rate constant of modified first-order desorption (min-^{1}) and k_2 is the rate constant of modified second-order desorption.

3.6.3 Thermodynamics

The effect of temperature on metal sorption using algae was determined by thermodynamic parameters of Gibbs free energy (ΔG), enthalpy$(\Delta H)$and entropy(ΔS). ΔG was determined from equation 3.15 while ΔH and ΔS was determined from Eq. 3.16.

$$\Delta G = -RT \ln K_C \tag{3.15}$$

$$\ln K_C = {}^{-\Delta H}\!/_{RT} + {}^{\Delta S}\!/_{R} \tag{3.16}$$

where ΔG is Gibbs free energy(kJ/mol), ΔH is enthalpy (kJ/mol), ΔS is entropy (kJmol^{-1}k^{-1}) and T is temperature in kelvin. K_C and R are equilibrium constants respectively.

3.6.4 Empirical Modelling of Multi-Metal Systems

Biosorption of multiple metals using algae was done using predictive models containing parameters from single metallic systems. The extended Langmuir was used in this study and is given by the equation as follows;

$$q_{e(i)} = \frac{q_{m.i} K_{L..i} \left(C_{e.i}\right)}{1 + \sum_{j=1}^{N} K_{L.j} \left(C_{e.j}\right)} \tag{3.17}$$

where $q_{e(i)}$ is equilibrium metal uptake for i metals (mg/g), $C_{e.i}$ is equilibrium concentration (mg/L), $q_{m.i}$ is maximum adsorption capacity (mg/g) and $K_{L..i}$ is Langmuir isotherm parameters (L/g)

For the ternary system, 3 metals were used and are represented by the following equations; Thallium is given by,

$$q_{e.Tl} = \frac{q_{m.Tl} K_{L.Tl} \left(C_{e.TL}\right)}{1 + K_{L.Tl} C_{e.Tl} + K_{L.La} C_{e.La} + K_{L.Cd} C_{e.Cd}} \tag{3.18}$$

Lanthanum is given by,

$$q_{e.La} = \frac{q_{m.La} K_{L.La} \left(C_{e.La}\right)}{1 + K_{L.Tl} C_{e.Tl} + K_{L.La} C_{e.La} + K_{L.Cd} C_{e.Cd}} \tag{3.19}$$

Cadmium is given by,

$$q_{e.Cd} = \frac{q_{m.Cd} K_{L.Cd} \left(C_{e.Cd}\right)}{1 + K_{L.Tl} C_{e.Tl} + K_{L.La} C_{e.La} + K_{L.Cd} C_{e.Cd}} \tag{3.20}$$

The Combined Langmuir-Freundlich model was also used and is given by the equation as follows;

$$q_{e.i} = \frac{q_{m.i} b_{LF.i} C_{e.i}^{1/nLF,i}}{1 + \sum_{a=1}^{N} b_{LF,a} C_{e.a}^{1/nLF.a}} \tag{3.21}$$

where $b_{LF.i}$ is the affinity constant for adsorption (L/mg), n is the index of heterogeneity

For the ternary system, 3 metals were used and are represented by the following equations; Thallium is given by,

$$q_{e.Tl} = \frac{q_m b_{LF.Tl} C_e^{1/nTl}}{1 + b_{LF.Tl} C_e^{1/nTl} + b_{LF.La} C_e^{1/nLa} + b_{LF.Cd} C_e^{1/nCd}} \tag{3.22}$$

Lanthanum is given by,

$$q_{e.La} = \frac{q_m b_{LF.La} C_e^{1/nLa}}{1 + b_{LF.Tl} C_e^{1/nTl} + b_{LF.La} C_e^{1/nLa} + b_{LF.Cd} C_e^{1/nCd}} \tag{3.23}$$

Cadmium is given by,

$$q_{e.Tl} = \frac{q_m b_{LF.Cd} C_e^{1/nCd}}{1 + b_{LF.Tl} C_e^{1/nTl} + b_{LF.La} C_e^{1/nLa} + b_{LF.Cd} C_e^{1/nCd}} \tag{3.24}$$

Biosorption preference and relative coverage of metals in ternary equilibrium system were assessed using the following equations as given by (Chang and Chen, 1998; Sağ and Aktay, 2002).

$$Y_i = {}^{C_s}\!/_{C_i} \times 100 \tag{3.25}$$

$$Y_t = {}^{C_s.t}\!/_{C_{i.t}} \times 100 \tag{3.26}$$

$$\theta = {}^{C_{s.m}}\!/_{C_{s.m.t}} \times 100 \tag{3.27}$$

where Y_i is the biosorption preference (%), Y_t is total biosorption efficiency (%), θ_i is the relative coverage (%), C_i is the initial concentration (mg/L), C_s is the concentration of metal sorbed(mg/L), $C_{s.m}$ is the moles of metal biosorbed, $C_{s.m.t}$ is the total moles of metal biosorbed.

3.6.4 Kinetic Modelling of Multi-Metal Systems

The experimental data from the kinetic studies of a ternary system of La, Tl and Cd was simulated using AQUASIM (Version 2). A non-linear Langmuir-Hinshelwood (L-H) which explains kinetics of heterogeneous surfaces was used to describe the sorption behaviour of metallic ions on the adsorbent (Jin, et al., 1996; Kumar, et al., 2007). The L-H expression is given by the following generic equation;

$$-{}^{dc}\!/_{dt} = {}^{qC}\!/_{K+C} \tag{3.28}$$

where q is the rate reaction constant (1/hr), C is the concentration at any time, t (mg/L), K is the adsorption equilibrium constant(mg/L).

The assumption of L-H equation is that complete degradation occurs which does not seem to be the case with biosorption. When the active surface binding sites become saturated, then no further reduction occurs. Therefore, the L-H kinetics was modified to the following equation;

$$-{}^{dc}\!/_{dt} = {}^{qC}\!/_{K+C}\left[A_o - \frac{(C_o - C)}{R_c}\right] \tag{3.29}$$

where A_o is the initial surface area (m^2/g), C_o is initial metal concentration and R_c is the reduction capacity (mg.g)/ (L.m^2).

3.6.5 Mechanistic Models

Mechanistic models are important in understanding the mechanisms involved in the adsorption process and these include;

The intraparticle diffusion model (Weber and Morris, 1962) which is given by the following equation,

$$q_t = K_i t^{0.5} + C \tag{3.30}$$

where K_i is the intraparticle diffusion rate constant (mgg-^{1}hr$^{0.5}$) and C is the intercept

The mass transfer model (Mc Kay et al, 1980) is given by the following equation,

$$\ln\left(\frac{c_t}{c_i} - \frac{1}{1+mK}\right) = \left(\frac{1+mK}{mK} B_L S_S\right) t + \left(\frac{mK}{1+mK}\right) \tag{3.31}$$

where c_i the initial concentration (mg/L) is, c_t is concentration at time t (mg/L), m is the mass of the adsorbent per volume of solution (g/L), B_L is the surface mass transfer co-efficient (ms^{-1}), S_S is the surface area of the adsorbent (m^2/g), K is the constant from multiplication of Langmuir constants of $q_{\max}$ and K_L.

CHAPTER 4

EQUILIBRIUM AND KINETICS OF LANTHANUM ADSORPTION AND RESOURCE RECOVERY

4.1 BACKGROUND

REE are considered as one of the most critical elements with a rising global demand, increasing prices and a high supply risk (Eg European Commission, 2010; Sonich-Mullin, 2012). REE are increasingly being used in high-tech industries due to the unique properties associated to these metals. For example lanthanum (La) is one of the most commonly utilised REE with 85% global production for making super alloys, catalysts, special ceramics, condensers, etc. (Sert, et al., 2008a; Kütahyali, et al., 2010; Du and Graedel, 2011).

Although REE have a wide range of applications in the industry, knowledge about the level of toxicity and recycling possibilities is limited. For example consumer electronics is one of the major industries using REE with supply of waste materials available for recycling (Sonich-Mullin, 2012). UNEP, 2011 also states that the end-life of recycling (percentage of metals in discards that is actually recycled) for REE is less than 1% (Graedel, et al., 2011). Urban mining (recycling of waste materials) is the current subject of interest especially using cost effective technologies as an incentive for recovery of valuable materials such as REE.

REE are more abundant than many known metals but their extraction involves several costly steps such as solvent extraction or ion exchange (Palmieri, et al., 2000). The major environmental concern of REEs is the release of mineral acids, chelating agents and fission products emanating from expired nuclear fuel into the environment (Andres, et al., 2003; Kanazawa and Kamitani, 2006). Biosorption using readily available dead biomass has proved to be effective in the removal of other metals at concentrations ≥ 100 mg/L (Kratochvil and Volesky, 1998; Ahalya, et al., 2003). In addition, recovery and re-use of REEs using biosorbents still remains largely unexplored (Korenevsky, et al., 1999; Andres, et al., 2003). The algal biomass collected from eutrophic freshwater dam was used to investigate the biosorption potential for removal and recovery of one of the ubiquitous lanthanides, lanthanum.

4.2 ALGAL CHARACTERISATION AND CLASSIFICATION

4.2.1 Species Identification

Molecular phylogenetic studies provides a better understanding of inter and intra specific relationships among similar species and mixed populations (Olmos, et al., 2000; Leliaert, et al., 2012). The results obtained from the BLAST tool after sequencing and comparison with the gene bank indicated an E-value of 0 for all the algal samples. When the E-value from the BLAST results is zero, then the results are good and can be accepted as the most probable species (Tempesta, et al., 2011). The Internal Transcribed Spacer (ITS) nucleotide sequences and 18S rRNA gene exhibited a high score ranging from 862.8- 1239.5 bits with no significant gaps, Table 4.1. The ITS sequences (ITS 1, ITS 2 including 5.8S rRNA) found between nuclear ribosomal genes are frequently used for predicting genetic similarities and phylogeny of the genus whereas 18S rRNA provide information on differentiation of various strains (Coleman and Mai, 1997; González, et al., 1998; Tempesta, et al., 2011). The algal species with similarities to the query samples were found to have 95-98 % sequence identities and these included; *Desmodesmus multivariabilis, Scenedesmus acuminutus, Chloroidium saccharophilum* and *Stichococcus bacillaris*, Table 4.1(Appendix J).

4.2.2 Taxonomic Classification

Algae are broadly classified under morphological, ultra structure and molecular concept. Traditionally morphological and ultra-structure were more widely applied for classification of orders and classes but with gaps in some cases (Proschold and Leliaert, 2007). Molecular data provides a better understanding of phylogenetic placement of taxa and greater resolution of many relationships complementing the other 2 classes (Lewis and McCourt, 2004). Taxonomic classification of species using the molecular concept was done based on results obtained from the BLAST search. The results indicated all species belonging to green algae with 2 distinct lineages; Chloropyta and Streptophyta.

Green algae is one of the most diverse algal group with over 500 genera and approximately 15,000 species found mainly in freshwater (Wehr, 2002). Chlorophyta has majority of the known green algae species with 4 classes; Chlorophyceae, Trebouxiophyceae, Ulvophyceae and Prasinophyceae (Leliaert, et al., 2012). From the results, *Desmodesmus multivariabilis* and *Scenedesmus acuminutus* belonged to Chlorophyceae whereas *Stichococcus bacillaris* and *Chloroidium saccharophilum* belonged to Trebouxiophyceae, Figure 4.1. It can also be noted that some species belonged to same class but with different genus. (Proschold and Leliaert, 2007), also confirmed that same species can belong to different classed with priority rule valid at the generic and species level.

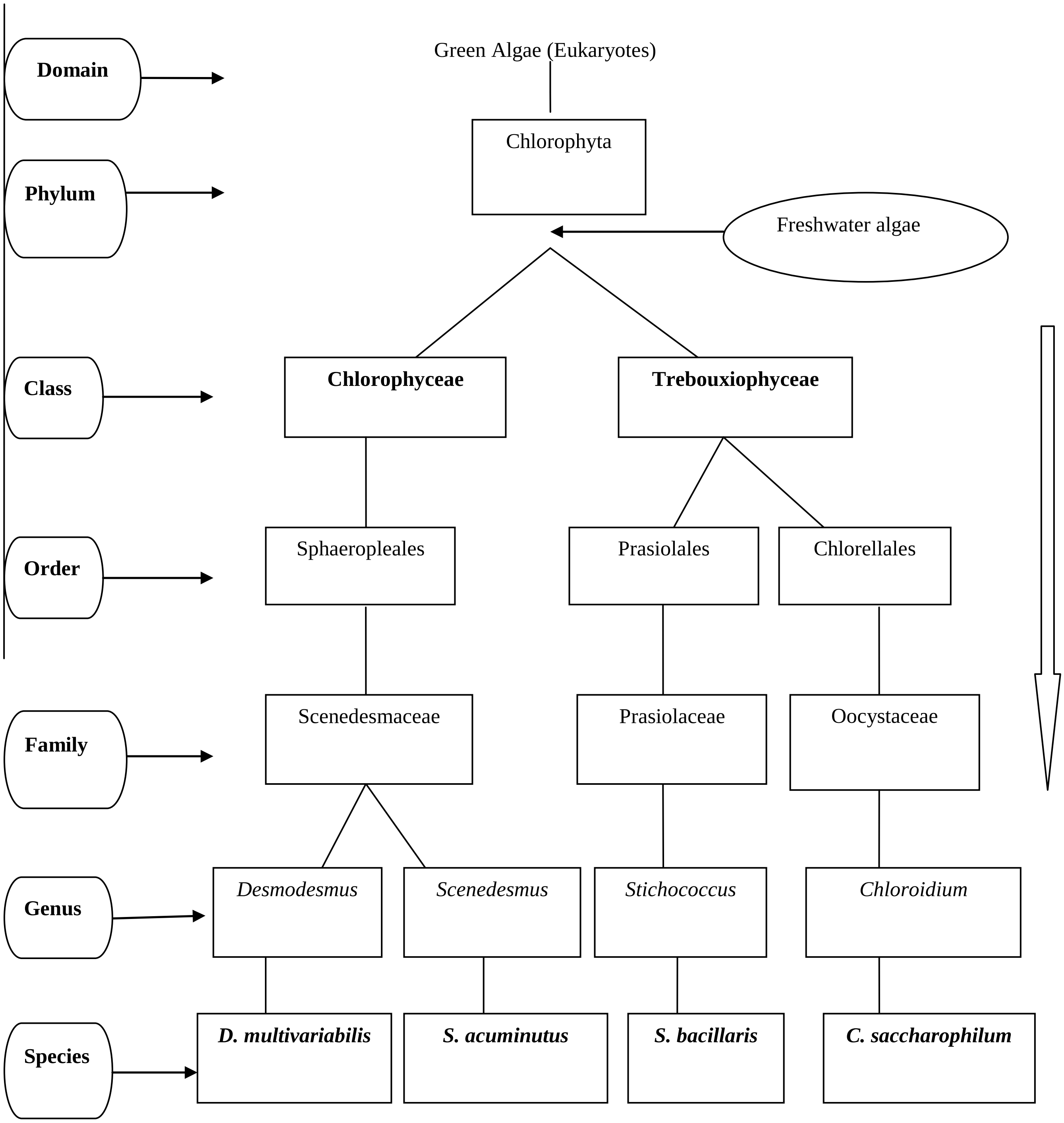


Figure. 4.1.Taxonomic Classification of Green Algae Based on Results from Molecular Identification

Table 4.1: Summary of BLAST Results for Molecular Identification of Micro Algae

Query samples	Score (bits)	Strand	Identities	% of identities	Gaps	Species	Description
1. A3	1079.2	++	601/615	98	5/615	*Desmodesmus multivariabilis*	18S rRNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene, and internal transcribed spacer 2, complete sequence; and 26S rRNA gene, partial sequence
2. A2-P	1048.2	++	544/558	98	1/558		
3. G2-3	1079.2	++	601/615	98	5/615		
4. G2-4	1239.5	++	670/685	98	0/685	*Chloroidium saccharophilum*	genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, culture collection CCAP 211/58
5. G1-4	2526	+-	1371/1395	98	1/1395		
6. A1-old	862.8	++	645/679	95	9/679	*Scenedesmus acuminutus*	5.8S rRNA gene, ITS1 and ITS2 strain UTEX 415
7. B1-b	1051.1	+-	581/598	97	0/598	*Stichococcus bacillaris*	18S rRNA gene, partial sequence; internal transcribed spacer 1, 5.8S rRNA gene, and internal transcribed spacer 2, complete sequence; and 26S rRNA gene, partial sequence

4.3 ADSORPTION OF LANTHANUM

4.3.1 Equilibrium and Adsorption Behaviour

Equilibrium experiments are an important step in the identification of species with the highest sorption capacity preceding any further biosorption studies. The Langmuir and Freundlich models are the most commonly used equilibrium isotherms in the biosorption studies due to their simplicity (Davis, et al., 2003; Wang and Chen, 2009). In this study, the linearised data from the Langmuir model (Eq. 3.2) and Freundlich model (Eq. 3.3) were plotted as shown in Figure 4.2 and 4.3 respectively. From the results, the Langmuir model showed a better fit compared to Freundlich model with a higher correlation co-efficient (R^2) of ≥ 0.9 , Table 4.2. The Langmuir parameters q_{max} and b represent the maximum sorption capacity and affinity respectively. *Chlamydomonas reinhardtii* had the highest sorption capacity with a q_{max} of 142.86 mg/g and a low affinity b of 0.25 L/g emerging the best adsorbent for lanthanum. *Stichococcus bacillaris* was the least effective adsorbent with the lowest q_{max} of 51.02 mg/g and highest b of 4.56 L/g.

Table 4.2: Langmuir and Freundlich parameters from monometallic experiments of lanthanum on tested algal species

Algal species	**Langmuir constants**			**Freundlich constants**		
	q_{max}**(mg/g)**	***b*** **(L/g)**	R^2	***n***	***K***	R^2
Stichococcus bacillaris	51.02	4.56	0.997	9.04	4.51	0.894
Desmodesmus multivariabilis	100	4.55	0.984	4.17	4.93	0.782
Chlorella vulgaris	74.60	0.178	0.995	3.41	3.66	0.779
Scenedesmus acuminutus	111.1	0.120	0.953	3.50	4.259	0.83
Chloroidium saccharophilum	129.87	0.142	0.913	4.06	4.81	0.807
Chlamydomonas reinhardtii	142.86	0.25	0.908	3.35	4.721	0.896

The maximum sorption capacity (q_{max}) of lanthanum for the tested algae was compared to other biosorbents from previous studies. *Chlamydomonas reinhardtii* had a higher q_{max} of 142.86 mg/g for La as compared to other biosorbents except for *Turbinaria conoides* with a q_{max} of 150.5 mg/g, Table 4.3. It was also noted that *Chlamydomonas reinhardtii* was a more effective adsorbent than some brown algae such as *sargassum* species with a q_{max} of 91.68

and 139 mg/g of which is lower than 140 mg/g obtained from this study (Diniz and Volesky, 2005a; Oliveira and Garcia Jr, 2009).

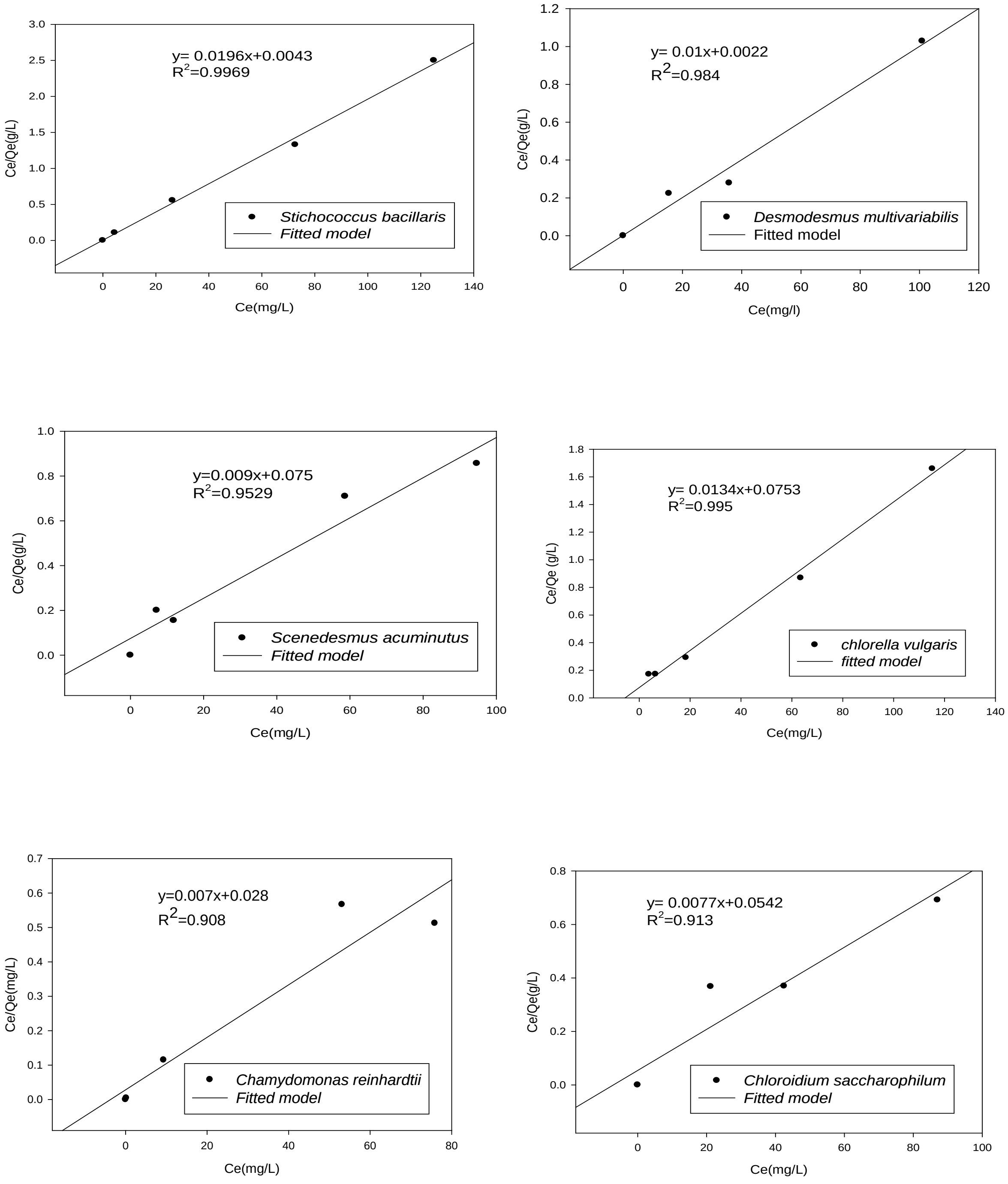


Figure.4.2: Langmuir isotherm models with linear plots of lanthanum uptake for the six tested algal species

On the other hand, *Desmodesmus multivariabilis* was found to have both a high removal and recovery rate at q_{max} of 100 mg/g and b of 4.55 L/g for La compared to other tested algae. Selection of biosorbents depends on the intended use of the species. Therefore, the study aimed at finding sorbents with a high sorption and recovery rate for La was achieved. Other authors noted that a high q_{max} and lower b implies sorbents with the highest sorption capacity regardless of the recovery rate. Also a higher q_{max} and a higher b imply sorbents with both a high sorption and recovery rate at low equilibrium concentration (Volesky and Holan, 1995; Davis, et al., 2003; Romera, et al., 2007).

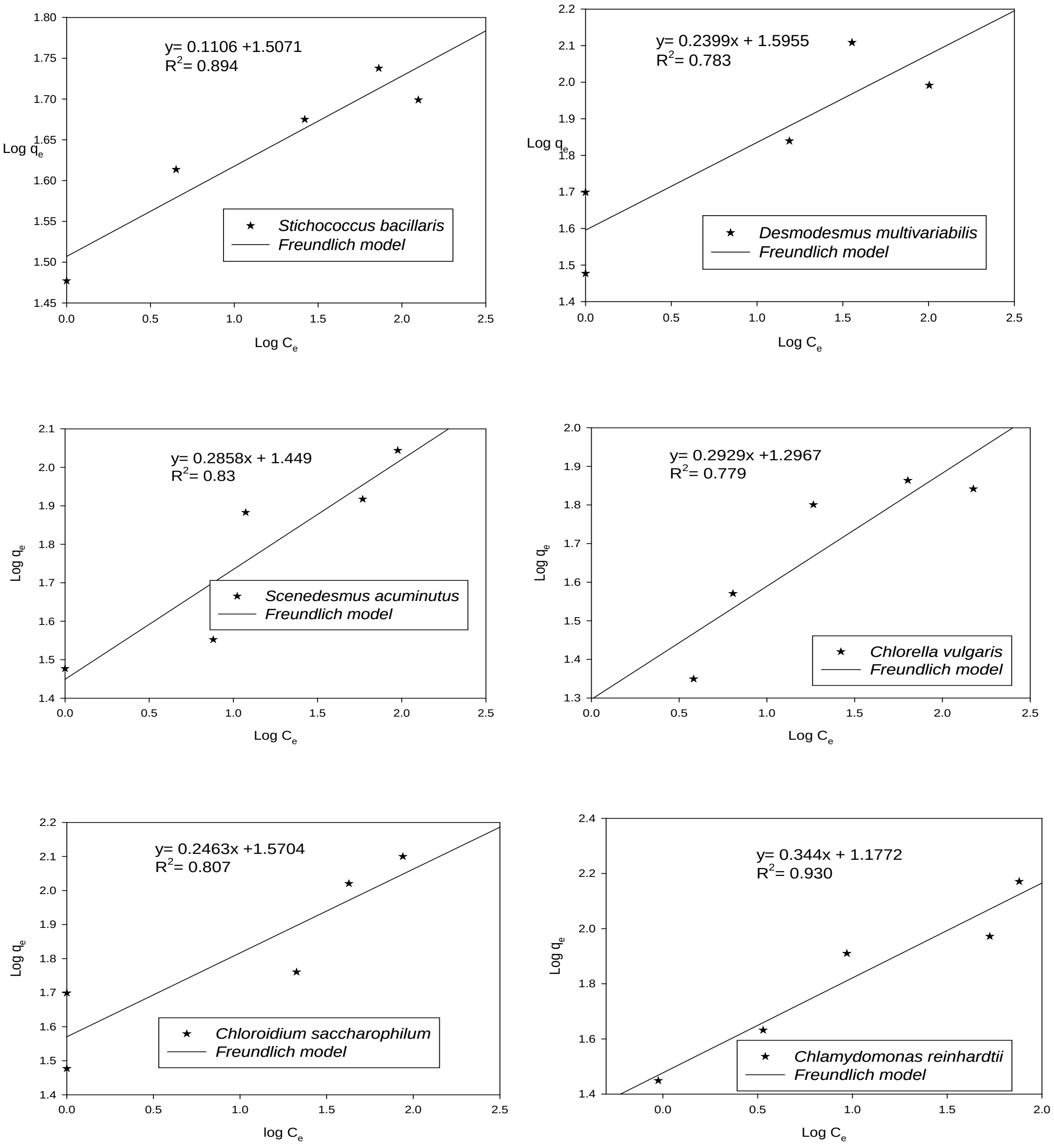


Figure.4.3: Freundlich isotherm models with linear plots of lanthanum uptake for the six tested algal species

Table 4.3: Maximum uptake (q_{max}) of lanthanum using different biomass from previous studies

Biosorbent	**q_{max} in mg/g**	**References**
Platanus orientalis leaf powder	28.65	(Sert, et al., 2008a)
Agrobacterium sp. HN1	34.29	(Shuxia, et al., 2011)
Crab shell	140.1	(Vijayaraghavan, et al., 2009)
Bamboo charcoal	120	(Qing, 2010)
Magnetic alginate chitosan gel beads	97.1	(Wu, et al., 2010)
Sargassum species	91.68	(Oliveira and Garcia Jr, 2009)
Sargassum polycystum	139	(Diniz and Volesky, 2005b)
Iron oxide loaded calcium alginate beads	123.5	(Wu, et al., 2010)
Turbinaria conoides (brown algae)	150.5	(Vijayaraghavan, et al., 2010)
Chlamydomonas reinhardtii	142.86	From this study

4.3.2 Effect of Initial Concentration on the Removal of La

Initial experiments were carried out to determine the effect of contact time on the concentration of lanthanum removal. The effect of initial concentration on the removal of lanthanum was high in the first 15- 30 minutes followed by a slow reduction until equilibrium was attained for all the tested algae. From the results, it can be noted that *Chloroidium saccharophilum* had the highest removal of La at low concentration of 15 mg/L with over 94.34% removal in less than 15 minutes, Figure 4.4a. At high concentrations of 50 mg/L and 150 mg/L, *Chlamydomonas reinhardtii* had the highest removal of La with final concentration after adsorption at 9.3 mg/L and 75.9 mg/L respectively, Figure 4.4b and 4.4c. Sorption efficiency decreases with increase in metal ion concentration which is due to saturation of adsorption sites (Kütahyali, et al., 2010).

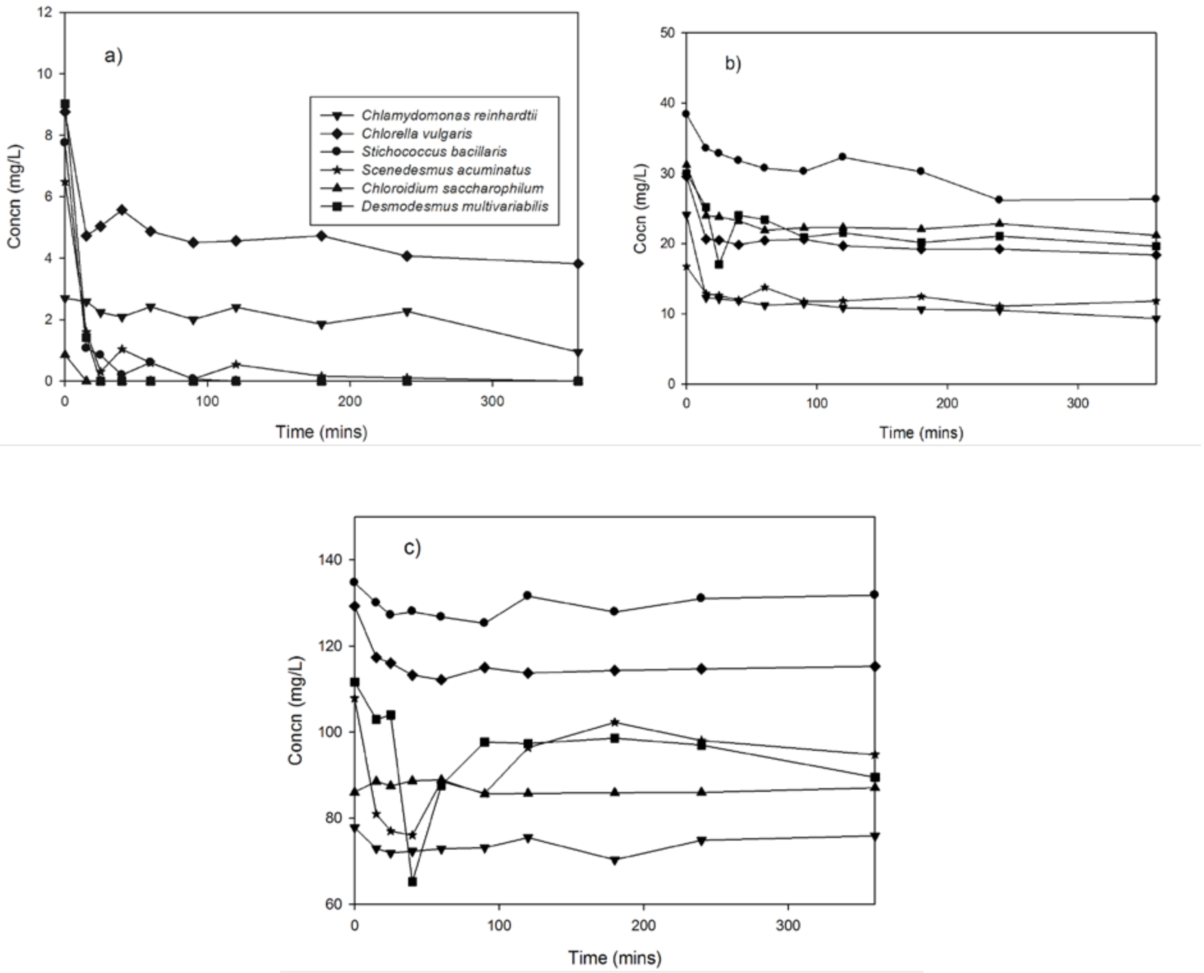
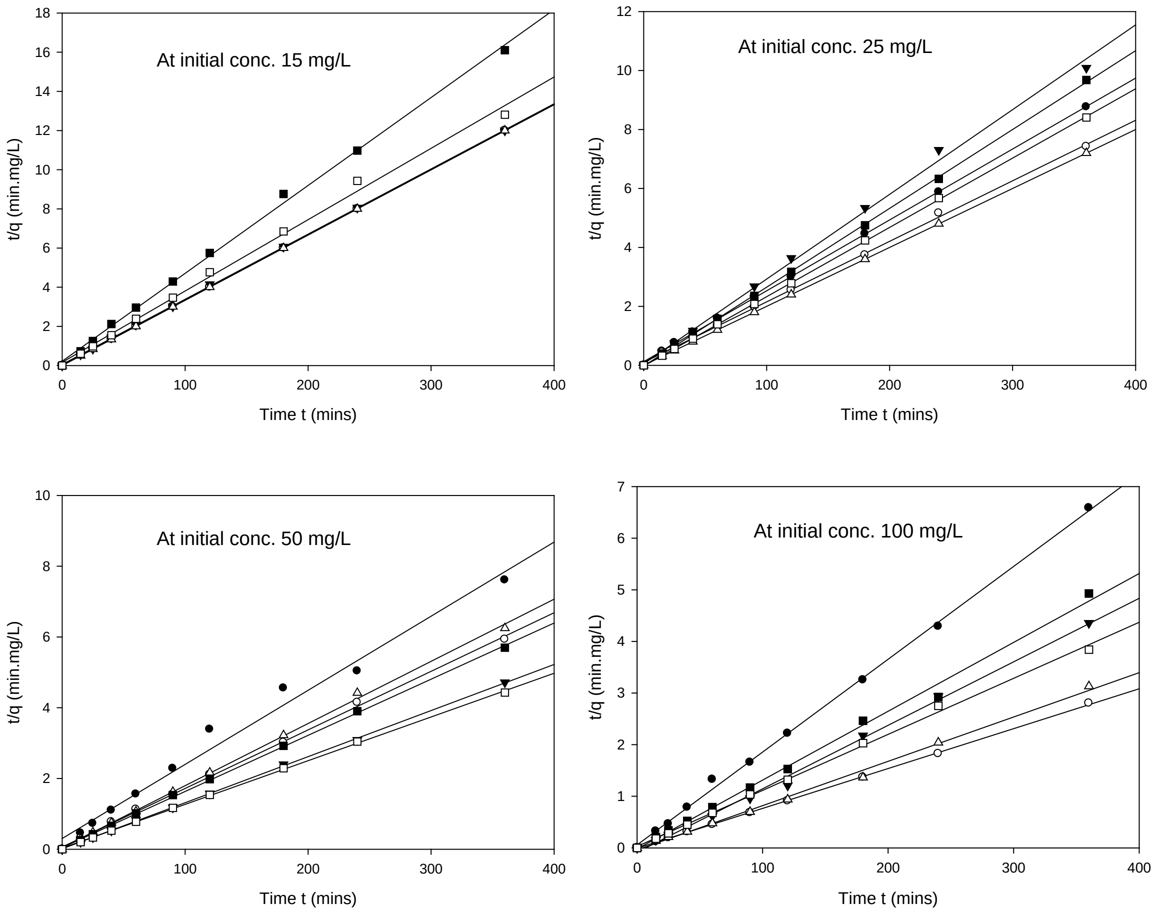


Figure. 4.4: Effect of Concentration Vs Time for removal of La in 6 algal species at, a) 15 mg/L, b) 50 mg/L, and c) 150 mg/L.

Biosorption is known to be a rapid process with equilibrium reached only after a few minutes and the reaction mechanism especially related to ion exchange or surface precipitation (Chojnacka, et al., 2005; Lee and Chang, 2011). Adsorption mechanism depends on the concentration of anions and cations found in the solution. From the results obtained from FTIR, the most active functional groups found in all tested algal biomass were carboxyl and hydroxyl ions. The metal salt of lanthanum was prepared from nitric acid forming La $(NO_3)_3$. The functional groups (anions) act as sites for ion exchange with metals (cations) in this case $La^{3+.}$ Ion exchange occurs at rapid kinetics with possible surface precipitation when the cell wall becomes saturated. Diniz and Volesky, 2005a also found ion exchange as the main mechanism for La adsorption using nitrate salt.

4.3.3 Adsorption Kinetic Models

Batch kinetic models provide insight on the potential rate controlling steps that are useful when designing a full scale biosorption process. The first and Pseudo-second order kinetic models are the most commonly encountered models in biosorption studies, and were used to evaluate experimental kinetic data in this study. The kinetic models were plotted from linearised equations (Eq. 3.9 and 3.10) from which the slope and intercept were determined, Figure 4.5. The Pseudo-second order model produced a stronger R^2 of ≥ 0.99 than the first order model with ≤ 0.8 for all the biosorbents tested, Table 4.4.



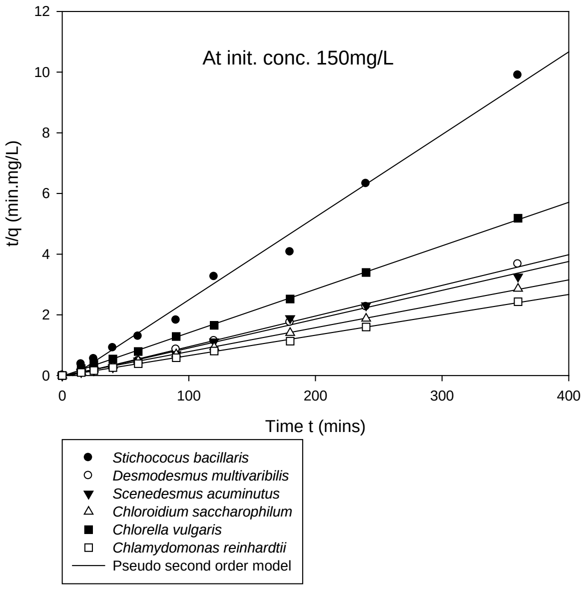


Figure 4.5: Linearised graphs for Pseudo- Second Order Model for all tested algae on adsorption of lanthanum

In addition there was no significant difference between the experimental sorption capacity (q_{exp}) and the calculated sorption capacity (q_{cal}) for Pseudo-second order kinetics. For example at low initial concentration of 15 mg/L of La, q_{exp} and q_{cal} was 30 mg/g for *Stichococcus bacillaris, Desmodesmus multivariabilis, Scenedesmus acuminutus* and *Chloroidium saccharophilum,* Table 4.4. At higher initial concentrations of 150 mg/L, the difference between q_{exp} and q_{cal} was minimal, for example *Desmodesmus multivariabilis* had 98.12 mg/g and 100 mg/g respectively. The rate of reaction (k_2) was highest at 1 min^{-1} for *Chloroidium saccharophilum* at very low concentrations of 15 mg/L of La, Table 4.4. This can be attributed to the highest rate of removal of La at 100 % in less than 15 minutes for *Chloroidium saccharophilum* as compared to other tested algae, Figure 4.4a.

Table 4.4: Pseudo-second order adsorption kinetic parameters for all the tested algae at concentrations of 15-150 mg/L

Algal species	C_i **(mg/L)**	$k_{2,\mathrm{ads}}$ **min^{-1}**	R^2	q_e **Experimental (exp) (mg/g)**	q_e **Calculated (cal) (mg/g)**
Stichococcus bacillaris	15	0.033	1.000	30.00	30.30
Desmodesmus multivariabilis	15	0.109	1.000	30.00	30.30
Scenedesmus acuminutus	15	0.018	0.999	30.00	30.30
Chloroidium saccharophilum	15	1.000	1.000	30.00	30.30
Chlorella vulgaris	15	0.009	0.998	22.36	22.73
Chlamydomonas reinhardtii	15	0.004	0.996	28.60	29.41
Stichococcus bacillaris	50	0.001	0.984	47.40	50.00
Desmodesmus multivariabilis	50	0.004	0.998	69.12	62.50
Scenedesmus acuminutus	50	0.015	0.999	76.43	76.92
Chloroidium saccharophilum	50	0.007	0.998	57.62	58.82
Chlorella vulgaris	50	0.005	0.999	63.20	66.67
Chlamydomonas reinhardtii	50	0.005	0.999	81.32	83.33
Stichococcus bacillaris	100	0.006	0.998	54.60	55.56
Desmodesmus multivariabilis	100	0.008	0.999	128.44	142.00
Scenedesmus acuminutus	100	0.002	0.996	82.66	83.33
Chloroidium saccharophilum	100	0.002	0.996	115.00	125.00
Chlorella vulgaris	100	0.009	0.995	73.05	76.92
Chlamydomonas reinhardtii	100	0.005	0.998	93.77	100
Stichococcus bacillaris	150	0.003	0.991	49.40	37.04
Desmodesmus multivariabilis	150	0.002	0.996	98.12	100.00
Scenedesmus acuminutus	150	0.002	0.991	110.54	111.11
Chloroidium saccharophilum	150	0.156	0.999	125.91	126.58
Chlorella vulgaris	150	0.009	0.999	69.46	71.43
Chlamydomonas reinhardtii	150	0.003	0.999	148.20	166.67

4.3.4 Effect of Initial Concentration on the Recovery of La

The concentration of lanthanum recovery increased with increase in time for all the species until a steady state was attained. At low concentrations of ≤ 15 mg/L, desorption of La was highest for *Stichococcus bacillaris* and *Desmodesmus multivariabilis* with a final concentration recovered at 13.57 and 11.73 mg/L respectively, Figure 4.6a. At higher concentration of ≤ 50 mg/L, *Chloroidium saccharophilum* and *Desmodesmus multivariabilis* had the highest recovery of 28.9 mg/L and 33.48 mg/L at initial concentration of 29 and 34.56 mg/L respectively, Figure 4.6b. Nitric acid was used as a desorbent with a high level of H^+ which was replaced with La^{3+} found mostly in the carboxyl binding sites. Higher efficiency removal is due ion exchange of excess H^+ from nitric acid (Vijayaraghavan, et al., 2010).

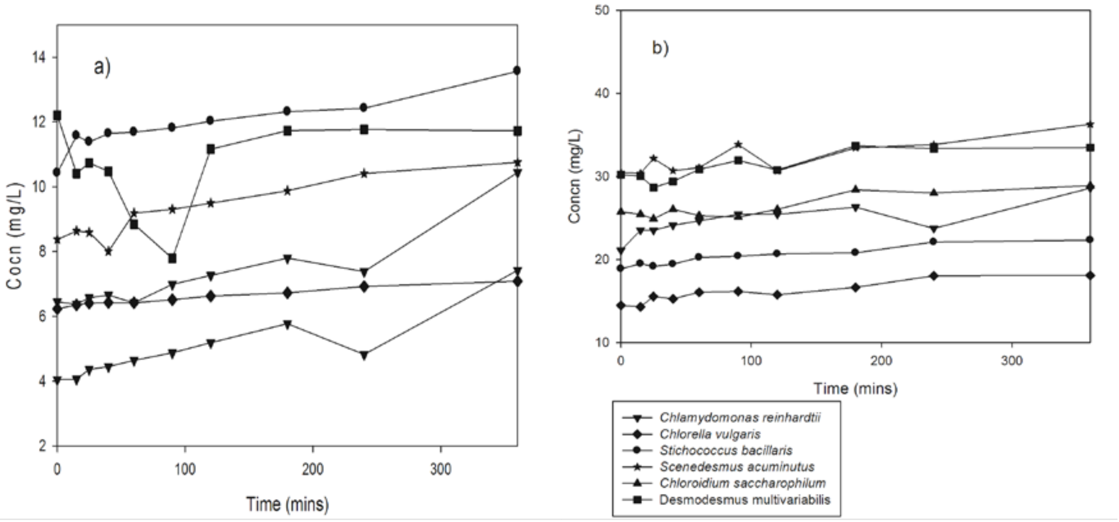


Figure 4.6: Effect of Concentration Vs Time for recovery of La in 6 algal species at, a) 15 mg/L and b) 50 mg/L

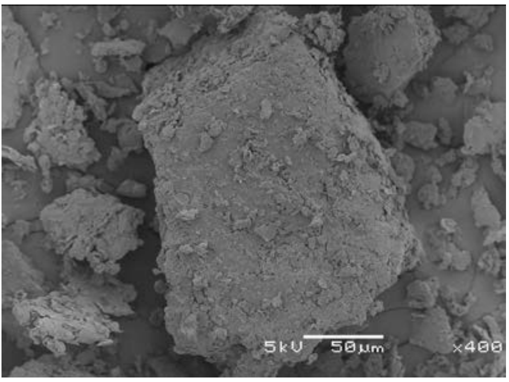
4.3.5. Surface Characterisation of Algal Cell wall

Surface electron microscope was used in identification of the morphology of the surface on the cell wall of the algae before and after biosorption. The SEM micrographs before adsorption were found compact and smooth. There was a significant difference in the biomass surface of the cell wall after adsorption with the walls appearing rough, porous and scattered implying interaction of the metal and algal surface, Figure 4.7.

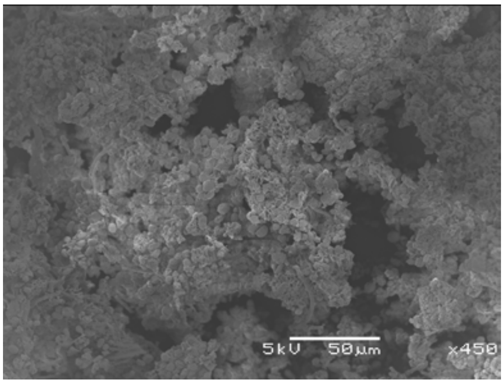
The FTIR analysis provides insight on the functional groups responsible for the biosorption mechanisms. *Chloroidium saccharophilum, Scenedesmus acuminutus* and *Stichococcus bacillaris* displayed a shift from 1629 cm^{-1} before adsorption to 1649 cm^{-1} after adsorption indicating an N-H medium bond for primary amines. *Desmodesmus multivariabilis* showed a shift from 1226-1247 cm^{-1} which is a C-H bond for aliphatic amines.

At higher frequencies, *Chloroidium saccharophilum* and *Desmodesmus multivariabilis* had a similar shift from 3258 cm^{-1} to 3300 cm^{-1} which is a medium O-H stretch for carboxylic acids. *Stichococcus bacillaris* and *Desmodesmus multivariabilis* also showed a higher frequency of 2898-2941 cm^{-1}, a C-H bond for alkanes. *Scenedesmus acuminutus* had a functional group of N-H stretch between 3351-3373 cm^{-1} for primary and secondary amines, Figure 4.8.

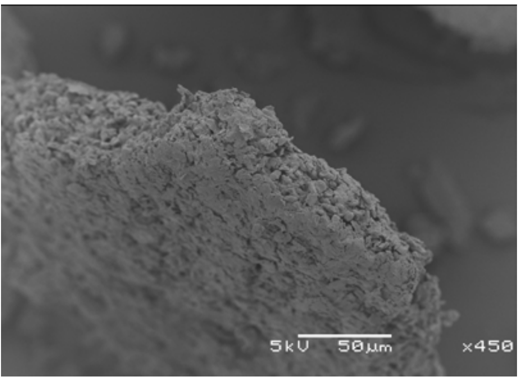
In general, all the tested algae appear to have transmittance within the same wave length region with differences in adsorption volume. The gap in the adsorption volume could be due to the different components found in the biomass like proteins, carbohydrates and lipids (Lee and Chang, 2011). Wu, et al., 2010 also found *chlorella vulgaris* and *Chlamydomonas reinhardtii* with similar functional groups to the tested algae of carboxyl, hydroxyl and amines.



a(i)



a(ii)



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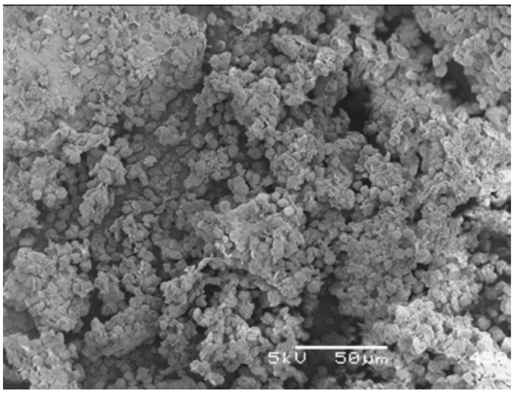

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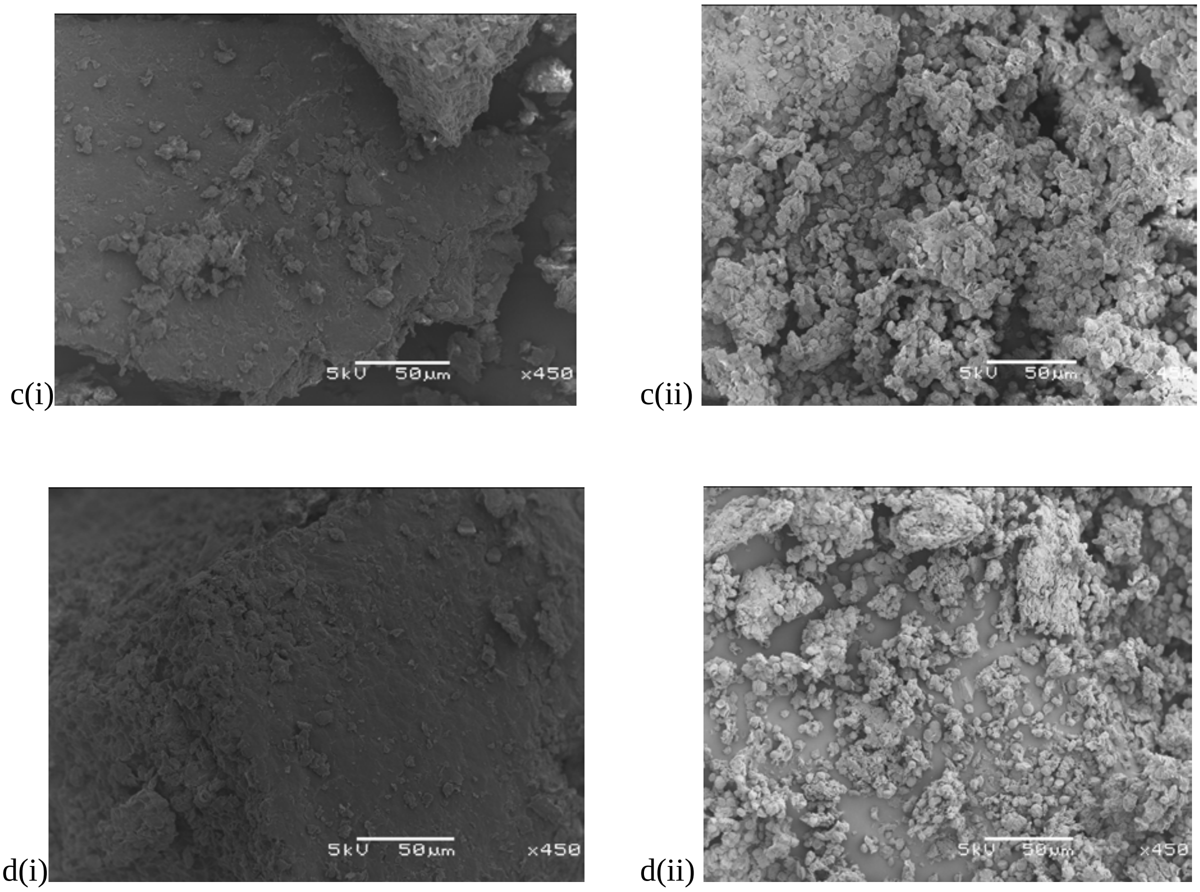
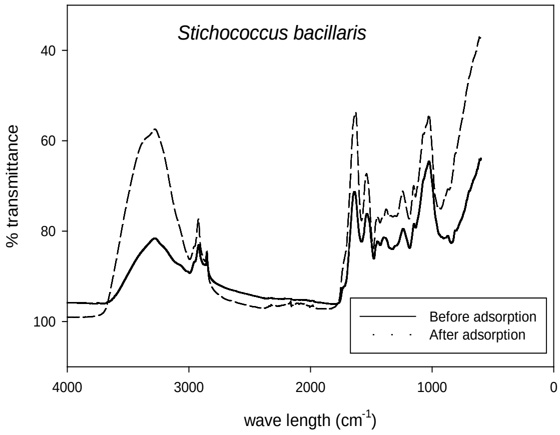
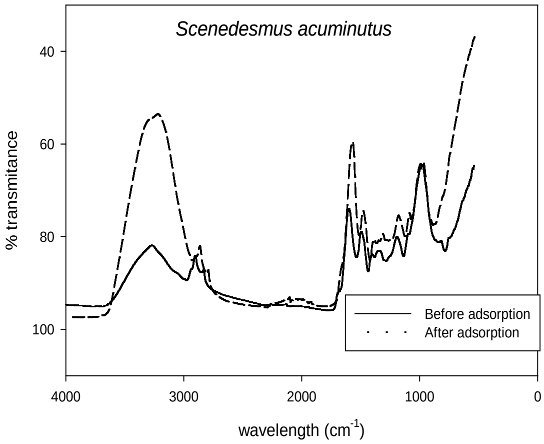


Figure 4.7: SEM micrographs a, b, c, d (i) are biomass before adsorption and a, b, c, d (ii) after adsoprtion for *Scenedesmus acuminutus, Desmodesmus multivariabilis, Chloroidium saccharophilum* and *Stichococcus bacillaris* respectively.





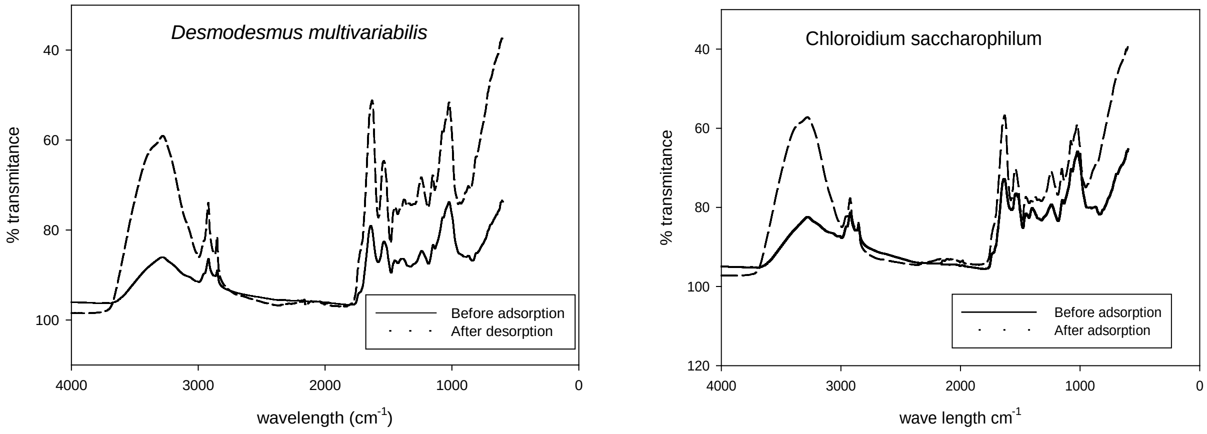


Figure 4.8: FTIR images of the four tested algae showing transmittance of wavelength before and after adsorption.

4.4 DESORPTION OF LANTHANUM

4.4.1. Desorption Kinetics of Lanthanum Recovery

The adsorption kinetic models of first order and pseudo second-order were modified to fit desorption kinetics with the amount of lanthanum remaining in the algae as the rate determining concentration, Eq. 3.12-3.14. The results from the modified model showed a better R^2 of ≤ 0.98 for the pseudo second-order model than the first order of ≤ 0.7. In addition, the experimental(q_n) and calculated (q_{n+1}) recovery values of lanthanum were closer for pseudo second- order kinetics, Table 4.5. In the modified model, it is assumed that the rate of desorption is dependent of the quantity of metal-filled binding sites (Njikam and Schiewer, 2012).

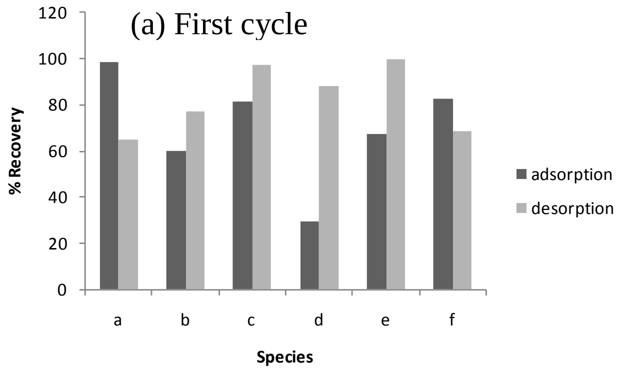
Table 4.5: Modified Pseudo-second order desorption kinetic parameters for tested algae

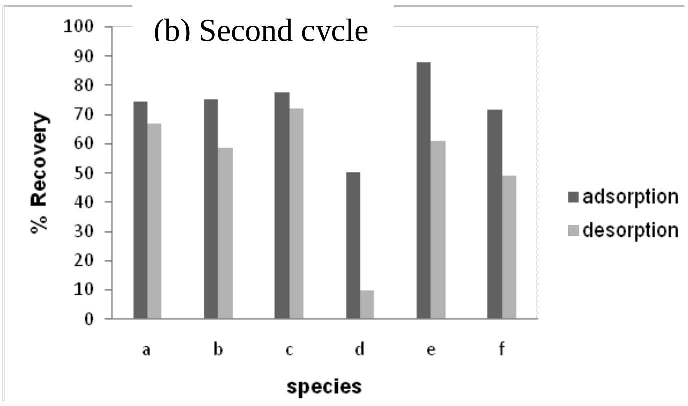
Algal species	C_i (mg/L)	$k_{2,,des}$ ($\times 10^{-4}$) Min^{-1}	R^2	q_n (mg/g)	q_{n+1} (mg/g)
Stichococcus bacillaris	15	0.7	0.955	26.083	25
Desmodesmus multivariabilis	15	0.4	0.911	41.652	40
Scenedesmus acuminutus	15	0.4	0.945	41.44	41.67
Chloroidium saccharophilum	15	0.2	0.885	63.024	66.67
Chlorella vulgaris	15	0.4	0.982	12.843	12.821
Chlamydomonas reinhardtii	15	0.6	0.881	18.586	19.23
Stichococcus bacillaris	50	1	0.894	37.244	35.71
Desmodesmus multivariabilis	50	0.7	0.963	42.619	41.67
Scenedesmus acuminutus	50	0.7	0.868	84.357	83.33
Chloroidium saccharophilum	50	0.2	0.891	86.529	90.90
Chlorella vulgaris	50	0.2	0.878	40.775	40.00
Chlamydomonas reinhardtii	50	0.4	0.911	43.461	40
Stichococcus bacillaris	100	0.2	0.894	72.537	76.92
Desmodesmus multivariabilis	100	0.3	0.921	62.981	66.67
Scenedesmus acuminutus	100	0.2	0.877	64.21	71.43
Chloroidium saccharophilum	100	0.3	0.878	52.498	50
Chlorella vulgaris	100	0.3	0.857	29.319	31.25
Chlamydomonas reinhardtii	100	0.2	0.943	56.463	58.82
Stichococcus bacillaris	150	0.2	0.981	80.155	83.33
Desmodesmus multivariabilis	150	0.9	0.855	60.793	76.92
Scenedesmus acuminutus	150	0.9	0.903	39.34	45.45
Chloroidium saccharophilum	150	0.09	0.859	193.718	200
Chlorella vulgaris	150	0.4	0.920	85.992	66.67
Chlamydomonas reinhardtii	150	0.03	0.936	96.433	100

4.4.2 Efficiency of Lanthanum Recovery

The algae were tested for efficiency in regeneration and reuse. In the first cycle, recovery was highest for *Desmodesmus multivariabilis* at initial concentration of 100 mg/L of lanthanum with recovery up to 99.63%, followed by *Chloroidium saccharophilum* at 97.17%, Figure 4.9a. In the subsequent cycles, the biomass was still reusable with a slight reduction in efficiency for *Desmodesmus multivariabilis* upto 77.2%, Figure 4.9b and c. In the 1st cycle, however *Chlamydomonas reinhardtii* showed the highest rate of removal of up to 98.44%. In some instances, there was low rate of removal for some species in the 1st cycle compared to

2nd and 3rd cycle which could be attributed to the contact time allocated being lower than required. *Stichococcus bacillaris* had the lowest adsorption and desorption efficiency in all the cycles.





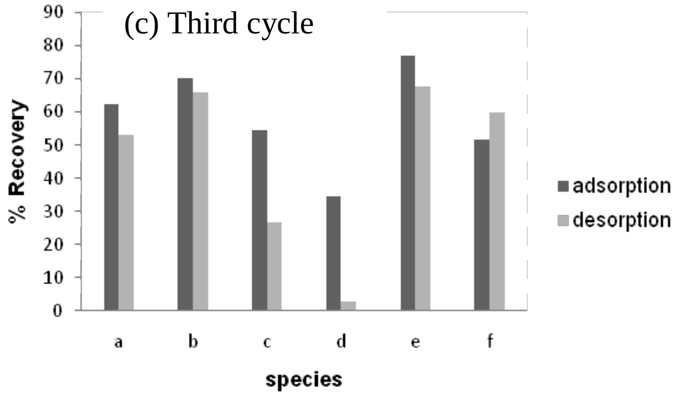


Figure. 4.9: Adsorption- desorption cycle of lanthanum for the six algal species; a) *Chlamydomonas reinhardtii*, b) *Scenedesmus acuminutus*, c) *Chloroidium saccharophilum*, d) *Stichococcus bacillaris*, e) *Desmodesmus multivariabilis*, f) *Chlorella vulgaris*

4.5 Summary

In this study, the internal transcribed spacer (ITS) and 18S ribosomal RNA gene (rRNA) were used successfully for molecular identification of the species. The algal species were found to have 95-98 % identities to *Desmodesmus multivariabilis, Scenedesmus acuminutus, Chloroidium saccharophilum* and *Stichococcus bacillaris*. The species were cultured and maintained easily in the laboratory on relatively cheap culture media. All the species identified were compared for their biosorption potential on lanthanum. *Desmodesmus multivariabilis* was found to be the most efficient at adsorbing lanthanum with a maximum sorption capacity of 100 mg/g and a high affinity of 4.55 L/g compared to other tested algae. Desorption of La was also highest in *Desmodesmus multivariabilis* with recovery up to 99.63% at initial concentration as high as 100 mg/L. *Stichococcus bacillaris* had the least performance with the removal capacity of 51.02 mg/g. Desorption data fitted best to the modified Pseudo second-order with a better correlation coefficient (R^2) of $\leq$ 0.98 than first order model. The possible biosorption mechanisms responsible for metal uptake on the algal surface were determined using FTIR. The results showed feasibility of lanthanum removal/recovery using tested algal sorbent.

CHAPTER 5

BIOSORPTION OF THALLIUM TOXICITY: EQUILIBRIUM AND KINETIC STUDIES

5.1 BACKGROUND

Thallium (Tl) is a non- essential trace metal often excluded from the list of priority metallic pollutants (Li, et al., 2012; Xiao, et al., 2012). Thallium is regarded to be more toxic to humans than mercury, lead and cadmium and notable for causing occupational, accidental and environmental hazards (Mulkey and Oehme, 1993; Xiao, et al., 2004). Thallium appears in two oxidation states; Tl (I) which is highly soluble in aqueous environments and resembles alkali metals and Tl (III) which is more stable forming complexes with sulphates, chlorides, carbonates and nitrates (Busev and Tiptsova, 1961; Ralph and Twiss, 2002; Antón, et al., 2013). Thallium deposits are generally scarcely distributed worldwide with many industrial applications such as catalyst for making certain alloys, molecular probes, thermometers, acusto-optical equipment, optical glass with refractive index, imitation jewellery, scintillation counters, and super-conductivity in ceramic compounds (Galván-Arzate and Santamaría, 1998; Nriagu, 2003). Information on the occurrence, mechanisms, behaviour, and bioremediation of thallium toxicity in the environment is still sketchy (Zhang, et al., 2003; Duan, et al., 2012).

One of the major concerns of Tl toxicity lies in the similar ionic radii of Tl^+ and monovalent cations especially potassium (K^+) in the crystal lattice which affects all organic ligands. In this case, the cell membrane has non- discriminatory uptake of Tl^+ over K^+ which alters the proper functioning of the biochemical reactions, for example pyruvate kinase, ATPase, stabilisation of ribosomes (Zitko, 1975a; Mulkey and Oehme, 1993; Karlsson, 2006). Thallium has been reported to cause acute and chronic poisoning with effects such as degenerative changes in the heart, liver and kidney, damage of the central nervous and cardiovascular systems, psychosis, insomnia, alopecia, peripheral neuropathy, gastroenteritis (Cavanagh, 1991; Meggs, et al., 1994; Herrero, et al., 1995).

U.S.EPA has approved various treatment technologies such as activated alumina and ion exchange for removal of thallium but the methods are seemingly costly and ineffective (Peter and Viraraghavan, 2005). Microbial organisms are promising sorbents due to the relatively cheap biomass and high removal efficiency associated with them (Babel and Kurniawan, 2003; Şenol and Ulusoy, 2010). This chapter focused on the biosorption potential of 6 green algal species. Equilibrium and kinetic models were used to estimate the sorption potential and the rate of reaction respectively.

5.2 REMOVAL OF THALLIUM

5.2.1 Isotherm Modelling

The Langmuir and Freundlich models were used to fit experimental data using linearised equation 3.2 and 3.3 respectively. The linearised graph C_e/q_e vs. C_e for Langmuir model and $\log q_e$ vs. $\log C_e$ for Freundlich model was plotted for all the tested algae, Fig. 5.1and 5.2. The models were compared and the Langmuir model generally exhibited a better correlation co- efficient (R^2) of ≤ 0.99 as compared to the Freundlich model with ≤ 0.94 for all the 6 tested species. The slope and intercept were calculated from the linearised equations for sorption capacity (q_{max}) and affinity of the metal (b). Depending on the interest of the study, biosorbents with a higher q_{max} and a lower b are reported to be better adsorbents regardless of recovery levels (Volesky and Holan, 1995; Kratochvil and Volesky, 1998; Herrero Rodríguez, et al., 2005). Biosorbents with both a high q_{max} and a high b usually have a better efficiency for recovery (Romera, et al., 2007).

Chlamydomonas reinhardtii and *Chloroidium saccharophilum* were the best adsorbents with the highest uptake and recovery levels for Tl. This was due to the higher q_{max} of 1000 mg/g and higher affinity of 1.667 L/g attained for both species, Table 5.1. *Scenedesmus acuminutus* had a lower q_{max} of 833.3 mg/g and lowest b of 0.290 L/g compared to other tested algae. The Langmuir isotherm was further evaluated using a separation factor, R_L given by the equation (Eq. 3.7). When R_L is >1 then it's unfavourable and favourable if 0< R_L <1 (Dang, et al., 2009). R_L was calculated for all the tested algae and found to be favourable for adsorption for all the species, Table 5.1.

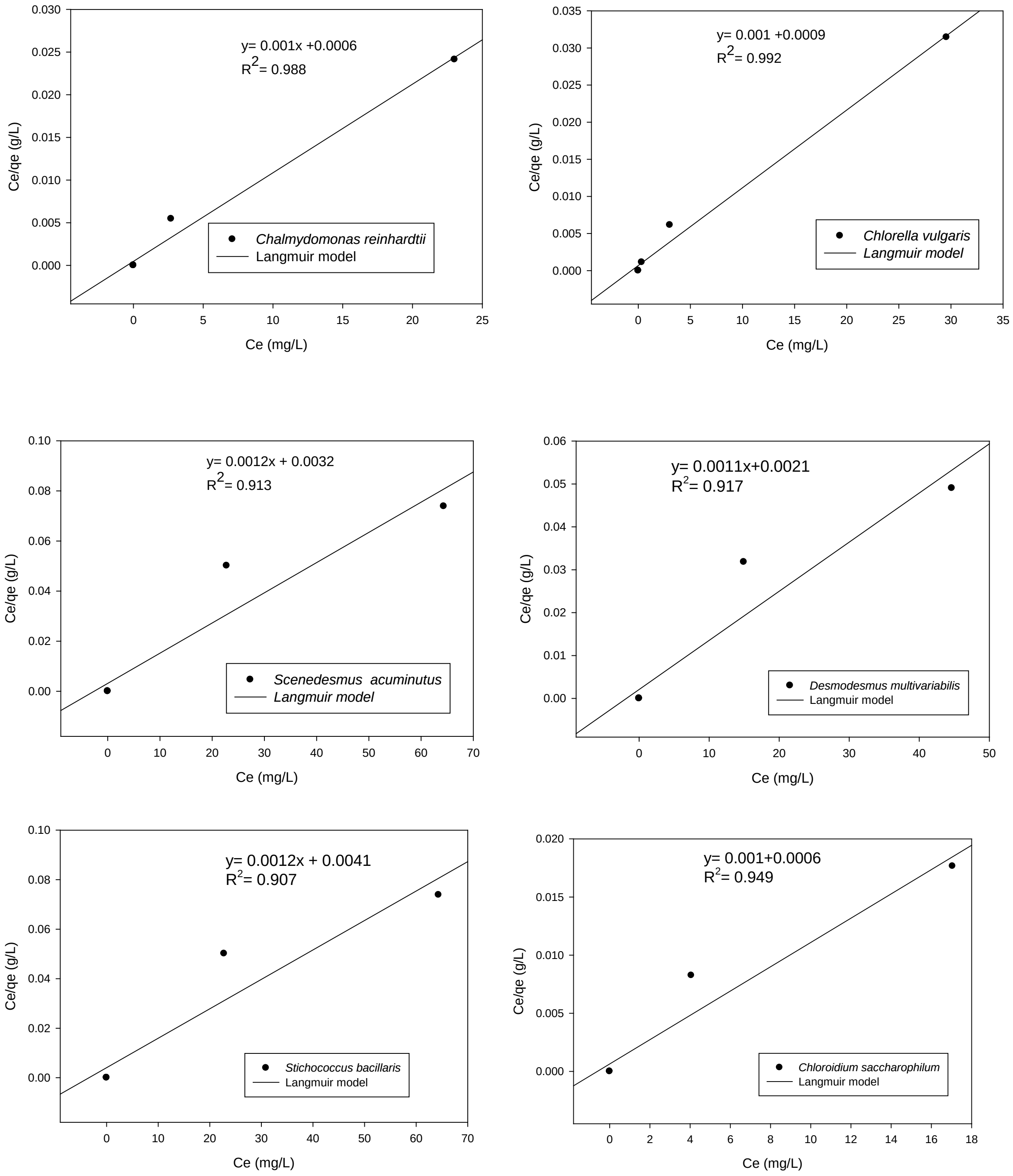


Figure 5.1: Linearised models of Langmuir for *C. vulgaris*, *C.reinhardtii*, *S. acuminutus*, *D. multivariabilis*, *C. saccharophilum* and *S. bacillaris*

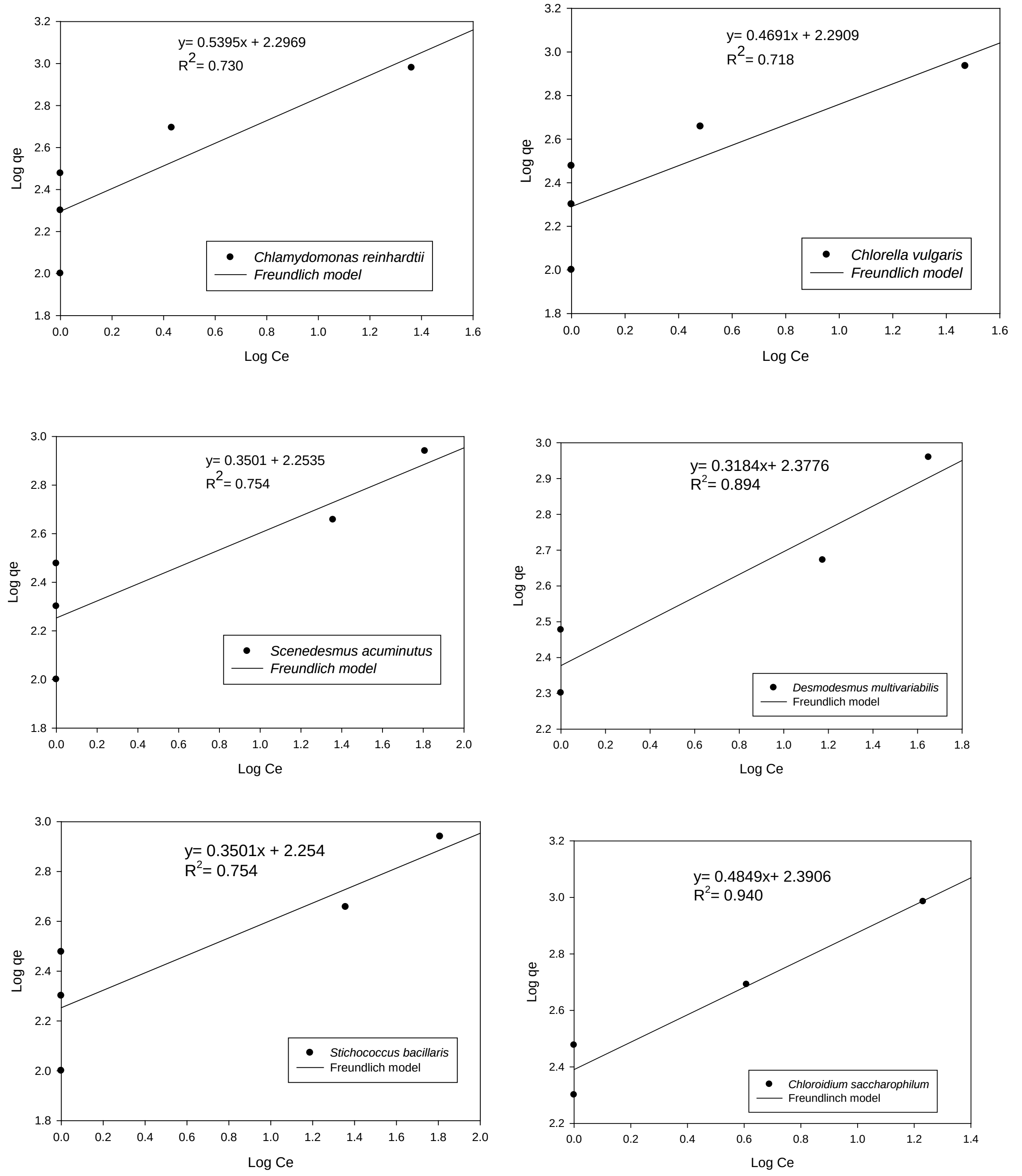


Figure 5.2: Linearised models of Freundlich for *C. vulgaris*, *C.reinhardtii*, *S. acuminutus*, *D. multivariabilis*, *C. saccharophilum* and *S. bacillaris*

Table 5.1: Model constants and regression coefficients for Langmuir and Freundlich isotherms

Algal species	**Langmuir constants**				**Freundlich constants**		
	q_{max} **(mg/g)**	b **(L/g)**	R_L	R^2	n	K	R^2
Chlorella vulgaris	1000	1.111	0.0018	0.992	2.132	9.884	0.718
Scenedesmus acuminutus	833.33	0.290	0.0069	0.907	2.856	9.521	0.754
Chlamydomonas reinhardtii	1000	1.667	0.0012	0.987	1.854	9.943	0.730
Desmodesmus multivariabilis	909.09	0.524	0.0038	0.917	3.141	10.779	0.894
Chloroidium saccharophilum	1000	1.667	0.0012	0.949	2.062	10.92	0.940
Stichococcus bacillaris	833.33	0.293	0.0067	0.907	2.856	9.526	0.754

The tested algal species were compared with some other potential adsorbents for Tl removal. All the 6 tested algal species had significant q_{max} in a range of 830-1000 mg/g compared to most adsorbents tested for thallium removal except for Prussian blue NP (PB) which had 1400 mg/g, Table 5.2. The high binding capacity for PB could be attributed to the physical chemical properties of the reaction medium such as particle size, moisture content and is usually used as an antidote for Tl poisoning (Yang, et al., 2008).

5.2.2 Adsorption Affinity of Tl on Test Algae

The adsorption affinity of metals can be determined using the distribution co- efficient (K_d) which expresses the effectiveness of the biosorbent for metal uptake. The higher the K_d, the stronger the binding affinity of the sorbents to sorbate. When the K_d value is more than 5000 mL/g, then it's considered good, and when the value is greater than 50000 mL/g then its excellent (Memon, et al., 2008; Sangvanich, et al., 2010). The K_d was calculated using equation (Eq. 3.8) for all the test algae. *Chloroidium saccharophilum* had the highest K_d value of 121092.07 and 56640.71 mL/g at initial concentration of 250 and 500 mg/L of Tl respectively, Table 5.3. These values where greater than 50000 mL/g hence the species considered excellent for Tl removal. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* also had high K_d values of 45934 and 41458 mL/g respectively which were very close to excellent value. Other tested species also had a high K_d value greater than 5000 mL/g which implied that all the algal species tested had a strong binding affinity for Tl.

Table 5.2: Comparison of thallium removal for a diversity of trial sorbents

Adsorbent	q_{max} **(mg/g)**	**References**
Sodium Polystyrene sulfonate	713	(Hoffman, et al., 1999)
Modified sugar beet pulp	185.2	(Zolgharnein, et al., 2011)
Pistachio hull	125	(Sheibani and Zare-Khormizi, 2012)
Prussian blue NP	1400	(Yang, et al., 2008)
Activated coal	59.7	(Zolgharnein, et al., 2011)
Modified eucalyptus	80.65	(Khavidaki, et al., 2013)
Alginate- PB	103	(Vincent, et al., 2014)
Saw dust	13.18	(Memon, et al., 2008; Khavidaki, et al., 2013)
Scenedesmus acuminutus	833.33	Current study
Chlorella vulgaris	1000	Current study
Chlamydomonas reinhardtii	1000	Current study
Desmodesmus multivariabilis	909.09	Current study
Chloroidium saccharophilum	1000	Current study
Stichococcus bacillaris	833.33	Current study

Table 5.3: Distribution co-efficient for 6 test algae at initial concentrations of 250 and 500 mg/L

Species	c_o **(mg/L)**	c_f **(mg/L)**	K_d **(mL/g)**
Scenedesmus acuminutus	250	22.784	19945.23
	500	64.350	13540.02
Chlorella vulgaris	250	28.043	15829.76
	500	20.862	45934.04
Chlamydomonas reinhardtii	250	16.221	28825.19
	500	23.011	41457.48
Desmodesmus multivariabilis	250	14.951	31442.58
	500	44.642	20400.44
Chloroidium saccharophilum	250	4.062	121092.07
	500	17.053	56640.71
Stichococcus bacillaris	250	21.614	21133.15
	500	62.967	13881.34

5.2.3 Effect of Initial Concentration on Removal of Tl

The effect of initial concentration on removal of Tl was studied in the range of 50-500 mg/L for three algal species. At very low concentration ≤ 150 mg/L, there was complete efficiency with 100% uptake in less than 5 minutes for all the species. At higher concentrations, maximum uptake was still attained in the first 25 minutes with initial concentration between 250- 500 mg/L reduced ≤ 55 mg/L, Fig. 5.3. Fluctuation in the removal of Tl were observed in the preceding period until equilibrium was attained which could be due to saturation of some active functional groups. *Chlorella vulgaris* showed the highest removal rate at 95.83% followed by *Chlamydomonas reinhardtii* at 95.4% in a period of 6 hours.

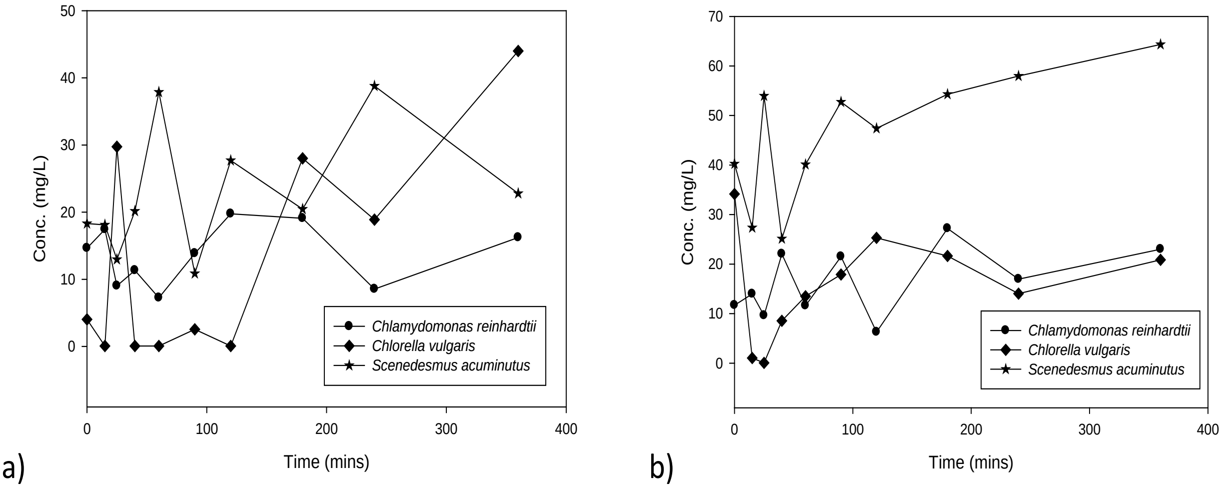


Figure 5.3: Dependence of high initial thallium concentration with time for the 3 algal species, a) at 250 mg/L and b) at 500 mg/L

5.2.4 Effect of pH on Removal of Tl

The variation in solution pH is an important factor which influences the binding of protons and cation for the active sites on the adsorbent (Mukhopadhyay, et al., 2007; Sarı and Tuzen, 2008a). At low pH of 2, there was a generally low uptake of Tl for all tested species. At lower pH, the hydrogen ions (H^+) are in excess and compete with Tl^+ for the functional groups on the algae. When the pH was increased to 4, uptake also increased due to deprotonation of active sites. At pH of 5, uptake increased for *Chlamydomonas reinhardtii* and *Scenedesmus acuminutus* but reduction was observed for *Chlorella vulgaris*. Between pH of 5 and 6, there was no significant change in uptake except an increase was observed for *Chlorella vulgaris*, Fig. 5.4. The sorption of Tl in other studies was also found to increase with an increase in pH (Peter and Viraraghavan, 2005; Pu, et al., 2013). The results indicated optimal pH to be between 5 and 6 for Tl adsorption. At pH above 7, there was a general reduction in removal except for *Chlamydomonas reinhardtii* which showed a minimal increase in uptake. At very high pH, precipitation usually occurs inhibiting any further uptake hence reduction in sorption.

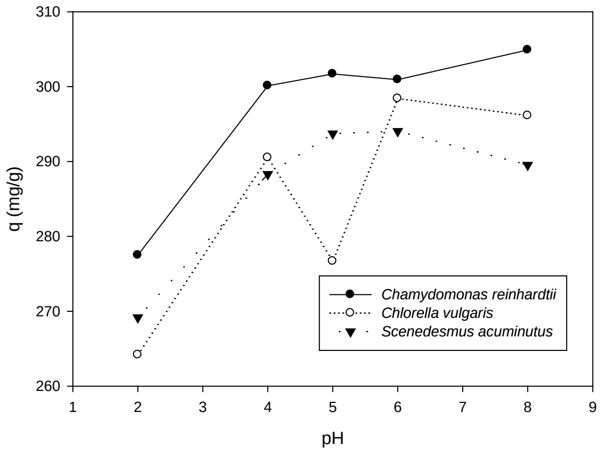


Figure 5.4: Effect of pH on uptake of Tl for all tested algae

5.2.5 Adsorption Kinetic Model for Thallium Removal

Kinetic models assist in understanding the adsorption mechanisms caused by variation in experimental conditions. In this study, the first and Pseudo- second order kinetic models were tested on 3 algal species. The linearised equations for first and Pseudo-second order model were used to estimate the slope and intercept, Eq. 3.9 and 3.10. The first order model fit well with only a few data points in the first 10 minutes and then the experimental data became scattered with a very low correlation co-efficient for the rest of the period. The experimental data fit well with the Pseudo-second order model with R^2 of ≥ 0.99 at initial concentrations of 250 and 500 mg/L of Tl, Fig. 5.5a and 5.5b.

Additional evidence for preference of Pseudo- second order model was provided in Table 5.4 with no pronounced difference between experimental q_{exp} and calculated q_{cal} data especially at initial concentration of 250 mg/L. At higher concentrations of 500 mg/L, the difference was slightly greater between q_{exp} and q_{cal}. This could be attributed to the rate of chemical reaction k_2 observed to be high especially for *Chlorella vulgaris* and *Chlamydomonas reinhardtii* at 0.002 and 0.001min^{-1} respectively.

Table 5.4: Pseudo-second order adsorption kinetic parameters for all the tested algae at concentrations of 250 and 500 mg/L for Thallium

Algal species	c_i (mg/L)	k_2 min^{-1}	R^2	q_{exp} (mg/g)	q_{cal} (mg/g)
Scenedesmus acuminutus	250	0.00084	0.998	454.432	454.546
Chlorella vulgaris	250	0.00032	0.994	412.005	416.67
Chlamydomonas reinhardtii	250	0.0074	0.9995	467.559	476.191
Scenedesmus acuminutus	500	0.000484	0.999	871.300	909.091
Chlorella vulgaris	500	0.002	0.999	958.276	1000
Chlamydomonas reinhardtii	500	0.00143	0.9998	953.978	1000

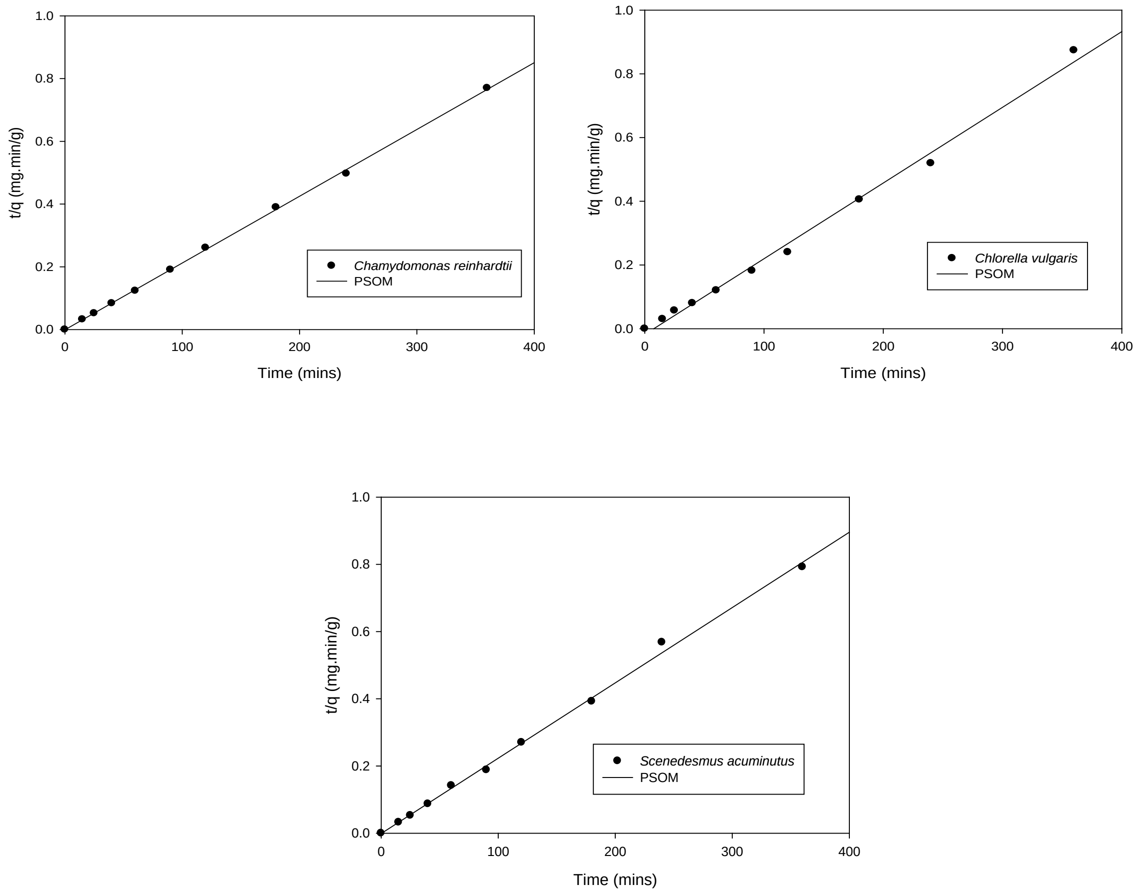


Figure 5.5a: Linearised plots of Pseudo- second order kinetic model for 3 algal species at 250 mg/L

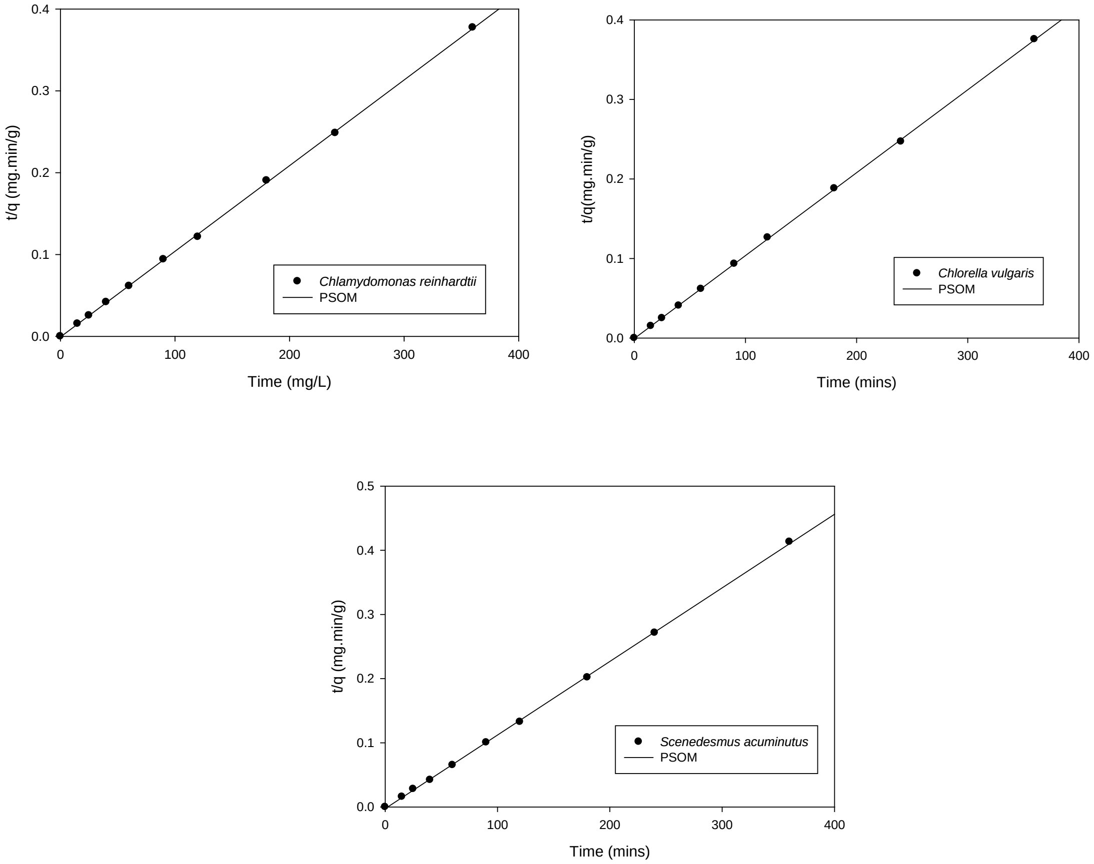


Figure 5.5b: Linearised plots of Pseudo- second order kinetic model for 3 algal species at 500 mg/L

5.3 RECOVERY OF THALLIUM

5.3.1 Effect of Contact Time on Thallium Recovery

The recovery of Tl was a much slower process than its uptake in the 3 tested algal species. The desorption efficiency was very low in the first 360 minutes with less than 40% recovery for all the algal species. This could be due to the formation of very strong complexes of Tl with the active functional groups which were not easily released. After 16 hours, the percentage recovery doubled for *Scenedesmus acuminutus* and *Chlorella vulgaris* to ≥ 66% respectively. In a period of 24 hours, there was a significant recovery of Tl with 93.26% and 85.52% achieved for *Chlorella vulgaris* and *Scenedesmus acuminutus*, Fig. 5.6. *Chlamydomonas reinhardtii* had the lowest recovery rate of 57.49% in the same period. The results implied that given more time, there is possibility of total recovery for some species. Some studies indicated that essentially all Thallium was recovered due to the cation exchange mechanism (reversible) as opposed to the precipitation or absorption (Lehn and Schoer, 1987; Sager, 1992).

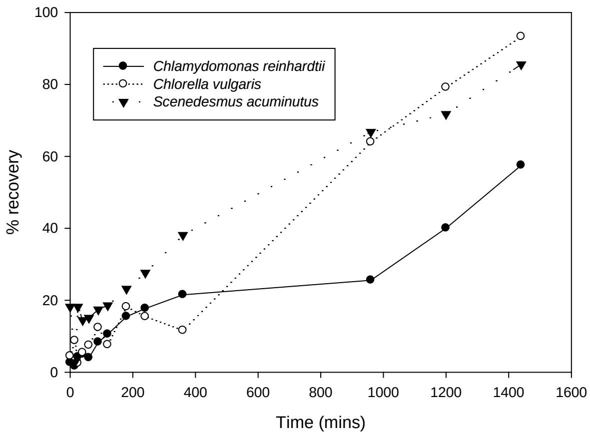


Figure 5.6: Efficiency of Tl recovery for *Chlorella vulgaris*, *Chlamydomonas reinhardtii* and *Scenedesmus acuminutus* at initial concentration of 250 mg/L

5.3.2 Desorption Kinetic Model for Thallium Removal

The modified desorption kinetic models of first order and Pseudo- second order model were used to estimate the rate of recovery (Njikam and Schiewer, 2012). The slope and intercept were estimated from desorption Eqns. 3.12, 3.12 and 3.14. In the modified first order model the data fit only for the first 15 minutes for the 3 tested algal species. The modified Pseudo-second order had a high $R^2 \geq 0.99$ as it fit well with the data with no significant difference between the experimental(q_n) and calculated (q_{n+1}), Table 5.5.

Table 5.5: Modified Pseudo-second order kinetic model parameters

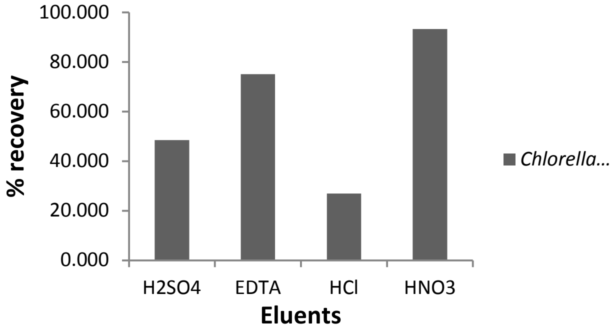
Algal species	C_i (mg/L)	$k_{2,,des}$ (Min^{-1})	R^2	q_n (mg/g)	q_{n+1} (mg/g)
Chlamydomonas reinhardtii	250	0.000002	0.9702	421.341	416.67
Chlorella vulgaris	250	0.0031	0.998	360.501	238.1
Scenedesmus acuminutus	250	0.000003	0.838	361.02	400
Chlamydomonas reinhardtii	500	0.0000006	0.898	926.161	909.091
Chlorella vulgaris	500	0.0000009	0.910	582.197	588.24
Scenedesmus acuminutus	500	0.000001	0.938	681.40	666.67

5.3.3 Comparison of Different Eluents for Tl Recovery

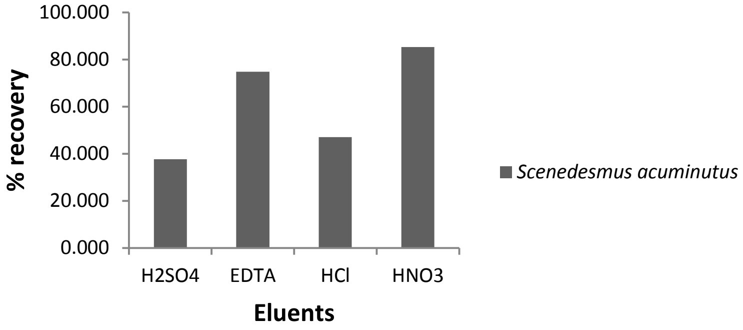
The recovery of Tl from biomass loaded with metal was tested using H_2SO_4, EDTA, HCl and HNO_3. Low concentration of 0.1M was used for all cases as the biomass degenerates with increase in concentration reducing the sorption efficiency (Chojnacka, et al., 2005). For *Chlorella vulgaris*, HNO_3 recovered the highest Tl of up to 85.2% followed by EDTA with

74.83%, Fig. 5.7a. *Scenedesmus acuminutus* had the highest sorption efficiency at 93.26% followed by EDTA at 75.12%, Fig. 5.7b. In *Chlamydomonas reinhardtii*, there was a slight difference in recovery between HNO_3 and EDTA at 57.49 and 61.93% respectively, Fig. 5.7c. In general, use of HCl and H_2SO_4 exhibited very low efficiencies in the recovery of Tl. The preference of eluents was in the order of HNO_3 > EDTA >HCl >H_2SO_4. Our results also coincide with (Chojnacka, et al., 2005), who also found HNO_3 as a better desorbing agent for heavy metals.

a)



b)



c)

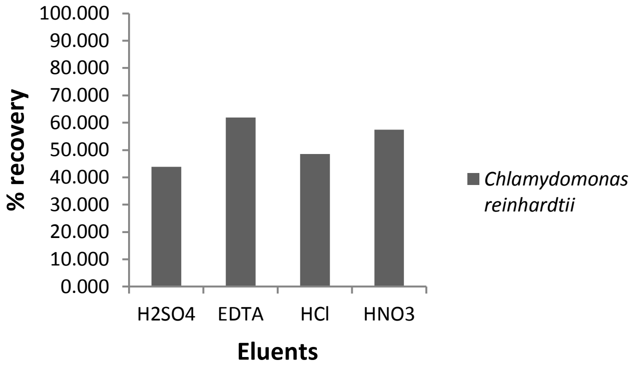


Figure 5.7: Comparison of different eluents for recovery of Tl for all the test algae

5.4. SURFACE CHARACTERISATION OF TEST ALGAE

5.4.1 Surface Electron Microscope

The SEM provided detailed information on surface topography through acquisition of a beam of electrons on the sample. All the algal samples before adsorption formed compact and irregular surface whereas after adsorption the surface was fragmented and indication of adsorption of metals on the surface wall, Fig. 5.8.

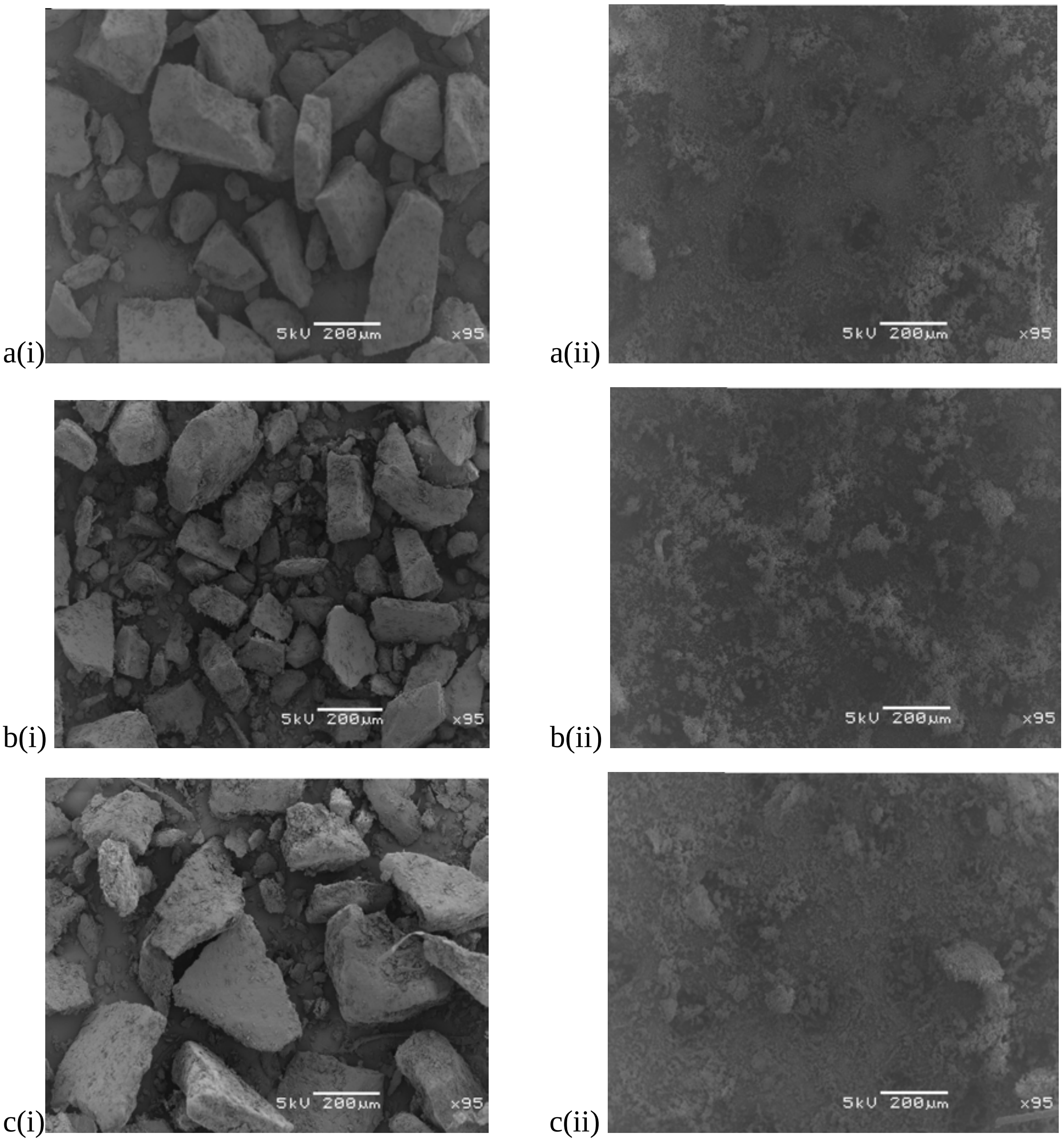
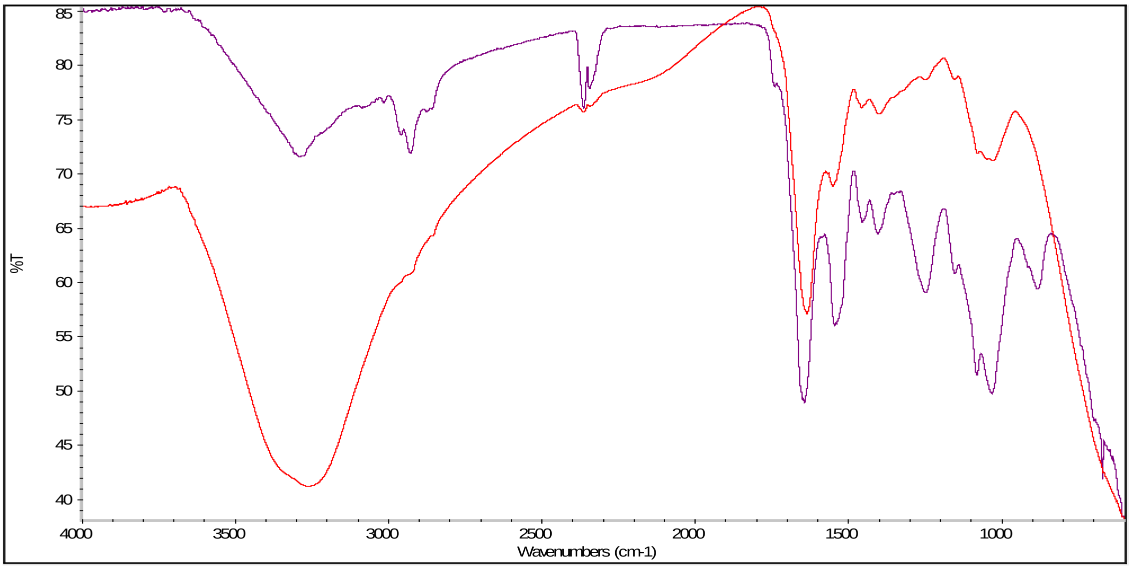


Figure 5.8: SEM images for a) *Chlamydomonas reinhardtii*, b) *Chlorella vulgaris* and c) *Scenedesmus acuminutus*- i) before, ii) after adsorption of Tl

5.4.2 Fourier Transform Infrared Spectroscopy

For FTIR analysis, *Chlamydomonas reinhardtii* showed pronounced peaks at 3273.82 cm^{-1} before adsorption and 3293.45 cm^{-1} after adsorption. This indicates a very strong O-H stretch which depicts phenols and alcohols. Another stretch was between 2916.56 and 2920.48 cm^{-1} which is indicative of an O-H bond for carboxylic acid. Other bonds ranged from 1637-1645, 1546-1539, 1024-1039.9cm^{-1} representative of primary amines, nitro compounds and aliphatic amines respectively, Fig. 5.9a**.**

For *Scenedesmus acuminutus* and *Chlorella vulgaris*, there was no significant difference in peaks with the highest attained between 3289 and 3301 cm^{-1} reflecting primary and secondary amines and amides, Fig. 5.9b and c. The carboxyl stretch also appeared for the 2 species in the range of 2854-2924 cm^{-1}. Other similar stretches were 1629-1652, 1534-1546, 1024-1048 cm^{-1} representing primary amines, nitro compounds and aliphatic amines respectively. It can be noted that there was slight difference in wavelength for all the species inferring similar function groups. The differences in uptake levels were due to variations in wave numbers which influences the transmittance.



a)

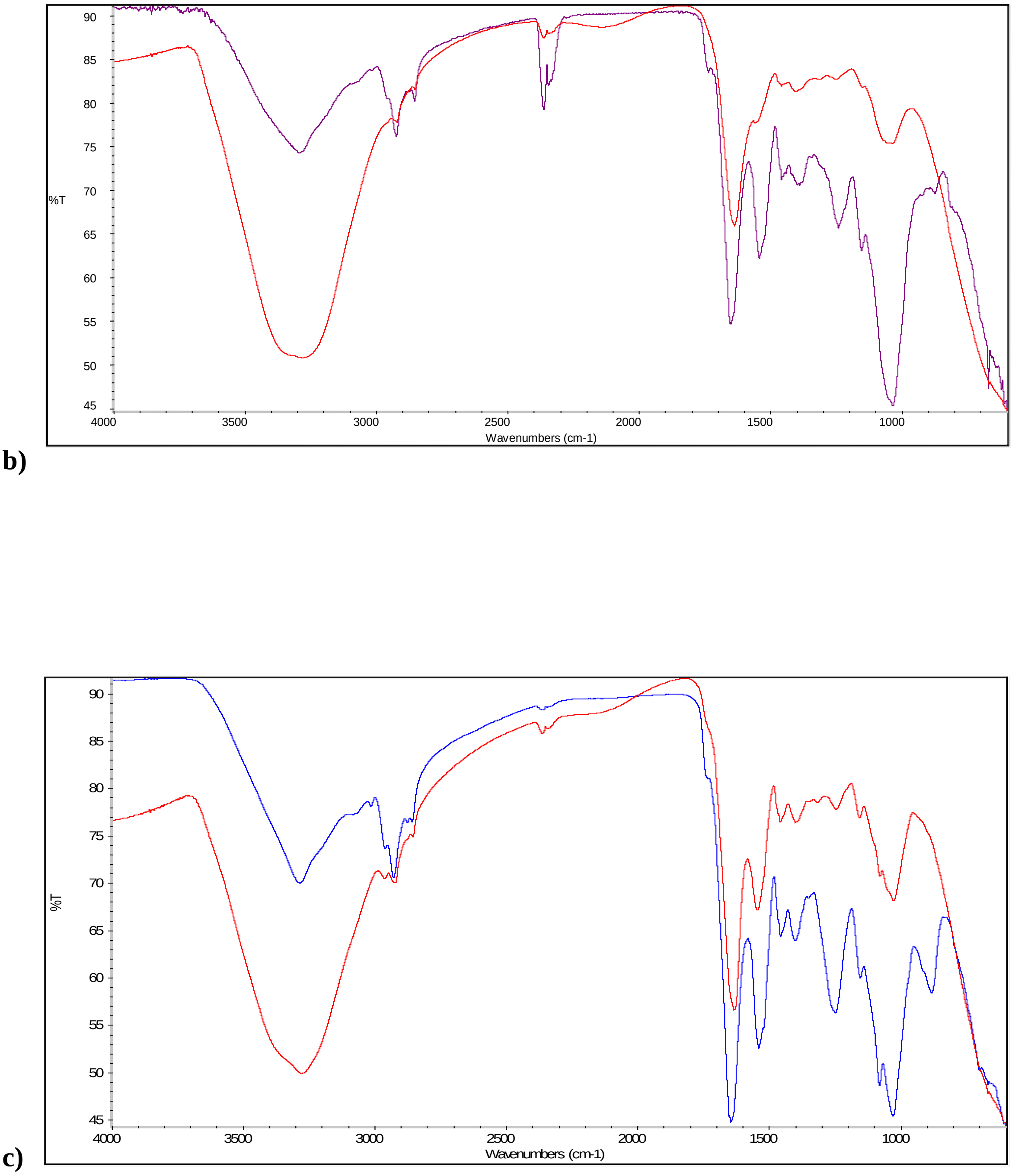


Figure 5.9: FTIR peaks of transmittance of Tl in a) *Chlamydomonas reinhardtii*, b) *Scenedesmus acuminutus*, c) *Chlorella vulgaris* and red representing before adsorption

5.5 Summary

Chlamydomonas reinhardtii and *Chloroidium saccharophilum* had the highest sorption capacity and affinity for Tl removal. Generally all the 6 tested algae showed great potential for adsorption of Tl as compared to other adsorbents. For lower concentrations of $\geq$ 150 mg/L of Tl removal efficiency was achieved at 100%. At higher concentrations in a range of 250-500 mg/L, the performance of algae was still higher with sorption capacity (q_{max}) between 830 and 1000 mg/g. The adsorption and desorption kinetic studies showed better correlation co-efficient of $\leq$ 0.99 for Pseudo-second order and modified Pseudo-second order models. The highest removal of Tl ($\leq$ 70%) is achieved the first few minutes but recovery takes a longer period with most removed after more than 15 hours. The strongest functional groups responsible for Tl binding on the algal cell wall were carboxyl and phenols. Different eluents were tested with HNO_3 acid emerging as the most efficient for recovery of Tl.

CHAPTER 6

BIOSORPTION OF CADMIUM USING EMPIRICAL AND KINETIC MODELS

6.1 BACKGROUND

Cadmium is one of the most ubiquitous toxic metals mobilised in the environment from natural and anthropogenic sources. The solubility of this metal is high forming complexes with chlorides, amines, thiols, halides, sulphhydryls, cyanides (Förstner, 1980a; McLaughlin and Singh, 1999). The sources of cadmium are numerous, e.g coal combustion, smelting, mining, corrosion of galvanised metal, pesticides, fertilisers, refining, battery and alloy manufacturing, pigments, ceramics, plastic stabilizers, electroplating, incineration of municipal waste (Alloway and Steinnes, 1999; Gupta and Rastogi, 2008b; Wu, et al., 2011). Cadmium toxicity is known to cause acute and long term effects like hypertension, renal dysfunction, bone lesions, cancer, lung edema, liver and kidney damage, reproductive toxicity (Sharma, 1995; Staessen, et al., 1999; Sarkar, 2002).

In order to control the release of cadmium in the environment, World Health Organisation (WHO) set standard permissible limits for Cd in wastewater and drinking water at 0.1 and 0.005 mg/L respectively. The treatment of cadmium using various physical and chemical methods has been applied widely. These methods are seemingly ineffective due to the high cost of operation and capital investment associated with treatment of toxic metals even at low concentrations (Ahalya, et al., 2003; Lesmana, et al., 2009). The biosorption potential of green algae from freshwater bodies has not been fully explored for treatment of heavy metals (Romera, et al., 2006; Wang and Chen, 2009). In this chapter the focus was to determine the adsorption potential of 3 green algal species for the removal /recovery of cadmium.

6.2 CADMIUM ADSORPTION

6.2.1 Equilibrium Modelling

Single metallic studies are a fundamental step in determining sorption efficiency for biosorbents prior to including variable environmental conditions like pH and ionic strength (Volesky and Holan, 1995; Volesky, 2003; Mehta and Gaur, 2005). Empirical models of Langmuir, Freundlich and Dubinin-Radushkevich (D-R) models were used to quantitatively describe sorption potential of algae using linear regression. In the Langmuir model, a graph of $^{C_e}\!/_{q_e}$ vs. C_e was plotted from the linearised equation (Eq. 3.2). The maximum sorption capacity ($q_{\max}$) and the affinity constant (b) were determined from the slope and intercept, Fig. 6.1a. The Freundlich model was plotted with values of $\log q_e$ vs. $\log C_e$ from the linearized Eq. 3.3. The Freundlich constants k and n were determined from the slope and intercept respectively, Fig. 6.1b. The D-R model was plotted from the linearised equation (Eq. 3.4). The D-R parameters β and X_m were determined from the slope and intercept respectively.

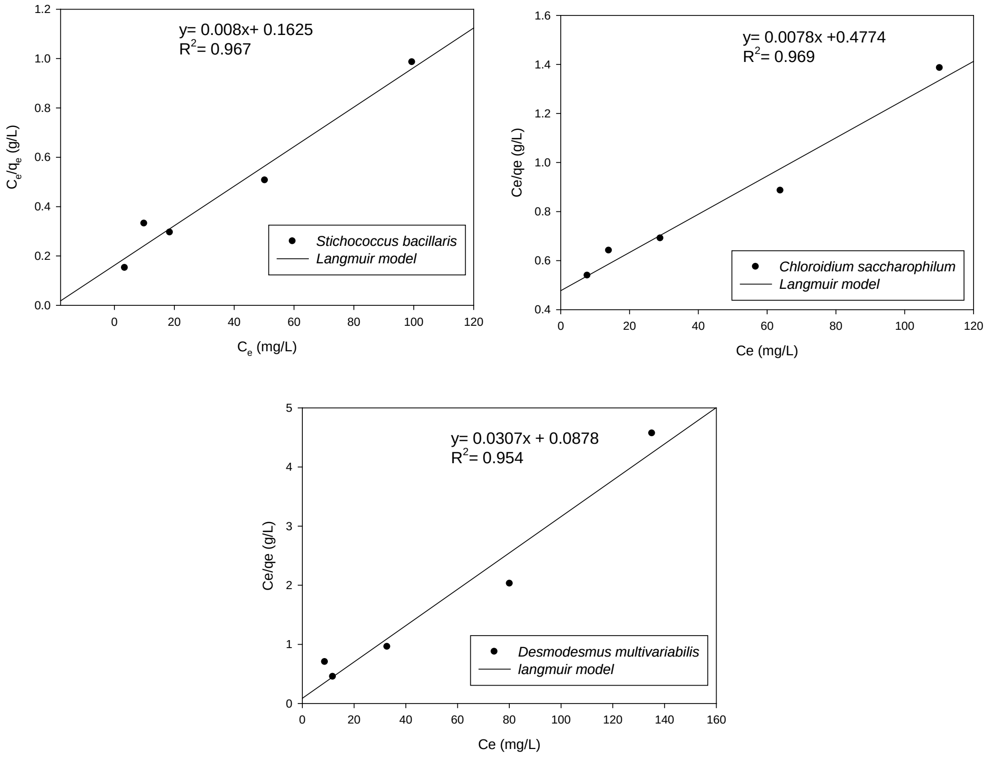


Figure 6.1a: Linearised graphs plotted from Langmuir model for *S. bacillaris, D. multivaribilis* and *C. saccharophilum*

The results from the models were compared and generally showed a better correlation co-efficient (R^2) for Langmuir model of 0.97 as compared to Freundlich and D-R models for all the tested algae, Fig. 6.1 a,b and c. *Chloroidium saccharophilum* had the highest $q_{\max}$ of 128.21 mg/g followed by *Stichococcus bacillaris* with 125 mg/g, Table, 6.1. *Desmodesmus multivariabilis* had the lowest $q_{\max}$ but the highest affinity b of 1.49 L/g. The results from the Freundlich constant $^1\!/_n$ were 0.495, 0.575 and 0.27 for *Stichococcus bacillaris,* *Chloroidium saccharophilum* and *Desmodesmus multivariabilis* respectively. When values of $\frac{1}{n}$ are in the range of 0-1, then the biosorbent is favourable, (Sarı and Tuzen, 2008b).

The D-R model is usually used to distinguish between physical and chemical adsorption through estimation of mean biosorption energy (E). When the value of E is between 8 and 16 kJ/mol, and the process follows chemical sorption. If E is less than 8, then it could be a physical adsorption process (Helfferich, 1962; Sarı and Tuzen, 2008b; Şeker, et al., 2008). All the 3 tested algae had E values between 8-16 kJ/mol indicating a dependence on chemisorption process, Table 6.1.

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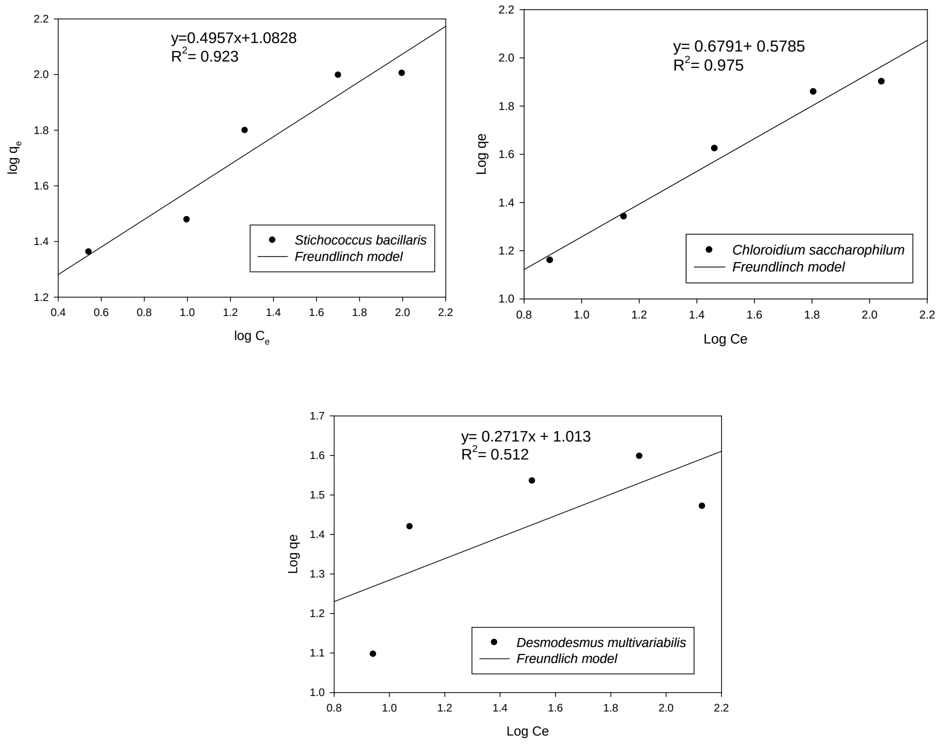
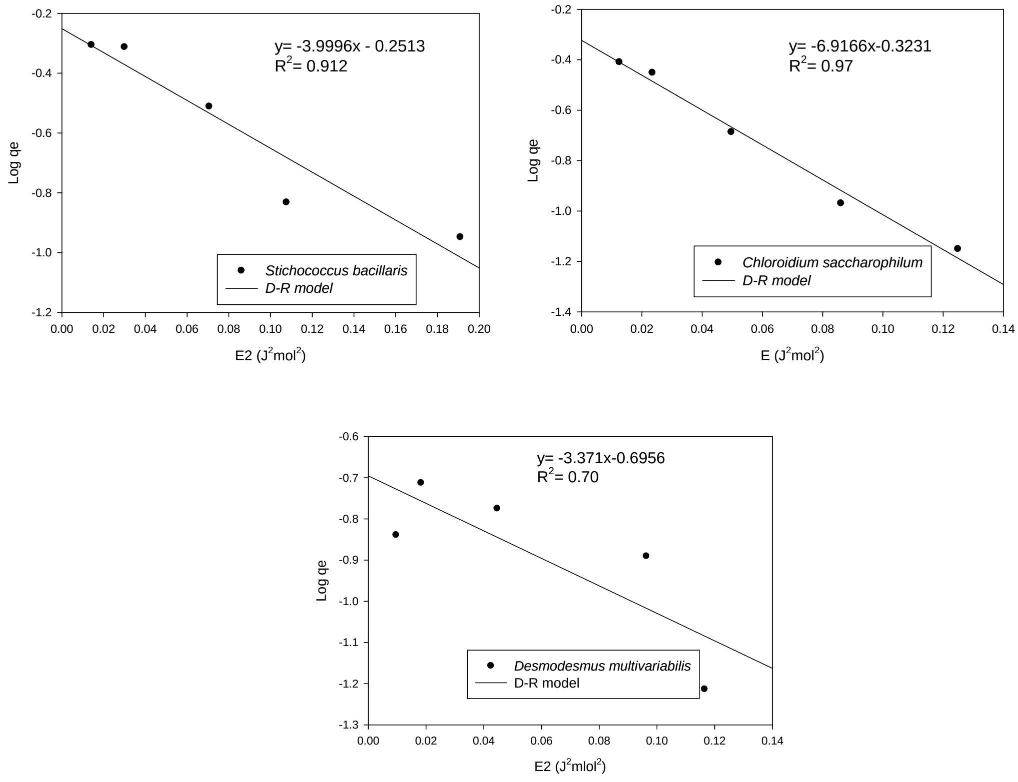


6.1b: Linearised graphs plotted from Freundlich model for *S. bacillaris, D. multivaribilis* and *C. saccharophilum*



6.1c: Linearised graphs plotted from D-R model for *S. bacillaris*, *D. multivaribilis* and *C. saccharophilum*

Table 6.1: Equilibrium model constants for Langmuir, Freundlich and D-R models

Species	**Langmuir constants**			**Freundlich constants**			**Dubinin-Radushkevich constants**			
	q_{max} (mg/g)	b (L/g)	R^2	k	n	R^2	X_m (mmol/g)	β (mol^{2}g^{-1}kJ^{-1})	E kJmol^{-1}	R^2
Stichococcus bacillaris	125	0.049	0.967	2.95	2.02	0.923	1.0003	-0.00399	11.18	0.912
Chloroidium saccharophilum	128.21	0.016	0.969	1.78	1.47	0.975	1.0003	-0.00692	8.50	0.970
Desmodesmus multivariabilis	32.57	1.490	0.954	2.75	3.68	0.512	1.0007	-0.00337	12.18	0.70

The sorption capacity from this study was compared with brown algae, green algae and other non-algal sorbents for removal of cadmium. From the results, *Chloroidium saccharophilum* and *Stichococcus bacillaris* had a relatively higher $q_{\max}$ of 128.21 and 125 mg/g respectively for removal of Cd than most brown algae, Table 6.2a. The sorption capacity is dependent on the physical-chemical conditions subjected to the biomass which influence the uptake, for example, the high $q_{\max}$ of *Durvillaea potatorum* could be due to pre-treatment of biomass which increases sorption capacity (Matheickal and Yu, 1999).

Table 6.2a: Comparison of sorption capacity of tested algae with brown algae

Biosorbent	$q_{\max}$ **(mg/g)**	**pH**	**References**
Sargassum sp.	85.43	5.5	(Sheng, et al., 2004)
Padina sp.	84.31	5.5	(Sheng, et al., 2004)
Sargassum siliquosum	82.06	5	(Hashim and Chu, 2004)
Sargassum vulgarie	88.80	4.5	(Davis, et al., 2000)
Fucus spiralis	114.9	6	(Romera, et al., 2006)
Sargassum filipendula	74.19-78.69	4.5	(Davis, et al., 2000)
Ascophyllum nodosum	78.69	4.5	(Lodeiro, et al., 2005)
Laminaria ochroleuca	62.95	4.5	(Lodeiro, et al., 2005)
Macrocystis pyrifera	100.05	3	(Cazón, et al., 2012)
Durvillaea potatorum	125.9	5.4	(Matheickal, et al., 1999)
Padina tetrastomatica	59.58	5	(Hashim and Chu, 2004)
Stichococcus bacillaris	**125**	**5.5**	**Findings from this study**
Chloroidium saccharophilum	**128.21**	**5.5**	**Findings from this study**
Desmodesmus multivariabilis	**32.57**	**5.5**	**Findings from this study**

It is evident from previous research, a few studies have been done on green micro-algae species, e.g *Chlamydomonas reinhardtii, Oedogonium sp. Scenedesmus sp. and Chlorella species*, Table 6.2b. When compared with other green algae and non-algal sorbents, *Chloroidium saccharophilum* and *Stichococcus bacillaris* still had higher sorption for Cd as shown in Tables 6.2b & 6.2c. Green algae was apparently ranked the worst in biosorption as compared to brown and red algae from previous studies (Romera, et al., 2006). Contrary, the findings using green algae from this study competed favourably with other algal and non-algal sorbents in Cd uptake except for *Desmodesmus multivariabilis* which had a lower $q_{\max}$,

Table 6.2 b and c. The differences in performance of green algae could be due to the available and active functional groups of different algal species.

Table 6.2b: Comparison of sorption capacity of tested algae with other green algae

Biosorbent	**q_{max} (mg/g)**	**pH**	**References**
Chlamydomona reinhardtii	42.6	6	(Tüzün, et al., 2005)
Chlorella vulgaris	85.3	4	(Aksu, 2001)
Oedogonium sp.	88.2	5	(Gupta and Rastogi, 2008b)
Scenedesmus subspicatus	7.3	6	(Schmitt, et al., 2001)
Chlorella minutissima	11.1	-	(Roy, et al., 1993)
Chlorella sorokiniana	32.2	-	(Akhtar, et al., 2003)
Codium vermilara	21.36	-	(Romera, et al., 2006)
Ulva lactuca	49	2-5.5	(Areco, et al., 2012)
Algal waste	9.7	5.3	(Vilar, et al., 2006)
Codium fragile	9.22	-	(Romera, et al., 2006)
Stichococcus bacillaris	**125**	**5.5**	**Findings from this study**
Chloroidium saccharophilum	**128.21**	**5.5**	**Findings from this study**
Desmodesmus multivariabilis	**32.57**	**5.5**	**Findings from this study**

Table 6.2c: Comparison of sorption capacity with other non- algal sorbents

Biosorbent	**q_{max} (mg/g)**	**pH**	**References**
Saccharomyces cerevisiae	28-38	4.5	(Volesky, et al., 1993)
Pine bark	28	7.5	(Al-Asheh and Duvnjak, 1998)
Endophytic fungus	247.5	6.5	(Xiao, et al., 2010)
Wheat straw	4.88	6	(Dang, et al., 2009)
Lactic acid bacteria	12.1-54.7	6	(Halttunen, et al., 2007)
Anaerobic granular bimass	64	5	(Hawari and Mulligan, 2006)
Fungal immobilised in loofa sponge	85.98	6	(Iqbal and Edyvean, 2005)
Rhodotorulla sp. Y11	14.09	5.8	(Li and Yuan, 2006)
Stichococcus bacillaris	**125**	**5.5**	**Findings from this study**
Chloroidium saccharophilum	**128.21**	**5.5**	**Findings from this study**
Desmodesmus multivariabilis	**32.57**	**5.5**	**Findings from this study**

6.2.2 Kinetic Modelling

The rate of sorption provides information on reaction pathways which are helpful when designing and optimising reactors under different conditions (Benguella and Benaissa, 2002; Zafar, et al., 2007; Chen, et al., 2008). *Stichococcus bacillaris* was selected for kinetic studies using the Pseudo- second order model (PSOM) and the Elovich model. The effect of initial concentration, pH and temperature with time were compared. The linearised form of PSOM and Elovich model were plotted from the equations, 3.10 and 3.11 respectively. PSOM had a better R^2 of ≥ 0.928 than Elovich model with ≥ 0.765 for a period of 120 minutes, Table 6.3 Other authors also confirmed cadmium sorption following Pseudo- second order model (Martínez, et al., 2006; Mata, et al., 2008; Sulaymon, et al., 2013).

Table 6.3: Pseudo-second order and Elovich model constants for kinetics of initial concentration, pH and temperature

	Pseudo second order model				**Elovich model**		
	R^2	q_{exp} **mg/g**	q_{cal} **mg/g**	k_2	R^2	α **mgg^{-1} min^{-1}**	β **gmg^{-1}**
Initial concentration							
15	0.999	22.620	23.256	0.0164	0.978	34.9715	0.1598
150	0.998	118.4	117.647	0.0041	0.847	21905.72	0.0825
pH							
2	0.981	68	70.42	0.0054	0.765	1.6544	16.809
5.5	0.999	202	204.08	0.003	0.8844	489.367	17.839
Temperature							
10°c	0.928	16.5	34.965	0.0032	0.987	0.2102	8.3773
50°c	0.987	53	59.172	0.0085	0.969	2426435512	2.2291
80°c	0.993	76.5	87.72	0.011	0.951	4069.062	8.2673

In Elovich model, the R^2 only fitted for the first 20 minutes deviating when the time was increased. At higher temperatures ≥ 80°c and pH ≥ 7, the experimental data did not fit well with the Elovich model but followed the PSOM better, Fig 6.2. An example can be observed for PSOM with no significant difference in the q_{exp} and q_{cal} at initial concentration of 150 mg/L with 118.4 and 117.65 mg/L respectively, Table 6.3.

Temperature had the least fit with R^2 of ≥ 0.92 for the same period of 120 minutes in PSOM. This could explain why temperature is not considered a significant factor in adsorption of cadmium.

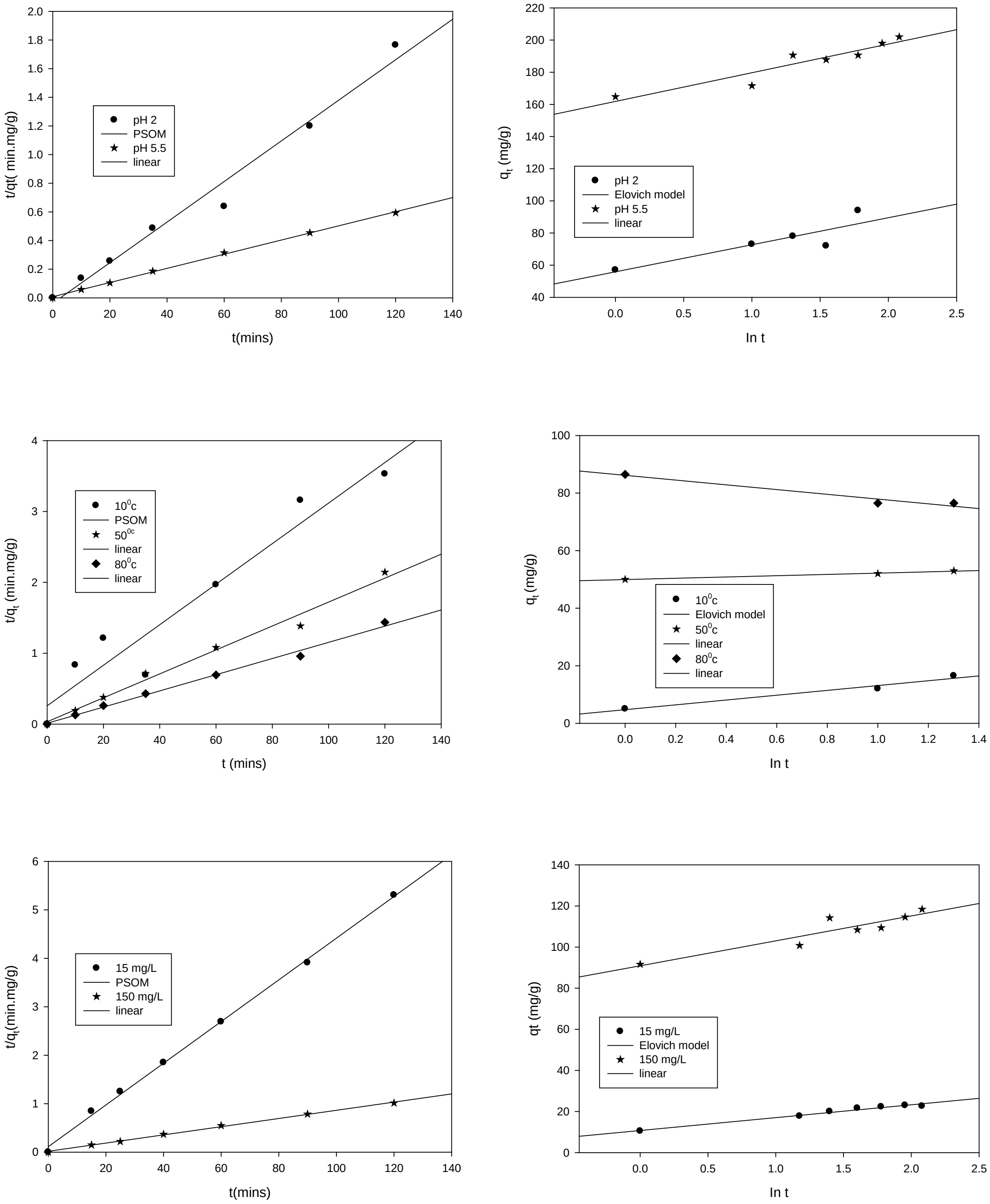


Figure 6.2: Linearised graphs of pseudo- second order and Elovich models for pH, temperature and initial concentration.

6.2.3 Effect of pH on Cd Adsorption

pH is one of the most important environmental factor affecting biosorption of cadmium (Vilar, et al., 2006). The study set out to establish the effect of pH on Cd adsorption at an initial concentration of 100 mg/L of Cd^{2+} in a pH range of 2-7 for *Stichococcus bacillaris*. At a low pH of 2, there was almost no observable sorption with only 14.4 mg/g attained, Fig.6.3. When the pH is very low, the protons (H^+) are increased greatly which compete with the cations for the adsorption site thus reducing the adsorption capacity (Blázquez, et al., 2005; Areco, et al., 2012). The most active functional groups on the cell surface of *Stichococcus bacillaris* were found to be carboxyl and phenols which were protonated at very low pH. When the pH was increased to 4, there was a significant change in sorption performance of 101 mg/g. At pH 5, the difference in uptake was very minimal but steadily increased at pH of 5.5 to 210 mg/g. This implied that as the pH increased, the surface site was deprotonated and Cd adsorption increased. In Dang, et al., 2009, sorption of cadmium increased with increase in pH from 4-7. At higher pH of 7, uptake was reduced to 200 mg/g, Fig. 6.3. As the pH increases from 7 and above, there is formation of soluble hydroxide complexes which affects adsorption (Lodeiro, et al., 2006; Gupta and Rastogi, 2008b). It can be noted that the optimal pH for Cd dasorption using tested algae was 5.5.

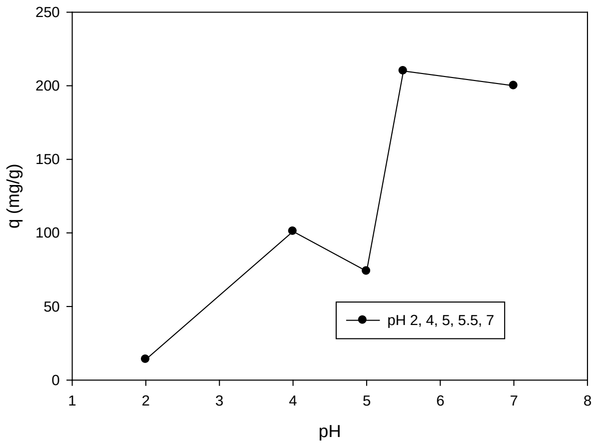


Figure 6.3: Effect of pH on Cd adsorption using *Stichococcus bacillaris*

6.2.4 Effect of Temperature on Cd Adsorption

The effect of temperature with time was studied in a range of 10-80°c at an initial concentration of 50 mg/L of Cd^{2+} for *Stichococcus bacillaris*. At low temperature of 10°c, there was a slight increase in uptake in the first 35 minutes to 50 mg/g and there after a general decrease to 15.5mg/g in a period of 4 hours. When the temperature was increased to 25°c, the uptake increased to 53.5 m/g in the same period of 4hours, a value which was greater than at 50°c with 43 mg/g. At higher temperatures of 80°c, there was an increase in sorption in the first 100 minutes followed by a drastic decline for the rest of the period, Fig.6. 4. As the temperature increases, there is a possibility of weakening the energy bond between adjacent molecules and deactivation of some active sites therefore reducing the uptake levels (Özer and Özer, 2003; Sulaymon, et al., 2013). From the results, there was a general increase in sorption with increase in temperature although the changes were not significant. This could mean that the process could be partially dependant on temperature, therefore endothermic. Some studies also showed an increase in sorption with increase in temperature (Wehrheim and Wettern, 1994; Tüzün, et al., 2005; Chen, 2012).

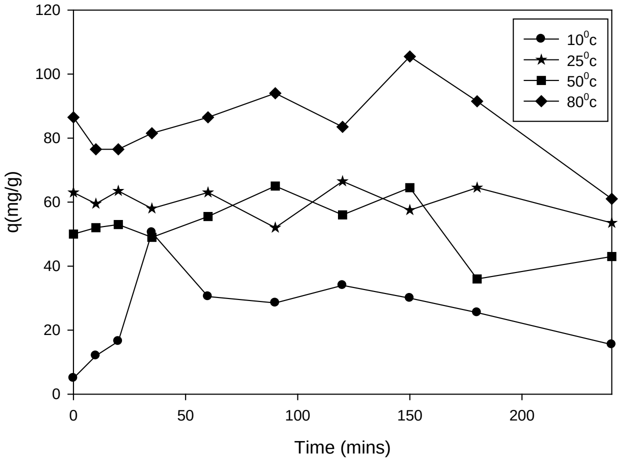


Figure 6.4: Effect of Temperature with time on adsorption of Cd in *Stichococcus bacillaris*

6.2.5 Effect of Initial Concentration on Cd Adsorption

The effect of concentration on cadmium removal by *Stichococcus bacillaris* was tested at initial concentration of 15-100 mg/L. At low concentrations of 15 mg/L of Cd, there was a fast reduction at initial time (t_0) to 9.75 mg/L, followed by a slow reduction for a period of 4 hours to 8.05 mg/L, Fig. 6.5. At higher concentrations of 50 and 100 mg/L, reduction was highest in the first 15 minutes and slowed down at 18.55 and 50.3 mg/L remaining in solution at equilibrium respectively. Higher sorption rate was also found at low concentration and lower at higher concentrations (Vilar, et al., 2006; Memon, et al., 2007). When metal concentration increases, sorption increases until all the available sites become saturated (Romera, et al., 2007; Rajfur, et al., 2010).

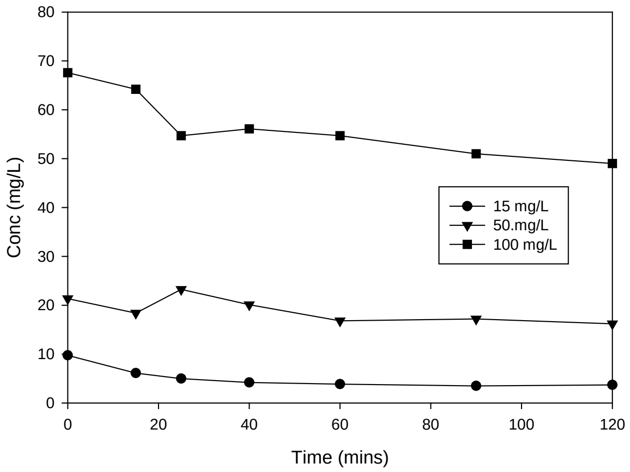


Figure 6.5: Effect of concentration with time on adsorption of Cd in *Stichococcus bacillaris*

6.2.6 Thermodynamics of Algal Sorption

The study of thermodynamics gives an indication of the energy exchanged in the system commonly known as Gibbs free energy ΔG. *Stichococcus bacillaris* was used in thermodynamic studies on biosorption. A graph of $\ln K_C$ vs $\frac{1}{T}$ was plotted from the linearised equation, 3.16 from which the slope and intercept was determined, Fig. 6.6**.** At temperatures of 298.15, 323.15 and 353.15k, the value of ΔG were all negative at -0.2934, -0.658, -1.4065 kJ/mol respectively except at very low temperature of 283.15K which was positive. When the value of ΔG is negative and decreases with increase in temperature, then the adsorption reaction is spontaneous and feasible (Memon, et al., 2007; Sarı and Tuzen, 2008a). The values obtained for ΔH and ΔS were positive at 7.398 kJ/mol and 0.0251 kJmol^{-1}k^{-1} respectively. For positive values for ΔH, the process could be endothermic with an increase in sorption at high temperature. Positive values of ΔS could imply an increase in the interaction of solid and liquid phase (Şeker, et al., 2008). Some authors also found the reaction to be endothermic in thermodynamic studies on Cd (Benguella and Benaissa, 2002; Shen and Duvnjak, 2004).

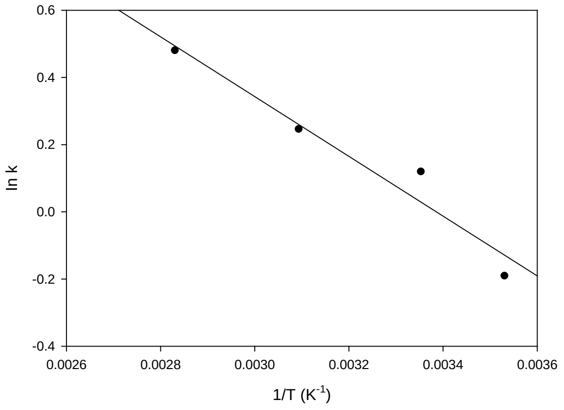


Figure 6.6**:** Linearised plot of $\ln K_C$ vs $\frac{1}{T}$ for Gibbs free energy

6.3. COMPOSITION AND TOPOGRAPHY OF ALGAE

6.3.1 Scanning Electron Microscopy / Energy Dispersive Spectroscopy (SEM-EDS)

SEM-EDS was used to examine cell morphology and elemental composition before and after adsorption for *Stichococcus bacillaris*. The algal sample before adsorption had smooth and intact lumps (Fig.6.7a) whereas after adsorption the surface was rough and crumbled (Fig.6.7b). The EDS images also showed the differences with presence of Cd detection after adsorption which confirmed the biosorption process, Fig. 6.7a, b. The BET surface area for *Stichococcus bacillaris* was found to be 1.966 m^2/g.

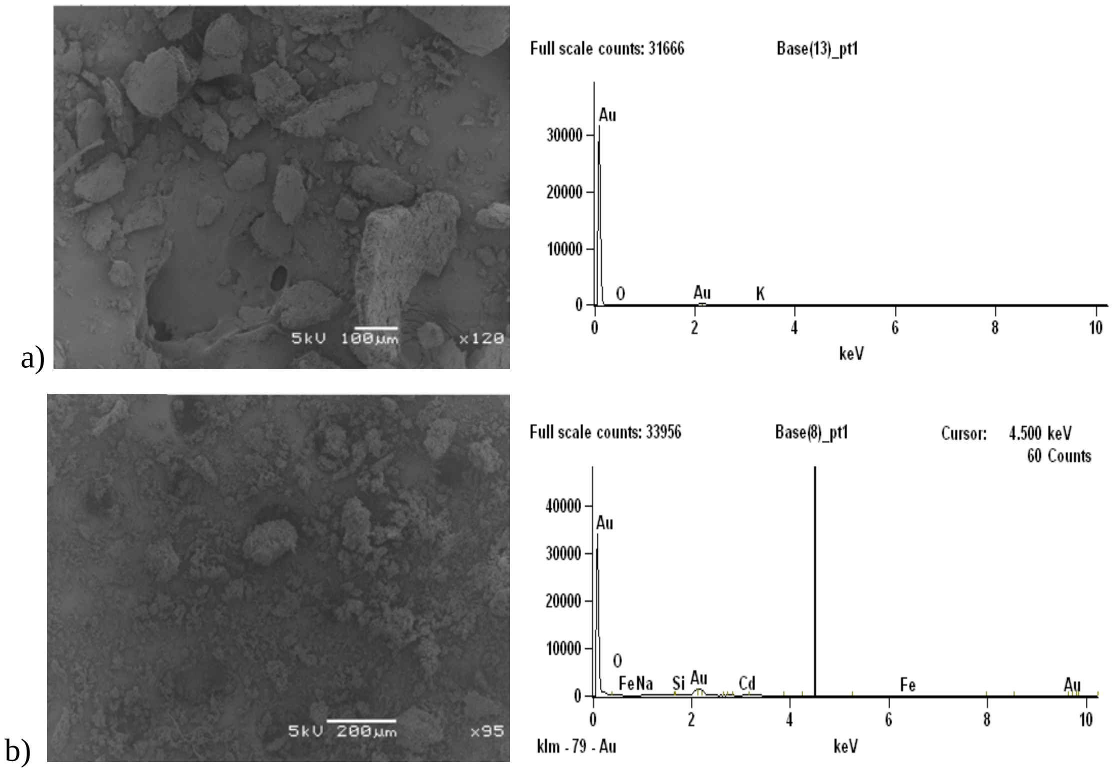


Figure 6.7: SEM_EDS images with a) before sorption, b) after sorption of Cd on algae

6.3.2 Fourier Transform Infrared Spectrum

The functional groups for *Stichococcus bacillaris* showed strong and sharp O-H stretch which shifted from 3294.01 to 3299.57 cm^{-1} representing phenols and alcohols, Fig. 6.8. The carboxyl group was a medium O-H stretch from 2915.94 to 2918.72 cm^{-1}. Gupta and Rastogi, 2008b also found a strong relationship of carboxylic and phenol bands in green algae. This study found that other active groups included a C-H stretch representing alkanes from 2846.44-2849.22 cm^{-1}; N-H bend for primary amines from 1642.72-1634.38 cm^{-1} and aliphatic amines of C-N stretch from 1025.57-1028.35 cm^{-1}. It can be noted that when the active acidic functional group increases, the total proton exchange capacity also increases due to cation exchange mechanisms (Ahmady-Asbchin, et al., 2008; Zakhama, et al., 2011).

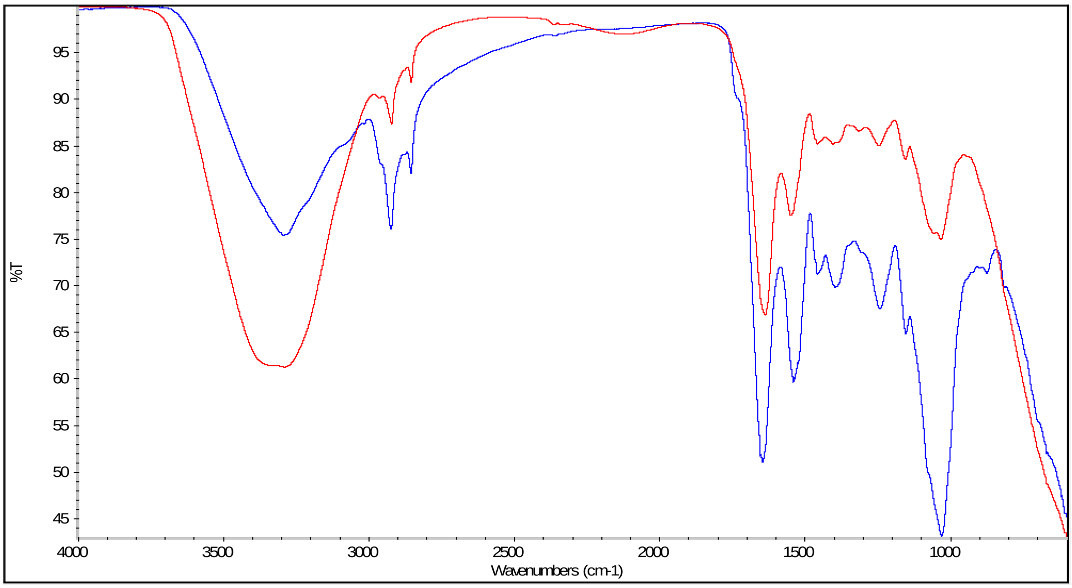


Figure 6.8: FTIR transmittance and wave number before (red) and after adsorption (blue) for *Stichococcus bacillaris*

6.4 RECOVERY OF CADMIUM

6.4.1 Regeneration and re-use of algae

Stichococcus bacillaris was used in three cycles for the removal and recovery of cadmium to check for re-usability. In the first cycle, removal and recovery rate was high at 95.8% and 73.49% respectively, Fig. 6.9. In the sub-sequent cycles, there was a slight reduction in removal and recovery with increase in cycles. In the third cycle, removal was attained at 61.4% and recovery at 50 % an indication that the species studied could actually be re-used efficiently for some period.

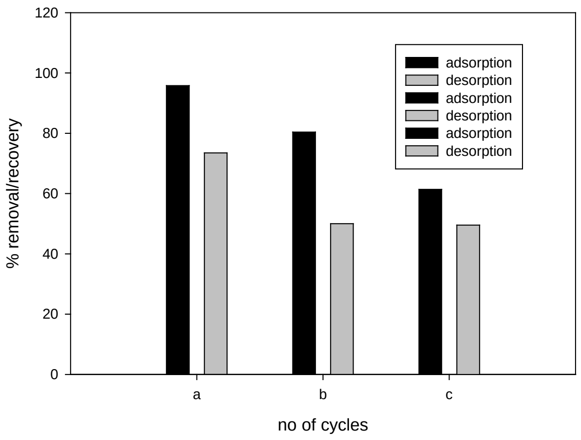


Figure 6.9: Adsorption and desorption cycles for Cd using *Stichococcus bacillaris*, a) 1st cycle, b) 2nd cycle, c) 3rd cycle

6.5 Summary

Chloroidium saccharophilum and *Stichococcus bacillaris* competed favourably with other tested algae and non-algal sorbents with a higher sorption capacity of 128.21 and 125 mg/g. Despite the lower $q_{\max}$ obtained for *Desmodesmus multivariabilis*, the affinity of Cd was high an indication of potential metal recovery. The E values indicated a chemisorption process influencing the adsorption process. Gibbs free energy ΔG showed most negative values implying the reaction was spontaneous. The Langmuir and PSOM models displayed a better fit with a correlation co-efficient (R^2) of 0.97 and 0.99 respectively. The most active functional groups for *Stichococcus bacillaris* were phenols, carboxyl, hydroxyl, alkanes and aliphatic amines. The tested algae showed possibility for re-use of algae in the adsorption/desorption cycles with the highest values attained at 95.8% and 73.49% respectively.

CHAPTER 7

ADSORPTION OF MULTI-METALLICS USING SIMULATED AND ACTUAL WASTEWATER

7.1 BACKGROUND

Industrial wastewater contains a mixture of different heavy metals, metalloids, radionuclides and organic pollutants under varying environmental conditions. The presence of competing ions creates effects on selectivity and affinity of metals for the binding sites which maybe synergistic or antagonistic (Mukhopadhyay, et al., 2011; Fomina and Gadd, 2014). The mutual and inhibitory effects are attributed to the classification of metals. The border-line metals usually belonging to the same class have a higher ionic competition for the binding sites like Cd^{2+}, Ni^{2+}, Zn^{2+} with less competition for metals belonging to different classes, e.g. Pb^{2+} and Cd^{2+}. Actual wastewater also contains light metals like Na^{+}, Ca^{2+}, K^{+}, Mg^{2+} and inorganic chemicals like NaCl, KCl, $MgCl_2$, $CaCl_2$ which bind lightly through electrostatic attraction (Tsezos, et al., 1996; Arief, et al., 2008).

Most reviews in biosorption research on algae showed a tendency to focus on single metallic systems with the aim of finding better adsorbents in the relatively abundant biomass (Davis, et al., 2003; Mehta and Gaur, 2005; Wang and Chen, 2009). These studies are crucial in determining sorption capacity and metal affinity prior to consideration of other biosorption processes. The use of more than one metal in biosorption studies is a more representative system as it reflects the actual wastewater with varying environmental conditions like pH, metal concentration, ionic strength and temperature (Sağ, et al., 2001; Vijayaraghavan and Yun, 2008a; Acheampong, et al., 2010). A few studies have tackled removal and recovery of multi-metallic systems using different biosorbents (Chong and Volesky, 1995; Bakatula, et al., 2014; Tabaraki and Nateghi, 2014). The challenge in multi metallics lies in modelling a system with more than one metal as it becomes complicated as the adsorbent binding site has competing ions (Pagnanelli, et al., 2001; Pradhan and Rai, 2001; Lu, et al., 2008).

Most biomass are capable of metal sequestration but only those with high sorption capacity and selectivity for metals are efficient for use in industrial effluent (Volesky and Prasetyo, 1994). Previous chapters addressed the sorption potential of 6 green algal sorbents from fresh water sources including *Chlamydomonas reinhardtii, Chlorella vulgaris, Desmodesmus multivariabilis, Scenedesmus acuminutus, Stichococcus bacillaris and Chloroidium saccharophilum*. Out of all the tested algae, *Desmodesmus multivariabilis* was found have both a high sorption capacity and ability to recover rare earth elements (Birungi and Chirwa, 2014). In this study, *Desmodesmus multivariabilis* was used to study the removal efficiency of a ternary system of La, Tl and Cd. The effect of using combo biosorbents and actual industrial wastewater were also investigated.

7.2 EMPIRICAL MODELLING OF TERNARY SYSTEM

Industrial wastewater contains a complex system of inorganic and organic pollutants under varying environmental conditions which can best be represented using competitive adsorption models for biosorption equilibrium (Chong and Volesky, 1996; Pagnanelli, 2011; Reynel-Avila, et al., 2011). The Extended Langmuir Model (ELM) and Combined Langmuir and Freundlich model (CLFM) were used to evaluate the experimental data from a ternary metallic system using MATLAB (Version 4). In the ELM, Eq. 3.17 was used and further transformed to represent metals individually into Eqns. 3.18, 3.19 and 3.20 for Tl, La and Cd respectively. Four parameters for the ELM were determined for each metal and these included $q_m, K_{L.Tl}, K_{L.La}, K_{L.Cd}$. The ELM assumes the surface to be homogeneous with the sorbate ions competing for the same binding site (Sağ and Aktay, 2002; Srivastava, et al., 2006). In the CLFM, Eq. 3.21 was used which was also transformed into Eqns. 3.22, 3.23, 3.24 for the 3 metals. Seven parameters q_m, b_{Tl}, b_{La}, b_{Cd}, n_{Tl}, n_{La}, n_{Cd} were determined for each metal. The CLM assumes both homogeneous and heterogeneity nature of binding sites for the competing ions (Sips, 1948; Turiel, et al., 2003).

The results from the extended Langmuir model for multi-metals showed the highest q_{max} for Tl at 77.278 mg/g compared to La and Cd in the order Tl> La> Cd, Table 7.1. The q_{max} for multi-metal system was compared to that of single-metal systems in previous chapters using *Desmodesmus multivariabilis*. The difference in q_{max} for Tl was significant with 909.09 mg/g in single metallics as compared to 77.278 mg/g in multi metallics. The q_{max} for La decreased from 100 in single metallics to 77.307 mg/g in multi-metallics whereas that of Cd from 48.54 to 40.075 mg/g. The lower values in the multi-metallic studies are an indication of competing ions and metal preference for the binding sites. In other studies, also found a lower q_{max} value in multi-metals compared to monometallic studies (Kumar, et al., 2008; Hossain, et al., 2014).

The affinity (K_L) for the different metals were determined and three values each representing a metal were given. The K_L values for Tl was highest in the order of Tl> La> Cd; Lanthanum La>Tl> Cd and Cadmium La> Tl > Cd, table 7.1. Generally, the K_L value for Lanthanum was highest at 0.403 L/g and lowest for Cd at -0.0013 L/g. The higher the K_L value, the greater the affinity of the metal for the biosorbent and possibility of metal recovery (Sağ and Aktay, 2002; Fagundes-Klen, et al., 2007; Romera, et al., 2007).

Table 7.1 ELM parameters for the ternary adsorption of Tl, La and Cd

Metals	$q_{\max}$ **(mg/g)**	K_{Tl} **(L/g)**	K_{La} **(L/g)**	K_{Cd} **(L/g)**	R^2	RMSE
Thallium	77.278	0.2440	- 0.0250	- 0.095	0.99	26.21
Lanthanum	71.307	0.0470	0.4030	0.2160	0.95	69.79
Cadmium	40.075	0.0006	- 0.0013	0.0014	0.99	16.35

The ELM generally performed better with a higher correlation co-efficent of ≤ 0.99 than the CLM with ≤ 0.95, Tables 7.1& 7.2. The RMSE was highest for La in the ELM and highest for Tl in the CLM. The higher the RMSE, the less accurate the results which also confirms that the ELM generally perfomed better than the CLM.

The CLM for multi-metals showed significantly lower $q_{\max}$ values as compared to ELM with the highest value from Tl at 5.089 mg/g, Table 7.2. The highest affinity (b) was 0.445 L/g for Tl but also had the lowest heterogeneity index (n) at -0.758. Cadmium was observed to have the lowest b of -0.356 L/g but the highest n of 0.546. When the n value is less than 1, then the biosorbent surface is heterogeneous, and n=1 implies homogeneous surface (Turiel, et al., 2003; Jeppu and Clement, 2012). All the n values from this study were less than 1, Table 7.2. Generally from both models, it can be noted that Tl and La showed better affinity for the adsorbent compared to Cd. This could be related to the physical-chemical properties, surface properties and solution chemistry which affects biosorption preference for competing metals (Mahamadi and Nharingo, 2010).

Table 7.2: CLF parameters for the ternary adsorption of Tl, La and Cd

Metals	$q_{\max}$ **(mg/g)**	b_{Tl} **(L/g)**	b_{La} **(L/g)**	b_{Cd} **(L/g)**	n_{Tl}	n_{La}	n_{Cd}	R^2	RMSE
Thallium	5.089	0.445	- 0.076	- 0.356	- 0.758	0.076	0.546	0.50	564.35
Lanthanum	4.300	0.083	0.005	- 0.075	0.207	0.011	0.034	0.95	69.79
Cadmium	4.694	0.077	- 0.005	- 0.063	- 0.209	0.017	0.069	0.95	69.79

The ternary system was further evaluated based on the atomic weight, electronegativity, ionic radius, standard reduction potential and electron configuration, Table 7.3. The results showed that an increase in biosorption capacity increased with an increase in atomic weight in the order of Tl> La>Cd. The reduction potential was also highest for Tl with -0.34V, Table7.3. Metals with a higher atomic weight generate higher energy which increases the molar adsorption capacity. A higher reduction potential also increases the interaction of ions between the adsorbate and adsorbent (Tobin, et al., 1984; Sağ, et al., 2001; Iqbal and Edyvean, 2004).

An increase in electronegativity increases the number of available ions for adsorption and a smaller ionic radius increases adsorption capacity (Sağ, et al., 2001). The results did not seem to agree as Tl had a lower electronegativity (1.62) than Cd (1.69) and a higher ionic radius of 1.47^{0}A than the other metals. The differences could be due to other interaction factors, for example, the adsorbent surface was not considered in this comparison. In addition the atomic weight for Tl was significant as compared to other metals which probably played a role in the general adsorption preference for Tl.

Table 7.3: Physical-chemical properties for biosorption preference in multi- metallics

Metals	**Atomic weight**	**Electro-negativity**	**Ionic radius (^{0}A)**	**Standard reduction potential (V)**	**Electron configuration**	**Adsorption capacity ,** q_m **(mg/g)**
Thallium	204.38	1.62	1.47	-0.34	$4f^{14}5d^{10}6s^{2}6p^{1}$	72.278
Lanthanum	138.906	1.1	1.061	-2.52	$5d^{1}s^{2}$	71.307
Cadmium	112.411	1.69	0.97	-0.40	$4d^{10}5s^{2}$	40.075

The biosorption effect on competing metals in the ternary system was also evaluated using biosorption preference factor (Y_i), and the distribution of metals on the biosorbent (θ_i). The equations used for Y_i, Y_t and θ_i were given as Eqn. 3.25, 3.26 and 3.27 respectively. The trend generally showed an increase in Y_i with an increase in initial concentration for Tl and vice versa for Cd, Table 7.4. At lower concentrations of ≤ 20 mg/L, Y_i seemed to favour Cd and La with efficiencies obtained at ≤ 98.4 and 95.7% respectively. This could be due to the smaller ionic radius of Cd and La over Tl which improves sorption efficiency at that level. The relative coverage of metals generally increased with increasing concentration. The distribution of metals on the biosorbent strongly depends on the molar concentration ratio of the solution (Sağ and Aktay, 2002). Tl had the highest θ_i which increased with an increase in initial concentration except at 1000 mg/L, Table 7.4. The reduction in θ_i for Tl could be due the release of Tl ions back into the solution creating some free binding sites for the other 2 metals. The total adsorption preference (Y_t) was generally high and increased with an increase in concentration implying that adsorbent (*Desmodesmus multivariabilis*) is a promising adsorbent for the treatment of actual wastewater.

Table 7.4: Comparison of biosorption preference (Yi) and distribution (Ө) of metasl on the biosorbent

$C_{i.Cd}$ **(mg/L)**	Y_i **(Cd) %**	Y_i **(Tl) %**	Y_i **(La) %**	θ_i **(Cd) %**	θ_i **(Tl) %**	θ_i **(La) %**	Y_t **(%)**
5	98.40	72.740	91.60	26.411	53.692	19.897	79.100
10	96.75	81.88	89.75	24.521	57.070	18.408	85.129
15	92.567	85.7125	94.467	22.017	59.801	18.183	87.841
20	93.175	80.735	95.725	23.729	56.543	19.728	84.654
35	83.386	91.4875	98.843	18.493	63.768	17.740	91.391
80	78.662	94.834	98.756	20.338	59.000	20.663	92.913
120	74.433	96.254	94.063	17.925	63.744	18.331	92.824
250	76.192	96.905	96.726	21.999	55.400	22.601	93.174
500	70.981	92.034	82.449	30.349	41.122	28.528	84.178
1000	73.458	96.449	74.239	39.370	28.431	32.199	81.382

7.3 KINETIC MODELLING OF TERNARY SYSTEM

The kinetics of multi-metallic system for the removal of three metals (La, Tl and Cd) simultaneously using *Desmodesmus multivariabilis* was studied. AQUASIM program (version 2.0), was used for simulation of aquatic systems (Reichert, 1998). The model has four mutually dependant sub-systems which include variables, processes, compartments and links. Input of data into the sub-systems enables model simulation, parameter estimation and sensitivity analysis. The model consists of ordinary and partial differential and algebraic equations which describes the behaviour of important state variables in the system. The advantage of using this model lies in the user friendliness and flexibility to specify transformation processes (Reichert, 1998).

7.3.1 Model Simulation and Parameter Estimation

Experimental data from the ternary system was used for model optimisation and simulation with non-linear least square algorithm using Eqns. (3.28 and 3.29). Parameter estimations were carried out for variables q, K and R_c to determine the most important parameters influencing the prediction. The experimental data for Cd generally showed a good fit to the model with reduction occurring until a stable state was attained with no further adsorption, Fig. 7.1. At lower concentration in the range of 25-50 mg/L of Cd, most of the experimental data showed a good fit with the model especially in a period of 4 hours which had the highest data points, Fig 7.1

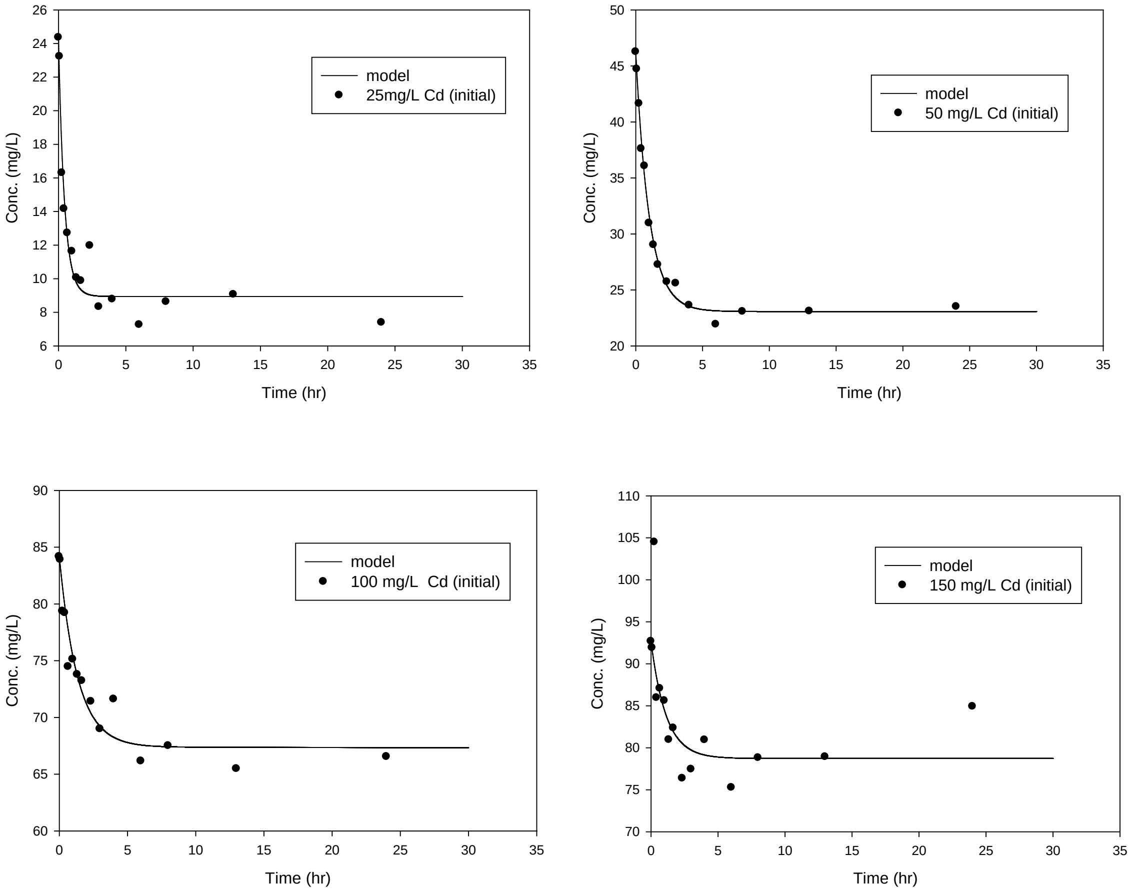


Figure 7.1: Experimental and model validation of cadmium adsorption at initial concentration of 25-150mg/L.

At higher Cd concentration, the measured data slightly scattered away from the model with an increase in time which could be due to the complete saturation of the binding sites. The results are further justified by the parameter estimations of which q clearly indicated that the rate of reaction decreased with an increase in initial concentration, Table 7.5. In addition the rate of reaction occurs fastest in minutes and then slows down to hours until saturation is attained. In most biosorption studies, the rate of reaction is rapid in the first 15-30 minutes due to the abundance in the available active functional groups (Chojnacka, 2010; Oliveira, et al., 2012). The adsorption equilibrium constant, K increased with an increase initial concentration while R_c showed an increase with concentration and then decreased at higher concentration, Table 7.5.

Table 7.5: Parameter estimations for cadmium adsorption

C_0 **(mg/L)**	q **(1/hr)**	K **(mg/L)**	R_c **(mg.g)/(L.m^2)**
25	6.621	0.698	2.801
50	4.805	1.226	4.700
100	2.614	3.222	3.494
150	2.0063	7.456	2.0902

At low concentrations ≤ 50 mg/L of La, the measured data closely fit the model well in the first 5 hrs and then deviated for the rest of the study period, Fig. 7.2. This could be due to competition of distinct ions for same site especially with Tl which showed the highest reduction in all experiments for a given period of time. At higher concentration of La, most of the experimental data fit well with the model, Fig 7.2. The rate of reaction (q) was highest at a lower concentration of 25 mg/L and then significantly decreased with increase in concentration except at 150 mg/L. A sudden increase in q at higher concentrations could be due to fact that Tl reduced and then was desorbed back into the ternary system. This could probably imply that some binding sites became available which increased the rate of reaction in the system. Parameter K was very low at a lower concentration of 25 mg/L and then generally showed a decrease with an increase in concentration ≥ 50 mg/L, Table 7.6.

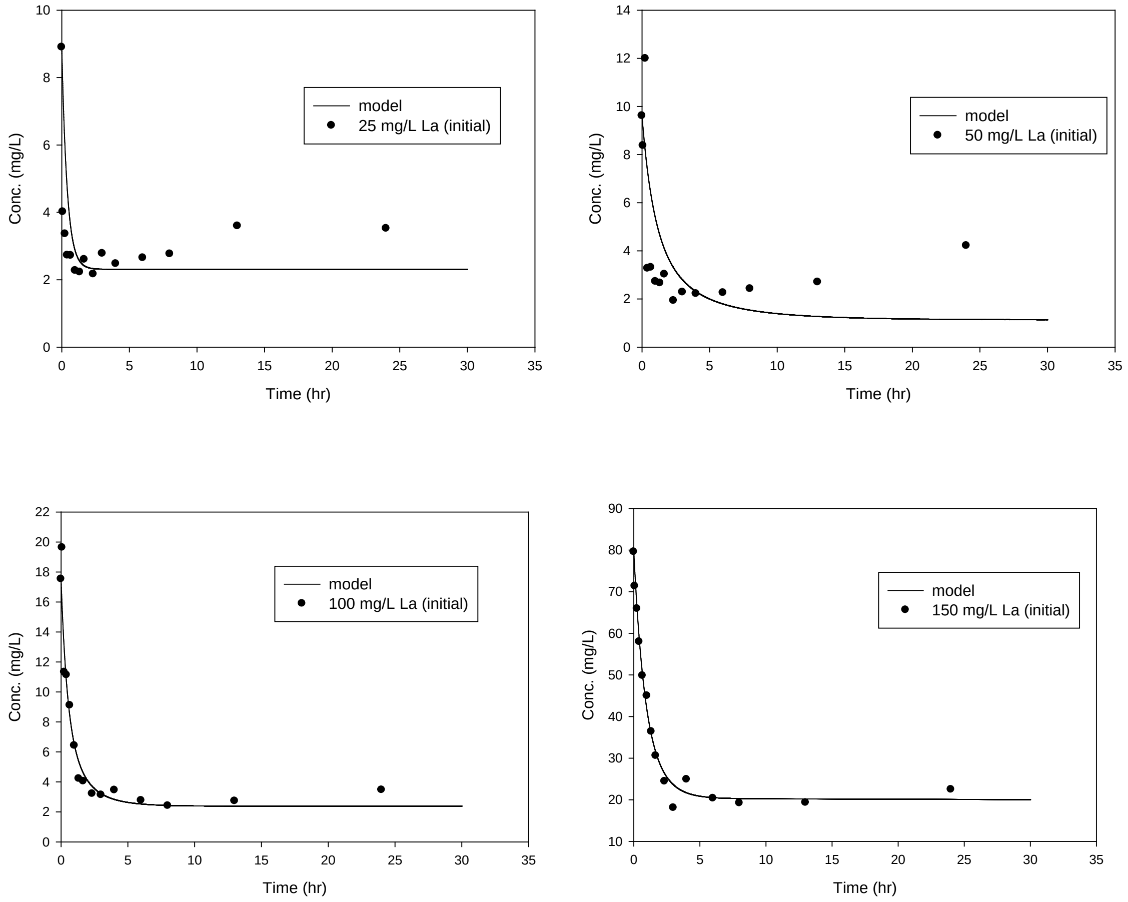


Figure 7.2: Experimental and model validation of lanthanum adsorption at initial concentration of 25-150mg/L

Table 7.6: Parameter estimations for lanthanum adsorption

C_0 **(mg/L)**	q **(1/hr)**	K **(mg/L)**	R_c **(mg.g)/(L.m^2)**
25	9.949	0.00725	3.965
50	1.0987	9.2955	0.6766
100	0.01511	8.9921	0.00608
150	4.055	7.565	3.4583

Higher concentrations in a range of 150- 800 mg/L of Tl were used to study kinetics due to the complete removal attained at lower concentrations ≤ 100 mg/L. The experimental data only relatively fit well with the model for the period studied, Fig. 7.3. For all the initial concentrations used, a significant reduction was attained from ≤ 800 mg/L to ≤ 35 mg/L. An increase in time had an antagonistic effect on Tl as it desorbed back into the system for the period of study. This could imply that probably more time was required to monitor Tl desorption into the system until equilibrium was attained. The q and R_c values increased with an increase in initial concentration, Table 7.7. The K values for the initial concentration were in the same range of 9.8-1.0 which implied that the initial concentration had no significant influence on the equilibrium.

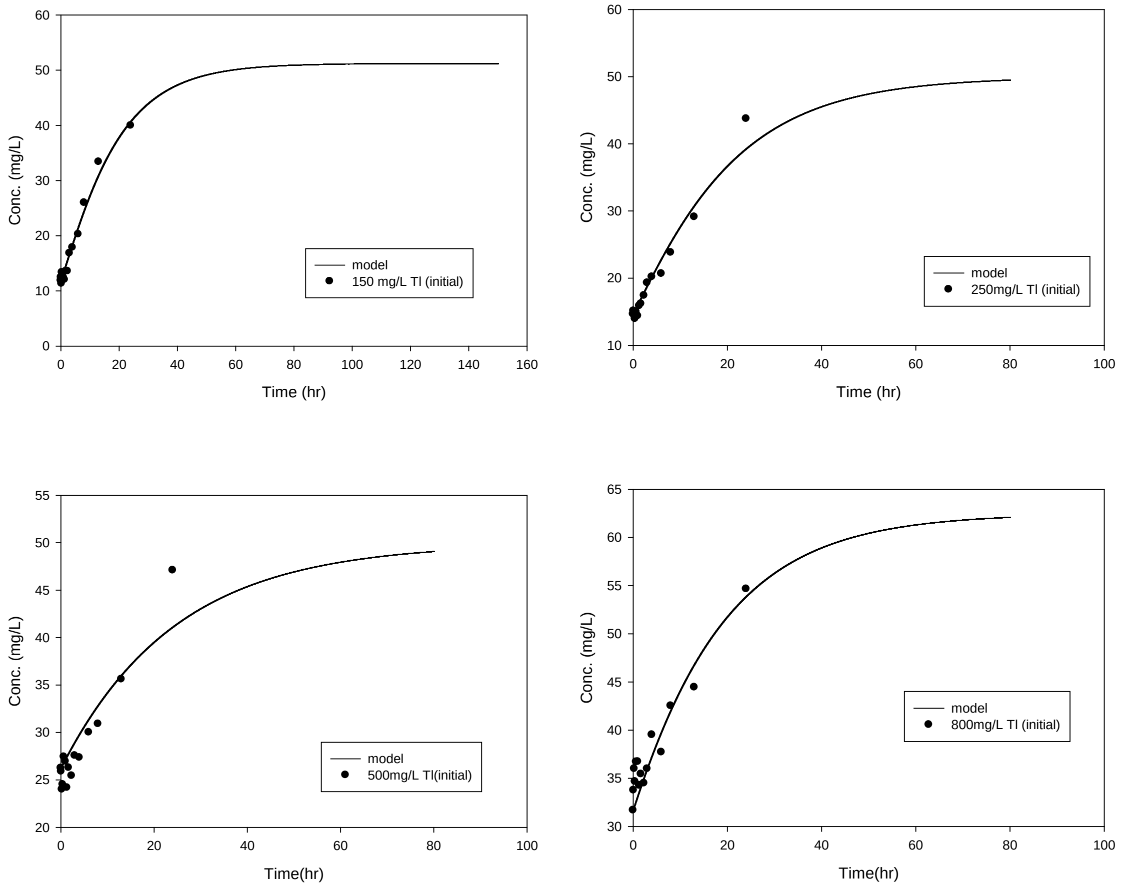


Figure 7.3: Experimental and model validation of Thallium adsorption at initial concentration of 150-800 mg/L

Table 7.7: Parameter estimations for thallium adsorption

C_0 **(mg/L)**	q **(1/hr)**	K **(mg/L)**	R_c **(mg.g)/(L.m^2)**
150	0.000559	9.9071	0.0074002
250	0.000427	9.999	0.006282
500	0.000108	1.0000021	0.00256
800	0.1929	9.8123	2.9686

7.3.2 Sensitivity Analysis

Sensitivity analysis is used for estimation of uncertainty through calculation of linear sensitivity functions of arbitrary variable with respect to selected parameters. The most important sensitivity functions are absolute-relative sensitivity function (measures absolute change in y for a 100% change in p) and relative-relative sensitivity function (measures relative change in y for a change in p) which do not depend on the unit of the parameter (Reichert, 1998). In this study, the absolute-relative sensitivity function was used on parameters q, K and R_c after model validation. The adsorption of Cd showed that parameters q and R_c influenced the biosorption process, Fig. 7.4. Sensitivity for q on Cd was highest in the first 1.47 hours showing a fast reduction and then decreased to a stationery phase after a period of 8.44 hours. Parameter R_c had a significant impact on Cd adsorption as reduction occurred for up to 10 hours before attaining a stationery phase. Uptake of Cd showed no dependency on K as the parameter remained at zero for a period.

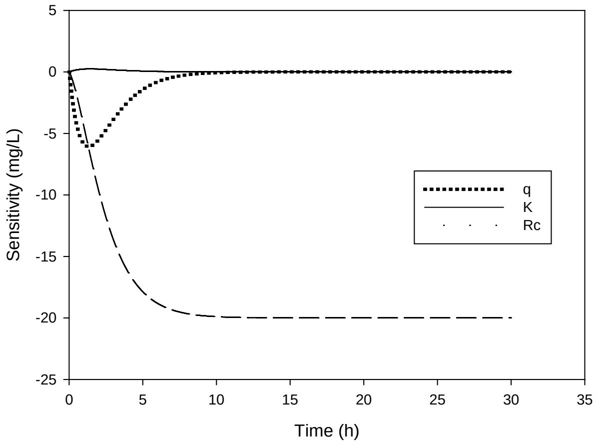


Figure 7.4: Sensitivity test for Cd adsorption on the algal surface at initial concentration of 100 mg/L

A sensitivity test on lanthanum showed that all 3 the parameters q, K and R_c had a significant influence on biosorption. Parameter q showed a significant reduction of La as it remained at zero for some period and then increased to a stationery phase after 2.7 hours, Fig 7.5. K initially showed an increase in uptake of La for a period of 2.05 hours and then decreased to a stationery phase after 11 hours. R_c showed a slight increase in uptake of La for 2.2 hours and then steadily decreased to a stationery phase, Fig. 7.5. The behaviour of the parameters is indicative of the actual processes that occur during biosorption as most adsorption occurs in the first few minutes and then decreases until no further adsorption. The fast reaction could be due to ion exchange and or complexation/coordination of the metallic ions with the anions on the surface of the biosorbents (Veglio and Beolchini, 1997; Fomina and Gadd, 2014).

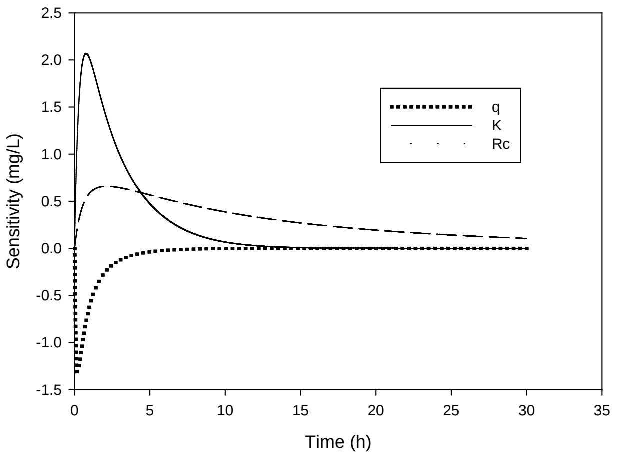


Figure 7.5: Sensitivity test for La adsorption on the algal surface at initial concentration of 100 mg/L

A sensitivity test on Tl showed that parameter q had a minimal effect on the adsorption of Tl as it remained steadily close to zero throughout the period of study, Fig. 7.6. Parameters K and R_c had a significant influence on the removal of Tl. K showed a significant reduction in Tl concentration for a period of 14.2 hours and then desorption occurred until steadiness was attained close to zero. R_c also showed a pronounced reduction for a period greater than 24 hours and then stabilised after about 38 hours, Fig. 7.6.

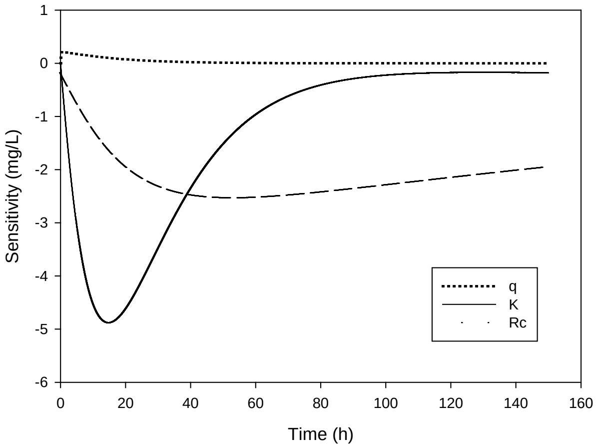


Figure 7.6: Sensitivity test for Tl adsorption on the algal surface at initial concentration of 150 mg/L

7.3.3 Mechanisms in Adsorption Kinetics

The most common technique for understanding the mechanisms involved in the adsorption of molecules from the solution to the sorbent material is the Weber and Moris intraparticle diffusion model (Aguilar-Carrillo, et al., 2006; Boparai, et al., 2011; Tabaraki and Nateghi, 2014). The model assumes that sorption process involves two or three steps; i) surface diffusion which is significant at the initial stage and involves transport from bulk solution to external surface, ii) pore diffusion/intraparticle diffusion which involves transport into the interior of the pores and usually considered the rate limiting step in the adsorption processes (Doğan, et al., 2004; Kavitha and Namasivayam, 2007a) and iii) adsorption of molecules into the interior. A linearised equation, Eq. 3.30 was used to determine the intraparticle diffusion rate constant (K_i) and the intercept (C) in the ternary system of Cd, La and Tl.

When the experimental data is linear and passes through the origin/ zero point, then the intraparticle diffusion is the only rate limiting step (Ozcan, et al., 2007). The linear plot for all the 3 metals did not pass through the zero point an indication that intraparticle diffusion is not the only rate limiting step, Fig. 7.7. The experimental data also showed deviation from the linear plot for Cd and La in two phases which could be due to external mass transfer and intra particle diffusion. Some authors also found a multi-step process dependant on two main phases of adsorption (Gupta, et al., 1990; Bhattacharyya and Sharma, 2004; Aguilar-Carrillo, et al., 2006). The regression co-efficient (R^2) for Cd was better than for other metals with an increase in concentration at ≤ 0.91. It can also be noted that the R^2 for La was more valid at higher concentration range of 100- 150 mg/L (≤ 0.89) while that of Tl was better at lower concentrations (≤ 0.8). Thallium showed a linear plot in the opposite direction reflecting the desorption behaviour of Tl after initial rapid adsorption.

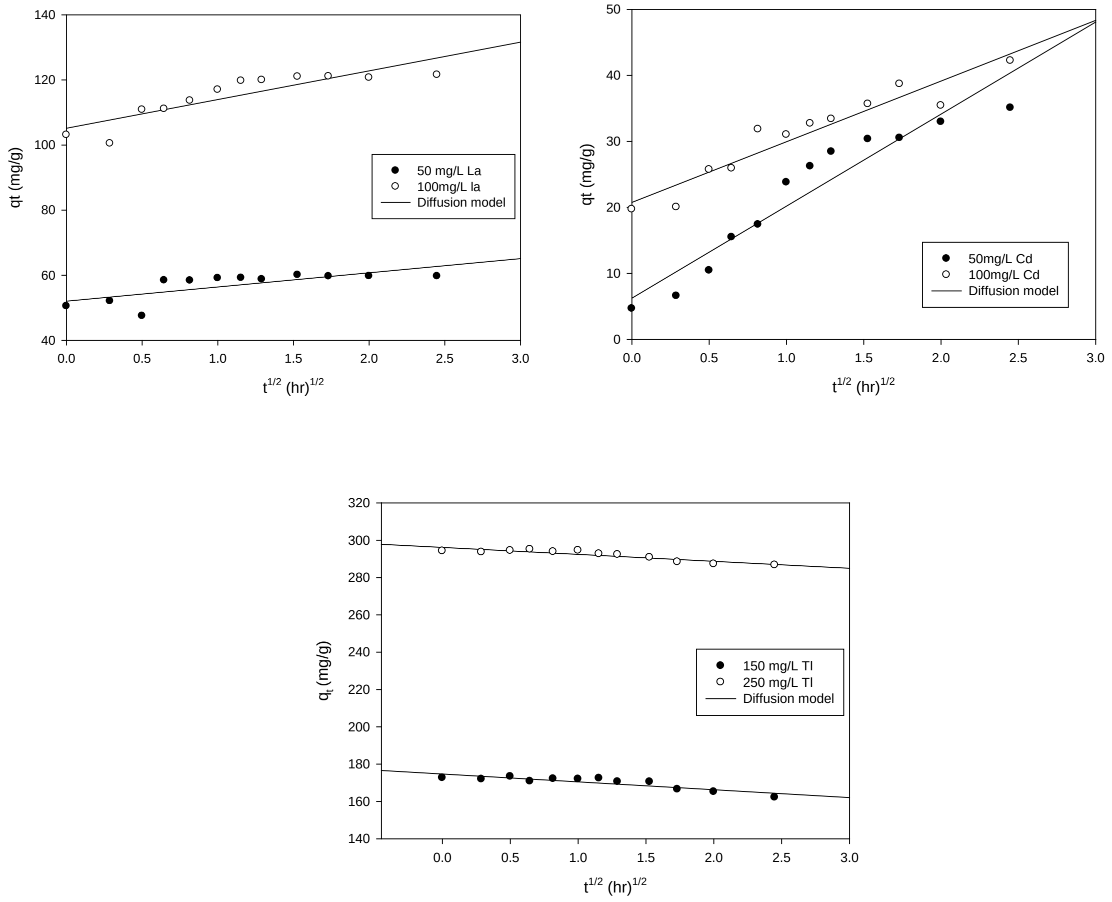


Figure 7.7: The intraparticle diffusion linearised plots for the ternary adsorption of Cd, Tl and La

The intraparticle rate parameters K_i and C generally increased with increase in concentration except for Tl, Table 7.8. It can also be noted that there was a slight reduction of K_i for Cd at 100mg/L but then increased at higher concentration of 150 mg/L. An increase in concentration increases the driving force of adsorption processes (Allen, et al., 1989; Vilar, et al., 2006). The low K_i value of Tl could be attributed to the desorption effect observed with an increase in time. The intercept, C provides information on the thickness of the boundary layer. The larger the intercept, the greater is the boundary layer effect (Mall, et al., 2005; Kavitha and Namasivayam, 2007b). Thallium had the lowest K_i but the largest C which increased with increase in concentration followed by lanthanum in the order Tl> La>Cd, Table 7.8.

Table 7.8: The intraparticle diffusion parameters for ternary adsorption of Cd, La and Tl

Cadmium				Lanthanum				Thallium			
C_i **(mg/L)**	K_i **(mgg-^{1}hr$^{0.5}$)**	C	R^2	C_i **(mg/L)**	K_i **(mgg-^{1}hr$^{0.5}$)**	C	R^2	C_i **(mg/L)**	K_i **(mgg-^{1}hr$^{0.5}$)**	C	R^2
25	8.41	5.33	0.80	**25**	1.92	25.06	<0.7	**150**	4.21	174.72	0.76
50	13.95	6.25	0.91	**50**	4.36	52.04	<0.7	**250**	3.73	296.14	0.82
100	9.19	20.77	0.91	**100**	8.82	105.15	0.77	**500**	1.83	594.14	<0.7
150	11.44	68.35	0.7	**150**	34.49	93.98	0.89	**800**	2.581	958.3	<0.7

In order to assess the validity of the diffusion model, a mass transfer model was used. The model is given by the linearised equation, Eq. 3.31. A plot of $\ln\left(\frac{c_t}{c_o} - \frac{1}{1+mK}\right)$ vs. t was used to determine the slope from which the surface mass transfer co-efficient (B_L) was calculated. The results indicated deviation of the experimental data from the linearised model with a double phase, Fig. 7.8. The R^2 for Cd relatively showed a better fit for all tested initial concentrations. Tl showed a better R^2 at lower concentrations (≤ 0.89) whereas R^2 for La was better at higher concentration. An increase in concentration increases the driving force of adsorption processes (≤ 0.75). The B_L indicates the velocity of mass transfer and was highest for Cd at lower initial concentration of 25 mg/L at 0.013ms^{-1} but decreased with an increase in concentration. The B_L value for La and Tl had the same magnitude at lower concentration (25 mg/L) of 0.007 ms-1 but then an increase in concentration increased the B_L for La and decreased for Tl, Table 7.9. Generally the results both show two possible phases and do pass through the origin implying that intraparticle diffusion is not the only rate limiting step in the reaction.

Table 7.9: The mass transfer parameters for ternary adsorption of Cd, La and Tl

Cadmium			Lanthanum			Thallium		
C_i **(mg/L)**	B_L (ms^{-1})	R^2	C_i **(mg/L)**	B_L (ms^{-1})	R^2	C_i **(mg/L)**	B_L (ms^{-1})	R^2
25	0.013	0.70	**25**	0.007	<0.70	**150**	0.007	0.89
50	0.009	0.78	**50**	0.018	<0.70	**250**	0.006	0.88
100	0.003	0.77	**100**	0.024	0.73	**500**	0.002	<0.70
150	0.003	<0.70	**150**	0.018	0.75	**800**	0.002	<0.70

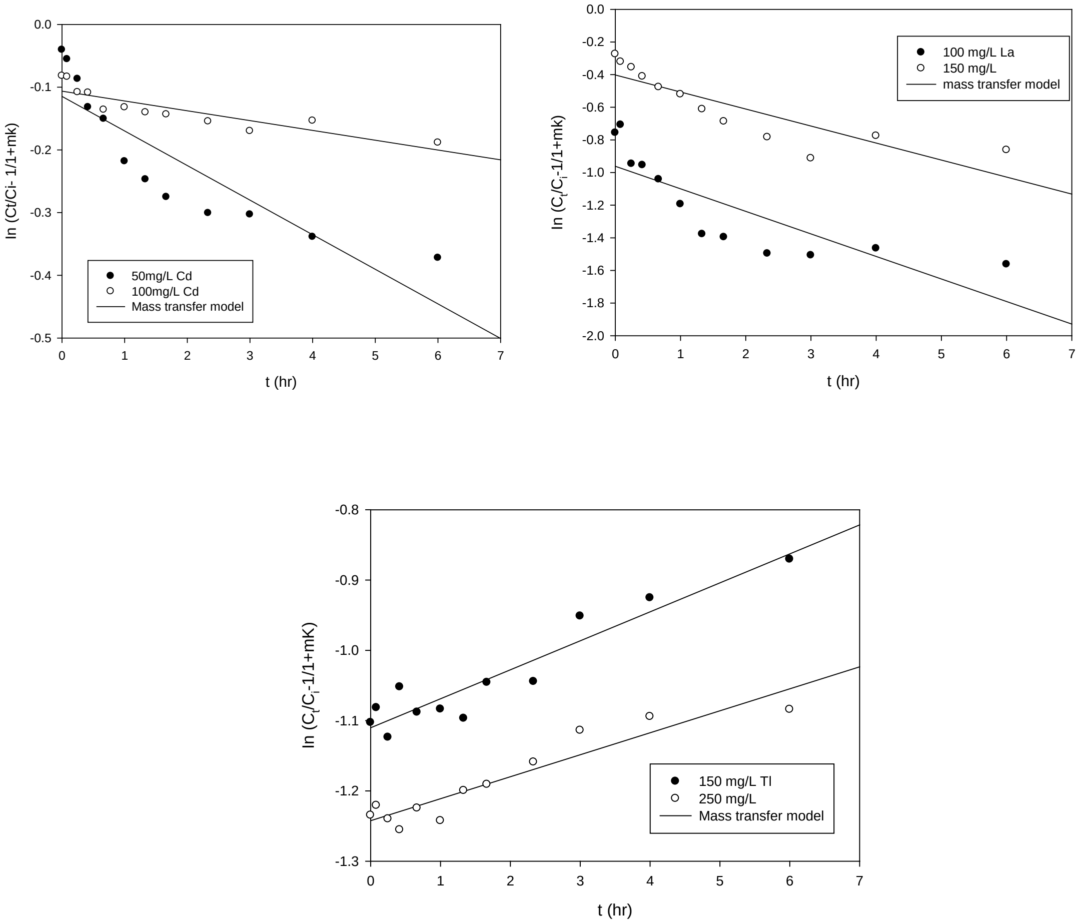


Figure 7.8: The mass transfer linearised plots for the ternary adsorption of Cd, Tl and La

7.4. EFFECT OF ENVIRONMENTAL FACTORS

7.4.1 Effect of Ionic Strength on Ternary System

The effect of ionic strength on the uptake of Tl, La and Cd was tested using a low and high molarity of sodium (Na^+) standard solution. At low levels of 0.65M Na^+, uptake of heavy metals significantly decreased with the highest value attained for Tl at 46.34%. La and Cd uptake was attained at the same efficiency of 35%. At higher levels of 4.35M Na^+, the sorption efficiency slightly improved for Tl at 52.61% and La at 38.98%, Fig.7.9 a. It can also be noted that at low molarity of Na^+, uptake of Na^+ was achieved at 100% which had a significant impact on other metals due to competition for the same adsorbent sites. (Schiewer and Volesky, 1997) also found that an increase in ionic strength of Na^+ reduces the uptake of heavy metals due to the electrostatic effect.

A mixture of different light and heavy metals was also studied at the same molarity. At the molar concentration of 0.65M, 100% removal rate was achieved for K^+, Na^+ and Mg^{2+} except Ca^{2+} (60.67%). The removal rate of Tl, La and Cd was generally low at 53.9, 47.5 and 47.995% respectively, Fig. 7.9b. When the concentration of light ions was increased to 4.35M, Na^+ and Mg^{2+} still showed removal efficiency of 100% with a slight reduction for K^+ at 90.78% and slight increase for Ca^{2+} at 84.8%. The removal of heavy metals was still lower at ≤ 43% for all metals. Other studies also showed a similar trend with a general decrease of heavy metal uptake when the ionic strength of light metals is increased due to metal activity change (Komy, et al., 2006; Ghodbane, et al., 2008). Bivalent ions are assumed to have a stronger displacing effect than monovalent cations (Wilke, et al., 2006). This does not necessarily apply to this study and could be due to variations in the nature of biomass and availability of surface ligands.

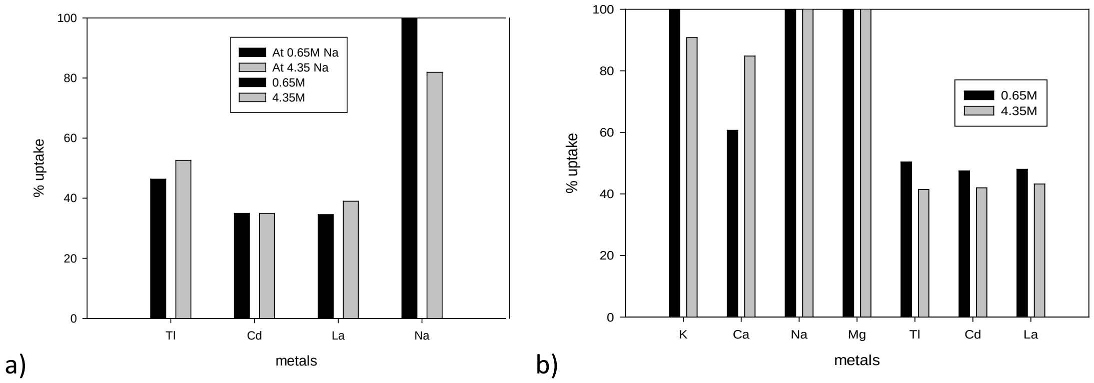


Figure.7.9: Effect of ionic strength on uptake of Tl, La and Cd, a) using Na+ only. b) combination of light metals

7.4.2 Effect of Initial Concentration on Ternary System

At initial concentration of 250 mg/L of Tl and 50 mg/L of La and Cd, reduction was significant at time (t_0) for Tl with 11.88 mg/L left in solution using *D. multivariabilis*. (Lu, et al., 2013) noted that the initial metal concentration provided a driving force to overcome mass transfer resistances between the adsorbent and adsorbate. It can be noted that Tl concentration was higher than other metals due to the higher atomic weight which improves biosorption efficiency. It was observed that an increase in time seemed to slightly increase Tl at 33.39 mg/L left in solution after a period of 13 hrs.

Lanthanum was reduced from 50mg/L to 9mg/L at the beginning of the experiment (t_0) and further reduction with time until equilibrium was attained at 2.7 mg/L after a period of 13 hrs. Initial reduction for Cd at t_0 was lowest at 45.14 mg/L and the value was still high after the same period of 13 hours at 22.59 mg/L, Fig. 7.10.a. When the initial concentration for Tl, was doubled from 250 to 500 mg/L, reduction was still very significant with 26.255 mg/L left in solution at t_0. There was minimal fluctuation of Tl with increase in time and no significant difference after 13 hrs. For the case of La and Cd, when the initial concentration was increased from 50 to 100 mg/L, La showed no difference with same value of 2.7mg/L left in solution after a period of 13 hours. Cd reduction was still the lowest with a significant concentration of 65mg/L left in solution, Fig. 7.10b. (Kwan and Smith, 1991) also found an inhibitory effect of Cd uptake with an increase in the concentration of Tl and La. This could be due to the different physical-chemical properties of these metals which influences the metal affinity for the adsorbent.

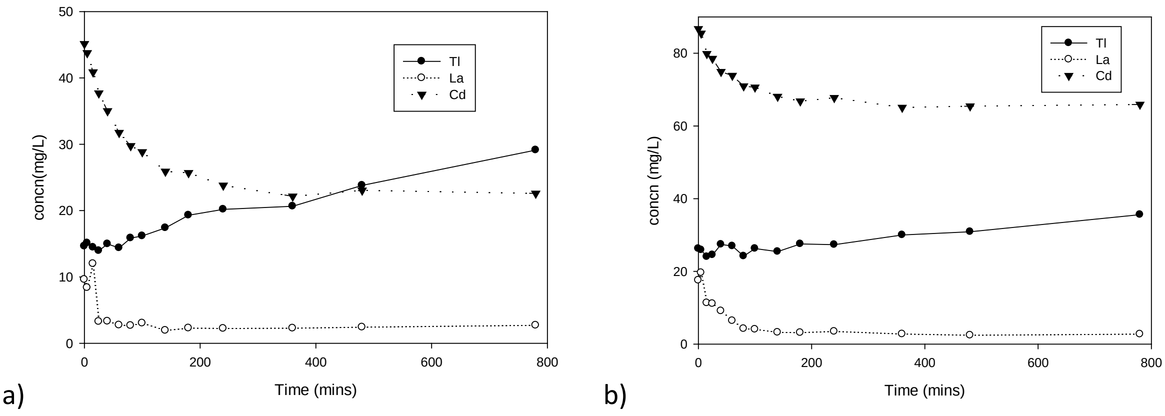


Figure 7.10: Effect of initial concentration with time in the ternary system of Tl, La and Cd; a) 50 mg/L Cd, La and 250 mg/L Tl, b) 100 mg/L Cd, La and 500 mg/L Tl

7.4.3 Effect of pH on Ternary System

The effect of pH on adsorption for mixed system of Tl, La and Cd was studied. At very low pH of 2, initial uptake of Tl was attained at 83.67%, Fig.7.11. At low pH, presence of excess H+ inhibits uptake of metal ions in solution due to competition (Ahmaruzzaman, 2011). Sorption efficiency for Tl increased with increase in pH upto 96.42% at pH of 6. The experiment was carried out at pH $\geq$ 8 as precipitation tends to occur at higher pH. At pH of 8, there was a slight reduction in uptake of Tl to 94.07% which could be due to beginning of precipitation. Lanthanum showed no removal at very low pH of 2 but an increase in pH improved sorption efficiency with maximum uptake attained between pH of 6 and 8 at 77.53

and 92.2 respectively. The uptake of Cd was generally low from pH of 2-5 then increased from pH of 5-8 with a significant rise at pH 8 of 70%, Fig. 7.11.Other studies also showed that an increase in pH increases the uptake of multi-metals under limited condition like constant temperature and biomass (Guo, et al., 2008; Hossain, et al., 2014). Generally the optimum pH for the 3 metals was found to be between 6 and 8. (Kwan and Smith, 1991) also found the optimal pH of Tl, Cd and La to be 6. The optimal pH varies from one species to another due to different solution chemistry and adsorption sites (Bakatula, et al., 2014).

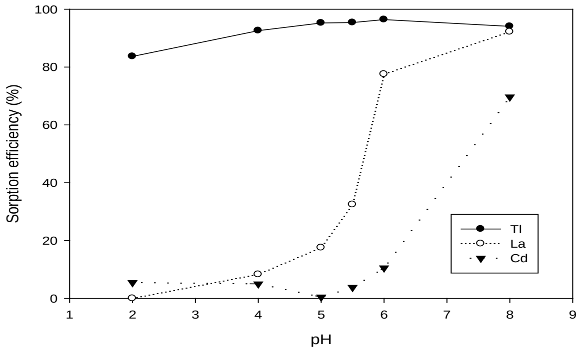


Figure 7.11: The effect of pH on the uptake of metals in a ternary system

7.5 RATE OF REMOVAL/RECOVERY IN TERNARY SYSTEM

The ability of the algal sorbent, *D. multivariabilis* was tested for the adsorption/desorption efficiency of a ternary system. Initial concentration of 250 mg/l Tl and 50mg/L of La and Cd was used. Lanthanum showed the highest removal and recovery at 94.04 and 90.92% respectively. The removal rate for Tl was also relatively high at 83.84% but recovery was very low at 23.77%. The removal rate of La was higher than for Tl and this could be due to the effect of contact time. An increase in time tends to release some adsorbed Tl back in solution therefore some binding sites become available for other metals. In addition the low recovery of Tl could be due the short time allocated for desorption studies. Despite the low uptake of Cd attained at 52.98%, recovery was relatively high at 90.15%, Fig. 7.12.

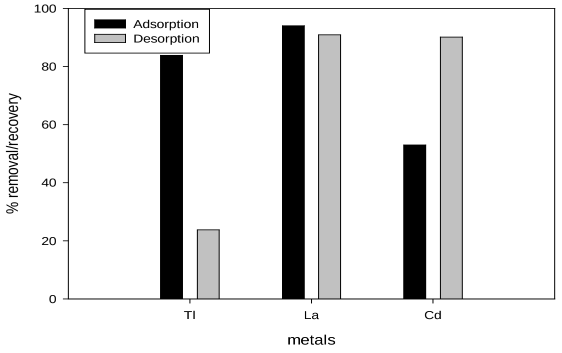


Figure. 7.12: Adsorption/Desorption efficiency for Tl, La and Cd using *D. multivariabilis*

7.6 COMPARISON OF METAL UPTAKE USING COMBO BIOSORBENTS

A mixture of different algal species was tested for their sorption efficiency at a constant biomass and initial concentration of Tl, La and Cd. *C. saccharophilum* and *D. multivariabilis* showed the highest uptake for Tl at 378.93mg/g and La at 164.67mg/g. A mixture of *C. vulgaris* and *D. multivariabilis* was second in position with an uptake of 372.88 and 151.38mg/g for Tl and La respectively. For the removal of Cd, a mixture of *C. reinhardtii* and *D. multivariabilis* had the highest uptake attained at 99.183mg/g. When all the four species were used for adsorption, the uptake reduced significantly to 187.58, 59.48 and 66.08 mg/g for Tl, La and Cd respectively, Fig. 7.13. Ahluwalia and Goyal, 2007 also suggested the removal efficiency of a variety of metals could be improved by use of combo biosorbents.

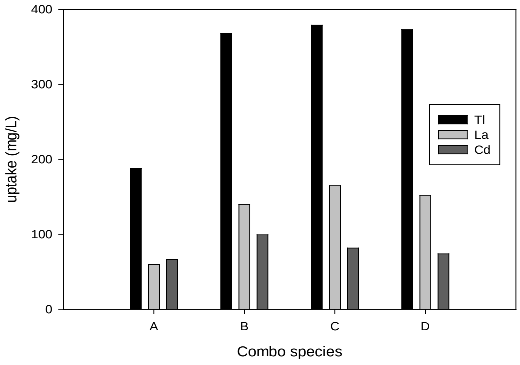


Figure. 7.13: Uptake of metals in ternary system using combo biosorbents; **A**- *C. reinhardtii*, *D. multivariabilis*, *C. saccharophilum* & *C. vulgaris* ., **B**- *C. reinhardtii* & *D. multivariabilis*., **C**- *C. saccharophilum* & *D. multivariabilis*., **D**- *C. vulgaris* & *D. multivariabilis*

7.7 TREATMENT OF ACTUAL INDUSTRIAL WASTEWATER

Industrial wastewater samples were subjected to some tested algal species to check for feasibility of application in the removal of toxic metals. Various metals including zinc (Zn), copper (Cu), iron(Fe), manganese (Mn), magnesium (Mg), calcium (Ca), lead (Pb), cobalt (Co), sodium (Na) , Potassium (K), nickel (Ni), Tl and La were tested in the industrial sample before carrying out adsorption studies. Only metals with pronounced initial concentration were considered for comparison of rate of removal using different species. Four species were tested for their ability to remove and or reduce the concentration of metals in the industrial wastewater.

Wastewater from the mine beneficiary plant showed the highest heavy metal concentration in Mn which was best reduced from 0.36 to 0.02mg/L by *D. multivariabilis*. Fig. 7.14. *S. acuminutus* removed the highest Zn concentration from 0.0561 to 0.003 mg/L. Cobalt was completely reduced to zero from an initial concentration of 0.316 mg/L by *C. vulgaris* and

D. multivariabilis. Wastewater from mine beneficiary plant had Na as the highest light metal concentration which was most removed by *C. vulgaris.* and *D. multivariabilis*, Fig. 7.14

Industrial water from smelter clave tailings showed Tl as the highest heavy metal concentration of 2.386 mg/L which was better reduced to 0.021 and 0.053 mg/L using *C. vulgaris.* and *D. multivariabilis* respectively, Fig. 7.15. Zinc was also greatly reduced from 1.37 to 0.02 mg/L by *D. multivariabilis*. Sodium was found in significant quantities and was highly reduced from 3740.30 to 2237.9 mg/L by *D. multivariabilis*. Potassium was reduced significantly by *C.reinhardtii* from 1036 to 744.7 mg/L, Fig. 7.15

In the smelter return water, Tl was found to have the highest concentration of 2.302 mg/L which completely removed by *C.vulgaris*, Fig.7.16. Zinc was the lowest concentration in this industrial sample and generally no significant difference in the rate of removal for all the algal species. For the light metals, Na was best removed using *C.reinhardtii* and least removed by *C.vulgaris*, Fig. 7.16. Calcium was relatively low compared to other light metals and no pronounced difference in the removal among all the algal species.

Chromium was analysed separately as it was found in significantly higher concentration compared to other heavy metals except for mine beneficiary water. This could be due to the fact that some samples were collected from chrome mine expected to have high Cr concentration. In the smelter clave tailings, Cr was greatly reduced from 78.365 to 43.47 and 38.14 mg/L using *S. acuminutus* and *D. multivariabilis*, Fig. 7.17. Smelter return water had the highest level of Cr at 135.56 mg/L and was best reduced to 45.67mg/l by *C.vulgaris*, Fig.7.17.

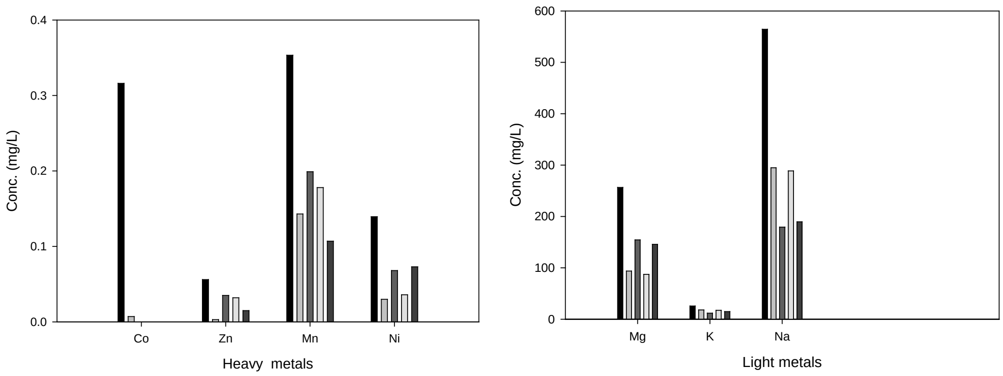


Figure 7.14: Treatment of industrial wastewater from mine beneficiary plant using 4 algal species

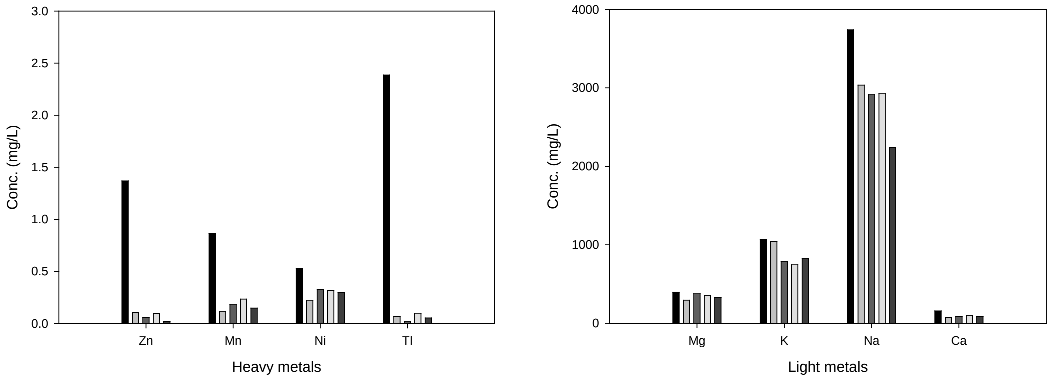


Figure 7.15: Treatment of industrial wastewater from smelter clave tailings using 4 algal species

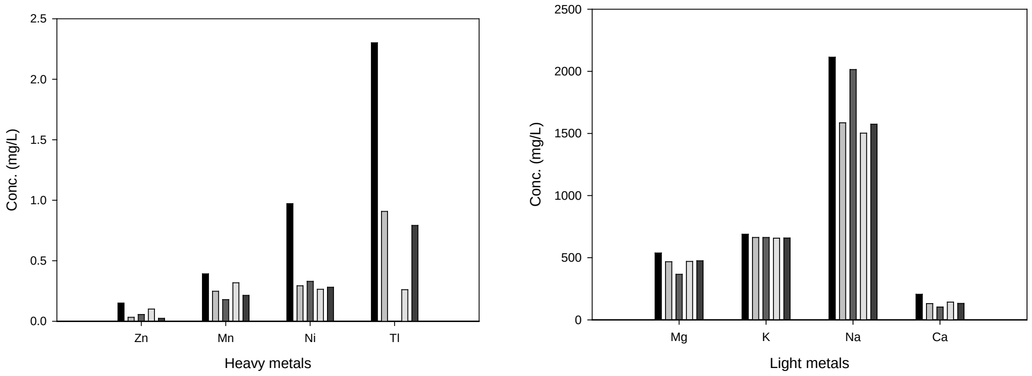


Figure 7.16: Treatment of industrial wastewater from smelter return water using 4 algal species

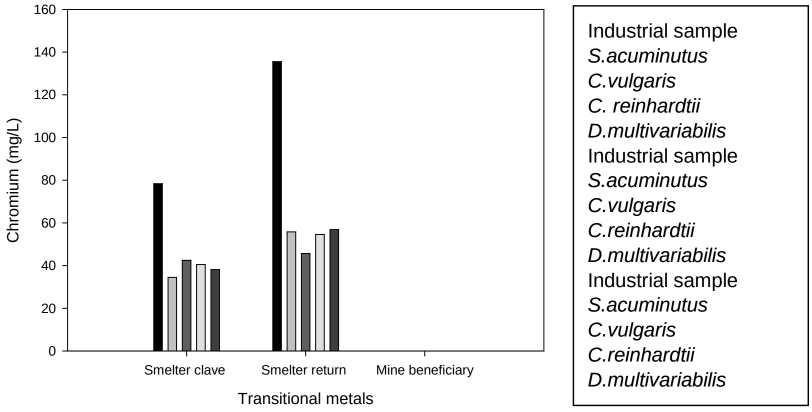


Figure 7.17: Treatment of chromium from smelter clave tailings and smelter return water using 4 algal species

7.8 SUMMARY

The competitive adsorption models represented the ternary system well providing information of the adsorption capacity and affinity of the metals on the biosorbent. Thallium generally showed the highest $q_{\max}$ and affinity b followed by lanthanum. The experimental data from the kinetic studies relatively fit well the predicted model in AQUASIM. Intraparticle diffusion was not found to be the only rate limiting step as most experimental data did not pass through the origin or zero point and 2 phases were found to be the controlling steps. Environmental factors were found to influence the adsorption process, for example, an increase in ionic strength of Na^+ reduced the uptake of heavy metals due to electrostatic effect.. Very low and high pH seemed to have a negative effect on adsorption with the optimu found pH 6. Recovery of the heavy metals by *D. multivariabilis* was achieved highest for La at 90.92% followed by Cd at 90.15%. Combo biosorbents showed the ability to improve sorption efficiency with a mixture of *C. saccharophilum* and *D. multivariabilis* found to be the best compared to other tested combos. The tested algal sorbents showed potential for the treatment of actual wastewater with the best adsorbent performance from *C. vulgaris.* and *D. multivariabilis*.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 SUMMARY AND CONCLUSIONS

Micro-algae consist of a diversity of algal species mostly found in freshwater bodies. In most cases, algae is considered as a nuisance as it forms algal blooms on the surface of water bodies causing eutrophication. The algal samples were collected from a eutrophied dam as waste biomass and utilized for biosorption potential. These species are environmentally friendly and relatively cheap due to their relative abundance.

The study was successful in isolating and identifying 4 algal species which are relatively new in biosorption studies especially for the selected metals. The species were found to have 95-98% identities and included *Desmodesmus multivariabilis*, *Chloroidium saccharophilum*, *Stichococcus bacillaris* and *Scenedesmus acuminutus*. These species were able to attain fast growth rate under controlled laboratory conditions.

All the species tested including *Chlorella vulgaris* and *Chlamydomonas reinhardtii* obtained from CCAP showed potential for removal and recovery of heavy metal and rare earth elements in single metallic systems. The level of efficiency varied with the highest removal being ≤ 70% at lower concentrations of ≥ 25 mg/L for Cd and La. The removal of Tl was unbelievably significant with 100% removal for concentrations ≤ 100 mg/L. Equilibrium models tested generally showed a better fit for Langmuir model with a regression coefficient of ≥ 0.9 compared to Freundlich and D-R models.

Environmental factors like pH, initial metal concentration, temperature were tested for their effect on biosorption potential. The results confirmed that these factors actually influence the sorption efficiency, for example, at low and high pH sorption reduces and generally increases at optimum pH between 5 and 6, sorption capacity also generally reduces with an increase in initial concentration.

The most active functional groups responsible for most binding were carboxyl, phenols, hydroxyl and amines. This implies that ion exchange was one of the main mechanisms involved in the biosorption processes in addition to physical adsorption. Other possible mechanisms included coordination/complexation and precipitation.

Generally all the algal species showed the highest metal uptake in the first 15-30 minutes, a process common in biosorption process followed by a slow uptake in kinetic studies. The Pseudo-second order fitted best for all the single metallic studies with R^2 of 0.9 than first order and Elovich models for all the algal species.

Generally, the study aimed at selecting species with both a high sorption capacity and recovery. In the study for removal and recovery of La, *Chloroidium saccharophilum* and *Chlamydomonas reinhardtii* had the highest q_{max} but *Desmodesmus multivariabilis* was the best adsorbent as it had both a high q_{max} of 100 mg/g and affinity b of 4.56 L/g. Recovery and re-use studies for *Desmodesmus multivariabilis* also had the highest recovery for La at 99.63% implying possible recycling of the biosorbent.

In the adsorption studies on single metals in the removal and recovery of Tl, *Chlamydomonas reinhardtii* and *Chloroidium saccharophilum* had the highest q_{max} and b of 1000mg/g and 1.776 L/g. Generally, it can be noted that all the tested species had a significant q_{max} for Tl removal compared to some other adsorbents in literature.

Chloroidium saccharophilum and *Stichococcus bacillaris* had significantly higher q_{max} for the removal of Cd but *Desmodesmus multivariabilis* had the highest affinity of 1.49 L/g. It can be noted that all the algal species showed adsorption potential and possible re-use of biomass. The study aimed at selecting species with both high uptake and recovery of the tested metals. Therefore, *Chloroidium saccharophilum* and *Desmodesmus multivariabilis* were generally the best adsorbents based on the intended use of the species.

In the ternary system, the extended Langmuir model performed better than the combined Langmuir model for the removal of Tl, La and Cd. The q_{max} values in the ELM was in the order of Tl > La > Cd. The q_{max} values from multi-metallics were much lower than the value for single metallics for all tested metals which implied competition for the binding sites. The higher the K_L value, the greater the affinity of the metal for the biosorbent which was generally highest for Tl and La. It can be noted that atomic weight of these metals partially contributed to higher sorption capacity. An increase in atomic weight increases the energy of ions hence improving the sorption efficiency. The biosorption preference factor for the ternary system increased with an increase in metal concentration for Tl and vice versa for Cd.

To better represent actual industrial effluents, equilibrium competitive models were used. Experimental data from multi metallic kinetic studies showed a relatively good fit with the predicted model in AQUASIM. The rate of reaction was highest in the first 30 minutes at lower concentration of ≤ 25 mg/L and reduced with an increase in concentration for La and Cd.

The sensitivity analysis showed that parameters q (rate of reaction) and K (adsorption equilibrium constant) generally influenced the adsorption process of Cd and La. A mixture of light and heavy metals reduced sorption efficiency due to competiting cations for the binding sites, e.g. ionic strength effect was observable due to electrostatic effect, pH and initial concentration also influenced sorption capacity. Chemisorption and or intraparticle diffusion are some of the rate limiting steps in the reaction kinetics.

The use of combo biosorbents greatly improved sorption efficiency for the adsorption of ternary metal system with *Desmodesmus multivariabilis* and *Chloroidium saccharophilum* emerging as the best. In the treatment of actual wastewater, the tested biosorbents showed great potential with the best performance from *Chlorella vulgaris* and *Desmodesmus multivariabilis*.

8.2. RECOMMENDATIONS

Based on the findings from the this study, the following outlines are recommended for further research;

- There is need for further investigation into the actual mechanisms involved in biosorption in addition to the proposed ones of ion exchange mechanism, physical adsorption, coordination/complexation and precipitation.

- There is need for further research into understanding the behavior of Tl as it significantly reduces in the first few minutes and then desorbs back into the system with time.

- It is important to determine the effect of optimizing agents for pH like NaOH and HCl which could probably have an effect on biosorption process. In equilibrium experiments, pH is kept constant by using an alkali or acid and yet these ions were found to reduce sorption capacity due to competition.

- There is need to carry out more research on recovery and re-use of biomass in competing metal systems.

- In the kinetic modelling of multi-metallics, the system contained excess Tl than the counterpart metals of La and Cd. The model is likely to over predict the degree of uptake inhibition. It is therefore important to test higher equimolar concentrations for the multi-metallics with Tl and check the effect on the model.

- In order to better understand the unique behaviour of Tl adsorption, more time is required for kinetic studies such that equilibrium is attained after desorption of this metal back into solution.

- Before further application into continuous or fixed columns, there is need to improve the robustness of the free micro algae cells. Generally free cells have a lower mechanical strength which can be improved by using immobilizing agents. Most of the available immobilizing agents are costly which contradicts with the main advantage of biosorption. There is therefore need to investigate on finding cheaper immobilizing agents.

- There is need to move from laboratory trials to actual industrial application through pilot scale testing. This can be done concurrently with cost evaluation of capital costs and operation and maintenance costs (O&M) which can provide an estimate of actual costs involved in biosorption.

BIBLIOGRAPHY

Acheampong, M.A., Meulepas, R.J., Lens, P.N., 2010. Removal of heavy metals and cyanide from gold mine wastewater. Journal of chemical technology and biotechnology 85, 590-613.

Aguilar-Carrillo, J., Garrido, F., Barrios, L., García-González, M.T., 2006. Sorption of as, cd and tl as influenced by industrial by-products applied to an acidic soil: Equilibrium and kinetic experiments. Chemosphere 65, 2377-2387.

Ahalya, N., Ramachandra, T., Kanamadi, R., 2003. Biosorption of heavy metals. Res. J. Chem. Environ. 7, 71-79.

Ahluwalia, S.S., Goyal, D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour. Technol. 98, 2243-2257.

Ahmady-Asbchin, S., Andres, Y., Gérente, C., Le Cloirec, P., 2008. Biosorption of cu (II) from aqueous solution by fucus serratus: Surface characterization and sorption mechanisms. Bioresour. Technol. 99, 6150-6155.

Ahmaruzzaman, M., 2011. Industrial wastes as low-cost potential adsorbents for the treatment of wastewater laden with heavy metals. Adv. Colloid Interface Sci. 166, 36-59.

Ahmed, S., Chughtai, S., Keane, M.A., 1998. The removal of cadmium and lead from aqueous solution by ion exchange with na □Y zeolite. Se
13, 57-64.

Akhtar, N., Iqbal, M., Zafar, S.I., Iqbal, J., 2008. Biosorption characteristics of unicellular green alga< i> chlorella sorokiniana</i> immobilized in loofa sponge for removal of cr (III). Journal of Environmental Sciences 20, 231-239.

Akhtar, N., Saeed, A., Iqbal, M., 2003. Chlorella sorokiniana immobilized on the biomatrix of vegetable sponge of luffa cylindrica: A new system to remove cadmium from contaminated aqueous medium. Bioresour. Technol. 88, 163-165.

Aksu, Z., 2001. Equilibrium and kinetic modelling of cadmium (II) biosorption by C. vulgaris in a batch system: Effect of temperature. Separation and Purification Technology 21, 285-294.

Aksu, Z., Açikel, Ü., 2000. Modelling of a single-staged bioseparation process for simultaneous removal of iron (III) and chromium (VI) by using< i> chlorella vulgaris</i>. Biochem. Eng. J. 4, 229-238.

Al-Asheh, S., Duvnjak, Z., 1998. Binary metal sorption by pine bark: Study of equilibria and mechanisms. Sep. Sci. Technol. 33, 1303-1329.

Allen, S., McKay, G., Khader, K., 1989. Intraparticle diffusion of a basic dye during adsorption onto sphagnum peat. Environmental Pollution 56, 39-50.

Allen, S.J., Brown, P.A., 1995. Isotherm analyses for single component and multi-component metal sorption onto lignite. Journal of Chemical Technology and Biotechnology 62, 17-24.

Alloway, B., Steinnes, E., 1999. Anthropogenic Additions of Cadmium to Soils. In: Anonymous Cadmium in Soils and Plants. Springer, , pp. 97-123.

Amuda, O., Giwa, A., Bello, I., 2007. Removal of heavy metal from industrial wastewater using modified activated coconut shell carbon. Biochem. Eng. J. 36, 174-181.

Andersen, R., Berges, J., Harrison, P., Watanabe, M., 2005. Recipes for freshwater and seawater media. Algal culturing techniques.Elsevier, Amsterdam 429-538.

Andres, Y., Texier, A., Le Cloirec, P., 2003. Rare earth elements removal by microbial biosorption: A review. Environ. Technol. 24, 1367-1375.

Antón, M.A.L., Spears, D.A., Somoano, M.D., Tarazona, M.R.M., 2013. Thallium in coal: Analysis and environmental implications. Fuel 105, 13-18.

Aoyama, H., Yoshida, M., Yamamura, Y., 1988. Induction of lipid peroxidation in tissues of thallous malonate-treated hamster. Toxicology 53, 11-18.

Areco, M.M., Hanela, S., Duran, J., dos Santos Afonso, M., 2012. Biosorption of cu (II), zn (II), cd (II) and pb (II) by dead biomasses of green alga ulva lactuca and the development of a sustainable matrix for adsorption implementation. J. Hazard. Mater. 213, 123-132.

Arıca, M.Y., Kaçar, Y., Genç, Ö., 2001. Entrapment of white-rot fungus< i> trametes versicolor</i> in ca-alginate beads: Preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresour. Technol. 80, 121-129.

Arief, V.O., Trilestari, K., Sunarso, J., Indraswati, N., Ismadji, S., 2008. Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: Characterization, biosorption parameters and mechanism studies. CLEAN–Soil, Air, Water 36, 937-962.

Awwad, N., Gad, H., Ahmad, M., Aly, H., 2010. Sorption of lanthanum and erbium from aqueous solution by activated carbon prepared from rice husk. Colloids and Surfaces B: Biointerfaces 81, 593-599.

Ayoub, G., Semerjian, L., Acra, A., Fadel, M.E., Koopman, B., 2001. Heavy metal removal by coagulation with seawater liquid bittern. J. Environ. Eng. 127, 196-207.

Aziz, H.A., Adlan, M.N., Ariffin, K.S., 2008. Heavy metals (cd, pb, zn, ni, cu and cr (III)) removal from water in malaysia: Post treatment by high quality limestone. Bioresour. Technol. 99, 1578-1583.

Babel, S., Kurniawan, T.A., 2003. Low-cost adsorbents for heavy metals uptake from contaminated water: A review. J. Hazard. Mater. 97, 219-243.

Babel, S., Kurniawan, T.A., 2004. Cr (VI) removal from synthetic wastewater using coconut shell charcoal and commercial activated carbon modified with oxidizing agents and/or chitosan. Chemosphere 54, 951-967.

Bakatula, E., Cukrowska, E., Weiersbye, I., Mihaly-Cozmuta, L., Peter, A., Tutu, H., 2014. Biosorption of trace elements from aqueous systems in gold mining sites by the filamentous green algae (oedogonium sp.). J. Geochem. Explor. 144, 492-503.

Balkaya, N., Cesur, H., 2008. Adsorption of cadmium from aqueous solution by phosphogypsum. Chem. Eng. J. 140, 247-254.

Barnes, J., Edwards, J., 1982. Solvent extraction at inco's acton precious metal refinery. Chemistry and Industry(London) 151-155.

Benaissa, H., Benguella, B., 2004. Effect of anions and cations on cadmium sorption kinetics from aqueous solutions by chitin: Experimental studies and modeling. Environmental Pollution 130, 157-163.

Benguella, B., Benaissa, H., 2002. Cadmium removal from aqueous solutions by chitin: Kinetic and equilibrium studies. Water Res. 36, 2463-2474.

Berman, E., 1980. Toxic metals and their analysis.

Beveridge, T.J., Doyle, R., 1989. Metal Ions and Bacteria. .

Bhattacharyya, K.G., Sharma, A., 2004. Adsorption of pb (II) from aqueous solution by azadirachta indica (neem) leaf powder. J. Hazard. Mater. 113, 97-109.

Bidoglio, G., Gibson, P., O'Gorman, M., Roberts, K., 1993. X-ray absorption spectroscopy investigation of surface redox transformations of thallium and chromium on colloidal mineral oxides. Geochim. Cosmochim. Acta 57, 2389-2394.

Binnemans, K., Pontikes, Y., Jones, P.T., Van Gerven, T., Blanpain, B., 2013. Recovery of rare earths from industrial waste residues: A concise review. 191-205.

Birungi, Z., Chirwa, E., 2014. The kinetics of uptake and recovery of lanthanum using freshwater algae as biosorbents: Comparative analysis. Bioresour. Technol. 160, 43-51.

Bishnoi, N.R., Garima, A., 2005. Fungus-an alternative for bioremediation of heavy metal containing wastewater: A review. J. Sci. Ind. Res. 64, 93-100.

Bishnoi, N.R., Kumar, R., Kumar, S., Rani, S., 2007. Biosorption of cr (III) from aqueous solution using algal biomass< i> spirogyra</i> spp. J. Hazard. Mater. 145, 142-147.

Blázquez, G., Hernáinz, F., Calero, M., Ruiz-Núñez, L., 2005. Removal of cadmium ions with olive stones: The effect of somes parameters. Process Biochemistry 40, 2649-2654.

Boparai, H.K., Joseph, M., O'Carroll, D.M., 2011. Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zerovalent iron particles. J. Hazard. Mater. 186, 458-465.

Borda, M., Sparks, D., 2008. Kinetics and mechanisms of sorption–desorption in soils: A multiscale assessment. Biophysico-Chemical Processes of Heavy Metals and Metalloids in Soil Environments 97-124.

Brierley, C.L., 1990. Bioremediation of metal-contaminated surface and groundwaters. Geomicrobiol. J. 8, 201-223.

Brunauer, S., Emmett, P.H., Teller, E., 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60, 309-319.

Bulatovic, S., 2010. Flotation of REO minerals. Handbook of Flotation Reagents: Chemistry, Theory and Practice: Flotation of Gold, PGM and Oxide Minerals (1st edn.), Elsevier Science, Amsterdam, NL 151.

Busev, A., Tiptsova, V., 1961. Analytical chemistry of thallium. VI. new complexometric methods for the determination of thallium. Zhur.Anal.Khim 16, 275-278.

Carlsson, A.S., Bowles, D.J., 2007. Micro-and Macro-Algae: Utility for Industrial Applications: Outputs from the EPOBIO Project, September 2007. CPL Press, .

Cavanagh, J., 1991. What have we learnt from graham frederick young? reflections on the mechanism of thallium neurotoxicity. Neuropathol. Appl. Neurobiol. 17, 3-9.

Cavanagh, J.B., Fuller, N.H., Johnson, H.R., Rudge, P., 1974. The effects of thallium salts, with particular reference to the nervous system changes. A report of three cases. Q. J. Med. 43, 293-319.

Cazón, J.P., Bernardelli, C., Viera, M., Donati, E., Guibal, E., 2012. Zinc and cadmium biosorption by untreated and calcium-treated macrocystis pyrifera in a batch system. Bioresour. Technol. 116, 195-203.

Chang, J., Chen, C., 1998. Quantitative analysis and equilibrium models of selective adsorption in multimetal systems using a bacterial biosorbent. Sep. Sci. Technol. 33, 611-632.

Chang, Q., Wang, G., 2007. Study on the macromolecular coagulant PEX which traps heavy metals. Chemical engineering science 62, 4636-4643.

Chen, G., Zeng, G., Tang, L., Du, C., Jiang, X., Huang, G., Liu, H., Shen, G., 2008. Cadmium removal from simulated wastewater to biomass byproduct of lentinus edodes. Bioresour. Technol. 99, 7034-7040.

Chen, G., 2004. Electrochemical technologies in wastewater treatment. Separation and purification Technology 38, 11-41.

Chen, J., Lin, J., Wu, X., Yuan, X., Zhou, J., 1998. Study on adsorption of cadmium with N 503 levextrel resin. Chinese Journal of Nonferrous Metals(China) 8, 507-510.

Chen, J.P., 2012. Decontamination of Heavy Metals: Processes, Mechanisms, and Applications. CRC Press, .

Choi, S.B., Yun, Y., 2006. Biosorption of cadmium by various types of dried sludge: An equilibrium study and investigation of mechanisms. J. Hazard. Mater. 138, 378-383.

Chojnacka, K., 2010. Biosorption and bioaccumulation–the prospects for practical applications. Environ. Int. 36, 299-307.

Chojnacka, K., Chojnacki, A., Górecka, H., 2005. Biosorption of cr< sup> 3 </sup>, cd< sup> 2 </sup> and cu< sup> 2 </sup> ions by blue–green algae< i> Spirulina</i> sp.: Kinetics, equilibrium and the mechanism of the process. Chemosphere 59, 75-84.

Chong, K., Volesky, B., 1995. Description of two-metal biosorption equilibria by Langmuir-type models. Biotechnol. Bioeng. 47, 451-460.

Chong, K., Volesky, B., 1996. Metal biosorption equilibria in a ternary system. Biotechnol. Bioeng. 49, 629-638.

Coleman, A.W., Mai, J.C., 1997. Ribosomal DNA and ITS-2 sequence comparisons as a tool for predicting genetic relatedness. J. Mol. Evol. 45, 168-177.

Collins, Y.E., Stotzky, G., 1989. Factors affecting the toxicity of heavy metals to microbes. Metal ions and bacteria 31-90.

Costa, A.C.A., Leite, S.G.F., 1990a. Cadmium and zinc biosorption byChlorella homosphaera. Biotechnol. Lett. 12, 941-944.

Costa, A.C.A., Leite, S.G.F., 1990b. Cadmium and zinc biosorption byChlorella homosphaera. Biotechnol. Lett. 12, 941-944.

Cotton, F., Wilkinson, G., 1977. Advanced inorganic chemistry (interscience, new york, 1972). LD Brown and WN Lipscomb, J.Am.Chem.Soc 99, 226.

Craig, P., 1980. Metal Cycles and Biological Methylation. In: Anonymous The Natural Environment and the Biogeochemical Cycles. Springer, , pp. 169-227.

Crist, R.H., Martin, J.R., Carr, D., Watson, J., Clarke, H.J., Carr, D., 1994. Interaction of metals and protons with algae. 4. ion exchange vs adsorption models and a reassessment of scatchard plots; ion-exchange rates and equilibria compared with calcium alginate. Environ. Sci. Technol. 28, 1859-1866.

Dang, V., Doan, H., Dang-Vu, T., Lohi, A., 2009. Equilibrium and kinetics of biosorption of cadmium (II) and copper (II) ions by wheat straw. Bioresour. Technol. 100, 211-219.

Das, N., Vimala, R., Karthika, P., 2008. Biosorption of heavy metals-an overview. Indian J Biotechnol 7, 159-169.

Das, N., 2010. Recovery of precious metals through biosorption—a review. Hydrometallurgy 103, 180-189.

Davis, J.A., Leckie, J.O., 1978. Surface ionization and complexation at the oxide/water interface II. surface properties of amorphous iron oxyhydroxide and adsorption of metal ions. J. Colloid Interface Sci. 67, 90-107.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37, 4311-4330.

Davis, T., Volesky, B., Vieira, R., 2000. Sargassum seaweed as biosorbent for heavy metals. Water Res. 34, 4270-4278.

Del Valls, T., Saenz, V., Arias, A.M., Blasco, J., 1999. Thallium in the marine environment: First ecotoxicological assessments in the guadalquivir estuary and its potential adverse effect on the doñana european natural reserve after the aznalcóllar mining spill (sw spain). Cienc. Mar. 25, 161-175.

Delgado, A., Anselmo, A., Novais, J., 1998. Heavy metal biosorption by dried powdered mycelium of fusarium flocciferum. Water Environ. Res. 70, 370-375.

Deng, L., Su, Y., Su, H., Wang, X., Zhu, X., 2007. Sorption and desorption of lead (II) from wastewater by green algae< i> cladophora fascicularis</i>. J. Hazard. Mater. 143, 220-225.

Diniz, V., Volesky, B., 2005a. Effect of counterions on lanthanum biosorption by sargassum polycystum. Water Res. 39, 2229-2236.

Diniz, V., Volesky, B., 2005b. Effect of counterions on lanthanum biosorption by< i> sargassum polycystum</i>. Water Res. 39, 2229-2236.

Doğan, M., Alkan, M., Türkyilmaz, A., Özdemir, Y., 2004. Kinetics and mechanism of removal of methylene blue by adsorption onto perlite. J. Hazard. Mater. 109, 141-148.

Donath, E., Walther, D., Shilov, V., Knippel, E., Budde, A., Lowack, K., Helm, C., Möhwald, H., 1997. Nonlinear hairy layer theory of electrophoretic fingerprinting applied to consecutive layer by layer polyelectrolyte adsorption onto charged polystyrene latex particles. Langmuir 13, 5294-5305.

Dönmez, G., Aksu, Z., 2002. Removal of chromium (VI) from saline wastewaters by< i> Dunaliella</i> species. Process Biochemistry 38, 751-762.

Du, X., Graedel, T.E., 2011. Global in-use stocks of the rare earth elements: A first estimate. Environ. Sci. Technol. 45, 4096-4101.

Duan, L., Song, J., Li, X., Yuan, H., Li, N., Xu, Y., 2012. Thallium concentrations and sources in the surface sediments of bohai bay. Mar. Environ. Res. 73, 25-31.

Dubinin, M., 1960. The potential theory of adsorption of gases and vapors for adsorbents with energetically nonuniform surfaces. Chem. Rev. 60, 235-241.

Eg European Commission, 2010. Critical raw materials for the EU. Report of the Ad-Hoc Working Group on Defining Critical Raw Materials.(EC, Ed.).Brussels .

Eriksson, P., 1988. Nanofiltration extends the range of membrane filtration. Environ. Prog. 7, 58-62.

Fagundes-Klen, M., Ferri, P., Martins, T., Tavares, C., Silva, E., 2007. Equilibrium study of the binary mixture of cadmium–zinc ions biosorption by the sargassum filipendula species using adsorption isotherms models and neural network. Biochem. Eng. J. 34, 136-146.

Faust, S.D., Aly, O.M., 1998. Chemistry of Water Treatment. CRC Press, .

Febrianto, J., Kosasih, A.N., Sunarso, J., Ju, Y., Indraswati, N., Ismadji, S., 2009. Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: A summary of recent studies. J. Hazard. Mater. 162, 616-645.

Fergusson, J.E., 1990. Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon, .

Figueira, M., Volesky, B., Ciminelli, V., 1997. Assessment of interference in biosorption of a heavy metal. Biotechnol. Bioeng. 54, 344-350.

Figueira, M., Volesky, B., Ciminelli, V., Roddick, F.A., 2000. Biosorption of metals in brown seaweed biomass. Water Res. 34, 196-204.

Fleischer, M., Sarofim, A.F., Fassett, D.W., Hammond, P., Shacklette, H.T., Nisbet, I.C., Epstein, S., 1974. Environmental impact of cadmium: A review by the panel on hazardous trace substances. Environ. Health Perspect. 7, 253-323.

Fomina, M., Gadd, G.M., 2002. Influence of clay minerals on the morphology of fungal pellets. Mycol. Res. 106, 107-117.

Fomina, M., Gadd, G.M., 2014. Biosorption: Current perspectives on concept, definition and application. Bioresour. Technol. 160, 3-14.

Forster, C., Wase, D., 1997. Biosorption of heavy metals: An introduction. Biosorbents for metal ions edited by Wase, J.and Forster, C 1-10.

Förstner, U., 1980a. Cadmium in the handbook of enviromental chemistry: Anthropogenic compounds. ed., O. hutzinger. edt.

Förstner, U., 1980b. Inorganic pollutants, particularly heavy metals in estuaries. Chemistry and Biochemistry of Estuaries (Eds.Olausson, E.& Cato, I.), John Wiley & Sons Ltd., Chichester 307-348.

Forstner, U., Wittmann, G.T., 1979. Metal pollution in the aquatic environment.

Freundlich, H., 1906. Over the adsorption in solution. J. Phys. Chem. 57, 1100-1107.

Friberg, L., Piscator, M., Nordberg, G.F., Kjellström, T., 1974a. Cadmium in the Environment. .

Friberg, L., Piscator, M., Nordberg, G.F., Kjellström, T., 1974b. Cadmium in the Environment. .

Fritz Ullmann, W., Yamamoto, Y., Campbell, F., 1985. Ullmann’s encyclopedia of industrial chemistry.

Fritz, W., Schluender, E., 1974. Simultaneous adsorption equilibria of organic solutes in dilute aqueous solutions on activated carbon. Chemical Engineering Science 29, 1279-1282.

Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters: A review. J. Environ. Manage. 92, 407-418.

Gadd, G.M., White, C., 1993. Microbial treatment of metal pollution—a working biotechnology? Trends Biotechnol. 11, 353-359.

Gadd, G.M., 2009. Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. Journal of Chemical Technology and Biotechnology 84, 13-28.

Gadd, G.M., 1992. Metals and microorganisms: A problem of definition. FEMS Microbiol. Lett. 100, 197-203.

Galván-Arzate, S., Santamaría, A., 1998. Thallium toxicity. Toxicol. Lett. 99, 1-13.

Gaur, N., Flora, G., Yadav, M., Tiwari, A., 2014. A review with recent advancements on bioremediation-based abolition of heavy metals. Environmental Science: Processes & Impacts 16, 180-193.

Ghodbane, I., Nouri, L., Hamdaoui, O., Chiha, M., 2008. Kinetic and equilibrium study for the sorption of cadmium (II) ions from aqueous phase by eucalyptus bark. J. Hazard. Mater. 152, 148-158.

Gilfillan, S.C., 1965. Lead poisoning and the fall of rome. Journal of Occupational and Environmental Medicine 7, 53-60.

Gipps, J., Coller, B., 1982. Effect of some nutrient cations on uptake of cadmium by chlorella pyrenoidosa. Marine and Freshwater Research 33, 979-987.

Glauert, A.M., Reid, N., 1975. Fixation, dehydration and embedding of biological specimens.

Godlewska-Żyłkiewicz, B., 2006. Microorganisms in inorganic chemical analysis. Analytical and bioanalytical chemistry 384, 114-123.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., Groneberg, D.A., 2006. The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 1, 1-6.

Göksungur, Y., Üren, S., Güvenç, U., 2005. Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. Bioresour. Technol. 96, 103-109.

Goldschmidt, V.M., 1954. Geochemistry. Soil Sci. 78, 156.

Gong, R., Ding, Y., Liu, H., Chen, Q., Liu, Z., 2005. Lead biosorption and desorption by intact and pretreated< i> spirulina maxima</i> biomass. Chemosphere 58, 125-130.

González, M.A., Gómez, P.I., Montoya, R., 1998. Comparison of PCR-RFLP analysis of the ITS region with morphological criteria of various strains of dunaliella. J. Appl. Phycol. 10, 573-580.

Gonzalez-Chavez, C., D'Haen, J., Vangronsveld, J., Dodd, J., 2002. Copper sorption and accumulation by the extraradical mycelium of different glomus spp.(arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240, 287-297.

Gorman, M.E., 2002. Levels of expertise and trading zones: A framework for multidisciplinary collaboration. Soc. Stud. Sci. 933-938.

Goyal, M., Rattan, V., Aggarwal, D., Bansal, R., 2001. Removal of copper from aqueous solutions by adsorption on activated carbons. Colloids Surf. Physicochem. Eng. Aspects 190, 229-238.

Graedel, T., Allwood, J., Birat, J., Reck, B., Sibley, S., Sonnemann, G., Buchert, M., Hagelüken, C., 2011. UNEP (2011) recycling rates of metals-A status report. A Report of the Working Group on the Global Metal Flows to the International Resource Panel, United Nations Environment Programme .

Grosse, F., 2010. Is recycling “part of the solution”? the role of recycling in an expanding society and a world of finite resources. SAPI EN.S.Surveys and Perspectives Integrating Environment and Society .

Guo, X., Zhang, S., Shan, X., 2008. Adsorption of metal ions on lignin. J. Hazard. Mater. 151, 134-142.

Gupta, G., Prasad, G., Singh, V., 1990. Removal of chrome dye from aqueous solutions by mixed adsorbents: Fly ash and coal. Water Res. 24, 45-50.

Gupta, R., Saxena, R., Mohapatra, H., Ahuja, P., 2002. Microbial variables for bioremediation of heavy metals from industrial effluents. Prog. Ind. Microbiol. 36, 189-229.

Gupta, V.K., Rastogi, A., 2008a. Biosorption of lead (II) from aqueous solutions by non-living algal biomass oedogonium sp. and nostoc sp: A comparative study. Colloids and surfaces.B, Biointerfaces 64, 170-178.

Gupta, V., Rastogi, A., 2008b. Equilibrium and kinetic modelling of cadmium (II) biosorption by nonliving algal biomass oedogonium sp. from aqueous phase. J. Hazard. Mater. 153, 759-766.

Halttunen, T., Salminen, S., Tahvonen, R., 2007. Rapid removal of lead and cadmium from water by specific lactic acid bacteria. Int. J. Food Microbiol. 114, 30-35.

Han, X., Wong, Y.S., Tam, N.F.Y., 2006. Surface complexation mechanism and modeling in cr(III) biosorption by a microalgal isolate, chlorella miniata. J. Colloid Interface Sci. 303, 365-371.

Hart, B.A., Bertram, P.E., Scaife, B.D., 1979. Cadmium transport by chlorella pyrenoidosa. Environ. Res. 18, 327-335.

Hashim, M., Chu, K., 2004. Biosorption of cadmium by brown, green, and red seaweeds. Chem. Eng. J. 97, 249-255.

Haskin, L.A., Paster, T., 1979. Geochemistry and mineralogy of the rare earths. Handbook on the physics and chemistry of rare earths 3, 1-80.

Hawari, A.H., Mulligan, C.N., 2006. Biosorption of lead (II), cadmium (II), copper (II) and nickel (II) by anaerobic granular biomass. Bioresour. Technol. 97, 692-700.

Helfferich, F.G., 1962. Ion Exchange. Courier Corporation, .

Herrero Rodríguez, R., Cordero Pérez, B., Lodeiro Fernández, P., Rey Castro, C., De Vicente, S., Esteban, M., 2005. Interactions of cadmium (II) and protons with dead biomass of marine algae fucus sp.

Herrero, F., Fernandez, E., Gomez, J., Pretel, L., Canizares, F., Frias, J., Escribano, J.B., 1995. Thallium poisoning presenting with abdominal colic, paresthesia, and irritability. Clin. Toxicol. 33, 261-264.

Hewitt, T., Wield, D., 1992. Industrialization and Development. Oxford University Press, .

Ho, Y., John Wase, D., Forster, C., 1995. Batch nickel removal from aqueous solution by sphagnum moss peat. Water Res. 29, 1327-1332.

Ho, Y., Wase, D., Forster, C., 1996. Kinetic studies of competitive heavy metal adsorption by sphagnum moss peat. Environ. Technol. 17, 71-77.

Ho, Y., McKay, G., 1999. Pseudo-second order model for sorption processes. Process Biochemistry 34, 451-465.

Hoffman, R.S., Hoffman, R., Stringer, J.A., Feinberg, R.S., Goldfrank, L.R., 1999. Comparative efficacy of thallium adsorption by activated charcoal, prussian blue, and sodium polystyrene sulfonate. Clin. Toxicol. 37, 833-837.

Holan, Z., Volesky, B., Prasetyo, I., 1993. Biosorption of cadmium by biomass of marine algae. Biotechnol. Bioeng. 41, 819-825.

Hossain, M., Ngo, H., Guo, W., Nghiem, L., Hai, F., Vigneswaran, S., Nguyen, T., 2014. Competitive adsorption of metals on cabbage waste from multi-metal solutions. Bioresour. Technol. 160, 79-88.

Hu, J., Chen, G., Lo, I., 2005. Removal and recovery of cr (VI) from wastewater by maghemite nanoparticles. Water Res. 39, 4528-4536.

Huff, J., Lunn, R.M., Waalkes, M.P., Tomatis, L., Infante, P.F., 2007. Cadmium-induced cancers in animals and in humans. International journal of occupational and environmental health 13, 202-212.

Hughes, M.N., Poole, R.K., 1991. Metal speciation and microbial growth--the hard(and soft) facts. Microbiology 137, 725-734.

Iqbal, M., Edyvean, R., 2004. Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of phanerochaete chrysosporium. Minerals Eng 17, 217-223.

Iqbal, M., Edyvean, R., 2005. Loofa sponge immobilized fungal biosorbent: A robust system for cadmium and other dissolved metal removal from aqueous solution. Chemosphere 61, 510-518.

Jacobson, A.R., McBride, M.B., Baveye, P., Steenhuis, T.S., 2005. Environmental factors determining the trace-level sorption of silver and thallium to soils. Sci. Total Environ. 345, 191-205.

Jensen, S., Jernelöv, A., 1969. Biological methylation of mercury in aquatic organisms.

Jeppu, G.P., Clement, T.P., 2012. A modified langmuir-freundlich isotherm model for simulating pH-dependent adsorption effects. J. Contam. Hydrol. 129, 46-53.

Jianlong, W., 2002. Biosorption of copper (II) by chemically modified biomass of< i> saccharomyces cerevisiae</i>. Process Biochemistry 37, 847-850.

Jin, X., Bailey, G., Yu, Y., Lynch, A., 1996. Kinetics of single and multiple metal ion sorption processes on humic substances. Soil Sci. 161, 509-520.

Johannesson, M., 2002. A review of risks associated to arsenic, cadmium, lead, mercury and zinc. Appendix A in Johannesson, M.(Ed.) et al 115.

Jonas, I., Norden, B., 1974. Induced optical-activity in co (nh3) 63 by outer-sphere association with chiral anions. Acta chemica scandinavica series a-physical and inorganic chemistry 289-293.

Kadirvelu, K., Palanival, M., Kalpana, R., Rajeswari, S., 2000. Activated carbon from an agricultural by-product, for the treatment of dyeing industry wastewater. Bioresour. Technol. 74, 263-265.

Kalfa, O.M., Yalçinkaya, Ö., Türker, A.R., 2009. Synthesis of nano B< sub> 2</sub> O< sub> 3</sub>/TiO< sub> 2</sub> composite material as a new solid phase extractor and its application to preconcentration and separation of cadmium. J. Hazard. Mater. 166, 455-461.

Kanazawa, Y., Kamitani, M., 2006. Rare earth minerals and resources in the world. J. Alloys Compounds 408, 1339-1343.

Kang, S.Y., Lee, J.U., Moon, S.H., Kim, K.W., 2004. Competitive adsorption characteristics of Co2+, Ni2+, and Cr3+ by IRN-77 cation exchange resin in synthesized wastewater. Chemosphere 56, 141-147.

Karlsson, U., 2006. Environmental levels of thallium: Influence of redox properties and anthropogenic sources.

Kasuya, M., Teranishi, H., Aoshima, K., Katoh, T., Horiguchi, H., Morikawa, Y., Nishijo, M., Iwata, K., 1992. Water pollution by cadmium and the onset of itai-itai disease. Water Science and Technology 25, 149-149.

Kavitha, D., Namasivayam, C., 2007a. Experimental and kinetic studies on methylene blue adsorption by coir pith carbon. Bioresour. Technol. 98, 14-21.

Kavitha, D., Namasivayam, C., 2007b. Experimental and kinetic studies on methylene blue adsorption by coir pith carbon. Bioresour. Technol. 98, 14-21.

Kazantzis, G., 2000. Thallium in the environment and health effects. Environ. Geochem. Health 22, 275-280.

Kazy, S.K., Das, S.K., Sar, P., 2006. Lanthanum biosorption by a pseudomonas sp.: Equilibrium studies and chemical characterization. Journal of Industrial Microbiology and Biotechnology 33, 773-783.

Khajeh, M., Laurent, S., Dastafkan, K., 2013. Nanoadsorbents: Classification, preparation, and applications (with emphasis on aqueous media). Chem. Rev. 113, 7728-7768.

Khavidaki, H.D., Aghaie, M., Shishehbore, M., Aghaie, H., 2013. Adsorptive removal of thallium (III) ions from aqueous solutions using eucalyptus leaves powders. Indian J. Chem. Technol. 20, 380-384.

Komy, Z.R., Gabar, R.M., Shoriet, A.A., Mohammed, R.M., 2006. Characterisation of acidic sites of pseudomonas biomass capable of binding protons and cadmium and removal of cadmium via biosorption. World Journal of Microbiology and Biotechnology 22, 975-982.

Korenevsky, A.A., Sorokin, V.V., Karavaiko, G.I., 1999. Biosorption of rare earth elements. Process Metallurgy 9, 299-306.

Kotrba, P., 2011. Microbial Biosorption of metals—General Introduction. Springer, .

Kratochvil, D., Volesky, B., 1998. Advances in the biosorption of heavy metals. Trends Biotechnol. 16, 291-300.

Kratochvil, D., Volesky, B., 2000. Multicomponent biosorption in fixed beds. Water Res. 34, 3186-3196.

Ku, Y., Jung, I., 2001. Photocatalytic reduction of cr (VI) in aqueous solutions by UV irradiation with the presence of titanium dioxide. Water Res. 35, 135-142.

Ku, Y., Wu, M., Shen, Y., 2002. A study on the cadmium removal from aqueous solutions by zinc cementation. Sep. Sci. Technol. 37, 571-590.

Kula, I., Uğurlu, M., Karaoğlu, H., Celik, A., 2008. Adsorption of cd (II) ions from aqueous solutions using activated carbon prepared from olive stone by ZnCl< sub> 2</sub> activation. Bioresour. Technol. 99, 492-501.

Kumar, D., Singh, A., Gaur, J., 2008. Mono-component versus binary isotherm models for cu (II) and pb (II) sorption from binary metal solution by the green alga pithophora oedogonia. Bioresour. Technol. 99, 8280-8287.

Kumar, K.V., Porkodi, K., Selvaganapathi, A., 2007. Constrain in solving Langmuir–Hinshelwood kinetic expression for the photocatalytic degradation of auramine O aqueous solutions by ZnO catalyst. Dyes and Pigments 75, 246-249.

Kurniawan, T., Babel, S., 2003. A research study on cr (VI) removal from contaminated wastewater using low-cost adsorbents and commercial activated carbon. 2, 1110-1117.

Kurniawan, T.A., Chan, G., Lo, W., Babel, S., 2006. Comparisons of low-cost adsorbents for treating wastewaters laden with heavy metals. Sci. Total Environ. 366, 409-426.

Kütahyali, C., Sert, Ş., Çetinkaya, B., Inan, S., Eral, M., 2010. Factors affecting lanthanum and cerium biosorption on pinus brutia leaf powder. Sep. Sci. Technol. 45, 1456-1462.

Kuyucak, N., Volesky, B., 1989. Accumulation of gold by algal biosorbent. Biorecovery. 1, 189-204.

Kwan, K., Smith, S., 1991. Some aspects of the kinetics of cadmium and thallium uptake by fronds of lemna minor L. New Phytol. 117, 91-102.

Lagergren, S., 1898. About the theory of so-called adsorption of soluble substances. Kungliga Svenska Vetenskapsakademiens Handlingar 24, 1-39.

Langmuir, I., 1916. The constitution and fundamental properties of solids and liquids. part i. solids. J. Am. Chem. Soc. 38, 2221-2295.

Lauwerys, R., Roels, H., Regniers, M., Buchet, J., Bernard, A., Goret, A., 1979. Significance of cadmium concentration in blood and in urine in workers exposed to cadmium. Environ. Res. 20, 375-391.

Lee, Y., Chang, S., 2011. The biosorption of heavy metals from aqueous solution by spirogyra and cladophora filamentous macroalgae. Bioresour. Technol. 102, 5297-5304.

Lehn, H., Schoer, J., 1987. Thallium-transfer from soils to plants-correlation between chemical form and plant uptake. Plant Soil 97, 253-265.

Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F., De Clerck, O., 2012. Phylogeny and molecular evolution of the green algae. Crit. Rev. Plant Sci. 31, 1-46.

Léonard, A., Gerber, G., 1997. Mutagenicity, carcinogenicity and teratogenicity of thallium compounds. Mutation Research/Reviews in Mutation Research 387, 47-53.

Lesmana, S.O., Febriana, N., Soetaredjo, F.E., Sunarso, J., Ismadji, S., 2009. Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. Biochem. Eng. J. 44, 19-41.

Leusch, A., Volesky, B., 1995. The influence of film diffusion on cadmium biosorption by marine biomass. J. Biotechnol. 43, 1-10.

Lewis, L.A., McCourt, R.M., 2004. Green algae and the origin of land plants. Am. J. Bot. 91, 1535-1556.

Leyva-Ramos, R., Rangel-Mendez, J., Mendoza-Barron, J., Fuentes-Rubio, L., Guerrero-Coronado, R., 1997. Adsorption of cadmium (II) from aqueous solution onto activated carbon. Water Science and Technology 35, 205-211.

Li, Q., Wu, S., Liu, G., Liao, X., Deng, X., Sun, D., Hu, Y., Huang, Y., 2004. Simultaneous biosorption of cadmium (II) and lead (II) ions by pretreated biomass of< i> phanerochaete chrysosporium</i>. Separation and Purification Technology 34, 135-142.

Li, S., Xiao, T., Zheng, B., 2012. Medical geology of arsenic, selenium and thallium in china. Sci. Total Environ. 421, 31-40.

Li, Z., Yuan, H., 2006. Characterization of cadmium removal by rhodotorula sp. Y11. Appl. Microbiol. Biotechnol. 73, 458-463.

Lin, X., Burns, R.C., Lawrance, G.A., 2005. Heavy metals in wastewater: The effect of electrolyte composition on the precipitation of cadmium (II) using lime and magnesia. Water Air Soil Pollut. 165, 131-152.

Liu, A., Hidajat, K., Kawi, S., Zhao, D., 2000. A new class of hybrid mesoporous materials with functionalized organic monolayers for selective adsorption of heavy metal ions. Chem.Commun. 1145-1146.

Lodeiro, P., Barriada, J., Herrero, R., De Vicente, M.S., 2006. The marine macroalga cystoseira baccata as biosorbent for cadmium (II) and lead (II) removal: Kinetic and equilibrium studies. Environmental pollution 142, 264-273.

Lodeiro, P., Cordero, B., Barriada, J., Herrero, R., De Vicente, M.S., 2005. Biosorption of cadmium by biomass of brown marine macroalgae. Bioresour. Technol. 96, 1796-1803.

Low, K., Lee, C., Tan, K., 1995. Biosorption of basic dyes by water hyacinth roots. Bioresour. Technol. 52, 79-83.

Lu, M., Liu, Y., Hu, X., Ben, Y., Zeng, X., Li, T., Wang, H., 2013. Competitive adsorption of cu (II) and pb (II) ions from aqueous solutions by ca-alginate immobilized activated carbon and saccharomyces cerevisiae. Journal of Central South University 20, 2478-2488.

Lu, W., Kao, W., Shi, J., Chang, J., 2008. Exploring multi-metal biosorption by indigenous metal-hyperresistant enterobacter sp. J1 using experimental design methodologies. J. Hazard. Mater. 153, 372-381.

MacFarland, H., 1979. Pulmonary effects of cadmium. Cadmium toxicity 15, 113.

Mack, C., Wilhelmi, B., Duncan, J., Burgess, J., 2007. Biosorption of precious metals. Biotechnol. Adv. 25, 264-271.

Madigan, M.T., Jung, D.O., Woese, C.R., Achenbach, L.A., 2000. Rhodoferax antarcticus sp. nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an antarctic microbial mat. Arch. Microbiol. 173, 269-277.

Madigan, M.T., Martinko, J.M., Parker, J., Brock, T.D., 1997. Biology of Microorganisms. prentice hall Upper Saddle River, NJ, .

Madyiwa, S., 2006. Modelling lead and cadmium uptake by star grass under irrigation with treated wastewater .

Mahamadi, C., Nharingo, T., 2010. Competitive adsorption of pb 2 , cd 2 and zn 2 ions onto eichhornia crassipes in binary and ternary systems. Bioresour. Technol. 101, 859-864.

Malik, P., 2004. Dye removal from wastewater using activated carbon developed from sawdust: Adsorption equilibrium and kinetics. J. Hazard. Mater. 113, 81-88.

Malkoc, E., Nuhoglu, Y., 2007. Determination of kinetic and equilibrium parameters of the batch adsorption of cr (VI) onto waste acorn of< i> quercus ithaburensis</i>. Chemical Engineering and Processing: Process Intensification 46, 1020-1029.

Mall, I.D., Srivastava, V.C., Agarwal, N.K., Mishra, I.M., 2005. Removal of congo red from aqueous solution by bagasse fly ash and activated carbon: Kinetic study and equilibrium isotherm analyses. Chemosphere 61, 492-501.

Malla, M.E., Alvarez, M.B., Batistoni, D.A., 2002. Evaluation of sorption and desorption characteristics of cadmium, lead and zinc on amberlite IRC-718 iminodiacetate chelating ion exchanger. Talanta 57, 277-287.

Martínez, M., Miralles, N., Hidalgo, S., Fiol, N., Villaescusa, I., Poch, J., 2006. Removal of lead (II) and cadmium (II) from aqueous solutions using grape stalk waste. J. Hazard. Mater. 133, 203-211.

Martins, T.S., Isolani, P.C., 2005. Rare earths: Industrial and biological applications. Química Nova 28, 111-117.

Masciangioli, T., Zhang, W., 2003. Peer reviewed: Environmental technologies at the nanoscale. Environ. Sci. Technol. 37, 102A-108A.

Mata, Y., Blazquez, M., Ballester, A., Gonzalez, F., Munoz, J., 2008. Characterization of the biosorption of cadmium, lead and copper with the brown alga fucus vesiculosus. J. Hazard. Mater. 158, 316-323.

Matheickal, J.T., Yu, Q., 1999. Biosorption of lead (II) and copper (II) from aqueous solutions by pre-treated biomass of australian marine algae. Bioresour. Technol. 69, 223-229.

Matheickal, J.T., Yu, Q., Woodburn, G.M., 1999. Biosorption of cadmium (II) from aqueous solutions by pre-treated biomass of marine alga< i> DurvillAea potatorum</i>. Water Res. 33, 335-342.

McHugh, D.J., 2003. A Guide to the Seaweed Industry. Food and Agriculture Organization of the United Nations Rome, .

McLaughlin, M.J., Singh, B.R., 1999. Cadmium in Soils and Plants. Springer, .

Meggs, W.J., Hoffman, R.S., Shih, R.D., Weisman, R.S., Goldfrank, L.R., 1994. Thallium poisoning from maliciously contaminated food. Clin. Toxicol. 32, 723-730.

Mehta, S., Gaur, J., 2005. Use of algae for removing heavy metal ions from wastewater: Progress and prospects. Crit. Rev. Biotechnol. 25, 113-152.

Mehta, S., Tripathi, B., Gaur, J., 2000. Influence of pH, temperature, culture age and cations on adsorption and uptake of ni by< i> chlorella vulgaris</i>. Eur. J. Protistol. 36, 443-450.

Mehta, S., Tripathi, B., Gaur, J., 2002. Enhanced sorption of Cu2 and Ni2 by acid-pretreated chlorella vulgaris from single and binary metal solutions. J. Appl. Phycol. 14, 267-273.

Memon, G.Z., Bhanger, M., Akhtar, M., Talpur, F.N., Memon, J.R., 2008. Adsorption of methyl parathion pesticide from water using watermelon peels as a low cost adsorbent. Chem. Eng. J. 138, 616-621.

Memon, S.Q., Memon, N., Shah, S., Khuhawar, M., Bhanger, M., 2007. Sawdust—a green and economical sorbent for the removal of cadmium (II) ions. J. Hazard. Mater. 139, 116-121.

Memon, S.Q., Memon, N., Solangi, A.R., 2008. Sawdust: A green and economical sorbent for thallium removal. Chem. Eng. J. 140, 235-240.

Meyer, L., Bras, B., 2011. Rare earth metal recycling. 1-6.

Michalak, I., Saeid, A., Chojnacka, K., 2013. The effect of increase in concentration of na (I) ions on biosorption of cr (III) ions by enteromorpha prolifera and spirulina sp. Central European Journal of Chemistry 11, 313-319.

Ming-Ho, Y., 2005. Environmental toxicology: Biological and health effects of pollutants, chap 12.

Mirbagheri, S., Hosseini, S., 2005. Pilot plant investigation on petrochemical wastewater treatmentfor the removal of copper and chromium with the objective of reuse. Desalination 171, 85-93.

Modak, J., Natarajan, K., 1995. Biosorption of metals using nonliving biomass-A review. Minerals and Metallurgical Processing 12, 189-196.

Mohamad, O.A., Hao, X., Xie, P., Hatab, S., Lin, Y., Wei, G., 2012. Biosorption of copper (II) from aqueous solution using non-living mesorhizobium amorphae strain CCNWGS0123. Microbes and Environments 27, 234-241.

Mohapatra, H., Gupta, R., 2005. Concurrent sorption of zn (II), cu (II) and co (II) by< i> oscillatoria angustissima</i> as a function of pH in binary and ternary metal solutions. Bioresour. Technol. 96, 1387-1398.

Monteiro, C.M., Castro, P.M., Malcata, F.X., 2012. Metal uptake by microalgae: Underlying mechanisms and practical applications. Biotechnol. Prog. 28, 299-311.

Mooiman, M.B., Miller, J.D., Hiskey, J.B., Hendriksz, A.R., 1984. Comparison of process alternatives for gold recovery from alkaline cyanide solutions. Heap and Dump Leaching Practice 93-108.

Moore, J.W., Ramamoorthy, S., 1984. Heavy Metals in Natural Waters. Applied Monitoring and Impact Assessment. Springer-Verlag, .

Mortaheb, H.R., Kosuge, H., Mokhtarani, B., Amini, M.H., Banihashemi, H.R., 2009. Study on removal of cadmium from wastewater by emulsion liquid membrane. J. Hazard. Mater. 165, 630-636.

Mukhopadhyay, M., Noronha, S., Suraishkumar, G., 2007. Kinetic modeling for the biosorption of copper by pretreated aspergillus niger biomass. Bioresour. Technol. 98, 1781-1787.

Mukhopadhyay, M., Noronha, S., Suraishkumar, G., 2011. A review on experimental studies of biosorption of heavy metals by aspergillus niger. The Canadian Journal of Chemical Engineering 89, 889-900.

Mulkey, J.P., Oehme, F.W., 1993. A review of thallium toxicity. Vet. Hum. Toxicol. 35, 445-453.

Muraleedharan, T., Venkobachar, C., 1990. Mechanism of cobalt biosorption. Biotechnol. Bioeng. 33, 823-831.

Murata, I., Hirono, T., Saeki, Y., Nakagawa, S., 1970. Cadmium enteropathy, renal osteomalacia ("itai itai" disease in japan). Bull. Soc. Int. Chir. 29, 34-42.

Murray, M.G., Thompson, W.F., 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8, 4321-4325.

Naja, G.M., Murphy, V., Volesky, B., 2010. Biosorption, metals. Encyclopedia of Industrial Biotechnology .

Naja, G., Mustin, C., Berthelin, J., Volesky, B., 2005. Lead biosorption study with< i> rhizopus arrhizus</i> using a metal-based titration technique. J. Colloid Interface Sci. 292, 537-543.

Naja, G., Volesky, B., 2011. The Mechanism of Metal Cation and Anion Biosorption. In: Anonymous Microbial Biosorption of Metals. Springer, , pp. 19-58.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17, 372-386.

Ngah, W., Hanafiah, M., 2008. Biosorption of copper ions from dilute aqueous solutions on base treatedrubber (< i> hevea brasiliensis</i>) leaves powder: Kinetics, isotherm, and biosorption mechanisms. Journal of Environmental Sciences 20, 1168-1176.

Ngwenya, B.T., 2007. Enhanced adsorption of zinc is associated with aging and lysis of bacterial cells in batch incubations. Chemosphere 67, 1982-1992.

Nixson, F., 1991. Industrialization and development. Journal of International Development 3, 79-85.

Njikam, E., Schiewer, S., 2012. Optimization and kinetic modeling of cadmium desorption from citrus peels: A process for biosorbent regeneration. J. Hazard. Mater. 213–214, 242-248.

Nriagu, J.O., 1980. Cadmium in the Environment. Part 1: Ecological Cycling. John Wiley & Sons, Inc., .

Nriagu, J.O., 1983. Lead and lead poisoning in antiquity.

Nriagu, J.O., 1998. Thallium in the environment. Adv. Environ. Sci. Technol. .

Nriagu, J.O., 2003. Thallium. Chemical & engineering news 81, 153-153.

Ofomaja, A.E., Ho, Y., 2007. Effect of pH on cadmium biosorption by coconut copra meal. J. Hazard. Mater. 139, 356-362.

Oliveira, R.C., Guibal, E., Garcia Jr, O., 2012. Biosorption and desorption of lanthanum (III) and neodymium (III) in fixed-bed columns with sargassum sp.: Perspectives for separation of rare earth metals. Biotechnol. Prog. .

Oliveira, R., Garcia Jr, O., 2009. Study of biosorption of rare earth metals (la, nd, eu, gd) by sargassum sp. biomass in batch systems: Physicochemical evaluation of kinetics and adsorption models. Advanced Materials Research 71, 605-608.

Olmos, J., Paniagua, J., Contreras, R., 2000. Molecular identification of dunaliella sp. utilizing the 18S rDNA gene. Lett. Appl. Microbiol. 30, 80-84.

Ozcan, A., Ozcan, A., Gok, O., 2007. Adsorption kinetics and isotherms of anionic dye of reactive blue 19 from aqueous solutions onto DTMA-sepiolite. Hazardous Materials and Wastewater—Treatment, Removal and Analysis, Nova Science Publishers, New York .

Özer, A., Özer, D., 2003. Comparative study of the biosorption of pb (II), ni (II) and cr (VI) ions onto S. cerevisiae: Determination of biosorption heats. J. Hazard. Mater. 100, 219-229.

Padmavathy, V., 2008. Biosorption of nickel (II) ions by baker's yeast: Kinetic, thermodynamic and desorption studies. Bioresour. Technol. 99, 3100-3109.

Pagnanelli, F., Trifoni, M., Beolchini, F., Esposito, A., Toro, L., Veglio, F., 2001. Equilibrium biosorption studies in single and multi-metal systems. Process Biochemistry 37, 115-124.

Pagnanelli, F., 2011. Equilibrium, Kinetic and Dynamic Modelling of Biosorption Processes. In: Anonymous Microbial Biosorption of Metals. Springer, , pp. 59-120.

Pagnanelli, F., Toro, L., Veglio, F., 2002. Olive mill solid residues as heavy metal sorbent material: A preliminary study. Waste Manage. 22, 901-907.

Palmieri, M.C., Volesky, B., Garcia Jr, O., 2002. Biosorption of lanthanum using< i> sargassum fluitans</i> in batch system. Hydrometallurgy 67, 31-36.

Palmieri, M.C., Garcia Jr, O., Melnikov, P., 2000. Neodymium biosorption from acidic solutions in batch system. Process Biochemistry 36, 441-444.

Palmieri, M., Garcia, O., 2001. Biosorption of erbium and ytterbium using biomass of microorganisms. PROCESS METALLURGY 11, 137-144.

Park, D., Yun, Y., Park, J.M., 2010a. The past, present, and future trends of biosorption. Biotechnology and Bioprocess Engineering 15, 86-102.

Park, D., Yun, Y.-., Park, J.M., 2010b. The past, present, and future trends of biosorption. Biotechnology and Bioprocess Engineering 15, 86-102.

Partington, J.R., 1970. A history of chemistry.

Patterson, C., Shirahata, H., Ericson, J.E., 1987. Lead in ancient human bones and its relevance to historical developments of social problems with lead. Sci. Total Environ. 61, 167-200.

Pehlivan, E., Altun, T., 2006. The study of various parameters affecting the ion exchange of cu 2 , zn 2 , ni 2 , cd 2 , and pb 2 from aqueous solution on dowex 50W synthetic resin. J. Hazard. Mater. 134, 149-156.

Peter, A., Viraraghavan, T., 2005. Thallium: A review of public health and environmental concerns. Environ. Int. 31, 493-501.

Pradhan, S., Rai, L., 2001. Biotechnological potential of microcystis sp. in cu, zn and cd biosorption from single and multimetallic systems. Biometals 14, 67-74.

Prescott, L.M., Harley, J.P., Klein, D.A., 2002. Microbiology. Wm C Brown Publishers .

Proschold, T., Leliaert, F., 2007. Systematics of the green algae: Conflict of classic and modern approaches. SYSTEMATICS ASSOCIATION SPECIAL VOLUME 75, 123.

Pu, Y., Yang, X., Zheng, H., Wang, D., Su, Y., He, J., 2013. Adsorption and desorption of thallium (I) on multiwalled carbon nanotubes. Chem. Eng. J. 219, 403-410.

Qing, C., 2010. Study on the adsorption of lanthanum (III) from aqueous solution by bamboo charcoal. Journal of Rare Earths 28, 125-131.

Qu, X., Alvarez, P.J., Li, Q., 2013. Applications of nanotechnology in water and wastewater treatment. Water Res. 47, 3931-3946.

Rajfur, M., Kłos, A., Wacławek, M., 2010. Sorption properties of algae spirogyra sp. and their use for determination of heavy metal ions concentrations in surface water. Bioelectrochemistry 80, 81-86.

Ralph, L., Twiss, M., 2002. Comparative toxicity of thallium (I), thallium (III), and cadmium (II) to the unicellular alga chlorella isolated from lake erie. Bull. Environ. Contam. Toxicol. 68, 261-268.

Ramakrishna, S., Fujihara, K., Teo, W., Yong, T., Ma, Z., Ramaseshan, R., 2006. Electrospun nanofibers: Solving global issues. Materials Today 9, 40-50.

Ramsden, D., 2002. Thallium. Mol Death 304-311.

Rao, C.N.R., Müller, A., Cheetham, A.K., 2007. Nanomaterials Chemistry: Recent Developments and New Directions. John Wiley & Sons, .

Reichert, P., 1998. AQUASIM 2.0—user manual. Swiss Federal Institute for Environmental Science and Technology.Dubendorf, Switzerland .

Rengaraj, S., Yeon, K., Moon, S., 2001. Removal of chromium from water and wastewater by ion exchange resins. J. Hazard. Mater. 87, 273-287.

Reynel-Avila, H.E., Mendoza-Castillo, D.I., Hernández-Montoya, V., Bonilla-Petriciolet, A., 2011. Multicomponent removal of heavy metals from aqueous solution using low-cost sorbents. Water production and Wastewater Treatment, 1st edn.Editorial Nova Science Publishers 69-99.

Rezaee, A., Ramavandi, B., Ganati, F., Ansari, M., Solimanian, A., 2006. Biosorption of mercury by biomass of filamentous algae spirogyra species. Journal of Biological Sciences 6, 695-700.

Roginsky, S., Zeldovich, Y.B., 1934. The catalytic oxidation of carbon monoxide on manganese dioxide. Acta Phys.Chem.USSR 1, 554.

Romera, E., Gonzalez, F., Ballester, A., Blazquez, M., Munoz, J., 2006. Biosorption with algae: A statistical review. Crit. Rev. Biotechnol. 26, 223-235.

Romera, E., González, F., Ballester, A., Blázquez, M., Munoz, J., 2007. Comparative study of biosorption of heavy metals using different types of algae. Bioresour. Technol. 98, 3344-3353.

Romero-González, M.E., Williams, C.J., Gardiner, P.H., 2001. Study of the mechanisms of cadmium biosorption by dealginated seaweed waste. Environ. Sci. Technol. 35, 3025-3030.

Rosengrant, L., Craig, R., 1990. Final best demonstrated available technology (BDAT) background document for P and U thallium wastes. US EPA.Office of Solid waste, Washington, DC.EPA/530-sw-90-059R, National Technical Information Services PB90-234188l .

Roy, D., Greenlaw, P.N., Shane, B.S., 1993. Adsorption of heavy metals by green algae and ground rice hulls. Journal of Environmental Science & Health Part A 28, 37-50.

Ruchhoft, C., 1949. The possibilities of disposal of radioactive wastes by biological treatment methods. Sewage works journal 877-883.

Sablani, S., Goosen, M., Al-Belushi, R., Wilf, M., 2001. Concentration polarization in ultrafiltration and reverse osmosis: A critical review. Desalination 141, 269-289.

Sağ, Y., Akçael, B., Kutsal, T., 2001. Evaluation, interpretation, and representation of three-metal biosorption equilibria using a fungal biosorbent. Process Biochemistry 37, 35-50.

Sağ, Y., Aktay, Y., 2002. Kinetic studies on sorption of cr (VI) and cu (II) ions by chitin, chitosan and rhizopus arrhizus. Biochem. Eng. J. 12, 143-153.

Sager, M., 1992. Speciation of thallium in river sediments by consecutive leaching techniques. Microchimica Acta 106, 241-251.

Sahl, K., Albuquerque, C., Shaw, D., 1978. Thallium. Handbook of geochemistry, Springer-Verlag, Berlin, Heidelberg, New York .

Sahmoun, A.E., Case, L.D., Jackson, S.A., Schwartz, G.G., 2005. Cadmium and prostate cancer: A critical epidemiologic analysis. Cancer Invest. 23, 256-263.

Saleem, M., Pirzada, T., Qadeer, R., 2007. Sorption of acid violet 17 and direct red 80 dyes on cotton fiber from aqueous solutions. Colloids Surf. Physicochem. Eng. Aspects 292, 246-250.

Sangvanich, T., Sukwarotwat, V., Wiacek, R.J., Grudzien, R.M., Fryxell, G.E., Addleman, R.S., Timchalk, C., Yantasee, W., 2010. Selective capture of cesium and thallium from natural waters and simulated wastes with copper ferrocyanide functionalized mesoporous silica. J. Hazard. Mater. 182, 225-231.

Sarı, A., Tuzen, M., 2008a. Biosorption of cadmium (II) from aqueous solution by red algae (< i> ceramium virgatum</i>): Equilibrium, kinetic and thermodynamic studies. J. Hazard. Mater. 157, 448-454.

Sarı, A., Tuzen, M., 2008b. Biosorption of cadmium (II) from aqueous solution by red algae (ceramium virgatum): Equilibrium, kinetic and thermodynamic studies. J. Hazard. Mater. 157, 448-454.

Sarkar, B., 2002. Heavy Metals in the Environment. CRC Press, .

Schiewer, S., Volesky, B., 1997. Ionic strength and electrostatic effects in biosorption of divalent metal ions and protons. Environ. Sci. Technol. 31, 2478-2485.

Schiewer, S., Volesky, B., 2000. Biosorption processes for heavy metal removal. Environmental microbe–metal interactions 14, 329-362.

Schmitt, D., Müller, A., Csögör, Z., Frimmel, F.H., Posten, C., 2001. The adsorption kinetics of metal ions onto different microalgae and siliceous earth. Water Res. 35, 779-785.

Schoer, J., 1984. Thallium. In: Anonymous Anthropogenic Compounds. Springer, , pp. 143-214.

Şeker, A., Shahwan, T., Eroğlu, A.E., Yılmaz, S., Demirel, Z., Dalay, M.C., 2008. Equilibrium, thermodynamic and kinetic studies for the biosorption of aqueous lead (II), cadmium (II) and nickel (II) ions on< i> spirulina platensis</i>. J. Hazard. Mater. 154, 973-980.

Selatnia, A., Bakhti, M., Madani, A., Kertous, L., Mansouri, Y., 2004. Biosorption of cd< sup> 2 </sup> from aqueous solution by a NaOH-treated bacterial dead< i> streptomyces rimosus</i> biomass. Hydrometallurgy 75, 11-24.

Semerjian, L., Ayoub, G., 2003. High-pH–magnesium coagulation–flocculation in wastewater treatment. Adv. Environ. Res. 7, 389-403.

Şenol, Z.M., Ulusoy, U., 2010. Thallium adsorption onto polyacryamide–aluminosilicate composites: A tl isotope tracer study. Chem. Eng. J. 162, 97-105.

Sert, Ş., Kütahyali, C., İnan, S., Talip, Z., Çetinkaya, B., Eral, M., 2008a. Biosorption of lanthanum and cerium from aqueous solutions by< i> platanus orientalis</i> leaf powder. Hydrometallurgy 90, 13-18.

Sert, Ş., Kütahyali, C., İnan, S., Talip, Z., Çetinkaya, B., Eral, M., 2008b. Biosorption of lanthanum and cerium from aqueous solutions by< i> platanus orientalis</i> leaf powder. Hydrometallurgy 90, 13-18.

Shan, X., Lian, J., Wen, B., 2002. Effect of organic acids on adsorption and desorption of rare earth elements. Chemosphere 47, 701-710.

Sharma, V., Shukla, R.K., Saxena, N., Parmar, D., Das, M., Dhawan, A., 2009. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol. Lett. 185, 211-218.

Sharma, Y., 1995. Economic treatment of cadmium (II)-rich hazardous waste by indigenous material. J. Colloid Interface Sci. 173, 66-70.

Sheibani, A., Zare-Khormizi, M., 2012. Application of factorial design for adsorption of thallium (III) ion from aqueous solutions by pistachio hull. Indian J. Chem. Technol. 19, 48-51.

Shen, J., Duvnjak, Z., 2004. Effects of temperature and pH on adsorption isotherms for cupric and cadmium ions in their single and binary solutions using corncob particles as adsorbent. Sep. Sci. Technol. 39, 3023-3041.

Sheng, P.X., Ting, Y., Chen, J.P., Hong, L., 2004. Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: Characterization of biosorptive capacity and investigation of mechanisms. J. Colloid Interface Sci. 275, 131-141.

Shuxia, X., ZHANG, S., Ke, C., Jinfeng, H., Huashan, L., Kun, W., 2011. Biosorption of la 3 and ce 3 by agrobacterium sp. HN1. Journal of Rare Earths 29, 265-270.

Singh, K., Talat, M., Hasan, S., 2006. Removal of lead from aqueous solutions by agricultural waste maize bran. Bioresour. Technol. 97, 2124-2130.

Sips, R., 1948. On the structure of a catalyst surface. J. Chem. Phys. 16, 490-495.

Skowronski, T., Pirszel, J., Pawlik-Skowronska, B., 2001. Heavy metal removal by the waste biomass of penicillium chrysogenum. Water Qual. Res. J. Can. 36, 793-803.

Smith, I.C., Carson, B.L., 1977. Trace metals in the environment.

Sonich-Mullin, C., 2012. Rare earth elements: A review of production, processing, recycling, and associated environmental issues.

Srivastava, V.C., Mall, I.D., Mishra, I.M., 2006. Equilibrium modelling of single and binary adsorption of cadmium and nickel onto bagasse fly ash. Chem. Eng. J. 117, 79-91.

Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R., 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: Prospective population study. The Lancet 353, 1140-1144.

Stirk, W., Van Staden, J., 2000. Removal of heavy metals from solution using dried brown seaweed material. Bot. Mar. 43, 467-473.

Stumm, W., Morgan, J., Aquatic chemistry, 1996.

Sulaymon, A.H., Mohammed, A.A., Al-Musawi, T.J., 2013. Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environmental Science and Pollution Research 20, 3011-3023.

Tabaraki, R., Nateghi, A., 2014. Multimetal biosorption modeling of zn 2 , cu 2 and ni 2 by sargassum ilicifolium. Ecol. Eng. 71, 197-205.

Tam, M.S., Antal, M.J., 1999. Preparation of activated carbons from macadamia nut shell and coconut shell by air activation. Ind Eng Chem Res 38, 4268-4276.

Taty-Costodes, V.C., Fauduet, H., Porte, C., Delacroix, A., 2003. Removal of cd (II) and pb (II) ions, from aqueous solutions, by adsorption onto sawdust of< i> pinus sylvestris</i>. J. Hazard. Mater. 105, 121-142.

Temkin, M., Pyzhev, V., 1940. Kinetics of ammonia synthesis on promoted iron catalysts. Acta physiochim.URSS 12, 217-222.

Tempesta, S., Paoletti, M., Pasqualetti, M., 2011. Morphological and molecular identification of a strain of the unicellular green alga dunaliella sp. isolated from tarquinia salterns. Transitional waters bulletin 4, 60-70.

Thayer, J.S., Brinckman, F., 1982. The biological methylation of metals and metalloids. Advances in Organometallic Chemistry 20, 313-356.

Thornton, I., 1986. Geochemistry of Cadmium. In: Anonymous Cadmium in the Environment. Springer, , pp. 7-12.

Tien, C., 2002. Biosorption of metal ions by freshwater algae with different surface characteristics. Process Biochemistry 38, 605-613.

Tobin, J.M., Cooper, D., Neufeld, R., 1984. Uptake of metal ions by rhizopus arrhizus biomass. Appl. Environ. Microbiol. 47, 821-824.

Treybal Robert, E., 1981. Mass-Transfer Operations. McGraw-Hill Book Company, .

Tsezos, M., Remoudaki, E., Angelatou, V., 1996. A study of the effects of competing ions on the biosorption of metals. Int. Biodeterior. Biodegrad. 38, 19-29.

Tsezos, M., Volesky, B., 1981. Biosorption of uranium and thorium. Biotechnol. Bioeng. 23, 583-604.

Tsuchiya, K., 1981. Clinical signs, symptoms, and prognosis of cadmium poisoning. JOHN WILEY & SONS, INC., 605 THIRD AVE., NEW YORK, NY 10158. 39-54.

Tsuruta, T., 2007. Accumulation of rare earth elements in various microorganisms. Journal of Rare Earths 25, 526-532.

Turiel, E., Perez-Conde, C., Martin-Esteban, A., 2003. Assessment of the cross-reactivity and binding sites characterisation of a propazine-imprinted polymer using the langmuir-freundlich isotherm. Analyst 128, 137-141.

Tüzün, İ., Bayramoğlu, G., Yalçın, E., Başaran, G., Çelik, G., Arıca, M.Y., 2005. Equilibrium and kinetic studies on biosorption of hg (II), cd (II) and pb (II) ions onto microalgae< i> chlamydomonas reinhardtii</i>. J. Environ. Manage. 77, 85-92.

Twidwell, L., Williams-Beam, C., 2002. Potential technologies for removing thallium from mine and process wastewater: An abbreviated annotation of the literature. ejmp & ep (European Journal of Mineral Processing and Environmental Protection) 2, 1-10.

Unuabonah, E.I., Taubert, A., 2014. Clay–polymer nanocomposites (CPNs): Adsorbents of the future for water treatment. Appl. Clay. Sci. 99, 83-92.

Urík, M., Kramarová, Z., Ševc, J., Čerňanský, S., Kališ, M., Medveď, J., Littera, P., Kolenčík, M., Gardosova, K., 2010. Biosorption and bioaccumulation of thallium (I) and its effect on growth of neosartorya fischeri strain. Polish Journal of Environmental Studies 19, 457-460.

Uslu, G., Tanyol, M., 2006. Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto< i> pseudomonas putida</i>: Effect of temperature. J. Hazard. Mater. 135, 87-93.

Van der Bruggen, B., Vandecasteele, C., 2002. Distillation vs. membrane filtration: Overview of process evolutions in seawater desalination. Desalination 143, 207-218.

Vaněk, A., Komárek, M., Vokurková, P., Mihaljevič, M., Šebek, O., Panušková, G., Chrastný, V., Drábek, O., 2011. Effect of illite and birnessite on thallium retention and bioavailability in contaminated soils. J. Hazard. Mater. 191, 170-176.

Veglio, F., Beolchini, F., 1997. Removal of metals by biosorption: A review. Hydrometallurgy 44, 301-316.

Veglio, F., Quaresima, R., Fornari, P., Ubaldini, S., 2003. Recovery of valuable metals from electronic and galvanic industrial wastes by leaching and electrowinning. Waste Manage. 23, 245-252.

Vieira, R.H., Volesky, B., 2010. Biosorption: A solution to pollution? International microbiology 3, 17-24.

Vijayaraghavan, K., Sathishkumar, M., Balasubramanian, R., 2010. Biosorption of lanthanum, cerium, europium, and ytterbium by a brown marine alga, turbinaria conoides. Ind Eng Chem Res 49, 4405-4411.

Vijayaraghavan, K., Mahadevan, A., Joshi, U.M., Balasubramanian, R., 2009. An examination of the uptake of lanthanum from aqueous solution by crab shell particles. Chem. Eng. J. 152, 116-121.

Vijayaraghavan, K., Palanivelu, K., Velan, M., 2006. Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. Bioresour. Technol. 97, 1411-1419.

Vijayaraghavan, K., Yun, Y., 2008a. Bacterial biosorbents and biosorption. Biotechnol. Adv. 26, 266-291.

Vijayaraghavan, K., Yun, Y., 2008b. Bacterial biosorbents and biosorption. Biotechnol. Adv. 26, 266-291.

Vilar, V.J., Botelho, C.M., Boaventura, R.A., 2006. Equilibrium and kinetic modelling of cd (II) biosorption by algae gelidium and agar extraction algal waste. Water Res. 40, 291-302.

Vincent, T., Taulemesse, J., Dauvergne, A., Chanut, T., Testa, F., Guibal, E., 2014. Thallium (I) sorption using prussian blue immobilized in alginate capsules. Carbohydr. Polym. 99, 517-526.

Vink, B., 1993. The behaviour of thallium in the (sub) surface environment in terms of eh and pH. Chem. Geol. 109, 119-123.

Volesky, B., 1987. Biosorbents for metal recovery. Trends Biotechnol. 5, 96-101.

Volesky, B., 2001. Detoxification of metal-bearing effluents: Biosorption for the next century. Hydrometallurgy 59, 203-216.

Volesky, B., May, H., Holan, Z., 1993. Cadmium biosorption by saccharomyces cerevisiae. Biotechnol. Bioeng. 41, 826-829.

Volesky, B., Prasetyo, I., 1994. Cadmium removal in a biosorption column. Biotechnol. Bioeng. 43, 1010-1015.

Volesky, B., 1990a. Biosorption and biosorbents. Biosorption of heavy metals 3-5.

Volesky, B., 1990b. Biosorption of Heavy Metals. CRC press, .

Volesky, B., 2003. Sorption and Biosorption. BV Sorbex, .

Volesky, B., 2007. Biosorption and me. Water Res. 41, 4017-4029.

Volesky, B., Holan, Z., 1995. Biosorption of heavy metals. Biotechnol. Prog. 11, 235-250.

Volokh, A., Gorbunov, A., Gundorina, S., Revich, B., Frontasyeva, M., Pal, C.S., 1990. Phosphorus fertilizer production as a source of rare-earth elements pollution of the environment. Sci. Total Environ. 95, 141-148.

Waalkes, M.P., 2003. Cadmium carcinogenesis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 533, 107-120.

Waalkes, M.P., Coogan, T.P., Barter, R.A., 1992. Toxicological principles of metal carcinogenesis with special emphasis on cadmium. CRC Crit. Rev. Toxicol. 22, 175-201.

Wang, D., Sun, W., Xu, Y., Tang, H., Gregory, J., 2004. Speciation stability of inorganic polymer flocculant–PACl. Colloids Surf. Physicochem. Eng. Aspects 243, 1-10.

Wang, J., Chen, C., 2006. Biosorption of heavy metals by< i> saccharomyces cerevisiae</i>: A review. Biotechnol. Adv. 24, 427-451.

Wang, J., Chen, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol. Adv. 27, 195-226.

Weber, W., Morris, J., 1962. Advances in water pollution research: Removal of biologically resistant pollutants from waste waters by adsorption. 2, 231-266.

Weber, T.W., Chakravorti, R.K., 1974. PORE AND SOLID DIFFUSION MODELS FOR FIXED-BED ADSORBERS. AIChE J. 20, 228-238.

Wehr, J.D., 2002. Freshwater Algae of North America: Ecology and Classification. Academic Press, .

Wehrheim, B., Wettern, M., 1994. Biosorption of cadmium, copper and lead by isolated mother cell walls and whole cells of chlorella fusca. Appl. Microbiol. Biotechnol. 41, 725-728.

Wilke, A., Buchholz, R., Bunke, G., 2006. Selective biosorption of heavy metals by algae. Environmental Biotechnology 2, 47-56.

Wingenfelder, U., Hansen, C., Furrer, G., Schulin, R., 2005. Removal of heavy metals from mine waters by natural zeolites. Environ. Sci. Technol. 39, 4606-4613.

Wu, D., Zhao, J., Zhang, L., Wu, Q., Yang, Y., 2010. Lanthanum adsorption using iron oxide loaded calcium alginate beads. Hydrometallurgy 101, 76-83.

Wu, Q., Wu, C., Wang, C., Lu, X., Li, X., Wang, Z., 2011. Sensitive determination of cadmium in water, beverage and cereal samples by a novel liquid-phase microextraction coupled with flame atomic absorption spectrometry. Analytical Methods 3, 210-216.

Xiao, T., Guha, J., Boyle, D., Liu, C., Chen, J., 2004. Environmental concerns related to high thallium levels in soils and thallium uptake by plants in southwest guizhou, china. Sci. Total Environ. 318, 223-244.

Xiao, T., Yang, F., Li, S., Zheng, B., Ning, Z., 2012. Thallium pollution in china: A geo-environmental perspective. Sci. Total Environ. 421, 51-58.

Xiao, T., Hong, Y., Zhang, B., Hong, B., 2000. Hydrogeochemistry of toxic metal elements in a au-as-hg-tl mineralized area of southwestern guizhou, china. Diqiu Huaxue 29, 571-577.

Xiao, X., Luo, S., Zeng, G., Wei, W., Wan, Y., Chen, L., Guo, H., Cao, Z., Yang, L., Chen, J., 2010. Biosorption of cadmium by endophytic fungus (EF) microsphaeropsis sp. LSE10 isolated from cadmium hyperaccumulator solanum nigrum L. Bioresour. Technol. 101, 1668-1674.

Xu, H., Liu, Y., Tay, J., 2006. Effect of pH on nickel biosorption by aerobic granular sludge. Bioresour. Technol. 97, 359-363.

Yang, Y., Faustino, P.J., Progar, J.J., Brownell, C.R., Sadrieh, N., May, J.C., Leutzinger, E., Place, D.A., Duffy, E.P., Lawrence, X.Y., 2008. Quantitative determination of thallium binding to ferric hexacyanoferrate: Prussian blue. Int. J. Pharm. 353, 187-194.

Yasuda, M., Miwa, A., Kitagawa, M., 1995. Morphometric studies of renal lesions in itai-itai disease: Chronic cadmium nephropathy. Nephron 69, 14-19.

Yiacoumi, S., Tien, C., 1995. Kinetics of Metal Ion Adsorption from Aqueous Solutions: Models, Algorithms, and Applications. Springer, .

Younesi, S., Alimadadi, H., Alamdari, E.K., Marashi, S., 2006. Kinetic mechanisms of cementation of cadmium ions by zinc powder from sulphate solutions. Hydrometallurgy 84, 155-164.

Zafar, M.N., Nadeem, R., Hanif, M.A., 2007. Biosorption of nickel from protonated rice bran. J. Hazard. Mater. 143, 478-485.

Zakhama, S., Dhaouadi, H., M’henni, F., 2011. Nonlinear modelisation of heavy metal removal from aqueous solution using ulva lactuca algae. Bioresour. Technol. 102, 786-796.

Zhang, X., Yin, G., Hu, Z., 2003. Extraction and separation of gallium, indium and thallium with several carboxylic acids from chloride media. Talanta 59, 905-912.

Zitko, V., 1975a. Toxicity and pollution potential of thallium. Sci. Total Environ. 4, 185-192.

Zitko, V., 1975b. Toxicity and pollution potential of thallium. Sci. Total Environ. 4, 185-192.

Zolgharnein, J., Asanjarani, N., Shariatmanesh, T., 2011. Removal of thallium (I) from aqueous solution using modified sugar beet pulp. Toxicological & Environmental Chemistry 93, 207-214.

APPENDICES

APPENDIX A: EQUILIBRIUM DATA FOR THE TESTED ALGAL SPECIES LANTHANUM

Stichococcus bacillaris

initial	Ci(mmol/L)	ce (mg/L) final	Ce(mmol/L)	qe (mg/L) uptake	qe(mmol/L)	ce/qe (mg/L)	ce/qe (mmol/L)
15	0.1080	**0**	0.00	30	0.2160	**0**	0.00
25	0.1800	**4.46**	0.032	41.08	0.2957	**0.108569**	0.11
50	0.3599	**26.338**	0.190	47.324	0.3407	**0.556546**	0.56
100	0.7199	**72.669**	0.523	54.662	0.3935	**1.329424**	1.33
150	1.0798	**125**	0.900	50	0.3599	**2.5**	2.50

Scenedesmus acuminutus

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1080	**0**	0.00	30	0.2160	**0**	0.00
25	0.1800	**7.16**	0.052	35.68	0.2569	**0.201**	0.20
50	0.3599	**11.83**	0.085	76.34	0.5496	**0.155**	0.15
100	0.7199	**58.67**	0.422	82.66	0.5951	**0.710**	0.71
150	1.0798	**94.7**	0.682	110.6	0.7962	**0.8562**	0.86

chloroidium saccharophilum

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1080	**0**	0.00	30	0.2160	**0.0000**	0.00
25	0.1800	**0**	0.00	50	0.3599	**0.0000**	0.00
50	0.3599	**21.19**	0.15	57.62	0.4148	**0.3678**	0.37
100	0.7199	**42.5**	0.31	115	0.8279	**0.3696**	0.37
150	1.0798	**87.05**	0.63	125.9	0.9063	**0.6914**	0.69

Desmodesmus multivariabilis

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1080	**0**	0.0000	30	0.2160	**0**	0.0000
25	0.1800	**0**	0.0000	50	0.3599	**0**	0.0000
50	0.3599	**15.44**	0.1112	69.12	0.4976	**0.22338**	0.2234
100	0.7199	**35.78**	0.2576	128.44	0.9246	**0.278574**	0.2786
150	1.0798	**100.94**	0.7267	98.12	0.7064	**1.02874**	1.0287

Chlorella vulgaris

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1080	**3.822**	0.0275	22.356	0.1609	**0.170961**	0.1710
25	0.1800	**6.404**	0.0461	37.192	0.2677	**0.172188**	0.1722
50	0.3599	**18.398**	0.1324	63.204	0.4550	**0.291089**	0.2911
100	0.7199	**63.475**	0.4570	73.05	0.5259	**0.868925**	0.8689
150	1.0798	**115.2705**	0.8298	69.459	0.5000	**1.659547**	1.6595

Chlamydomonas reinhardtii

	Ci(mmol/L)	ce	Ce(mmol/L)	qe	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
initial		final		uptake			
15	0.1080	**0**	0.0000	28.11	0.2024	**0**	0.0000
25	0.1800	**0.183**	0.0013	42.84	0.3084	**0.004272**	0.0000
50	0.3599	**9.343**	0.0673	81.32	0.5854	**0.114892**	0.0008
100	0.7199	**53.11**	0.3823	93.77	0.6750	**0.566386**	0.0041
150	1.0798	**75.901**	0.5464	148.198	1.0669	**0.512159**	0.0037

THALLIUM

Chlamydomonas

	Ci(mmol/L)	ce	Ce(mmol/L)	qe	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
initial		final		uptake			
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	0	0.0000	300	1.4679	**0.0000**	0.0000
250	1.2232	2.701	0.0132	494.598	2.4200	**0.0055**	0.0055
500	2.4464	23.011	0.1126	953.978	4.6677	**0.0241**	0.0241

Chlorella

	Ci(mmol/L)	ce	Ce(mmol/L)	qe	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
initial		final		uptake			
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	0.3365	0.0016	299.327	1.4646	**0.0011**	0.0011
250	1.2232	3.033	0.0148	493.934	2.4167	**0.0061**	0.0061
500	2.4464	29.579	0.1447	940.842	4.6034	**0.0314**	0.0314

Scenedesmus

	Ci(mmol/L)	ce	Ce(mmol/L)	qe	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
initial		final		uptake			
15	0.0734	0	0.0000	30	0.1468	**0.0000**	0.0000
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	0	0.0000	300	1.4679	**0.0000**	0.0000
250	1.2232	22.784	0.1115	454.432	2.2235	**0.0501**	0.0501
500	2.4464	64.35	0.3149	871.3	4.2631	**0.0739**	0.0739

Chloroidium

	Ci(mmol/L)	ce	Ce(mmol/L)	qe	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
initial		final		uptake			
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	0	0.0000	300	1.4679	**0.0000**	0.0000
250	1.2232	4.062	0.0199	491.876	2.4067	**0.0083**	0.0083
500	2.4464	17.053	0.0834	965.894	4.7260	**0.0177**	0.0177

Stichococcus

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	3.0684	0.0150	293.8632	1.4378	**0.0104**	0.0104
250	1.2232	21.614	0.1058	456.772	2.2349	**0.0473**	0.0473
500	2.4464	62.967	0.3081	874.066	4.2767	**0.0720**	0.0720

Desmodesmus

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
50	0.2446	0	0.0000	100	0.4893	**0.0000**	0.0000
100	0.4893	0	0.0000	200	0.9786	**0.0000**	0.0000
150	0.7339	0	0.0000	300	1.4679	**0.0000**	0.0000
250	1.2232	14.951	0.0732	470.098	2.3001	**0.0318**	0.0318
500	2.4464	44.642	0.2184	910.716	4.4560	**0.0490**	0.0490

CADMIUM

Chloroidium

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1334	7.78	0.0692	14.44	0.1285	**0.5388**	0.5388
25	0.2224	14.04	0.1249	21.92	0.1950	**0.6405**	0.6405
50	0.4448	29	0.2580	42	0.3736	**0.6905**	0.6905
100	0.8896	63.9	0.5684	72.2	0.6423	**0.8850**	0.8850
150	1.3344	110.215	0.9805	79.57	0.7078	**1.3851**	1.3851

Desmodesmus

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.1334	8.75	0.0778	12.5	0.1112	**0.700**	0.7000
25	0.2224	11.86	0.1055	26.28	0.2338	**0.451**	0.4513
50	0.4448	32.85	0.2922	34.3	0.3051	**0.958**	0.9577
100	0.8896	80.2	0.7135	39.6	0.3523	**2.025**	2.0253
150	1.3344	135.2	1.2027	29.6	0.2633	**4.568**	4.5676

Stichococcus

initial	Ci(mmol/L)	ce final	Ce(mmol/L)	qe uptake	qe(mmol/L)	ce/qe	ce/qe (mmol/L)
15	0.0734	3.49	0.017	23.02	0.1126	**0.152**	0.1516
25	0.1223	9.96	0.049	30.08	0.1472	**0.331**	0.3311
50	0.2446	18.55	0.091	62.9	0.3078	**0.295**	0.2949
100	0.4893	50.3	0.246	99.4	0.4863	**0.506**	0.5060
150	0.7339	99.5	0.487	101	0.4942	**0.985**	0.9851

APPENDIX B: ADSORPTION KINETIC DATA FOR THE TESTED ALGAL SPECIES

LANTHANUM

Stichococcus bacillaris

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	14.502	18.467	23.216	33.846	30.65
15	27.877	31.603	32.897	46.467	39.999
25	28.318	32.648	34.347	53.937	45.713
40	29.604	35.569	36.372	50.944	44.035
60	28.792	37.57	38.535	45.213	46.54
90	29.871	38.599	39.46	54.321	49.442
120	30	40.007	35.415	54.131	36.867
180	30	40.35	39.537	55.332	44.224
240	30	40.822	47.639	55.953	37.956
360	30	41.081	47.324	54.663	36.381

Scenedesmus acuminutus

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	17.028	28.372	66.528	94.689	84.32
15	26.81	34.578	74.224	98.485	138.08
25	29.395	33.381	74.738	99.523	145.98
40	27.921	34.174	75.994	99.444	147.9
60	28.806	37.67	72.48	97.239	122.9
90	29.871	33.608	76.418	93.731	128.48
120	28.923	32.992	76.292	99.399	107.2
180	29.678	33.729	75.06	82.777	95.5
240	29.801	32.865	77.792	81.768	103.94
360	30	35.691	76.344	82.669	110.54

Chloroidium saccharophilum

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	28.30	40.680	37.584	79.960	127.932
15	30	48.395	52.042	110.996	122.868
25	30	50.000	52.340	119.102	125.020
40	30	50.000	53.508	127.414	122.664
60	30	50.000	56.174	125.748	122.184
90	30	50.000	55.474	129.140	128.752
120	30	50.000	55.404	127.998	128.468
180	30	50.000	55.868	131.992	128.184
240	30	50.000	54.322	117.648	128.040
360	30	50.000	57.620	115.004	125.908

Desmodesmus multivariabilis

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/gat 150 mg/L
0	11.9530	19.94	40.032	118.908	76.725
15	27.1550	34.171	49.618	124.505	94.103
25	30.0000	41.234	65.876	128.425	92.011
40	30.0000	41.735	51.894	128.5725	169.46
60	30.0000	44.412	53.165	132.8525	124.768
90	30.0000	45.479	58.155	132.2615	104.625
120	30.0000	47.1	56.912	131.949	105.345
180	30.0000	48.137	59.657	131.5345	102.812
240	30.0000	46.471	57.861	131.59	106.072
360	30.0000	48.528	60.689	128.4325	98.114

Chlorella vulgaris

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	12.476	22.168	40.904	40.889	41.5
15	20.538	37.528	58.724	71.003	65.286
25	19.928	37.302	58.998	73.457	67.96
40	18.861	37.904	60.307	76.674	73.464
60	20.258	38.063	59.07	75.934	75.738
90	20.987	38.204	58.761	77.043	70.012
120	20.874	37.818	60.591	78.482	72.562
180	20.545	37.893	61.58	73.117	71.339
240	21.862	37.939	61.528	82.087	70.654
360	22.356	37.192	63.204	73.051	69.459

Chlamydomonas reinhardtii

Time(t)mins	q_t(mg/g) at 15	q_t(mg/g) at 25	q_t(mg/g) at 50	q_t(mg/g) at 100	q_t(mg/g) at 150
0	28.60	40.98	51.80	62.32	144.42
15	24.82	42.84	75.41	83.58	154.08
25	25.53	45.06	75.72	88.84	156.16
40	25.82	44.33	76.25	88.83	155.34
60	25.16	43.16	77.54	88.42	154.27
90	25.99	43.33	77.04	86.94	153.73
120	25.19	43.14	78.23	90.99	149.05
180	26.30	42.48	78.71	88.79	159.27
240	25.46	42.33	78.92	87.22	150.23
360	30.00	46.37	81.32	93.77	148.20

THALLIUM

Chlamydomonas

	250mg/L		500mg/L	
t min	qt	t/q	qt	t/q
0	470.738	0	976.590	0
15	465.136	0.03225	972.026	0.0154
25	481.945	0.05187	980.732	0.0255
40	477.363	0.08379	955.910	0.0418
60	485.504	0.12358	976.756	0.0614
90	472.297	0.19056	956.966	0.0940
120	460.488	0.26059	987.415	0.1215
180	461.841	0.38974	945.606	0.1904
240	482.905	0.49699	966.050	0.2484
360	467.559	0.76996	953.978	0.3774

Chlorella

	250mg/L		500mg/L	
t min	qt	1/qt	qt	t/q
0	491.925	0	931.673	0
15	499.854	0.0300	997.888	0.0150
25	440.500	0.0568	999.854	0.0250
40	499.854	0.0800	982.887	0.0407
60	499.854	0.1200	972.961	0.0617
90	494.899	0.1819	964.198	0.0933
120	499.854	0.2401	949.375	0.1264
180	443.974	0.4054	956.701	0.1881
240	462.221	0.5192	971.905	0.2469
360	412.005	0.8738	958.276	0.3757

Scenedesmus

	250mg/L		500mg/L	
t min	qt	t/q	qt	t/q
0	463.408	0	919.493	0
15	463.802	0.0323	945.299	0.01587
25	474.072	0.0527	892.090	0.02802
40	459.705	0.0870	949.769	0.04212
60	424.259	0.1414	919.787	0.06523
90	478.277	0.1882	894.518	0.10061
120	444.618	0.2699	905.186	0.13257
180	459.114	0.3921	891.420	0.20193
240	422.420	0.5682	884.050	0.27148
360	454.432	0.7922	871.300	0.41318

CADMIUM

Stichococcus

		15		50		150	
Time(t)mins	In t	q	t/q	q	t/q	q	t/q
0	0	10.500	0.000	57.3	0.000	91.6	0.000
15	1.176	17.780	0.844	63.2000	0.237	100.8	0.149
25	1.398	20.020	1.249	53.5000	0.467	114.2	0.219
40	1.602	21.640	1.848	59.8000	0.669	108.4	0.369
60	1.778	22.300	2.691	66.4000	0.904	109.4	0.548
90	1.954	23.020	3.910	65.6000	1.372	114.6	0.785
120	2.079	22.620	5.305	67.6000	1.775	118.4	1.014

APPENDIX C: DESORPTION KINETIC DATA FOR TESTED ALGAL SPECIES

LANTHANUM

Stichococcus bacillaris

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	22.310	25.696	34.785	57.868	29.461
15	16.705	23.761	30.258	57.553	31.617
25	17.637	24.076	32.673	56.597	22.783
40	16.388	20.899	30.658	55.715	23.656
60	16.173	20.366	24.822	55.548	22.911
90	15.551	19.428	23.669	46.893	27.961
120	14.527	18.023	21.785	45.814	21.422
180	13.095	12.637	20.767	43.926	18.206
240	12.578	5.414	11.302	34.732	14.100
360	7.002	5.014	9.658	20.841	9.433

Scenedesmus multivariabilis

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	41.438	44.273	38.010	64.211	39.336
15	39.809	45.352	40.024	67.196	34.718
25	40.069	45.232	35.600	67.537	57.236
40	43.731	45.229	39.383	63.766	36.382
60	36.328	43.619	38.374	62.584	35.173
90	35.603	44.022	30.338	65.171	33.255
120	34.403	44.375	37.812	61.631	31.173
180	32.028	38.000	29.257	56.746	27.491
240	28.694	36.787	27.979	54.593	22.118
360	26.534	27.340	19.093	41.917	18.491

Chloroidium saccharophilum

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	66.385	85.791	7.637	29.774	213.549
15	66.297	55.905	13.538	26.915	148.202
25	64.464	54.568	14.757	26.494	153.887
40	63.955	53.387	11.403	22.774	154.892
60	62.764	52.789	14.215	16.051	139.621
90	61.367	51.305	17.003	16.391	147.364
120	59.470	54.479	16.591	15.638	145.046
180	55.906	53.226	17.003	19.591	134.185
240	61.667	60.589	11.065	12.226	115.026
360	45.933	49.089	10.960	7.936	106.338

Desmodesmus multivariabilis

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	24.261	17.195	16.417	61.217	60.793
15	39.904	7.725	16.981	58.987	58.236
25	37.104	6.862	22.245	59.837	55.895
40	39.313	8.425	19.423	57.135	52.683
60	53.548	4.570	13.992	56.174	58.243
90	62.670	19.429	9.908	57.794	56.479
120	33.391	4.496	14.277	53.404	54.410
180	28.417	3.918	3.257	53.901	41.912
240	28.122	0.607	4.587	53.888	33.171
360	28.478	0.000	4.075	53.484	26.850

Chlorella vulgaris

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	12.843	9.525	40.775	29.319	130.772
15	12.527	9.098	41.180	29.495	58.631
25	12.384	9.074	38.227	29.510	56.592
40	12.339	9.063	38.893	29.433	56.530
60	12.356	8.877	36.976	29.100	54.110
90	12.113	8.558	36.758	29.670	51.341
120	11.826	7.973	37.710	26.769	49.361
180	11.568	7.277	35.585	25.601	45.338
240	11.057	7.315	32.268	26.634	39.445
360	10.638	5.734	32.160	21.326	33.115

Chlamydomonas reinhardtii

Time(t)mins	q_t(mg/g) at 15 mg/L	q_t(mg/g) at 25 mg/L	q_t(mg/g) at 50 mg/L	q_t(mg/g) at 100 mg/L	q_t(mg/g) at 150 mg/L
0	16.532	32.402	43.461	56.463	0
15	16.695	32.525	38.070	53.295	0.153
25	16.267	32.422	38.089	56.107	0.257
40	16.088	32.285	36.729	53.538	0.414
60	16.601	31.879	35.538	52.443	0.618
90	15.366	31.533	33.813	54.711	0.950
120	14.766	31.024	33.810	48.137	1.257
180	13.596	29.786	31.896	45.533	1.920
240	14.508	28.572	37.570	43.830	2.614
360	7.853	26.927	26.796	40.053	4.120

THALLIUM

Chlamydomonas

	250mg/L		**500mg/L**	
t min	qt	1/qt	qt	1/qt
0	421.3407	0.00237	926.1608	0.00108
15	425.7176	0.00235	902.8706	0.00111
25	414.6222	0.00241	895.7706	0.00112
40	411.3065	0.00243	859.0882	0.00116
60	415.6741	0.00241	889.9127	0.00112
90	396.8	0.00252	865.3451	0.00116
120	387.2611	0.00258	882.8873	0.00113
180	366.037	0.00273	841.8618	0.00119
240	356.3083	0.00281	825.1039	0.00121
360	339.7111	0.00294	760.55	0.00131

Chlorella

	250mg/L		**500mg/L**	
t min	qt	1/qt	qt	1/qt
0	360.5008	0.002774	582.1987	0.001718
15	344.3504	0.04356	584.5484	0.001711
25	368.0802	0.06792	581.3129	0.00172
40	357.026	0.112037	548.7632	0.001822
60	349.2015	0.171821	553.2387	0.001808
90	330.6992	0.272151	545.1116	0.001834
120	348.7252	0.344111	534.4555	0.001871
180	308.9702	0.58258	522.751	0.001913
240	319.2603	0.751738	503.6297	0.001986
360	333.6809	1.078875	494.1523	0.002024

	Scenedesmus			
	250mg/L		**500mg/L**	
t min	qt	1/qt	qt	1/qt
0	361.0146	0.00277	681.3974	0.001468
15	429.2282	0.00233	660.4948	0.001514
25	361.0602	0.00277	678.7052	0.001473
40	377.4524	0.002649	654.1552	0.001529
60	374.6825	0.002669	628.1043	0.001592
90	364.3864	0.002744	630.5017	0.001586
120	359.2621	0.002783	618.2198	0.001618
180	338.9951	0.00295	586.4017	0.001705
240	319.3252	0.003132	577.3422	0.001732
360	273.0495	0.003662	531.7871	0.00188

APPENDIX D: ADSORPTION/DESORPTION CYCLES FOR SOME TESTED ALGAE

LANTHANUM

3RD CYCLE

Species	**spp name**	**initial**	**Adsorption**	***10 Ad**	**% uptake**	**Desorption**	***10**
Chlamy	a	100	3.7534	37.534	62.466	5.3214	53.214
Scened	b	100	2.9748	29.748	70.252	6.5765	65.765
Chlorodium	c	100	4.5587	45.587	54.413	2.6804	26.804
Sticho	d	100	6.5436	65.436	34.564	0.288	2.88
Des	e	100	2.2883	22.883	77.117	6.7614	67.614
chlorella	f	100	1.8216	18.216	81.784	9.1883	91.883

2ND CYCLE

spp name	**Adsorption**	***10**	**% uptake**	**Desorption**	***10 recovery**
a	2.5389	25.389	74.611	6.6852	66.852
b	2.4601	24.601	75.399	5.866	58.66
c	2.2421	22.421	77.579	7.1927	71.927
d	4.9921	49.921	50.079	0.9664	9.664
e	1.1979	11.979	88.021	6.0983	60.983
f	2.8436	28.436	71.564	4.8928	48.928

1ST CYCLE

spp name	**Adsorption**	***10 Ads**	**% uptake**	**Desorption**	
a	0.1559	1.559	98.441	7.7085	65
b	4.0023	40.023	59.977	3.4574	77.44
c	1.8438	18.438	81.562	6.1771	97.17
d	7.0433	70.433	29.567	0.0013	88.37
e	3.2551	32.551	67.449	4.1776	99.63
f	1.7484	17.484	82.516	4.21	68.47

APPENDIX I: KINETIC MODELLING OF TERNARY SYSTEM USING AQUASIM

AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for Cadmium at 25
****

Date and time of listing: 02/12/2015 14:17:47
Number of parameters = 3
Number of data points = 15

```
Estimation method     = simplex

Parameters:
Name        Unit        Start       Minimum       Maximum
 K          mg/L        0.698253      0              10
 q          1/h         6.62172       0                10
 qc         (mg*g)/(L*m^2)2.90085       0            10

Calculations:
 K            q            qc           Chi^2
 [mg/L]    [1/h]   [(mg*g)/(L*m^2)]
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 50 mg/L Cd
**********************************************************************
Date and time of listing:  02/12/2015  14:38:34
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit        Start       Minimum       Maximum
 Co          mg/L        50             0                50
 K           mg/L        1.03957       0              10
 q           1/h         6.68382        0                10
 qc          (mg*g)/(L*m^2)2.80621      0            10
**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 100mg/L Cd
**********************************************************************
Date and time of listing:  02/13/2015  09:42:03
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit        Start       Minimum       Maximum
 Co          mg/L        86.8771       0            100
 K           mg/L        2.86397       0            10
 q           1/h         2.61383       0            10
 qc          (mg*g)/(L*m^2)3.439         0            10
**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 150 mg/L Cd
**********************************************************************
Date and time of listing:  02/12/2015  15:13:11

Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit        Start       Minimum       Maximum
 Co          mg/L        81.3101        0            150
 K           mg/L        6.91081        0            10
 q           1/h         2.03591       0            10
 qc          (mg*g)/(L*m^2)2.43276       0            10



**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 25 mg/L La
**********************************************************************
Date and time of listing:  02/12/2015  16:58:00
Number of parameters  = 3
Number of data points = 15
Estimation method     = simplex
```

```
Parameters:
Name        Unit        Start       Minimum      Maximum
 K          mg/L        0.0072474    0           10
 q          1/h         9.94904      0           10
 qc         (mg*g)/(L*m^2)3.96539      0           10

**********************************************************************\
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File at 50 mg/L La
**********************************************************************
Date and time of listing:  02/12/2015  16:40:29
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit        Start       Minimum      Maximum
 Co          mg/L        0.00260841    0           50
 K           mg/L        9.29554      0           10
 q           1/h         1.0987       0           10
 qc          (mg*g)/(L*m^2)0.676607     0           10


**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 100 mg/L La
**********************************************************************
Date and time of listing:  02/13/2015  10:35:18
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex

Parameters:
 Name        Unit        Start       Minimum      Maximum
 Co          mg/L        2.41995      0           100
 K           mg/L        9.99211      0           10
 q           1/h         0.0151054    0           10
 qc          (mg*g)/(L*m^2)0.00607788    0           10
**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 150 mg/L La
**********************************************************************
Date and time of listing:  02/12/2015  16:32:18
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit        Start       Minimum      Maximum
 Co          mg/L        39.5479      0           150
 K           mg/L        8.0631       0           10
 q           1/h         3.91611      0           10
 qc          (mg*g)/(L*m^2)3.35033      0           10



**********************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File at 150 mg/l Tl
**********************************************************************

Date and time of listing:  04/17/2015  17:42:47
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
```

```
Parameters:
 Name        Unit         Start       Minimum      Maximum
 Co           mg/L         51.2517      0            80
 K            mg/L         9.90711      0            10
 q           1/h         0.000559908   0            10
 qc         (mg*g)/(L*m^2)0.00740019    0            10
************************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 250 mg/l Tl
************************************************************************

Date and time of listing:  04/17/2015  17:28:29
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex

Parameters:
 Name        Unit         Start       Minimum      Maximum
 Co           mg/L         49.9933      0            50
 K            mg/L         9.99988      0            10
 q           1/h         0.000427064   0            10
 qc         (mg*g)/(L*m^2)0.00628164    0            10
************************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File at 500 mg/L Tl
************************************************************************
Date and time of listing:  04/17/2015  17:11:49

Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex

Parameters:
Name        Unit         Start       Minimum      Maximum
 Co           mg/L         49.95        0            50
 K            mg/L         2.10401e-006  0            10
 q           1/h         0.000108032   0            10
 qc         (mg*g)/(L*m^2)0.00255603    0            10

************************************************************************
AQUASIM Version 2.0 (win/mfc) - Parameter Estimation File for 800 mg/l Tl
************************************************************************
Date and time of listing:  04/16/2015  15:53:13
Number of parameters  = 4
Number of data points = 15
Estimation method     = simplex
Parameters:
 Name        Unit         Start       Minimum      Maximum
 Co           mg/L         5.00261      0              80
 K            mg/L         9.29554      0              10
 q           1/h         1.0987         0              10
 qc         (mg*g)/(L*m^2)0.676607     0            10
```

APPENDIX J: SPECIES IDENTIFICATION USING MOLECULAR CONCEPT SAMPLE QUERY A_2P

```
BLASTN 2.2.26+
Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped
BLAST and PSI-BLAST: a new generation of protein database search programs",
```

```
Nucleic Acids Res. 25:3389-3402.
RID: XGBDKG2X012
Database: nr
Query= A2_P_ITS_1_H09_24
(528 letters)
Score E
Sequences producing significant alignments: (Bits) Value
gi|374719759|gb|JQ315188.1| Desmodesmus sp. GTD9C2 18S riboso... 1113 0E00
gi|89515343|gb|DQ417525.1| Desmodesmus multivariabilis var. t... 1048 0E00
gi|338809991|gb|JF835988.1| Desmodesmus sp. F2 internal trans... 908 0E00
gi|338809990|gb|JF835987.1| Desmodesmus sp. F1 internal trans... 903 0E00
gi|338809993|gb|JF835990.1| Desmodesmus sp. F18 internal tran... 891 0E00
gi|89515348|gb|DQ417530.1| Desmodesmus sp. isolate Tow 8/18 P... 848 0E00
gi|89515383|gb|DQ417565.1| Desmodesmus sp. isolate Tow 10/11 ... 840 0E00
gi|359385310|emb|FR865727.1| Scenedesmus armatus var. subalte... 836 0E00
gi|89515382|gb|DQ417564.1| Desmodesmus sp. isolate Tow 6/16 T... 830 0E00
gi|89515381|gb|DQ417563.1| Desmodesmus sp. isolate Tow 6/16 T... 816 0E00
gi|385258200|gb|JQ710682.1| Desmodesmus sp. CB1 internal tran... 802 0E00
gi|344190134|gb|HQ335218.1| Desmodesmus sp. S7 internal trans... 791 0E00
gi|344190133|gb|HQ335217.1| Desmodesmus sp. S3 internal trans... 791 0E00
gi|89515349|gb|DQ417531.1| Desmodesmus sp. isolate Itas 6/3 T... 791 0E00
gi|338809992|gb|JF835989.1| Desmodesmus sp. F5 internal trans... 787 0E00
gi|169798015|gb|EU502832.1| Desmodesmus sp. MAT-2008a 18S rib... 785 0E00
gi|89515350|gb|DQ417532.1| Desmodesmus sp. isolate Tow 6/16 T... 779 0E00
gi|89515339|gb|DQ417521.1| Desmodesmus armatus var. subaltern... 775 0E00
gi|89515338|gb|DQ417520.1| Desmodesmus armatus var. subaltern... 773 0E00
gi|169798018|gb|EU502835.1| Desmodesmus sp. MAT-2008b 18S rib... 751 0E00
gi|379062656|gb|JQ313131.1| Desmodesmus sp. 2Cl66E internal t... 744 0E00
gi|379062658|gb|JQ313133.1| Desmodesmus sp. 1Hp86E-2 internal... 744 0E00
gi|379062657|gb|JQ313132.1| Desmodesmus sp. 3Dp86E-1 internal... 744 0E00
gi|379062659|gb|JQ313134.1| Desmodesmus sp. 1Pm66B internal t... 744 0E00
gi|89515366|gb|DQ417548.1| Desmodesmus sp. isolate Tow 6/16 T... 740 0E00
gi|40288331|gb|AY461360.1| Desmodesmus multivariabilis strain... 740 0E00
gi|89515365|gb|DQ417547.1| Desmodesmus armatus var. subaltern... 728 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 728 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 51 7E-03
gi|40288343|gb|AY461373.1| Desmodesmus multivariabilis var. t... 726 0E00
gi|89515380|gb|DQ417562.1| Desmodesmus komarekii isolate Tow ... 722 0E00
gi|89515376|gb|DQ417558.1| Desmodesmus bicellularis isolate N... 720 0E00
gi|40288336|gb|AY461366.1| Desmodesmus multivariabilis var. t... 718 0E00
gi|40288338|gb|AY461368.1| Desmodesmus multivariabilis strain... 712 0E00
gi|359385311|emb|FR865728.1| Scenedesmus armatus var. brevica... 679 0E00
gi|89515340|gb|DQ417522.1| Desmodesmus sp. isolate Tow 8/18 P... 667 0E00
gi|338223301|gb|HQ718787.1| Desmodesmus sp. SCU01 internal tr... 665 0E00
strain... 119 4E-23

ALIGNMENTS
3
>gb|JQ315188.1| Desmodesmus sp. GTD9C2 18S ribosomal RNA gene, partial sequence; internal
transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene, partial sequence
Length=1444
Score = 1113.4 bits (546), Expect = 0E00
Identities = 561/572 (98), Gaps = 0/572 (0)
Strand = Plus/Plus
Query 57 GACGTTMGGTCATAACCTTAACCCGGTTTGGCCTACTAACCTACACACCAKTGACCAACC 116
|||||| ||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct 557 GACGTTAGGTCATAACCTTAACCCGGTTTGGCCTACTAACCTACACACCATTGACCAACC 616
Query 117 ATTGCTTAAACCAAACTCTGAAGTTTCGGCTGCTGTTAATCGGCAGTTTTAACGAAAACA 176
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 617 ATTGCTTAAACCAAACTCTGAAGTTTCGGCTGCTGTTAATCGGCAGTTTTAACGAAAACA 676
Query 177 ACTCTCAACAACGGATATCTTGGCTCTCGCAACGATGAARAACGCAGCGAAATGCGATAC 236
||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct 677 ACTCTCAACAACGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATAC 736
Query 237 GTAGTGTGAATTGCAGAATTCCSTGAACCATCGAATCTTTGAACGCATATTGCGCTCGAC 296
|||||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct 737 GTAGTGTGAATTGCAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGCTCGAC 796
Query 297 TCCTCGGAGAAGAGCATGTCTGCCTCAKCGTCGGTTTACWCCCTCACCCCTCTTCCTTTW 356
||||||||||||||||||||||||||| ||||||||||| |||||||||||||||||||
Sbjct 797 TCCTCGGAGAAGAGCATGTCTGCCTCAGCGTCGGTTTACACCCTCACCCCTCTTCCTTTT 856
Query 357 CRAGGAAGCTTGTCGTGCTTGCTCAAGCCGGCATCAGGGGTGGATCTGGCCCTCCCAATC 416
| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 857 CAAGGAAGCTTGTCGTGCTTGCTCAAGCCGGCATCAGGGGTGGATCTGGCCCTCCCAATC 916
Query 417 GGAGTCACTTTCGGTTGGGTTGGCTGAAGCACAGAGGCTTAAACTGGGACCCCATTCGGG 476
|||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
```

```
Sbjct 917 GGAGTCACTTTCGGTTGGGTTGGCTGAAGCACAGAGGCTTAAACTGGGACCCGATTCGGG 976
Query 477 CTCAACTGGATAGGTAGCAACACCCTCGGGTGCCTACACGAAGTTGTGTCTGAGGACCTG 536
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 977 CTCAACTGGATAGGTAGCAACACCCTCGGGTGCCTACACGAAGTTGTGTCTGAGGACCTG 1036
Query 537 GTTAGGAGCCAAGCAGGAAACGCGTCTTTGGCGCGTATCTTTGTATTCGACCTGAGCTCA 596
|||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct 1037 GTTAGGAGCCAAGCAGGAAACGCGTCTTTGGCGCGTATCTCTGTATTCGACCTGAGCTCA 1096
Query 597 GGCAAGGCTACCCGCTGAASTTAAGCATATCA 628
||||||||||||||||||| ||||||||||||
Sbjct 1097 GGCAAGGCTACCCGCTGAACTTAAGCATATCA 1128
```

SAMPLE QUERY A3

```
BLASTN 2.2.26+
Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped
BLAST and PSI-BLAST: a new generation of protein database search programs",
Nucleic Acids Res. 25:3389-3402.
RID: Z7NR0B1N01N
Database: nr
Query= 2_A3
(659 letters)
Score E
Sequences producing significant alignments: (Bits) Value
gi|374719759|gb|JQ315188.1| Desmodesmus sp. GTD9C2 18S riboso... 1194 0E00
gi|89515343|gb|DQ417525.1| Desmodesmus multivariabilis var. t... 1079 0E00
gi|338809991|gb|JF835988.1| Desmodesmus sp. F2 internal trans... 962 0E00
gi|338809990|gb|JF835987.1| Desmodesmus sp. F1 internal trans... 954 0E00
gi|338809993|gb|JF835990.1| Desmodesmus sp. F18 internal tran... 946 0E00
gi|392328693|gb|JQ782747.1| Desmodesmus sp. IB-01 internal tr... 914 0E00
gi|390472947|gb|JQ867366.1| Desmodesmus sp. F32 internal tran... 904 0E00
gi|390472930|gb|JQ867365.1| Desmodesmus sp. F30 internal tran... 894 0E00
gi|89515348|gb|DQ417530.1| Desmodesmus sp. isolate Tow 8/18 P... 865 0E00
gi|89515381|gb|DQ417563.1| Desmodesmus sp. isolate Tow 6/16 T... 857 0E00
gi|359385310|emb|FR865727.1| Scenedesmus armatus var. subalte... 853 0E00
gi|338809992|gb|JF835989.1| Desmodesmus sp. F5 internal trans... 845 0E00
gi|89515382|gb|DQ417564.1| Desmodesmus sp. isolate Tow 6/16 T... 831 0E00
gi|89515383|gb|DQ417565.1| Desmodesmus sp. isolate Tow 10/11 ... 819 0E00
gi|89515350|gb|DQ417532.1| Desmodesmus sp. isolate Tow 6/16 T... 813 0E00
gi|89515349|gb|DQ417531.1| Desmodesmus sp. isolate Itas 6/3 T... 811 0E00
gi|344190133|gb|HQ335217.1| Desmodesmus sp. S3 internal trans... 807 0E00
gi|89515338|gb|DQ417520.1| Desmodesmus armatus var. subaltern... 791 0E00
gi|385258200|gb|JQ710682.1| Desmodesmus sp. CB1 internal tran... 787 0E00
gi|169798015|gb|EU502832.1| Desmodesmus sp. MAT-2008a 18S rib... 781 0E00
gi|169798018|gb|EU502835.1| Desmodesmus sp. MAT-2008b 18S rib... 773 0E00
gi|344190134|gb|HQ335218.1| Desmodesmus sp. S7 internal trans... 763 0E00
gi|89515339|gb|DQ417521.1| Desmodesmus armatus var. subaltern... 759 0E00
gi|89515339|gb|DQ417521.1| Desmodesmus armatus var. subaltern... 52 5E-03
gi|89515365|gb|DQ417547.1| Desmodesmus armatus var. subaltern... 753 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 749 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 50 2E-02
gi|379062656|gb|JQ313131.1| Desmodesmus sp. 2Cl66E internal t... 742 0E00
gi|379062659|gb|JQ313134.1| Desmodesmus sp. 1Pm66B internal t... 742 0E00
gi|390473016|gb|JQ867370.1| Desmodesmus sp. F51 internal tran... 740 0E00
gi|379062658|gb|JQ313133.1| Desmodesmus sp. 1Hp86E-2 internal... 738 0E00
gi|379062657|gb|JQ313132.1| Desmodesmus sp. 3Dp86E-1 internal... 738 0E00
ALIGNMENTS
>gb|JQ315188.1| Desmodesmus sp. GTD9C2 18S ribosomal RNA gene, partial sequence; internal
transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene, partial sequence
Length=1444
Score = 1194.5 bits (601), Expect = 0E00
Identities = 609/613 (99), Gaps = 0/613 (0)
Strand = Plus/Plus
Query 1 AGGATCATTGAATATGCAAACCACAACACGCACTCTTTACTTGTGTACCGACGTTAGGTC 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 508 AGGATCATTGAATATGCAAACCACAACACGCACTCTTTACTTGTGTACCGACGTTAGGTC 567
Query 61 ATAACCTTAACCCGGTTTGGCCTACTAACCTACACACCATTGACCAACCATTGCTTAAAC 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 568 ATAACCTTAACCCGGTTTGGCCTACTAACCTACACACCATTGACCAACCATTGCTTAAAC 627
Query 121 CAAACTCTGAAGTTTCGGCTGCTGTTAATCGGCAGTTTTAACGAAAACAACTCTCAACAA 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 628 CAAACTCTGAAGTTTCGGCTGCTGTTAATCGGCAGTTTTAACGAAAACAACTCTCAACAA 687
Query 181 CGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAAT 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 688 CGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAAT 747
```

```
Query 241 TGCAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGCTCGACTCCTYGGAGAA 300
||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct 748 TGCAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGCTCGACTCCTCGGAGAA 807
Query 301 GAGCATGTCTGCCTCAGCGTCGGTTTACACCCTCACCCCTCTTCCTTTTCAAGGAAGCTT 360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 808 GAGCATGTCTGCCTCAGCGTCGGTTTACACCCTCACCCCTCTTCCTTTTCAAGGAAGCTT 867
Query 361 GTCGTGCTTGCTCAAGCCGGCATCAGGGGTGGATCTGGCCCTCCCAATCGGAGTCACTTT 420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 868 GTCGTGCTTGCTCAAGCCGGCATCAGGGGTGGATCTGGCCCTCCCAATCGGAGTCACTTT 927
Query 421 CGGTTGGGTTGGCTGAAGCACAGAGGCTTAAAYTGGGACCCCATTCGGGCTCAACTGGAT 480
|||||||||||||||||||||||||||||||| |||||||| ||||||||||||||||||
Sbjct 928 CGGTTGGGTTGGCTGAAGCACAGAGGCTTAAACTGGGACCCGATTCGGGCTCAACTGGAT 987
Query 481 AGGTAGCAACACCCTCGGGTGCCTACACGAAGTTGTGTCTGAGGACCTGGTTAGGAGCCA 540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 988 AGGTAGCAACACCCTCGGGTGCCTACACGAAGTTGTGTCTGAGGACCTGGTTAGGAGCCA 1047
Query 541 AGCAGGAAACGCGTCTTTGGCGCGTATCTTTGTATTCGACCTGAGCTCAGGCAAGGCTAC 600
||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct 1048 AGCAGGAAACGCGTCTTTGGCGCGTATCTCTGTATTCGACCTGAGCTCAGGCAAGGCTAC 1107
4
Query 601 CCGCTGAACTTAA 613
|||||||||||||
Sbjct 1108 CCGCTGAACTTAA 1120
```

SAMPLE G2_3

```
BLASTN 2.2.26+
Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped
BLAST and PSI-BLAST: a new generation of protein database search programs",
Nucleic Acids Res. 25:3389-3402.
RID: Z7NR0B1N01N
Database: nr
Query= 6_G2
(659 letters)
Score E
Sequences producing significant alignments: (Bits) Value
gi|374719759|gb|JQ315188.1| Desmodesmus sp. GTD9C2 18S riboso... 1194 0E00
gi|89515343|gb|DQ417525.1| Desmodesmus multivariabilis var. t... 1079 0E00
gi|338809991|gb|JF835988.1| Desmodesmus sp. F2 internal trans... 962 0E00
gi|338809990|gb|JF835987.1| Desmodesmus sp. F1 internal trans... 954 0E00
gi|338809993|gb|JF835990.1| Desmodesmus sp. F18 internal tran... 946 0E00
gi|392328693|gb|JQ782747.1| Desmodesmus sp. IB-01 internal tr... 914 0E00
gi|390472947|gb|JQ867366.1| Desmodesmus sp. F32 internal tran... 904 0E00
gi|390472930|gb|JQ867365.1| Desmodesmus sp. F30 internal tran... 894 0E00
gi|89515348|gb|DQ417530.1| Desmodesmus sp. isolate Tow 8/18 P... 865 0E00
gi|89515381|gb|DQ417563.1| Desmodesmus sp. isolate Tow 6/16 T... 857 0E00
gi|359385310|emb|FR865727.1| Scenedesmus armatus var. subalte... 853 0E00
gi|338809992|gb|JF835989.1| Desmodesmus sp. F5 internal trans... 845 0E00
gi|89515382|gb|DQ417564.1| Desmodesmus sp. isolate Tow 6/16 T... 831 0E00
gi|89515383|gb|DQ417565.1| Desmodesmus sp. isolate Tow 10/11 ... 823 0E00
gi|89515350|gb|DQ417532.1| Desmodesmus sp. isolate Tow 6/16 T... 821 0E00
gi|89515349|gb|DQ417531.1| Desmodesmus sp. isolate Itas 6/3 T... 819 0E00
gi|344190133|gb|HQ335217.1| Desmodesmus sp. S3 internal trans... 815 0E00
gi|89515338|gb|DQ417520.1| Desmodesmus armatus var. subaltern... 791 0E00
gi|169798015|gb|EU502832.1| Desmodesmus sp. MAT-2008a 18S rib... 789 0E00
gi|385258200|gb|JQ710682.1| Desmodesmus sp. CB1 internal tran... 787 0E00
gi|344190134|gb|HQ335218.1| Desmodesmus sp. S7 internal trans... 775 0E00
gi|169798018|gb|EU502835.1| Desmodesmus sp. MAT-2008b 18S rib... 773 0E00
gi|89515339|gb|DQ417521.1| Desmodesmus armatus var. subaltern... 763 0E00
gi|89515339|gb|DQ417521.1| Desmodesmus armatus var. subaltern... 48 8E-02
gi|89515365|gb|DQ417547.1| Desmodesmus armatus var. subaltern... 753 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 749 0E00
gi|89515364|gb|DQ417546.1| Desmodesmus armatus var. subaltern... 50 2E-02
gi|379062656|gb|JQ313131.1| Desmodesmus sp. 2Cl66E internal t... 742 0E00
gi|379062658|gb|JQ313133.1| Desmodesmus sp. 1Hp86E-2 internal... 742 0E00
gi|379062657|gb|JQ313132.1| Desmodesmus sp. 3Dp86E-1 internal... 742 0E00
gi|379062659|gb|JQ313134.1| Desmodesmus sp. 1Pm66B internal t... 742 0E00
gi|390473016|gb|JQ867370.1| Desmodesmus sp. F51 internal tran... 740 0E00
gi|89515380|gb|DQ417562.1| Desmodesmus komarekii isolate Tow ... 738 0E00
gi|40288331|gb|AY461360.1| Desmodesmus multivariabilis strain... 738 0E00
gi|89515366|gb|DQ417548.1| Desmodesmus sp. isolate Tow 6/16 T... 734 0E00
gi|89515366|gb|DQ417548.1| Desmodesmus sp. isolate Tow 6/16 T... 60 2E-05
gi|89515376|gb|DQ417558.1| Desmodesmus bicellularis isolate N... 728 0E00
gi|40288343|gb|AY461373.1| Desmodesmus multivariabilis var. t... 724 0E00
gi|40288336|gb|AY461366.1| Desmodesmus multivariabilis var. t... 716 0E00
```

SAMPLE G1-4

```
Sequences producing significant alignments: (Bits) Value
gi|359385263|emb|FR865677.1| Chloroidium saccharophilum genom... 1239 0E00
gi|359385257|emb|FR865669.1| Chloroidium ellipsoideum genomic... 1192 0E00
gi|359385254|emb|FR865665.1| Chloroidium saccharophilum genom... 1192 0E00
gi|359385253|emb|FR865664.1| Chloroidium saccharophilum genom... 1154 0E00
gi|296315406|emb|FM946010.1| Chlorella saccharophila partial ... 1150 0E00
gi|296315403|emb|FM946007.1| Chlorella saccharophila partial ... 1150 0E00
gi|296315397|emb|FM946001.1| Chlorella saccharophila partial ... 1142 0E00
gi|296315398|emb|FM946002.1| Chlorella saccharophila partial ... 1142 0E00
gi|158325123|gb|EU038292.1| 'Chlorella' ellipsoidea strain Ce... 1140 0E00
gi|296315404|emb|FM946008.1| Chlorella saccharophila partial ... 1134 0E00
gi|296315405|emb|FM946009.1| Chlorella saccharophila partial ... 1134 0E00
gi|296315401|emb|FM946005.1| Chlorella saccharophila partial ... 1134 0E00
gi|296315402|emb|FM946006.1| Chlorella saccharophila partial ... 1134 0E00
gi|296315396|emb|FM946000.1| Chlorella saccharophila partial ... 1134 0E00
gi|296315400|emb|FM946004.1| Chlorella saccharophila partial ... 1126 0E00
gi|296315399|emb|FM946003.1| Chlorella saccharophila partial ... 1126 0E00
gi|119034594|gb|EF120783.1| 'Chlorella' saccharophila voucher... 1058 0E00
gi|119034592|gb|EF120782.1| 'Chlorella' saccharophila voucher... 1022 0E00
gi|119034608|gb|EF120784.1| 'Chlorella' saccharophila voucher... 921 0E00
gi|296315407|emb|FM946011.1| Chlorocloster engadinensis parti... 502 1E-138
gi|296315407|emb|FM946011.1| Chlorocloster engadinensis parti... 49 5E-02
gi|23476818|emb|AJ439400.1| Chlorella saccharophila 18S rRNA ... 456 1E-124
gi|296315415|emb|FM946019.1| Chlorella angustoellipsoidea par... 358 4E-95
gi|296315415|emb|FM946019.1| Chlorella angustoellipsoidea par... 105 6E-19
gi|296315415|emb|FM946019.1| Chlorella angustoellipsoidea par... 51 1E-02
gi|296315416|emb|FM946020.1| Chlorella angustoellipsoidea par... 358 4E-95
gi|296315416|emb|FM946020.1| Chlorella angustoellipsoidea par... 105 6E-19
gi|296315416|emb|FM946020.1| Chlorella angustoellipsoidea par... 51 1E-02
gi|296315417|emb|FM946021.1| Chlorella angustoellipsoidea par... 358 4E-95
gi|296315417|emb|FM946021.1| Chlorella angustoellipsoidea par... 105 6E-19
gi|296315417|emb|FM946021.1| Chlorella angustoellipsoidea par... 51 1E-02
gi|359385255|emb|FR865666.1| Chloroidium ellipsoideum genomic... 354 7E-94
gi|359385255|emb|FR865666.1| Chloroidium ellipsoideum genomic... 115 5E-22
gi|359385255|emb|FR865666.1| Chloroidium ellipsoideum genomic... 99 4E-17
gi|296315413|emb|FM946017.1| Chloroidium ellipsoideum partial... 350 1E-92
gi|296315413|emb|FM946017.1| Chloroidium ellipsoideum partial... 93 2E-15
ALIGNMENTS

>emb|FR865677.1| Chloroidium saccharophilum genomic DNA containing 18S rRNA gene,
ITS1, 5.8S rRNA
gene, ITS2, 28S rRNA gene, culture collection CCAP 211/58
Length=2672
Score = 1238.5 bits (617), Expect = 0E00
Identities = 665/683 (97), Gaps = 0/683 (0)
Strand = Plus/Plus
Query 36 CCCGCCAACCCAGACRTTRWGTTCCCTCGGTGCGGGCCCCTAGTGGGACCGCCCGAGCGC 95
||||||||||||||| || ||||||||||||||||||||| ||||||||||||||||||
Sbjct 1800 CCCGCCAACCCAGACGTTGTGTTCCCTCGGTGCGGGCCCCTCGTGGGACCGCCCGAGCGC 1859
Query 96 TTGTTCCTGGCCCAGCCGCTGGCCGACCCGTTCGTGGTCGGTGAAGCCTGGACAAAGGTC 155
||||||||||||||||||||||||||||| ||||||||||||||||||||| ||||||||
Sbjct 1860 TTGTTCCTGGCCCAGCCGCTGGCCGACCCATTCGTGGTCGGTGAAGCCTGGGCAAAGGTC 1919
Query 156 TTGCGGTGTGGGACCCTGCCTCTCCCCAGGGAGAGGGGGGCTGGCCTTCACGGTCGGCCT 215
|||||||||||||||||||||||||||||||||||||||||||||||||| ||| |||||
Sbjct 1920 TTGCGGTGTGGGACCCTGCCTCTCCCCAGGGAGAGGGGGGCTGGCCTTCATGGTTGGCCT 1979
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SAMPLE B1-b

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BLASTN 2.2.26+
Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped
BLAST and PSI-BLAST: a new generation of protein database search programs",
Nucleic Acids Res. 25:3389-3402.
```

```
RID: ZS8K40N9015
Database: nr
Query= Consensus B16
(1400 letters)
Score E
Sequences producing significant alignments: (Bits) Value
gi|18642518|gb|AF278751.1| Stichococcus bacillaris 16S small ... 1051 0E00
gi|18642518|gb|AF278751.1| Stichococcus bacillaris 16S small ... 248 1E-61
gi|306479467|emb|FR648163.1| Uncultured cyanobacterium partia... 1043 0E00
gi|371782133|emb|HE610165.1| Tetraselmis cordiformis plastid ... 1035 0E00
gi|371782133|emb|HE610165.1| Tetraselmis cordiformis plastid ... 351 1E-92
gi|290770765|emb|FN563077.1| Tetraselmis striata plastid 16S ... 1023 0E00
gi|290770765|emb|FN563077.1| Tetraselmis striata plastid 16S ... 377 2E-100
gi|312597851|gb|HQ396569.1| Uncultured eukaryote clone SD3 16... 1019 0E00
gi|285307007|emb|AM940509.1| Uncultured bacterium partial 16S... 1017 0E00
gi|371782134|emb|HE610166.1| Tetraselmis marina plastid 16S r... 1006 0E00
gi|371782134|emb|HE610166.1| Tetraselmis marina plastid 16S r... 336 6E-88
gi|307092198|gb|HM572446.1| Uncultured bacterium clone Rock2-... 1006 0E00
gi|364604043|gb|JN447524.1| Uncultured organism clone SBYC_52... 1004 0E00
gi|364604043|gb|JN447524.1| Uncultured organism clone SBYC_52... 343 2E-90
gi|21635230|gb|AF393609.1| Scherffelia dubia small subunit ri... 990 0E00
gi|21635230|gb|AF393609.1| Scherffelia dubia small subunit ri... 373 3E-99
gi|371782129|emb|HE610161.1| Stichococcus bacillaris plastid ... 982 0E00
gi|371782129|emb|HE610161.1| Stichococcus bacillaris plastid ... 458 6E-125
gi|371782129|emb|HE610161.1| Stichococcus bacillaris plastid ... 98 2E-16
gi|307566833|gb|HM745432.1| Uncultured phototrophic eukaryote... 982 0E00
gi|307566833|gb|HM745432.1| Uncultured phototrophic eukaryote... 387 2E-103
gi|307566833|gb|HM745432.1| Uncultured phototrophic eukaryote... 141 2E-29
gi|307566821|gb|HM745420.1| Uncultured phototrophic eukaryote... 982 0E00
gi|307566821|gb|HM745420.1| Uncultured phototrophic eukaryote... 387 2E-103
gi|307566821|gb|HM745420.1| Uncultured phototrophic eukaryote... 141 2E-29
gi|334690398|gb|JF817238.1| Uncultured bacterium clone Bac-3-... 974 0E00
gi|302027656|gb|HM565477.1| Uncultured bacterium clone MIGF11... 974 0E00
>gb|AF278751.1| Stichococcus bacillaris 16S small subunit ribosomal RNA gene,
partial sequence;
chloroplast gene for chloroplast product
Length=839
Score = 1051.1 bits (530), Expect = 0E00
Identities = 581/598 (97), Gaps = 0/598 (0)
Strand = Plus/Minus
Query 493 GATGTCAAGCCCTGGTAAGGTTCTTCGCGTTGCATCAGATTAAACCACATGCTCCACCGC 552
||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 598 GATGTCAAGTCCTGGTAAGGTTCTTCGCGTTGCATCAGATTAAACCACATGCTCCACCGC 539
Query 553 TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGAGCATACTCCCCAGGCGG 612
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 538 TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACTCTTGCGAGCATACTCCCCAGGCGG 479
Query 613 GATACTTCACGCGTTAGCTACAGCACTGGACGTTTTGAGTCGCACAGCACTTAGTATCCA 672
|||||||||||||||||||||||||||||||||||||| ||| |||||||||||||||||
Sbjct 478 GATACTTCACGCGTTAGCTACAGCACTGGACGTTTTGATTCGGACAGCACTTAGTATCCA 419
Query 673 TCGTTTACGGCTAGGACTACAGGGGTATCTAATCCCTTTTGCTCCCCTAGCTTTCGTCTC 732
||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct 418 TCGTTTACGGCTAGGACTACAGGGGTATCTAATCCCTTTCGCTCCCCTAGCTTTCGTCTC 359
Query 733 TCAATGTCAGTTTCGGCCCAGCAGAGTGCTTTCGCTATTGGTGTTCTTCTCGATATCTAC 792
|||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||
Sbjct 358 TCAATGTCAGTTTCGGCCCAGCAGAGTGCTTTCGCTTTTGGTGTTCTTCTCGATATCTAC 299
Query 793 GTATTTCACCACTACACCGAGAATTCCCTCTGCCCCTACCGTACTCAAGTCTACTAGTTT 852
||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct 298 GTATTTCACCACTACACCGAGAATTCCCTCTGCCCCTACCGTACTCAAGCCTACTAGTTT 239
Query 853 CTGTTGCCGGATCAGGGTTGAGCCCTAATTTTTAACAACAGACTTGGTAAACCACCTACA 912
||||||||| || | |||| ||||||||||||||||||||||||||||||||||||||||
Sbjct 238 CTGTTGCCGAATTAAGGTTAAGCCCTAATTTTTAACAACAGACTTGGTAAACCACCTACA 179
Query 913 GACGCTTTACGCCCAATTATTCCGGATAACACTTGCATCCCCCGTATTACCGCGGCTGCT 972
||||||||||||||||||||||||||||||||| ||||||||||| ||||||||||||||
Sbjct 178 GACGCTTTACGCCCAATTATTCCGGATAACACTCGCATCCCCCGTCTTACCGCGGCTGCT 119
```

```
Query 973 GGCACGGAGTTAGCCGATGCTTATTCCTCAGATACCGTCAAAATTTCTTCTCTGAGAAAA 1032
||||||||||||||||||||||||||||||||||||||||| | ||||||||||||||||
Sbjct 118 GGCACGGAGTTAGCCGATGCTTATTCCTCAGATACCGTCAAGAATTCTTCTCTGAGAAAA 59
Query 1033 GGAGTTTACAACCCATAGGCTTTCATCCTCCACGCGGCATTGCTCCGTCAGGCTTTCG 1090
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SAMPLE A1-OLD

```
BLASTN 2.2.26+
Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped
BLAST and PSI-BLAST: a new generation of protein database search programs",
Nucleic Acids Res. 25:3389-3402.
RID: Z7NR0B1N01N
Database: nr
Query= 3_A-old
(659 letters)
Score E
Sequences producing significant alignments: (Bits) Value
gi|6625510|emb|AJ249511.1| Scenedesmus acuminatus 5.8S rRNA g... 532 2E-147
gi|6625510|emb|AJ249511.1| Scenedesmus acuminatus 5.8S rRNA g... 82 6E-12
gi|46241853|gb|AY510468.1| Scenedesmus bajacalifornicus isola... 522 2E-144
gi|46241853|gb|AY510468.1| Scenedesmus bajacalifornicus isola... 74 2E-09
gi|46241852|gb|AY510467.1| Scenedesmus bajacalifornicus isola... 508 3E-140
gi|46241852|gb|AY510467.1| Scenedesmus bajacalifornicus isola... 74 2E-09
gi|46241854|gb|AY510469.1| Scenedesmus bajacalifornicus isola... 500 8E-138
gi|46241851|gb|AY510466.1| Scenedesmus dissociatus isolate UT... 423 1E-114
gi|46241851|gb|AY510466.1| Scenedesmus dissociatus isolate UT... 189 4E-44
gi|46241851|gb|AY510466.1| Scenedesmus dissociatus isolate UT... 44 1E00
```