Silver Biosorption Study by Using Seaweed-based Sorbents

by

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ABSTRACT

Biosorption is a green and effective alternative for metal recovery. Few researches on silver biosorption has been conducted by using seaweed species. This thesis conducted investigations into silver biosorption behavior of algae-based biosorbents. *Laminaria Japonica* and two *Fucus* species (*Fucus spiralis and Fucus vesiculosus*) was selected to experience some pretreatments. DOE (design of experiment) was employed to plan and analyze the tests for maximal silver capacity and selectivity between with copper ions. Seaweed species and pretreatments were verified to be the most significant factors in the biosorbent preparation. The silver biosorption on *Fucus* species was a rapid reaction, time-efficient for industrial application, which kinetic experiment data can be well described by Pseudo-second order equation. The mathematical maximal silver uptake by pretreated *Fucus spiralis* with acetone is around 78 mg/g at pH of 4.8 and is 120 mg/g at pH of 6.3, based on fitted Langmuir isotherm model. Characterization study of X-ray diffraction confirm the state of silver on the solid sorbent after tests, and Raman spectra and FTIR provided binding information between silver ions and functional groups on the targeted biosorbents. Compared with other reported biosorbent on silver recovery, this study demonstrates the utility of *Fucus* species as a promising potential candidate for silver biosorption in a competing environment.

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List of Abbreviations

ANOVA: Analysis of variance

BBD: Box-Behnken design

CCD: Central composite designs

DOE: Design of experiment

DSC: Differential scanning calorimetry

EDX: Energy dispersive X-ray

EDS: Energy dispersive X-ray spectroscopy

EDTA: Ethylenediaminetetraacetic acid

ESR: Electron spin resonance spectroscopy

FSA: *Fucus spiralis* treated by acetone washing

FTIR: Fourier transformed infrared spectroscopy

HSAB: Hard-soft acid-base

ICDD: International Centre for Diffraction Data

ICP-MS: Inductively Coupled Plasma Mass Spectrometer

ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometer

IR: Infra-red

MUN: Memorial University of Newfoundland

NL: Newfoundland and Labrador

NMR: Nuclear magnetic resonance

OFAT: One factor at a time

SDS: Sodium dodecyl sulfate

SEM: Scanning electron microscopy

TEM: Transmission electron microscope

TGA: Thermogravimetric analysis

Type J: *Laminaria japonica*

Type S: *Fucus spiralis*

Type V: *Fucus vesiculosus*

XAS: X-ray absorption spectroscopy

XPS: X-ray photoelectron spectroscopy

XRD: X-ray diffraction

Chapter 1 Introduction

Silver is one of the precious metals that have been widely used in the fields of aerospace, chemical, porcelain, photographic, electroplating and medical industries due to its excellent malleability, and extremely high electrical and thermal conductivity (Das, 2010). Silver, albeit its importance in modern industry is rarely mined alone, is usually distributed with relatively low contents in massive base metal sulfide ores. Development of novel cost-effective technology to recover silver metal from low grade ores is thus important. Moreover, the ever-increasing demand of silver metal from industry has attracted considerable concern towards the recovery and reuse of silver from industrial wastes. Silver recovery is also important from the environmental perspective. Silver ion is known to be released to the environment through its various industrial applications. The accumulation of silver ions in organisms (including human) through the food chain causes numerous diseases and disorders. It is therefore essential to recover silver ion from wastewater.

Several appropriate technologies are available for silver recovery from wastewater, such as electrolysis, metallic replacement, precipitation, and ion exchange (Songkroah *et al*., 2004). However, these conventional approaches have many disadvantages for their high capital costs, low efficiency, and secondary pollution to environment, etc (Mack *et al*., 2007). Biosorption is a green, efficient and low cost alternative method to separate targeted dissolved material from aqueous solution. This project aims to investigate the biosorption behavior of the chemically modified seaweeds in silver recovery from aqueous solutions.

1.1 Problem Statement

Biosorption is a developing technology that can be applied in environmental, chemical, biological, biochemical, material and many interdisciplinary engineering. Many research studies have been conducted and focused on the sequestration of heavy metals from wastewater. For the precious metal recovery, extensive research has been undertaken to develop the cost-effective biosorption process for gold recovery, but study on the green recovery of silver has been seldom reported (Mack *et al*., 2007). It is therefore important to explore the efficient method to recover metallic silver from dilute aqueous solutions through biosorption.

So far, most of the studies in this area are for the sorption of monometallic system. A major complication in the application of biosorption to industrial conditions is the presence of other metal ions in the wastewater to be treated. The effect of the co-existing metal ions on the selective biosorption of the metal of interest may be different for different cases. Usually the co-existing metal ions compete with the metal of interest for binding sites and thus lower the specificity of the biosorbent by binding to sites to which the metal ions of interest do not bind (Mack, *et al*., 2007). However, it is also reported that the presence of a hard acid had no effect on the biosorption of a soft acid based on hard and soft (Lewis) acids and bases theory，(Tsezos, *et al*., 1996; Mack et al., 2007). As usually copper, zinc and other metal ions coexist with silver ion either in mining effluent or in industrial wastewater; study the selective biosorption of silver ion from multi-metal ion solution environment is more important and valuable.

Another big challenge for the implementation of biosorption process for metal removal or metal recovery is to find the suitable biomass from the large biomass pools for a certain application. Investigated biomass types are of such diverse origins as bacterial, cyanobacterial, fungal (including filamentous fungi and unicellular yeasts), algal, plant (agricultural waste) or even animal (chitosan) (Kuyucak and Volesky, 1998). Important criteria for biomass screening are the cost and the availability. Newfoundland and Labrador is an oceanic island with abundant marine plants. Utilizing the abundant available seaweeds in this province as the raw biomass material for the selective recovery of Ag (I) is complementary to the current development in the region and will provide a source of more in-depth research in the fields of mineral processing and biosorption.

1.2 Objective and Scope of Study

Based on the information of natural resource in province of Newfoundland and Labrador, the silver amount in the copper and zinc mine can be considered valuable to extract as one mining product and the Atlantic province of Newfoundland and Labrador is a big pool of abundant marine organisms. Therefore, the topic of the current study is to explore the potential of non-living seaweed material as biosorbent for the green recovery of silver ion from multi-metallic system. The major objectives of this study are to:

- Recover silver in ionic form from multi-metallic solutions;
- Identify the sorption mechanism of silver on biosorbents;
- Determine the sorption kinetics and sorption equilibrium for silver ion from multi-metallic solutions; and
- Find the most important environmental parameters for biosorption of silver ion and optimize the recovery process.

The scope of the study is as follows:

1) *Sorbent Preparation and Screening*

Several species of local brown algae have been used as the raw biomass to prepare the biosorbents for Ag (I) uptake by chemical pretreatment. Design of experiments (DOE) was applied to screen the proper chemically modified seaweeds with high silver uptake capacity and high selectivity of Ag (I) from the multi-metal environment.

2) *Environmental Effects of Biosorption of Ag (I)*

Metal sorption performance depends greatly on the chemistry of solutions. Solution pH, solution temperature and the presence of competitive metal ions have remarkable effects on metal uptake. The effects of solution pH and the presence of competitive metal ions on the biosorption of Ag (I) have been studied.

3) *Sorption Equilibrium and Kinetic Studies*

Kinetic modeling is necessary to describe the response of the biosorption system to changes caused by variations in the experimental conditions and the properties of biosorbents. Kinetic studies give detailed information on adsorbate uptake rates and on rate-controlling steps such as external mass transfer, intraparticle mass transfer, and biosorptive reactions. Two preset-order models have been used to quantitatively describe kinetic behavior of the biosorption of Ag (I) in this study. In addition, Langmuir and Freundlich isotherm models have also been applied in this study to quantify the sorption equilibrium of Ag (I).

4) *Identification of Sorption Mechanism*

Metal ion uptake by biosorption is complex and may involve the contribution of diffusion, adsorption, chelation, complexaton, coordination or microprecipitation mechanisms, depending on the specific biomass or substrate. Therefore, identification of the sorption mechanism and quantification of adsorbate-sorbent interactions are essential for the evaluation of potential implementation strategies. The passive chemical sites/groups involved in Ag (I) binding will be explored by sophisticated instrumental analyses including Infra-red (IR) and Raman spectroscopy and X-ray diffraction (XRD) analysis.

1.3 Thesis Outline

This thesis is comprised of six chapters. Chapter 1 is the introductory chapter that consists of the problem statement, scope of research, and the objectives of the study. In chapter 2, literature review on the related research area has been carried out. Chapter 3 provides the biosorbent preparation by statistic experiment design method. The kinetic and isotherm study on the process are presented in Chapter 4. Chapter 5 introduced the analytical technologies to identify the biosorption mechanisms. Major observations from this study are summarized in Chapter 6.

Chapter 2 Literature Review

2.1 Silver Recovery and Biosorption

Recovery of low contents of precious metals from base metals products is important and necessary due to the scarcity and the widely applications of precious metals. Gold and silver have been found to be distributed with relatively low contents in massive base metal sulfide ores in the province of Newfoundland and Labrador (NL) (Outerbridge, 1909). It has been reported there would be up to 1600g silver, 50 g gold and a few amount of other precious metals present per ton of copper ore in NL. Table 2.1 presents the statistic data for silver and gold contents with other base metals in massive sulfide deposits. Copper, zinc, and lead are the major base metal elements in the mine ores. There are potential economic and industrial benefits to extract and recover those precious metals by green and cost-effective methods (Outerbridge, 1909; Mack *et al.*, 2007).

Table 2.1 Precious metal contents in some massive sulfide deposits (Modified from Hutchinson, 1990)

Ore type	Location	M.ton	Cu %	Zn %	Ag g/ton	Au g/ton
Yellow ore	Japan	-	high	low	27.90	0.47
Cons.Rambler	NL	0.4	1.3	2.15	27.8	4.8
Cons.Rambler	NL	2.2	3.04	p	13.7	1.64
Buchans	NL	15.5	1.32	14.6	126.8	1.47
Brunswick	NB	120	0.3	9.1	97	-

(NL is for the province of Newfoundland and Labrador in Canada, NB is for the province of New Brunswick in Canada)

Recovery of silver from variety of industrial wastes is also important. Wastewater from mining and photographic processing contains large content of silver. It has been reported that up to 6% silver content in industrial waste are released to the environment directly (Pethkar and Paknikar, 2003; Salunkhe, *et al*., 2011). This may pose severe environmental problems. Ionic silver is extremely toxic to aquatic plants and freshwater fish. Although it is a generally assumed that silver is more toxic towards prokaryotes than towards mammalian cells, the recent research by Greulich *et al*., (2012) clearly indicates that silver is harming or killing bacteria in the same concentration range where it harms human cells (as present in surrounding tissue). Removal and/or recovery of silver ion from wastewater are therefore essential from the environmental perspective.

The conventional technologies for silver recovery from aqueous solutions include: metal dust cementation, eletrodeposition (Das, 2010), adsorption on activated carbon (Schmidt *et al*., 1993), direct electrowinning (Juarez and Dutra, 2000), chemical precipitation, solvent extraction and ion exchange (Adhikari *et al.,* 2007). Especially, cation exchange resins and activated carbon have been employed commercially for precious metal preconcentration and purification in liquid solutions (Gurung *et al*., 2013). However, the major barriers in applying these methods for trace silver recovery are the high cost, incomplete metal removal, huge energy and chemical consumption, and secondary waste generated (Adhikari, *et al.,* 2007). The electrolytic recovery with high efficiency has been wildly employed in industrial silver recovery but still facing its application limitation as well as the high capital, operation and maintenance costs. For the trace amount silver recovery, electrolytic recovery is expensive and less efficient.

Recently, biosorption, a green, cost-effective and eco-friendly method has been applied for silver (precious metal) recovery from waste effluents (Das, 2010). Biosorption is a general property of living and dead biomass to rapidly bind and concentrate inorganic and organic compounds from diluted aqueous solutions (Kotrba *et al*., 2011). This term is defined more specifically as the process of concentrating a sorbate in the biomass, sequestering sorbates using a non-living biological sorbent. Different from bioaccumulation, biosorption can be reversible under different conditions, with a fairy quick rate (Chojnacka, 2010; Volesky, 2007).

The development of biosorption can be traced back to 1930's (Volesky and Holan, 1995; Adams and Holmes, 1935), with some finding of biological metal binding in bio-hydrometallurgy and biogeochemistry. After decades, biosorption was applied in wastewater treatment and became a branch of science. Currently, the use of biosorbents for the removal of toxic pollutants or for the recovery of valuable resources from aqueous wastewater has become one of the most promising developments in environmental or bioresource technology (Park *et al.*, 2010). The major advantages of biosorption over traditional treatment methods are: abundant material source, rapid process, low capital cost, high efficiency, less chemical consumption and less sludge discharged, available regeneration of biosorbents (Sud *et al.*, 2008; Farooq *et al.*, 2010).

2.2 Biosorbents

2.2.1 Available Biomass Resources for Biosorption

Biosorptive processes using non-living biomass are more applicable than the processes that use living microorganisms, since the latter requires a nutrient supply and complicated bioreactor system. Moreover, it is difficult to maintain a healthy microbial population due to toxicity of the pollutants being extracted and other unsuitable environmental factors such as temperature and pH of the solution being treated (Park *et al.,* 2010). Dead biomass thus offers some advantages over living microorganisms for metal sequestration. In the following review, we limit biosorbents to dead biomass materials. The major source categories for biosorbents are therefore divided into:

- Bacteria: gram-positive and gram-negative bacteria, cyanobacteria;
- Fungi: molds, mushrooms and yeast;
- Algae: micro-algae, macro-algae, brown and red seaweeds;
- Industrial waste: activated and anaerobic sludge, waste from fermentation, food or beverage industries;
- Agricultural waste: fruit or vegetable wastes, rice straws, wheat bran, soybean hulls, etc.;
- Natural residues: plant residues, sawdust, tree barks, weeds, etc., and
- Other biomaterials: derivatives of chitosan, cellulose, alginate (Park *et al*., 2010).

The use of bacteria as biosorbents is fast growing due to their small size, their ubiquity and their resilience to a wide range of environmental situations. Bacterial biomass (e.g., *Bacillus, Streptomyces, Citrobacter*) can be obtained as waste products from fermentation industries which makes it a cheap raw material (Naja and Volesky, 2011). However, it is usually necessary to immobilize the biomass before its application in sorption process, which adds to the cost. The potential of fungal biomass as adsorbents for the removal of toxic metals and recovery of precious metals from waste waters has been widely recognized. Fungi and yeast can be easily grown in substantial amounts using relatively unsophisticated fermentation techniques and inexpensive growth media. Thus, fungal biosorption can serve as an economical means for removal/recovery of metal ions from aqueous solutions (Pokhrel and Viraraghavan, 2006).

Algae, particularly brown algae stand out to be very good biosorbents for variety types of metal ions. In the majority of the cases biosorption of metals can be considered as rapid metabolism-independent ionic and covalent binding to the cell surfaces. But precipitation and crystallization of metals may occur within and around cell walls (Garnham, 1997). More detailed review on the preparation and application of algal based biosorbents will be presented in Section 2.2.3.

Most recently, agricultural residues have also attracted great attention for the biosorption of metal ions because they are widely and easily produced. In 2007, world wheat production was 610.6 million (Annual Report, 2009) which produced large amounts of wheat straw and bran. These agricultural by-products are mainly made up of lignin and cellulose and are rich in different kinds of functional groups such as alcohols, aldehydes, ketones, carboxylics, phenols and ethers (Demirbas, 2008). These groups have the ability to bind heavy metals by forming complexes with ionic species in solution via donation of an electron pair by the functional groups on the biosorbent. Farooq *et al.,* (2010) studied the feasibility of using ground straw and bran to remove metal ions and the process was quite fast and equilibrium reached in less than 20 minutes. Another example is buckwheat hull (Yin *et al.,* 2012).

Two criteria are widely used for sorbent screening. The first one is metal uptake capacity (q or q_e), i.e., the amount of metal ions sequestered per unit mass of sorbents. The second one is the remove percent R (%) which is defined as the percentage of metal ions removed from the solution to its original amount in the solution. The definitions of q_e and R are as follows,

$$R(\%) = \frac{C_i - C_e}{C_i} \times 100 \qquad \text{Eq. (2-1)}$$

and

$$q(q_e) = \frac{C_i - C_e}{m} \times v \qquad \text{Eq. (2-2)}$$

where C_i and C_e are initial and equilibrium metal ion concentrations in the solution, v is the volume, m is the dry weight of the biosorbent.

Selectivity is an important criterion for multi-metallic biosorption. When competing metal ions exist, the targeted metal ion uptake usually decreases. The capability of the biosorbent to selective bind the targeted metal becomes an important measurement in this scenario. It is ideal for a sorbent to possess both high metal uptake capacity and selectivity. But usually there is a compromise between these two objectives. For biosorption applications, it is also important that sorbents demand low-cost input and possess fast regeneration ability (Park *et al.,* 2010).

2.2.2 Modifications of Biomass

It has been widely reported that surface modification of the natural biomass materials can help to get a better biosorption performance. The modification of non-living biomass has mainly been classified into physical or chemical modifications (Park *et al*., 2010). Methods for physical modification include autoclaving, steaming, thermal drying, lyophilization, cutting, grinding, freezing, etc. These physical methods are simpler and cheaper, but are less effective and comparably more random on purpose than chemical approaches (Göksungur, *et al*., 2005).

Chemical washing or pretreatment, enhancement of binding groups, elimination of inhibiting groups and polymerization are the four major approaches for chemical modification (Park *et al.,* 2010). Among these methods, chemical washing is preferred due to its simplicity and efficiency. The washing with inorganic (acidic or alkaline) solutions and organic solvents has been applied frequently. Acid washing with hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, etc, can enhance the capacity of biosorbents for cationic metals or basic dyes through extraction of soluble organic or inorganic components from raw biomass and/or by changing its biochemistry (Park *et al.,* 2010). Alkaline solutions such as sodium hydroxide, potassium hydroxide, ammonia, calcium hydroxide and others have also been proved to be efficient in enhancing either the sorption capacity or selectivity of metal ions (Mack *et al.*, 2007). Organic solvent washing with methanol, ethanol, acetone, toluene, formaldehyde, epichorohydrin, salicylic acid, ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulfate (SDS) also shows its high efficiency in modifying the sorbents' surface (Basha *et al.,* 2008). However, some chemicals can cause serious mass losses of the biosorbent (i.e. structural damage), as well as a drop in the biosorptive capacity (Park *et al.*, 2005).

Remarkable improvements in the biosorptive capacity of a biosorbent can be obtained through enhancement or modification of its functional groups (Wan Ngah and Hanafish, 2008). Two alternative chemical modification methods, i.e., enhancement of binding groups and elimination of inhibiting groups have been widely applied for this purpose. Enhancement of binding groups of biosorbents involves different reactions, such as amination, carboxylation, and phosphorylation of hydroxyl groups, sulfonation, xanthanation, thiolation, halogenations, oxidation, etc. Elimination of inhibiting groups is usually implemented through decarboxylation of carboxyl group or elimination of amine group (Park *et al.,* 2010). These modifications improve the metal uptake capacity dramatically. However, modifications increase the costs of the biosorbents, bringing them closer to the price range of commercial ion-exchange resins.

Another import chemical modification method for sorbent capacity enhancement is polymer grafting. According to the methodology for creating active sites (free radical or ionic chemical groups) for initiating polymerization reactions, three types of typical initiation methods have been used, including high energy radiation, and photochemical and chemical initiation grafting (Park *et al.,* 2010). Many researchers are currently working in this area. Gurung *et al.,* (2013) applied this method to modify persimmon tannin gel by condensation reaction using concentrated sulfuric acid for the gold and silver recovery from electronic waste. Adhikari *et al.,* (2007) reported their study in modifying waste paper with p-aminobenzoic acid for a selective uptake (85-95%) of precious metals over other coexisting base metal ions in a competing environment. Most of the research results indicate that the amount of reagents used in the polymerization process has a significant influence on the final metal uptake capacity of the sorbents (Abd El-Ghaffar, Mohamed, and Elwakeel, 2009).

2.2.3 Algal Based Biosorbents

The term of algae is defined as a large group of organisms containing chlorophyll for photosynthesis (Davis *et al.,* 2003). Algae are important in marine, freshwater, and some terrestrial ecosystems. Perhaps the most well-known use of algae is in food or as additives in the cosmetic, pharmaceutical and food industries. In the past two decades, there has been an increasing interest for algae as biosorbent material due to its high sorption capacity and its almost unlimited availability in oceans (González *et al.*, 2011).

The biosorption capability of algae has been attributed mainly to the cell wall, which is composed of a fiber-like structure and an amorphous embedding matrix of various polysaccharides. Alginates and some sulfated polysaccharides such as fucoidan are important components of the cell walls of brown algae (González *et al.*, 2011). There are evidences that confirm that functional groups containing O-, N-, S-, or P-, participate directly in binding certain metals (Wang and Chen, 2009). The carboxyl, hydroxyl, sulphate and amino groups in algal cell wall polysaccharides act as binding sites for metals (Lesmana *et al*., 2009).

It has been proved by lots of research studies that most of the algal based biosorbents follow Langmuir-typed sorption isotherm. For the sorption of multi-metallic system, competitive isotherm models need to be used to describe the degree of interference and/or competence between different metals for the same active sites of the biosorebnts. From the knowledge of the sorption equilibrium it is possible to predict the behavior of different biomass with a given metal. Therefore, this could be a useful tool in order to make the right choice between different biosorbents for each occasion.

Most recently, binary and ternary combinations of different algae have been tested to check changes of behavior with respect to single-algal systems. Romera *et al*. (2008) studied the sorption performance of a ternary combination of a green (*Codium vermilara*), a red (*Chondrus crispus*) and a brown algae (*Ascophyllum nodosum*). It has been found that metal uptake was improved with any kind of mixture compared to the green algae but not for the brown alga. Again, these results confirmed that brown algae presented better biosorbent characteristics than red or green algae. The combination of biomass as biosorbent may benefit to the enhancement of the selectivity of certain target metal ion from multi-metallic system.

2.2.4 Application of Algal-Based Biosorbents

Table 2.2 summarizes that some research have investigated the metal biosorption capacity on brown, red and green alga. Great efforts have been invested on heavy metal biosorption, especially the application in wastewater treatment of lead, arsenic, cadmium, chromium, and copper (Jalali *et al*., 2002; Davis, Volesky, and Mucci, 2003; Pennesi *et al*., 2012). The criterion for the biosorption capacity is the metal uptake by unit of mmol metal per weight unit of biosorbents. However, some cross-field studies still use other units and make the comparison between sorbents complicated. Many chemical and physical factors can affect the metal capacity on the same biosorbents. pH should be provided at very first place when the metal uptake was calculated, and the flow rate should be provided for the capacity under column tests. From the review, brown algae have been studies with more emphasis than other two kinds and with more applications in metal uptake.

Table 2.3 shows different gold uptake by algal based biosorbents. The gold uptake capacity of the algal sorbents varies greatly. Gold uptake capacity of brown algae *Sargassum natans is* the highest among listed alge, which is roughly 410mg Au^{2+} per gram sorbent at pH 2.5 (Davis *et al.,* 2003).

Table 2.2 Applications of Algal-Based Biosorbents for Heavy Metal Removal and Rare Earth Elements.

Species	pH	Metal ion	Capacity (mg/g)	Reference
Brown Algae				
Chlorella vulgaris	2	Cr (VI)	79.30	Çetinkaya Dönmez *et al.,* 1999
Sargassum sp.	3.5	Cr (VI)	67.60	Cossich *et al.,* 2004
Ascophyllum nodosum	4.5	Ni (II)	135.95	Holan and Volesky, 1994
Chlorella vulgaris, free or immobilized	5	Ni (II)	31.00	Al-Rub *et al*., 2004
Chlorella vulgaris immobilized	5	Ni (II)	60.00	Akhtar *et al.,*2004
Cystoseira indica	6	Ni (II)	132.00	Basha, Murthy, and Jha, 2008
Fucus spiralis	6	Ni (II)	49.90	Romera *et al.,* 2006
Fucus vesiculosus	3.5	Ni (II)	23.01	Holan and Volesky, 1994
Padina sp.	5.5	Ni (II)	36.98	Sheng *et al*., 2004
Sargassum muticum	5	Ni (II)	70.00	Bermúdez *et al*., 2011
Sargassum sp.	5.5	Ni (II)	35.81	Sheng *et al*., 2004
Fucus vesiculosus	5.5	Cu (II)	23.40	Grimm *et al*., 2008
Fucus vesiculosus	4.5	Cu (II)	115.03	Ahmady - Asbchin *et al*., 2009
Laminaria japonica	4.5	Cu (II)	101.04	Fourest and Volesky, 1997
Padina sp.	5	Cu (II)	72.45	Sheng *et al*., 2004
Sargassum fluitans	4.5	Cu (II)	50.84	Davis, Volesky, and Vieira, 2000
Sargassum sp.	5	Cu (II)	62.91	Sheng *et al*., 2008
Sargassum vulgare	4.5	Cu (II)	59.10	Ahmady - Asbchin *et al*., 2009
Fucus spiralis	6	Zn (II)	52.96	Romera *et al*., 2006

Fucus vesiculosus	4.5	Zn (II)	52.30	Fourest and Volesky, 1997
Laminaria japonica	4.5	Zn (II)	91.53	Fourest and Volesky, 1997
Sargassum fluitans	4.5	Zn (II)	77.15	Fourest and Volesky, 1997
Cystoseira compressa	6–8	As (V)	53.93	Fourest and Volesky, 1997
Dictyopteris polypodioides	6–8	As (V)	74.90	Pennesi *et al*., 2012
Lessonia nigrescens	2.5	As (V)	3385.48	Mohan and Pittman, 2007
Ascophyllum nodosum	4.9	Cd (II)	215.04	Holan *et al.,* 1993
Fucus vesiculosus	3.5	Cd (II)	72.95	Holan *et al.,* 1993
Fucus spiralis	6	Cd (II)	114.66	Ahmady - Asbchin *et al*., 2009
Sargassum fluitans	4.5	Cd (II)	79.81	Davis, Volesky, and Vieira, 2000
Sargassum muticum	4.5	Cd (II)	76.44	Davis, Volesky, and Vieira, 2000
Sargassum sp.	6	Cd (II)	157.37	Tobin *et al.,* 1984
Sargassum vulgare	4.5	Cd (II)	88.80	Davis, Volesky, and Vieira 2000
Ascophyllum nodosum	6	Pb (II)	478.01	Holan and Volesky, 1994
Fucus spiralis	6	Pb (II)	203.06	Romera *et al.,* 2006
Fucus vesiculosus	6	Pb (II)	600.05	Holan and Volesky, 1994
Sargassum hystrix	4.5	Pb (II)	263.14	Ahmady - Asbchin *et al*., 2009
Turbinaria conoides	5	Ce (III)	42.04	Vijayaraghavan *et al*., 2011
Turbinaria conoides	5	Eu (III)	45.59	Vijayaraghavan *et al*., 2011
Turbinaria conoides	5	La (III)	34.73	Vijayaraghavan *et al*., 2011

Turbinaria conoides	5	Yb (III)	29.42	Vijayaraghavan *et al.*, 2011
Red Algae				
Chondrus crispus	6	Ni (II)	36.98	Romera *et al.*, 2006
Gracilaria caudata	5	Ni (II)	45.00	Bermúdez *et al.*, 2011
Gracillaria sp.	5.5	Ni (II)	15.85	Sheng *et al.*, 2004
Palmaria palmata	7	Ni (II)	29.35	Ahmady - Asbchin *et al.*, 2009
Chondrus crispus	6	Cu (II)	40.67	Romera *et al.*, 2006
Gracilaria sp.	5	Cu (II)	37.49	Sheng *et al.*, 2004
Palmaria palmata	7	Cu (II)	6.36	Ahmady - Asbchin *et al.,* 2009
Chondrus crispus	6	Zn (II)	45.77	Romera *et al.*, 2006
Palmaria palmata	7	Zn (II)	29.42	Ahmady - Asbchin *et al.,* 2009
Ceramium ciliatum	6–8	As (V)	92.88	Pennesi *et al.*, 2012
Gracilaria bursa-pastoris	6–8	As (V)	71.90	Pennesi *et al.*, 2012
Chondracanthus chamissoi	4	Cd (II)	85.20	Yipmantin *et al.*, 2011
Chondrus crispus	6	Cd (II)	75.31	Pennesi *et al.*, 2012
Mastocarpus stellatus	2.4	Cd (II)	55.08	Herrero *et al.*, 2006
Mastocarpus stellatus	4	Cd (II)	62.95	Herrero *et al.*, 2006
Mastocarpus stellatus	6	Cd (II)	66.32	Herrero *et al.*, 2006
Hypnea valentiae	6	Cd (II)	28.10	Rathinam *et al.*, 2010
Gracilaria sp.	5	Cd (II)	33.72	Sheng *et al.*, 2004
Chondracanthus chamissoi	4	Pb (II)	283.50	Yipmantin *et al.*, 2011
Galaxaura marginata	3.5	Pb (II)	26.94	Holan and Volesky 1994
Galaxaura	3.5	Pb (II)	317.02	Holan and Volesky

marginataa				1994
Gracilaria corticata	4.5	Pb (II)	53.87	Jalali *et al*., 2002
Gracilaria canaliculata	4.5	Pb (II)	41.44	Jalali *et al*., 2002
Polysiphonia violacea	4.5	Pb (II)	101.53	Jalali *et al*., 2002
Chondracanthus chamissoi	4–5	Pb (II)	279.72	Yipmantin *et al*., 2011
Chondrus crispus	6	Pb (II)	64.23	Romera *et al*., 2006
Gelidium sp.	5.3	Pb (II)	64.23	Ahmady - Asbchin *et al*., 2009
Palmaria palmata	6.5-7	Pb (II)	14.50	Ahmady - Asbchin *et al*., 2009
Green Algae				
Codium vermilara	6	Ni (II)	12.91	Romera *et al.,* 2006
Spirogyra insignis	6	Ni (II)	17.61	Romera *et al.,* 2006
Ulva sp.	5.5	Ni (II)	17.02	Sheng *et al.,* 2004
Codium vermilara	6	Cu (II)	17.16	Romera *et al.,* 2006
Spirogyra insignis	6	Cu (II)	19.07	Romera *et al.,* 2006
Ulva sp.	5	Cu (II)	47.66	Sheng *et al.,* 2004
Codium vermilara	6	Zn (II)	23.54	Romera *et al.,* 2006
Spirogyra insignis	6	Zn (II)	20.92	Romera *et al.,* 2006
Caulerpa racemosa	6–8	As (V)	33.71	Pennesi *et al.,* 2012
Maugeotia genuflexa	6	As (III)	57.48	Sari *et al.,* 2011
Ulva rigida	6–8	As (V)	56.92	Pennesi *et al.,* 2012
Zostera	6–8	As (V)	65.16	Pennesi *et al.,* 2012
Chaetomorha linum	5	Cd (II)	53.96	Hashim and Chu, 2008
Codium vermilara	6	Cd (II)	21.36	Romera *et al.,* 2006
Spirogyra insignis	6	Pb (II)	51.80	Romera *et al.,* 2006
Codium vermilara	6	Pb (II)	62.16	Romera *et al.,* 2006

Table 2.3 Algal-Based Biosrobent for Gold Recovery

Algae species	**pH**	**Capacity mg/g**	**Reference**
Fucus vesiculosus	7	68.95	Mata *et al.,* 2009
Dealginated Seaweed Waste	3	78.80	Romero-Gonz´alez *et al.,* 2003
Sargassum fluitans	2	0.63	Niu and Volesky, 2007
Sargassum natans	2.5	413.70	Kuyucak and Volesky, 1988
Ascophyllum nodosum	2.5	29.55	Kuyucak and Volesky, 1988

2.3 Environmental Parameters on Biosorption

Many factors influence the sorption capacity of a certain biosorbent. The most concerned parameters include: solution pH, sorbent particle size, ionic strength, initial solute concentration, agitation rate, process period and medium composition in feed solutions (Das, 2010).

Solution pH, is considered as one of most important factors which affect the chemical forms of targeted metal ions in solution, and also influence the dissociation state of binding sites on the material (Pokherl *et al.*, 2009; Das, 2010). Different pH of solution change the metal ions in terms of hydrolysis, complexation and redox reaction, so biosorption process was widely accepted as the pH-dependant process (Binupriya, *et al.*, 2010). In a biosorption study using modified chitosan, the silver uptake increased as the pH increased till gaining the highest value around pH 6. Donia, Atia and El-Boraey (2007) suggest the nitric and sulfuric atoms on the resin donor electron pairs to silver ions via complex formation (Abd El-Ghaffar, *et al.*, 2009).

Ionic strength mainly affects the biosorption performance on the physical mechanism by the competition between ions and changes in the properties of electrical double layers. When the biosorbent and solute are in contact, the electrical double layer will cover them due to electrostatic interaction (Aksu and Donmez, 2006). The presentation of 1-lysine in the medium has been reported to increase silver biosorption twice more than that in original tests.

Temperature of the solution is usually concerned by industrial process. Many reports found that temperature could not affect the biosorption behavior below 35 °C and a huge influence in gold biosorption by using the egg shell membrane study at 65 °C. The temperature is also a significant variable in desorption process in the downstream (Ishikawa, *et al.,* 2002).

Biosorbent dosage has been considered as an important factor in the capacity determine of biosorption extent. When it increases, the metal uptake per unite weight of sorbent decrease. Agitation rate and period have been studied mainly for determining the removal rate and equilibrium in biosorption kinetic study (Park *et al*., 2010; Das, 2010).

2.4 Kinetic and Isotherm Models of Biosorption

Many research focus on investigation of the biosorption behavior at equilibrium under different environmental conditions. Mathematic model can analyze the experimental data, provide prediction to the process, and transfer the technology from bench scale to industrial scale (Volesky and Holan 1995; Das, 2010). Batch sorption tests and dynamic continuous process tests are the basic two types of preliminary investigation for the biosorption system (Das, 2010).

For batch tests, the operation and experiment setup is comparably easy to conduct and less experiment runs. The batch test gives fundamental information of feed solution, information for later scale up (Gurung *et al.*, 2013). For continuous test, the whole process simulates practical operation conditions. The process goes in a column which is packed with immobilized biosorbent practices. The inlet flow rate can be adjusted and all the feed solutions can be prepared due to the requirements.

Most of these studies are kinetic and isotherm tests to simulate and predict the metal uptake behavior according to different operation conditions. Time efficiency is one significant practical and economic factor for industrial application. A kinetic study can interpret the change of biosorption properties of certain sorbent along the time. Psuedo first order and second order kinetic equations have been applied to describe the reaction or other adsorption processes. Based on many research, the biosorption of either base metal or precious metal, can be fitted with Langmuir and Frendlich models, which are the basic mathematic models in adsorption process, or certain modified version to each case (Volesky, 1995; Das, 2010).

2.4.1 Kinetic Study in the Batch System

The sorption rate is the main concern to design sorption systems and establish time dependence of biosorption under different parameters. In order to study the rate-controlling step of biosorption process, kinetic models have been applied to analyze the experimental results on mass transfer and chemical reaction. (Arica, 2001)

Wang and Chen, (2009) reviewed many kinetic models which were suggested mostly for the reaction order of biosorption system. The Pseudo second-order equation (Ho, 1998) has been proven to fit with experimental data for solid and liquid systems. Figure 2.1

depicts two widely applied kinetic models in a time-dependant adsorption study.

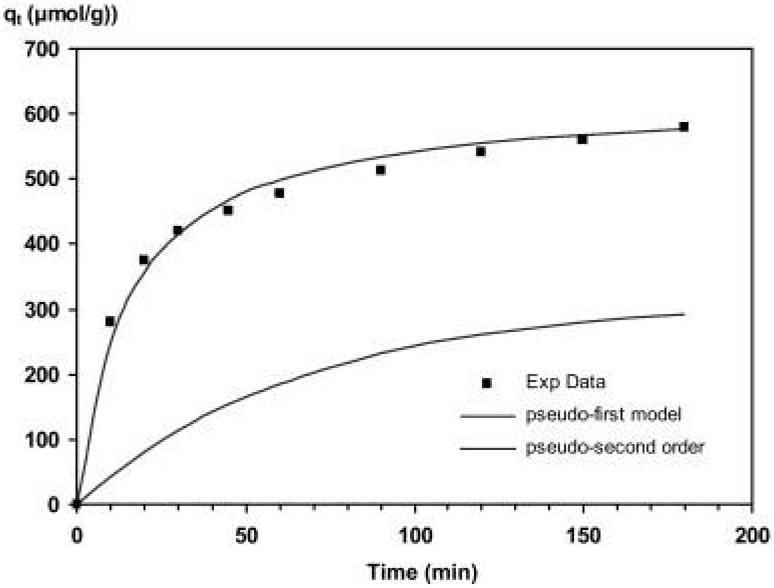

Figure 2.1 q_t vs. t Plots of Pseudo-first Model and Psedo-second Model Applied to a Kinetic Study (Alila, *et al.*, 2011; Zakeri *et al.,* 2010)

Table 2.4 Revised Table List for Applied Kinetic Equation in Biosorption Research (Park *et al*., 2010)

Expression	Equation form	Remarks
Second-order rate equation	$\frac{1}{C_t} = kt + \frac{1}{C_o}$	Based on residual concentration in early study
Lagergren equation (Pseudo first-order model)	$q_t = q_e\left(1 - e^{-kt}\right)$	Based on adsorption capacity
Ho equation (Pseudo second-order model)	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t$	Based on adsorption capacity

Table 2.4 summarizes the kinetic equations reported by Park *et al.,* (2010). These models have different mathematic forms and application scales. The reaction order in the kinetic study refers to the mechanism of biosorption including ion exchange or surface precipitation like metal hydroxide, sulfide or carbonate. The chemisorptions have been

reported as the rate-controlling step by sharing or exchanging electrons between sorbent and sorbate (Chojnacka, 2010).

The kinetic model can interpret the change of sorbate uptake rates, rate-controlling steps and biosorptive reaction, and provide some evidence for mechanism inside the process (Park *et al*., 2010).

2.4.2 Biosorption Equilibrium Study

As well as the kinetic study, process isotherm studies can also be divided into batch tests and continuous tests. Before studying within continuous column system, it is necessary to conduct batch test to gain apparent biosorption data, especially the basic important factors (range of pH, initial concentration, etc) for the future study design.

Biosorption has been applied as an equilibrium separation process to remove heavy metals in wastewater treatment. To design the system, a sorption isotherm study is necessary to provide the unit mass of sorbate that can be removed (capacity) under the system conditions (seen in Figure 2.2) (Aksu and Donmez, 2006).

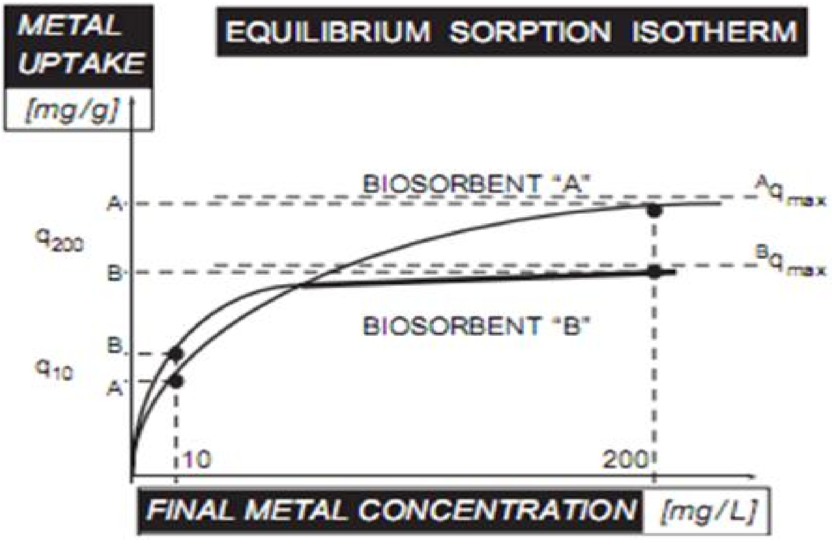

Figure 2.2 Comparison of a Sorption Isotherm Curves: sorbent B performs better than A at a lower equilibrium concentration of 10 mg/l (Volesky, 2007 review)

Park reviewed comprehensive published literature on biosorptions before 2010 and summarized various models for kinetic and isotherm study. Those applicable models can attribute to investigate the process mechanisms, predict responses to varying operation factors, and optimize the objectives (Park *et al.*, 2010).

Some isotherm models equations have been reported listed in Table 2.5. At a constant temperature, the Langmuir and Freundlich models are the most frequently applied for equilibrium data analysis on sorbate of the biosorbent and sorbate in the aqueous phase (Aksu and Donmez, 2006).

Table 2.5 Applied Equilibrium Isotherm Models for Biosorption (Park, *et al.,* 2010)

Single component	Langmuir	$q_t = \frac{q_m b C_e}{1 + bC_e}$	Monolayer sorption
	Freundlich	$q_i = kC_e^{1/n}$	Simple expression
	Sips	$q_i = \frac{aC_e}{1 + bC_e}$	complicated
Multi-component	Langmuir	$q_i = \frac{q_{mi} b_i C_i}{1 + \sum_{i=1}^{N} (b_i C_i)}$	Competitive
	Langmuir-Freundlich	$q_i = \frac{q_{mi} b_i C_i^{1/n_i}}{1 + \sum_{i=1}^{N} (b_i C_i^{1/n_i})}$	Competitive

The Langmuir model assumes that there are fixed amounts of binding sites on sorbent for monolayer sorption onto a homogeneous surface, providing a well described fit on the dominated passive biosorption process. The Freundlich isotherm is based on the assumption of sorption where there is not a homogeneous binding site. The exponential binding activity and sorbent surface are presented in this model.

For the plot of qe vs. Ce of Figure 2.3, some research point out that Freundlich model has been considered to be closer to biosorption theories for describing different heterogeneous functional groups on the surface of sorbents.

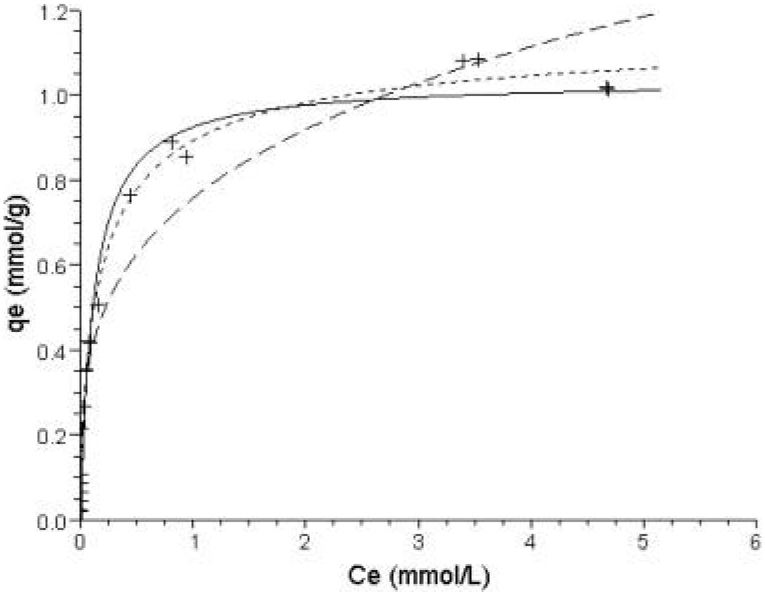

Figure 2.3 Isotherm Example: experimental data (+), Langmuir (solid line), Freundlich (long dashed line) and Langmuir–Freundlich (short dashed line; Luna *et al*., 2010)

2.5 Characterization of Sorption Mechanism

Although kinetic and isotherm studies provide empirical data of biosorption processes, neither of them can reveal the theories involving in metal – sorbent binding. Characteristic technologies can offer clues on the mechanisms of biosorption to help improve or design the sorbents on purpose (Volesky, 2007; Das, 2010).

Generally biosorption has been considered occurring by physical and chemical mechanisms. Von der Waals force, electrostatic force and adsorption contribute mainly in physical process (Volesky, 2001). Main chemical mechanisms include ion exchange, coordination, complexation, chelation and micro-precipitation. Ion exchange, involving the reversible interchanges of ions between sorbent and solution is widely accepted as the dominant mechanism for bio sorption. Coordination in which covalent bonds have been found between metal ions and donor atoms on sorbents, complexation, the combination of ligands and metal ions, or chelation which is the complexation of bi- or multi-dentate ligands with metal ions, and micro-precipitation *(*Bakir, McLoughlin, and Fitzgerald, 2010*)*. During the some studies, the dominant mechanism has been considered as ion exchange, but the complexity of cell walls in biomass probably causes some these processes exist simultaneously in the system (Volesky 1990; Göksungur *et al*., 2005).

Biomass cell walls contain polysaccharides, proteins and lipids that provide many functional groups to metal binding properties in aqueous solutions (Göksungur, *et al*., 2005). The complexity of cell walls' components could lead to all possible mechanisms taking place simultaneously in one system (Volesky, 1990).

Cationic silver ions can be coordinated or exchanged with the electron pairs provided by the surface of biosorbents, which have been reported possessing with many ionizable functional groups such as: phenolic hydroxyl groups, carbonyl groups, ethereal oxygen atoms, etc (Matsuo, 1978; Bakir, McLoughlin, and Fitzgerald*, 2010*). Moreover, reduction from silver ions to metallic form by some reducing functional groups of biosorbents has also been observed (Gurung *et al*., 2011). Thus biosorption can be a safe, simple, highly effective method for silver recovery from dilute effluents (Türker, 2012).

Table 2.6 Functional Groups Involved in Silver Biosorption (Bakir, McLoughlin, and Fitzgerald, 2010; Volesky 2007)

Functional groups	Structural formula	Ligand atom	Biomolecules
Hydroxyl	-OH	O	Polyscaccharides, uronic acid, sulfated, amino acids
Carbonyl	-HC=O	O	Peptide bond
Carboxyl	-(OH)C=O	O	Uronic acid, amino acid
Ethereal bond	-C-O-C-	O	Glucose
Sulfhydryl	-SH	S	Amino acids
Thioether	R-S-R	S	Amino acids
Amine	-NH2	N	Amino acids, chitosan
Amide	>NH	N	Amino acids
imine	=NH	N	Amino acids

Many sophisticated analytical techniques have been applied for revealing the mechanism of metal bio-binding on the active sites, including scanning electron microscopy (SEM), transmission electron microscope (TEM), infrared absorption spectroscopy of Fourier transformed infrared spectroscopy (IR or FTIR), energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD) analysis, electron spin resonance spectroscopy (ESR), nuclear magnetic resonance (NMR), X-ray photoelectron spectroscopy (XPS), X-ray absorption spectroscopy (XAS), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC), and so on.

SEM and TEM (transmission electron microscope) aim to confirm surface and inner morphology of the biosorbent; EDS can conduct element analysis and chemical characterize the metal bond with the sorbent; XRD can detect the crystallographic structure and chemical composition of targeted metal ion bound on the biosorbent; XPS and XAS can investigate oxidation state of targeted metal, ligand effects of the biosorbent and coordination environment; For determining the functional sties of the biosorbent, many technologies have been applied to study the mechanisms, including ESR,NMR, FT-IR and potentiometric titration (Park *et al*., 2010).

2.5.1 Function Groups on Carbon Chain

For investigating the mechanisms of gold and silver biosorption, some spectroscopic analyses have been taken. XPS and FTIR analysis indicated that protonated carbonyl and carboxyl groups played a key role in gold biosorption under acidic condition in one kind of fungus and the involvement of C–O and C–N bonds were noted in another fungus (Das, 2010). The cell wall hexosamine content was found to be 150 times greater than that in the Strain 2, supporting the role of hexosamines in precious metal binding (Das, 2010).

It was reported that the biosorbent could give a higher uptake percentage owing to a greater number of positive surface charges compared to others (Torres *et al.,* 2005). Due to more surface positivity, it could bind with silver thiosulfate. Mechanism of silver biosorption was reported by Tsezos *et al.,* (1996) with the inference drawn as a result of TEM microscopic and EDX analyses. A further in depth study was done by Kasthuri *et al.,* (2009) who used FTIR and TEM analysis to prove that silver was precipitated as nanoparticles as a result of interaction of Ag (I) with carbonyl groups. A similar phenomenon of interaction of silver ions with carboxylate groups on *Lactobacillus* sp. was investigated by Lin, *et al.,* (2005).

2.5.2 Electrostatic Interaction with Biosorbents

FTIR, XPS and XRD analysis proved that reduction of Ag (1) to Ag (0) accompanied in the process of biosorption. Donia *et al.,* (2007) reported that modification of chitosan by a reaction between chitosan and polymeric Schiff's base of thiourea/ glutaraldehyde in the presence of magnetite enhanced the process of silver uptake by modified chitosan. In case of platinum and palladium biosorption, electrostatic interaction plays a major role. Biosorption of platinum by chitosan cross-linked with glutaraldehyde was studied by Guibal *et al.,* (1999). Under an acidic condition, an uptake of 300 mg/g was reported. Presence of chloride and nitrate ions decreased the platinum uptake due to competition for positive sites thereby proving the phenomena of electrostatic binding mechanism. Similar work was done by Fujiwara *et al.,* (2007) on chitosan modified by cross linking with l-lysine. They reported that N atoms of amino group and O atoms of carboxyl group are mostly responsible for the biosorption. This was confirmed by FTIR analysis as C–N and C–O bond stretching. New chelating and reactive functional groups can readily be-grafted onto the chitosan.

Chapter 3 Preparation of Biosorbents from Brown Seaweeds for Selective Sorption of Ag (I)

3.1 Introduction

From the scientific point of view, physicochemical modifications of algae could lead to better sorption uptakes, better metal yields or higher mechanical stability. As discussed in Chapter 2, chemical modification of algae by acids, alkalis, salts, organic compounds (acetone, methanol, ethanol, formaldehyde, glutaraldeyde, etc), enzymes (lipase, lisozine, alkaline phosphatase) and metal chelates (EDTA) is capable to produce the desired effect. In this chapter, design of experiments (DOE) was applied to screen the proper chemically modified seaweed sorbents with high silver uptake capacity and high selectivity of Ag (I) from the multi-metal environment.

Sorbent screening for the chemically modified brown seaweeds has been carried out in a two phase design. Phase I design aims to find the most significant variables in sorbent preparation from brown seaweeds and the proper value of these parameters so that sorbent with high silver uptake capacity from monometallic system can be achieved. Based on the results of Phase I design, sorbent screening for Phase II focused on the use of various chemical solvents for the modification of brown seaweeds. The primary objective for Phase II design is to explore the best treatment of brown seaweeds to gain favorable affinity of silver uptake from multi-metal ion solutions. A two-level fractional factorial experiment design was employed for sorbent screening. Analysis of variance (ANOVA) has been applied to analyze the experimental data and get the statistic model to predict the biosorption performance.

3.2 Materials, Equipment and Methods

3.2.1 Seaweeds

Many kinds of seaweeds were available from the coast of North Atlantic Ocean along St. John's, NL. However, there were only a few types of seaweeds have adequate mass amount for practical application because most of seaweeds had huge weight loss after the drying. Three types of fresh seaweeds collected from the Middle Cove Beach, St. John’s (47.65 N 52.70W) and Portugal Cove, St. John’s (47.62 N 52.85W) were selected as the raw biomass materials for sorbent preparation. The images of the three types of seaweeds are demonstrated in Figure 3.1.

(A). Type J (B). Type V (C). Type S

Figure 3.1 Pictures of Three Types of Brown Seaweeds

- **Type J (*Laminaria Japonica*)**

Laminaria Japonica (Type J) grows wild in pure cold water. It is a sort of edible brown algae, usually grows from 2 to 5 meters long. *Laminaria Japonica* contains high level of alginate, organic iodine, fucoidan and laminarin (White, 2008).

- **Type V (*Fucus vesiculosus*)**

Fucus vesiculosus (Type V) is described as a brown and yellow-green seaweeds, Ochrophyta in phylum, belonging to the class of Phaeophyceae. It is also called bladder wrack which is growing in high densities on the middle shore. It fronds have a characteristic ridge along the edge of the receptacles (White, 2008).

- **Type S (*Fucus spiralis*)**

Fucus spiralis (Type S) is in the same family with Type V. It is also called spiral wrack, growing in intertidal zone on the high shore. It has air bladder, and looks very similar with Type V, and can grow up to 40 cm long (White, 2008).

3.2.2 Chemicals

For pretreatment of seaweeds:

Nitric Acid, HNO_3, (69%) was obtained from VWR International, Canada. Sodium Hydroxide, NaOH, (Pellets, 97+%, A.C.S. Reagent) was purchased from Sigma Aldrich Inc., Canada. Methanol (A.C.S Grade) was from VWR International, Canada. Acetone (A.C.S Grade) came from EMD chemicals Inc., Canada.

For metal sorption test:

Silver nitrate, $AgNO_3$, (0.01N A.C.S Grade), copper nitrate, $Cu(NO_3)_2$, (Hemipentahydrate, 98%, A.C.S Grade) and Iron Nitrate, $Fe(NO3)_3$, (Nonahydrate, 98%, A.C.S Grade) were purchased from Fisher Scientific, Canada. Distilled deionized water was prepared in the hydrometallurgy laboratory at Memorial University of Newfoundland (MUN).

3.2.3 Equipment and Methods

Equipment

Hammer grinder, Series 2000 (Crompton Cohtrols), was utilized for grinding the seaweeds into fine particles which later were separated and classified by mechanical sieve shaker, Retsch AS 200 (ATS). The New Brunswick incubator, Innova® 43, provides the experimental environment for all the experiments. The pH meter, AB 15+ (Fisher Scientific), were used for adjusting pH in the biosorbent preparation and biosorption tests.

Analytical techniques

Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), Optima 5300DV, and Perkin Elmer and Inductively Coupled Plasma Mass Spectrometer (ICP-MS) from CREAIT NETWORK-Core Research Equipment and Instrument Training Network at MUN were applied to analyze residual concentration of Ag (I) and other ions in the residual solutions.

3.3 Design of Experiment for Sorbent Preparation and Screening

OFAT, one factor at a time, the conventional experimental method varies one single factor, while keeping other factors at fixed values. Compared with OFAT, design of experiment (DOE) is an efficient method for planning experiments so that data obtained can be analyzed to yield valid objective conclusions (Khattar, 2009; Kleijinen, 2008; Patrón-Prado et al., 2012). Moreover, multiple factors (variables) and their interactions can be evaluated in a reduced number of experimental runs by using DOE (Khattar, 2009;

Romero-González *et al.,* 2003).

Factorial experimental designs investigate the effects of many different factors by varying them simultaneously instead of changing only one factor at a time. Factorial designs allow estimation of the sensitivity to each factor and also to the combined effect of two or more factors. Full two level factorial designs collect data from all of the vertices of a cube in *k*-dimensions (*k* is the number of factors being studied), requiring 2^k runs. While Fractional factorial designs collect data from a specific subset of all possible vertices and require a 2^{k-p} runs, with2^{k-p} being the fractional size of the design (Telford, 2007).

To efficiently find the optimal sorbent preparation methods and avoid the mode of OFAT, two-level half fractional factorial experiment design was applied in this study to find the significant parameters for sorbent preparation and their influences on the sorbent uptake capacity of Ag^+ from single- and multi-metallic solutions. Design-Expert® V8 (Stat-Ease Inc., MN, USA) was used for the design of experimental plans, statistical analysis and mathematical modeling (Khattar, 2009).

3.3.1 Sorbent Preparation and Screening for Single Ag (I) Sorption

- **Explanatory Variables Screening**

A fractional 2-level factorial experiment design was employed to make the competitive sorbent for Ag^+ uptake from single and multi-metallic solutions. The primary objective for our design is to find the most significant variables in sorbent preparation and the proper value of these parameters so that sorbent with high uptake capacity and selectivity of Ag^+ can be achieved. Many parameters are concerned in the sorbent preparation process, such as seaweed type, ground seaweed particle size, particular treatment, chemical dosage, treating temperature, treatment durance, pressure, and agitation rate. In this study, seaweed type, treatment, raw material particle size and reaction time were chosen as four factors for the trail designs. Table 3.1 lists the details of these four parameters.

Since the interactional effect, ABCD, is the highest order interaction which has rare interaction effect to the model in a real case. Thus, ABCD is chosen as the confounding factor in the design of experiment runs. Each main factor is aliased with higher order interaction factors for the trial tests. All the pairs of alias are listed in Table 3.2. The detailed 2-level fractional factorial design for modified sorbent preparation from brown algae is listed in Table 3.3.

Table 3.1Variable List to Optimize the Biosorbent Preparation Conditions

Code	Factor	Unit	Type
A	Seaweed type	-	Categoric
B	Chemical reagent	-	Categoric
C	Particle size	µm	Numeric
D	Reaction time	hour	Numeric

Table 3.2 Factorial Effect Alias List (confounding factor: ABCD)

	Term	Aliases
Require	Intercept	BD ABC ACD
Model	A-seaweed	BC CD ABD
Model	B-treat	BD ABC
Model	C-size	BC CD ABD BCD
Model	D-time	BD ACD
Error	AB	BC ABD BCD
Error	AC	BD ABC ACD
Error	AD	CD ABD BCD

Table 3.3 A 2^{4-1} Fractional Factorial Experimental Design for Biosorbent Preparation (via Design-Expert® V8.0)

Runs	**A**	**B**	**C**	**D**
1(6)	+	-	-	-
2(8)	+	+	+	-
3(5)	-	-	+	-
4(3)	-	+	-	-
5(14)	+	-	+	+
6(11)	-	+	-	+
7(16)	+	+	+	+
8(9)	-	-	-	+

- **Sorbent Preparation Procedure**

The fresh seaweeds collected from the coast were washed thoroughly with deionized water and then air-dried for 2 days. Afterwards, the dried seaweeds were grinded by a hammer mill into finer particles. Sizes of seaweed ranging from 0.2 to 1.0 mm were obtained by passing through standard sieves. 400mg or 800 mg of ground seaweed powders with different size ranges were weighted in a flask and mixed with 40ml of acidic or basic solution. Then, irrigating the flasks in the incubator was conducted with setting at: 200rpm and 80ºC. After cooling down, the solid phase of samples was filtered out of solution with WH41 filter paper and rinsed with deionized water for at least 5 times until the extract liquids reached pH around 7. After drying, the final sorbents were weighted, labeled and put into the storage for later testing.

3.3.2 Sorbent Preparation and Screening for Selective Sorption of Ag (I)

To recover silver from mining effluent or industrial waste, it is very important to test the selective uptake capacity of Ag$^+$ from competitive multi-metallic environment. Metal ions like copper, zinc, and iron have been reported to have similar aqueous chemistry to silver in nitric medium, and usually coexist in aqueous solutions. Hence, copper and iron were selected as competing metal ions in this study. The major objective of this part of work is to prepare the modified biosorbents by chemical pretreatment of brown seaweeds Types S and V with different chemical reagents so that sorbents with high uptake capacity and good selectivity of Ag$^+$ can be obtained. From the results of sorbent preparation and screening for single Ag$^+$ sorption, we realize that reaction temperature and reaction time of the chemical pretreatment process don't affect the sorbent capacity very much. Moreover, sorbent prepared from a bigger size of raw biomass shows better sorption performance.

Therefore, to prepare the sorbent with both high uptake capacity and selectivity of Ag^+, we majorly investigated the effect of chemical solvents on sorbent performance. Modified sorbents in this case were prepared with the particle size of the untreated seaweeds within 180-250 μm, with reaction time being 1.0 or 3.0 hours. Detailed design scheme for sorbent preparation is illustrated in Table 3.4.

- **Sorption Test**

The multiple-ion solution was prepared by mixing $AgNO_3$, $Cu(NO_3)_2$ and $Fe(NO_3)_3$ solutions. Batch sorption experiments were performed at room temperature with 20.0 mg of prepared sorbent suspended in 25.0 ml of tri-metallic solution with the concentration of 0.5 mmol/l of each metal. Suspensions were kept in constant agitation at 200rpm and an initial pH of 4.8. Experiments were carried out by duplicate for each sorbent. After 12 hours of agitation, biosorbent was separated from metal solutions by filtration. Afterwards, final metal concentrations in the filtered solutions were analyzed by ICP-OES. The values for initial and final metal cation concentrations were used to calculate the uptake capacity (mg/g) of each metal as the amount (in mg) of metal cation sorbed per gram of biosorbent.

Table 3.4 General Factorial Experiment Design on Two Algae Species for Ten Different Treatments on Cu (II), Ag (I) Uptake

Sample	Seaweed type	Treatment	Possible purposes	Operation*
1	S	85ºC NaOH	Biomass disintegration on components of seaweed S	Put 0.8g grinded seaweed S in 40ml 1M NaOH solution, keep heating and agitating the mixture at 85ºC for 1 hour.
2	V	85ºC NaOH	Biomass disintegration on components of seaweed V	Same as above
3	S	NaOH	Weaker biomass disintegration on components of seaweed S	Put 0.8g grinded seaweed S in 40ml 1M NaOH solution, keep agitating at 25ºC for 1 hour.
4	V	NaOH	Weaker biomass disintegration on components of seaweed V	Same as above.
5	S	HNO_3	Protonating seaweed S with H^+ , or oxidation (Figueira,M.M 2000),	Put 0.8g grinded seaweed S in 40ml 1M HNO_3, keep agitating at 25ºC for 1 hour.
6	V	HNO_3	Protonating seaweed V with H^+ ,or oxidation (Figueira,M.M 2000)	Same as above.
7	S	Methanol	Introducing more carboxyl groups on seaweed S via esterification	Put 0.8g grinded seaweed S in 40ml Methanol, add 2 drops of 1M HNO_3 into the mixture, keep agitating at 25ºC for 6 hours.
8	V	Methanol	Introducing more carboxyl groups on seaweed V via esterification	Same as above.
9	S	Acetone	To dissolve some protein and lipid, or introduce more	Put 0.8g grinded seaweed S in 40ml acetone; keep agitating at 25ºC for 3 hours.

			carbonyl and hydroxyl groups on seaweed S	
10	V	Acetone	To dissolve some protein and lipid, or introduce more carbonyl and hydroxyl groups on seaweed V	Same as above.

*Note: the different treatment duration times in operations were adjusted according to study of Ashkenazy *et al*., (1997), Brady and Duncan, (1994), and Park *et al*., (2005).

3.4 Results and Discussion

Analysis of variance (ANOVA), a mathematical process for separating the variability of a group of observations into assignable causes and setting up various significance tests was performed for the aforementioned experiments using the commercial software Design-Expert 8.0.

ANOVA is commonly applied to analyze experimental data as one statistical hypothesis testing. It bases on the null hypothesis, which is defined as all the treatments (factor levels) have the same relationship with response (Sahai and Ageel, 2000). The ANOVA results are considered statistically significant if test data are not due to chance.

$$\text{F value} = \frac{\text{Effect Variance}}{\text{Error Variance}} = \frac{\text{variance between treaments}}{\text{variance within treatments}} = \frac{SS_{\text{Treatments}}\ /(I-1)}{SS_{\text{Error}}\ /(n_T-1)} \qquad \text{Eq. (3-1)}$$

where SS is sum of square, I= number of treatments and n_T= total number of cases. The critical value of F is a function of the numerator degrees of freedom, the denominator degrees of freedom and the significance level (α). P value determines the levels of significance, which tells whether the data groups differ significantly. If it is statistically significant, the group of data differ for certain factors, not by chance. The null hypothesis is rejected, if the probability (p-value) is less than or equal to the significance level (α), 0.05. When $p < 0.05$, the result is said to be significant with a happen probability less than five times out of hundred (Turner and Thayer, 2001).

3.4.1 Single Ag (I) Biosorption

The results of the sorption tests using various sorbents prepared from brown algae are listed in Table 3.5. It is easily to find from Table 3.5 that seaweeds treated by HNO_3 shows lower Ag^+ uptake capacity than the sorbents prepared by alkaline saponification, which suggests that protonating seaweeds by HNO_3 have less enhancement in silver uptake than saponified seaweeds by NaOH. Moreover, modified type S seaweeds have higher capacity than type J on silver sorption.

Sorbent prepared by 1 hour alkali washing of Type S seaweeds with particle size of 180-250μm has a maximum silver uptake capacity of 82.14 g (silver) per kilogram sorbent. To date, 56.7mg/g of Ag uptake by alga *Chlorella vulgaris*, 52 mg/g of Ag uptake capacity by alginate bead loaded with Ca^{2+} at pH of 4 and 30.24mg/g of Ag uptake capacity by active carbons derived from coconut shell and polyacrylonitrile at pH of 10 have been reported from open literature. Compared with these results, the silver uptake of 82.14 mg/ g by modified *Fucus spiralis* seaweed in this study is quite competitive.

Table 3.5 Ag (I) Sorption Test Results Using Modified Seaweed Biosorbents

Run	Parameter Conditions				Ag uptake (mg/g)
	Seaweed	Treatment	Particle size (μm)	Reaction time (h)	
1	Type S	NaOH	150-180	1.0	82.1
2	Type S	HNO_3	180-250	1.0	24.3
3	Type J	NaOH	180-250	1.0	57.5
4	Type J	HNO_3	150-180	1.0	23.3
5	Type S	NaOH	180-250	4.0	69.3
6	Type J	HNO_3	150-180	4.0	15.1
7	Type S	HNO_3	180-250	4.0	11.7
8	Type J	NaOH	150-180	4.0	59.3

Results for the fractional factorial design of sorbent preparation for single Ag$^+$ uptake have been summarized in Table 3.6. Four factors i.e., A (seaweed type), B (chemical solvent), C (particle size) and D (reaction time) were considered in Design Matrix Evaluation for Factorial Reduced 2FI Model. The Model F-value of 65.64 implies the model is significant. There is only a 0.30% chance that a "Model F-Value" this large could occur due to noise. “p-value” (significance probabilities) results show that A and B are significant model terms in this case. The paired test results show each test have the same trend on the Ag (I) uptake. Therefore, we can conclude that particle size for the raw seaweeds and reaction times for the preparation process do not have significant effect on the sorbent performance for Ag$^+$ uptake.

Table 3.6 ANOVA for the Sorbent Preparation Experiments (Single Ag (I) Sorption)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	State
Model	5133.42	4	1283.35	65.64	0.0030	significant
A-seaweed	276.08	1	276.08	14.12	0.0329	
B-treatment	4694.81	1	4694.81	240.11	0.0006	
C-size	182.60	1	182.60	9.34	0.0552	
D-time	126.41	1	126.41	6.46	0.0845	
Residual	58.66	3	19.55			

Table 3.7 Design-Expert Output for Sorbent Preparation Experiments (Single Ag (I) sorption)

Std. Dev.	4.42	R-Squared	0.99
Mean	42.87	Adj R-Squared	0.97
C.V. %	10.32	Pred R-Squared	0.91
PRESS	457.03	Adeq Precision	20.02

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	42.87	1.56	37.89	47.84	
A-seaweed	6.78	1.81	1.04	12.53	1.33
B-treatment	-24.23	1.56	-29.20	-19.25	1.00
C-size	-5.52	1.81	-11.26	0.23	1.33
D-time	-3.98	1.56	-8.95	1.00	1.00

The summary of the model ANOVA for the experiments can be found in Table 3.7. The "Adj R-Squared" is a statistic that is adjusted for the size of the model, i.e., the number of factors. The adjusted R^2 decreases if non-significant terms are added to the model. The "Pred R-Squared" indicates how well the model will predict the new data. The "Pred R-Squared" of 0.91 is in reasonable agreement with the "Adj R-Squared" of 0.97 and suggests that the full model will be expected to explain about 91% of the variability in new data. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 20.02 indicates an adequate signal. Therefore, the model from Design-Expert can be used to navigate the design space.

The final equation in terms of Coded Factors is:

Ag conc. after sorption = 42.87+6.78* A-24.23* B-5.52* C-3.98* D

Final equations in terms of actual factors are produced from Design-Expert:

a) ***For Type J seaweed*:**

by treatment of NaOH: q_{Ag} = 127.58-0.37* size-2.65* time

by treatment of HNO_3: q_{Ag} = 79.13-0.37* size-2.65* time

b) For Type S seaweed:

by treatment of NaOH: q_{Ag} = 141.14-0.37* size-2.65 * time

by treatment of HNO_3: q_{Ag} = 92.70-0.37* size-2.65* time

For single silver sorption, it can be concluded that sorbent prepared from *Fucus spiralis* treated with NaOH solution for 1 hour provides the maximum silver uptake capacity. The seaweed species, and chemical solvent are the significant preparation factors on sorbent performance for silver binding.

3.4.2 Selective sorption of Ag (I)

Experimental results for selective sorption of Ag^+ from tri-metallic solution by sorbents prepared using the design scheme illustrated in Table 3.4 are interesting. For all the ten sorbent samples, iron precipitations were observed soon after the sorbents were added into the tri-metallic solutions as iron concentration in the residual solutions dropped rapidly due to chemical reaction and no evidence of iron binding was found on the surface of sorbent from the powder XRD results. Therefore, surface binding of Fe^{3+} was not considered under this scenario. Only Ag^+ and Cu^{2+} were considered to compete for binding sites on the sorbent surfaces. The measured uptake capacities of Ag^+ and Cu^{2+} for different modified seaweed sorbents are demonstrated in Table 3.8.

Table3.8 Copper and Silver Uptake Results of Modified Sorbents
(Selective Sorption of Ag (I))

Sample	Seaweed type	Treatment	q_{Cu} (mg/g)	q_{Ag} (mg/g)
1	S	85ºC NaOH	25.42	22.92
2	V	85ºC NaOH	26.21	25.62
3	S	NaOH	27.80	26.97
4	V	NaOH	28.60	25.62
5	S	HNO_3	10.33	18.88
6	V	HNO_3	13.50	17.53
7	S	Methanol	23.83	40.45
8	V	Methanol	26.21	37.75
9	S	Acetone	23.04	52.59
10	V	Acetone	24.63	44.50

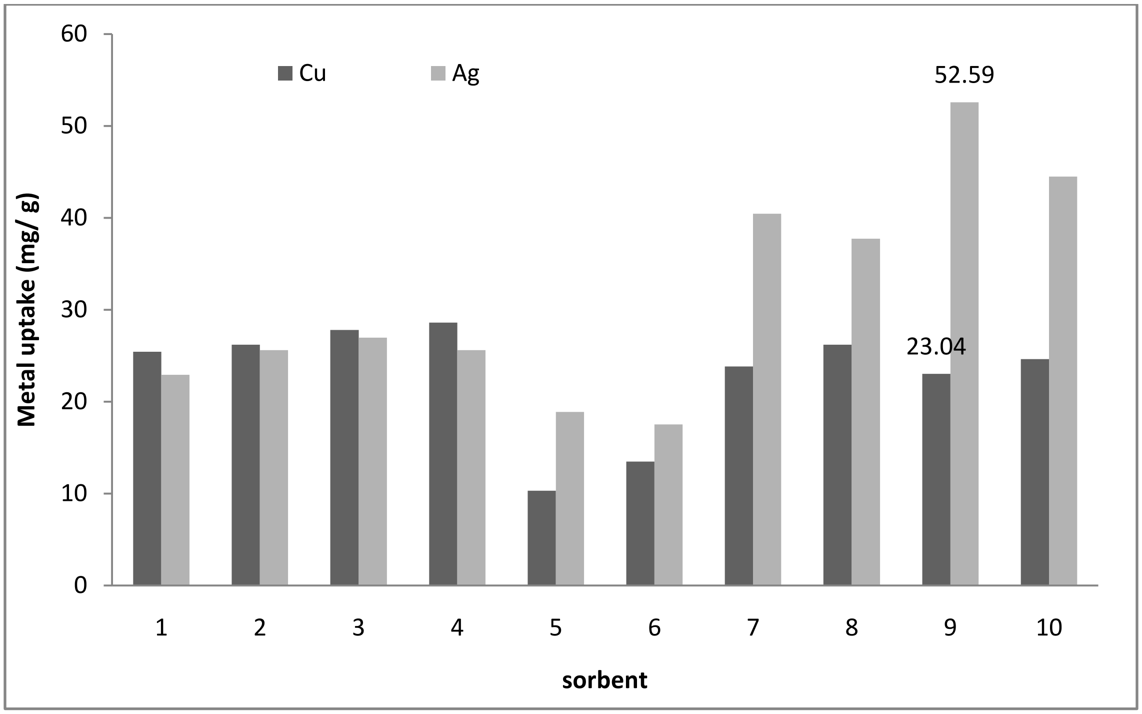

Figure 3.2 Cu and Ag Uptake Results on the Ten Modified Sorbents

Results from Figure 3.2 indicate that sorbent prepared from acetone washing of *Fucus spiralis* at room temperature gives the best selective uptake capacity of Ag^+. Although Fe^{3+} has been proven not to be involved in surface binding, it affects the sorbent selectivity of Ag^+. Discussion on the effect of Fe^{3+} on the sorbent selectivity of Ag^+ will be presented in Chapter 5.

- **Statistical Analysis of Sorbent Performance on Ag (I) Uptake**

Table 3.9 lists the AVONO results of factorial model with two factors for selective sorption of Ag^+ by chemical modified sorbents. The model F-value of 34.60 implies the model is significant. There is only a 0.22% chance that a "Model F-Value" this large could occur due to noise. “p-value” (significance probabilities) results show that in this case only B (chemical treatment) is significant, A (seaweed type) is not significant. Therefore, model with one factor as illustrated in Table 3.10 can be used. F-value of 36.03 for the one factor model implies the model is significant. There is only a 0.07% chance that a "Model F-Value" this large could occur due to noise. "p-value Prob > F" less than 0.05 indicate model term B is significant.

These ANOVA results indicate that the two *Fucus* seaweeds (Spiralis and Vesiculosus) have almost the same sorption ability for Ag^+, while the treatment from organic solvents (methanol and acetone) improves the sorption selectivity of Ag^+.

Table 3.9 ANOVA for Factorial Model of Two Factors (Selective Ag (I) Sorption) (by Design-Expert)

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F	State
Model	0.0677	5	0.0135	34.60	0.0022	significant
A-Seaweed	0.000757	1	0.000757	1.93	0.23	
B-Treatment	0.0670	4	0.0167	42.77	0.0015	
Residual	0.00157	4	0.000392			

Table 3.10 ANOVA for Factorial Model of One Factor (Selective Ag (I) sorption) (by Design-Expert)

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F	State
Model	0.067	4	0.016745	36.03	0.0007	significant
B-Treatment	0.067	4	0.016745	36.03	0.0007	
Residual	0.0023	5	0.000465			

Table 3.11 Design-Expert Output for Sorbent Preparation Experiments (Selective Ag (I) Sorption)

Std. Dev.	0.0216	R-Squared	0.966
Mean	0.267	Adj R-Squared	0.940
C.V. %	8.062	Pred R-Squared	0.866
PRESS	0.0093	Adeq Precision	14.72

Table 3.11 summarizes the model ANOVA for the experiments for selective sorption of Ag^+. The "Pred R-Squared" of 0.866 is in reasonable agreement with the "Adj R-Squared" of 0.9396. "Adeq Precision" of 14.72 indicates an adequate signal. This model can be used to navigate the design space to predict the final Ag^+ concentration after sorption.

- **Statistical Analysis of Sorbent Performance on Cu (II) Uptake**

Factorial model for Cu^{2+} sorption from tri-metallic solution was also obtained from ANOVA analysis by Design-Expert. As shown in Table 3.12, the model F-value of 161.50 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. . "p-value Prob > F" less than 0.05 indicates A and B are both significant model terms.

Table 3.12 ANOVA for Factorial Model with Two Factors (Cu (II) Sorption) (by Design-Expert)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	State
Model	0.055	5	0.011	161.497	0.0001	significant
A-Seaweed	0.0012	1	0.0012	17.36008	0.0141	
B-Treatment	0.054	4	0.013	197.5312	< 0.0001	
Residual	0.00027	4	6.78E-05			

Table3.13 generated by Design-Expert provides the optimal conditions for sorbent preparation to gain better selectivity for silver uptake.

Table 3.13 Optimal Conditions for Sorbent Preparation (Selective Ag (I) Sorption) (by Design-Expert)

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Seaweed	in range	S	V	1	1	3
Treatment	in range	85ºC NaOH	Acetone	1	1	3
Cu Uptake	maximize	0.136	0.373	1	1	3
Ag Uptake	minimize	0.110	0.366	1	1	3

Optimal conditions

Number	Seaweed	Treatment	Final Cu conc. (mmol/l)	Final Ag conc. (mmol/l)
1	S	Acetone	0.212	0.138

Note: Final Cu, Ag conc. (mmol/l) are the residual metal ions concentrations after biosorption

- **Sorbent Screening for Selective Sorption of Ag (I)**

Under multi-metal environment, the optimal preparation conditions for the sorbent which could provide the maximum final silver concentration and minimum final copper concentration in the residual solution can be found by Design-Expert. The summary of the optimal solutions under this scenario is given in Table 3.13. It can be seen from Table 3.13 that seaweed *Fucus spiralis* treated by acetone washing shows the highest selectivity of Ag^+ with the Ag^+ uptake capacity of 52.59 mg/g and Cu^{2+} uptake capacity of 23.04 mg/g.

3.5 Conclusion

Design of experiments (DOE) was applied in this study to prepare the modified biosorbents from brown algae for the Ag^+ sorption from both single metal and multi-metal ion solutions. Through a 2-level fractional factorial design, sorbents, prepared from *Fucus spiralis* treated with NaOH solution for 1 hour, possess the maximum uptake capacity (82 mg/g) of extracting silver from silver nitrate solution with a pH of 4.8. The ANOVA results demonstrated that seaweed type and chemical solvent are the significant preparation factors on sorbent performance for single Ag (I) binding.

For selective sorption of Ag^+ from the tri-metallic solution containing equal-molar concentration of Ag (I), Cu (II) and Fe (III) cations, sorbent prepared from acetone washing of *Fucus spiralis* at room temperature gives the highest selective uptake capacity of Ag^+. This modified sorbent has the highest silver uptake capacities at 52.59 mg/g sorbent and the lowest copper uptake capacity at 23.04 mg/g sorbent. All the factorial models obtained from ANOVA analyses can provide good predictions of the final metal concentrations in the residual solutions of sorption test.

Chapter 4 Sorption Isotherm and Kinetic Studies

4.1 Introduction

The biosorption of metal ions is affected by many experimental parameters such as the pH, ionic strength, biomass concentration, and the presence of different metallic ions in water solutions. The variability of these parameters in real wastewaters makes it necessary to know their influence on biosorption performance. From sorbent screening study, we found that solution pH has a huge influence on the Ag (I) uptake by brown seaweed. It is important to optimize the solution pH for a high uptake capacity of Ag (I).

Results from Chapter 3 indicate that modified brown seaweeds show a relative high uptake capacity of Ag (I). But a real implementation of biosorption for Ag (I) also requires a high sorption rate of Ag (I). Therefore, kinetic study for the biosorption of Ag (I) by acetone washed *Fucus spiralis* was performed using batch mode sorption tests.

It is known that equilibrium and kinetic analyses not only allow for the estimation of sorption capacities and rates, but also lead to express possible biosorption mechanisms. A detailed analysis of the kinetics and equilibrium of biosorption of Ag (I) binding by the acetone washed *Fucus spiralis* was conducted and the impacts of pH and the other competing metal ions on the effectiveness of the process were assessed.

In order to investigate the mechanism of biosorption and the potential rate controlling step, pseudo-first and pseudo-second order kinetic models have been used to fit the experimental data of the batch sorption process. Also, Langmuir and Freundlich isotherm models have been used to determine the suitable sorption equilibrium of Ag (I) on the modified seaweeds from competitive multi-meal environment. Although lots of work has been carried out on the kinetic and thermo-isotherm study of the biosorption of heavy metals, research on the kinetic and isotherm study of selective biosorption of Ag (I) from multi-metal system is seldom reported. Understanding the interference and competition phenomena on the binding sites involved should be addressed in the biosorption study.

Solution pH effect, kinetic and equilibrium isotherm studies of the biosorption of Ag (I) from competitive multi-metal system are presented in this chapter. This study would contribute to a better understanding of the biosorption phenomena and aid in the development of potential biosorbents using locally derived sorbents, which possess high capacities for silver ion uptake from aqueous solutions.

4.2 Material and Methods

4.2.1 Materials

The sorbent prepared from acetone washing of *Fucus spiralis* introduced in Chapter 3 was used for all the kinetic and sorption isotherm studies in this chapter. Figure 4.1 shows the modified *Fucus spiralis* sorbent after acetone washing.

Silver nitrate solution (0.01N A.C.S. Grade), copper nitrate (Hemipentahydrate, 98%, A.C.S. Grade) and iron nitrate (Nonahydrate, 98%, A.C.S. Grade) were purchased from Fisher Scientific, Canada. Distilled deionized water was prepared in the laboratory. To adjust the solution's pH, sodium hydroxide, (NaOH, Pellets, 97+%, A.C.S. Reagent) was purchased from Sigma Aldrich, Canada and nitric acid (HNO_3, 68%, A.C.S.) from VWR, Canada.

Figure 4.1 *Fucus spiralis* Sorbent after Acetone Washing

4.2.2 Instruments

Accumet AB 15+ pH/mV/°C meter (Fisher Scientific, Canada) was used for pH measurement. ICP-OES, Optima 5300DV was used to determine the residual concentration of silver (I) and copper (II) and Fe (III) in the tri-metallic solution.

4.2.3 Batch Sorption Tests

- **Effect of pH on silver biosorption**

The pH dependence of Ag^+ uptake by modified brown seaweeds Type J and Type S was studied through batch experiments in a pH range from 1 to 6.3, with 20.0 mg sorbents suspended in 25.0 ml $AgNO_3$ solution with an initial concentration of 0.1mM. The pH range was selected in order to avoid metal (hydr)oxides precipitation, and to ensure that the decay of metal concentration is due to adsorption processes. The pH was adjusted using diluted HNO_3 or NaOH. Sorption experiments were conducted at 30ºC with a shaking speed of 300 rpm in the incubator.

- **Kinetic Study**

Tri-metallic solution was prepared by mixing $AgNO_3$, $Cu(NO_3)_2$ and $Fe(NO_3)_3$ with the initial concentration of 0.5 mmol/l of each metal. Seven duplicates (25ml) in conical flasks were fully mixed with 20 mg sorbent (acetone washed *Fucus spiralis*) at 30ºC and 300 revolutions per minute (rpm) shaking speed in the incubator for seven different time periods. The first mixture was taken out after about 10 minutes of contact, and filtered for later metal concentration tests with ICP-OES, while the other six samples were taken out after 30 min, 1 h, 2h, 4h, 8h, and 12h. There are two duplicates for sample at 4 h in order to check experiment error.

- **Isotherm Determination**

Biosorption isotherms of Ag (I) and Cu (II) were obtained at solution pH of 4.8 and 6.3. The isotherm experiments were conducted with 20.0 mg of the biosorbent (acetone washed *Fucus spiralis*) in a working solution volume of 25 ml. The working solution contained equal molar Ag (I), Cu (II) and Fe (III). The initial metal concentration was varied between 0.1–5.0 mmol/L, which resulted in different final metal concentrations after the sorption equilibrium had been achieved. Following the addition of the biosorbent into the tri-metallic solutions, the solutions were kept in the incubator at 30ºC and 300 rpm shaking speed for 12 hours. After reaching the equilibrium, filtrates from suspensions were sent to CREIAT lab for ICP-OES analysis to test the final concentrations of Fe (III), Cu (II) and Ag (I) in the filtered solution. Batch sorption test has been duplicated for sample solution with the concentration of 2.5mmol/l to determine the reproducibility of the experiment.

4.3 Result and Discussion

4.3.1. Effect of pH on silver biosorption

Ag (I) uptake at different pH values was illustrated in Figure 4.2. As has been proven in Chapter 3, sorbent made from *Fucus spiralis* has greater capacity to absorb silver ions from the aqueous phase than sorbent J in the pH range from 1 to 6.3. Sorbent from Type J had a maximum silver uptake of 38 mg/g at pH of 4, while uptake capacity for the Type S sorbents increases as pH increase in the solution, especially (fast) from a pH of 2 to 4. It had an increase from 14 mg/g to 70 mg/g and then went up slowly when the medium became alkaline. It is different from other precious metals. For example, gold has been reported to have high uptake below a pH of 2 (Ogata and Nakano, 2005). For practical usage, the suggested solution pH range is 4 to 6 for further studies, because sorbent stability will decrease where pH is greater than 7 and the precipitation of Ag_2O will increase as the solution pH increases (Torres *et al.*, 2005).

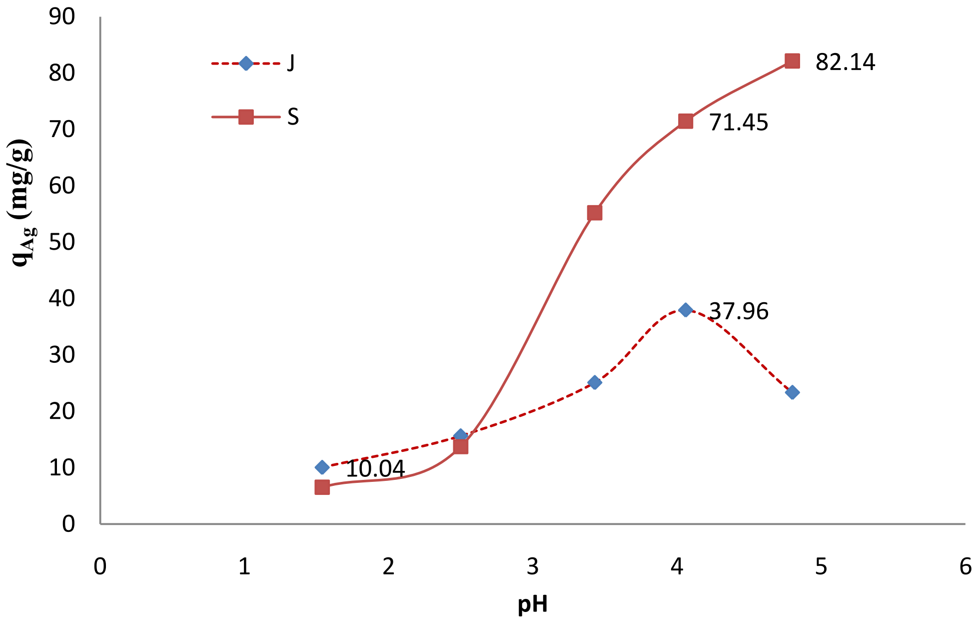

Figure 4.2 Silver Uptake on the Two Sorbents at Different pH

4.3.2. Kinetic Model

- **Measured Sorption Rates**

After 4-hour contact, the concentration of iron ions reached the lowest level of 10.4 mg/l, which is slower than copper and silver ions. Iron precipitate was observed during the sorption test. As discussed in Chapter 3, evidence of iron binding on sorbent surfaces has not been detected. Therefore, Fe (III) was considered to be an environmental factor in the kinetic and isotherm study for the selective sorption of Ag^+ from multi-metallic environment.

Figure 4.3 depicts that the residual silver and copper concentrations at different time of the sorption test. It shows that the rate of silver uptake was very fast, and the biosorption equilibrium was reached at 58.53mg/g, within 10 min after the sorbent addition. This rapid biosorption also has been reported on metal ions adsorbed on other algae to attain equilibrium in 30 minutes (Klimmek and Stan, 2001). For the copper ion, the uptake almost reached the equilibrium of 25.12 mg/g after two hours. These results show that the binding of metal ions onto the surface of sorbent are quite fast.

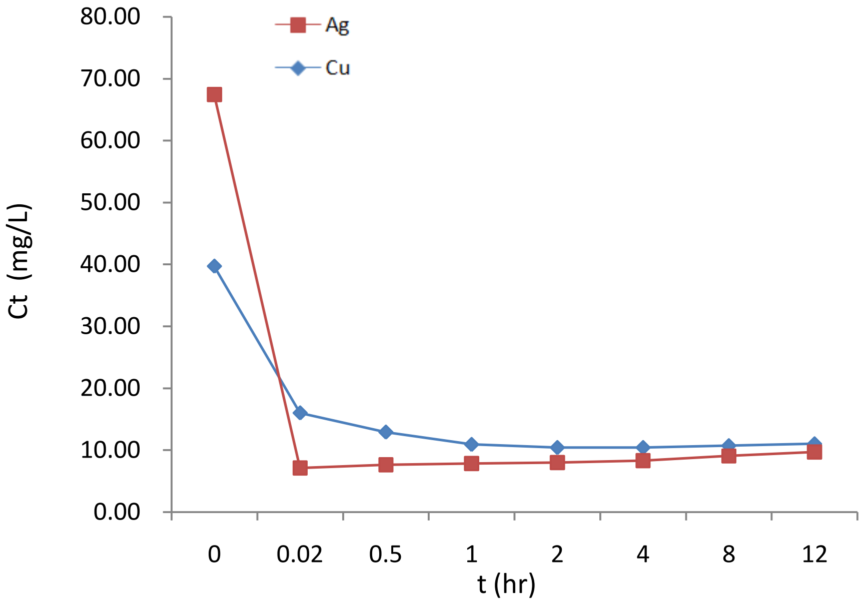

Figure 4.3 Copper and Silver Concentration vs. Time during Sorption Test

- **Sorption Rate Equations**

To elucidate the mechanisms on the biosorption process, the Pseudo first-order and the Pseudo second order kinetic equations were used to fit the experimental data obtained from batch experiments.

The Pseudo first order model can be expressed as:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \qquad \text{Eq. (4-1)}$$

where, q_e and q_t are metal uptakes at equilibrium and at a certain time *t,* respectively; k_1 is the first order rate constant. It is transferred into the linear form after integration:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad \text{Eq. (4-2)}$$

Experimental data can be converted to $\ln(q_e - q_t)$ vs. t in Table 4.1 to determine the rate constants q_e and k_1 by linear regression.

Table 4.1 Pseudo First Order Kinetic Model Data

	t (hr)	0.02	0.5	1	2	4	8	12
Ag	$\ln(q_e - q_t)$	0	0.585	1.169	1.512	1.800	2.085	2.291
Cu	$\ln(q_e - q_t)$	1.686	0.690	-2.724	0	-0.627	0.341	0.817

Linear forms of pseudo first order second rate equation are as follows:

For Ag (I): $\ln(q_e - q_t) = 0.155\, t + 0.7403$, $R^2 = 0.7083$ Eq. (4-3)

For Cu (II): $\ln(q_e - q_t) = 0.0575t - 0.1998$, $R^2 = 0.0336$ Eq. (4-4)

The extremely low R^2 implies that the Pseudo first order model is not suitable for describing the sorption rate of silver and copper. Therefore, the Pseudo second order rate equation as below was used:

$$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2 \qquad \text{Eq. (4-5)}$$

where k_2 is the second order rate constant. It is transferred into the linear form:

$$\frac{t}{q_t} = \frac{1}{k_2 (q_e)^2} + \frac{t}{q_e} \qquad \text{Eq. (4-6)}$$

The relationship of experimental $\frac{t}{q_t}$ vs. t for Ag (I) and Cu (II) are plotted in Figures 4.4 and 4.5, respectively.

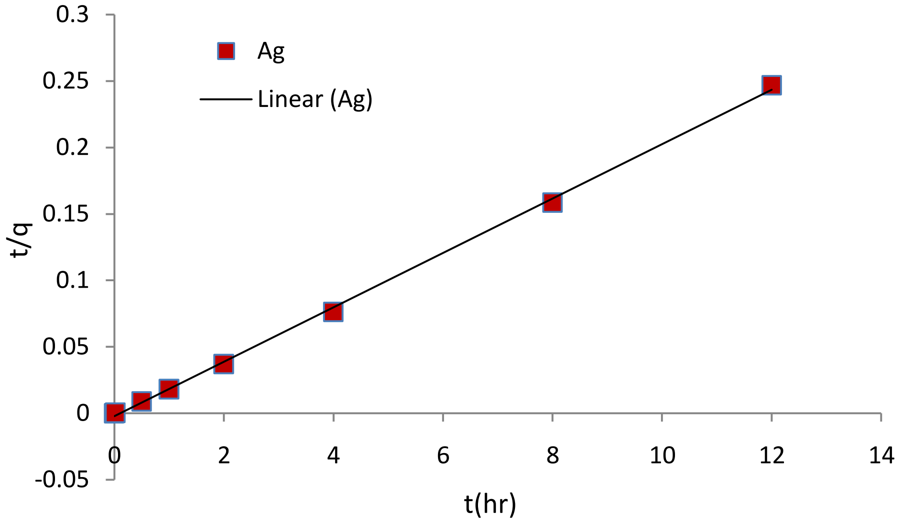

Figure 4.4 Linear Regression of the Pseudo Second Order Model for Silver Uptake

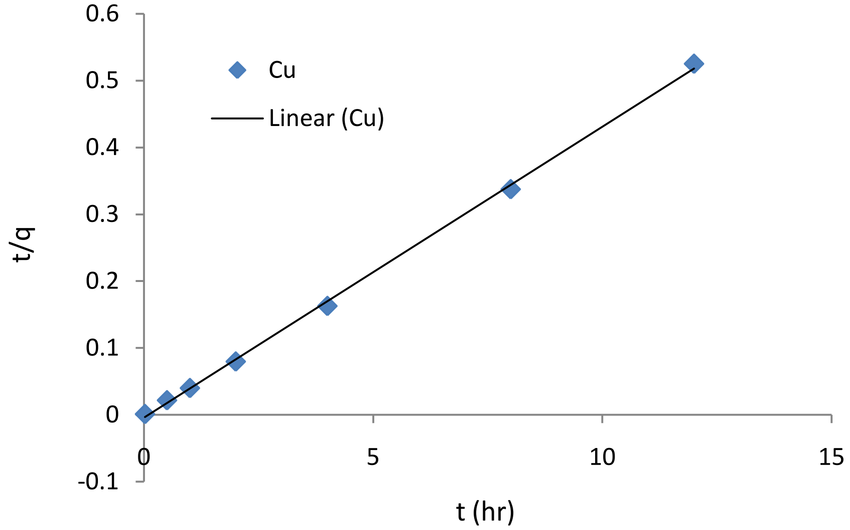

Figure 4.5 Linear Regression of the Pseudo Second Order Model for Copper Uptake

Linear forms of Pseudo second order rate equation are given below:

For Ag (I): $\frac{t}{q_t} = 0.0205t - 0.0021$, R^2 =0.9992 Eq. (4-7)

For Cu (II): $\frac{t}{q_t} = 0.0435t - 0.0044$, $R^2 = 0.9992$ Eq. (4-8)

Table 4.2 Rate Constants of the Pseudo First and Second Order Equations

Ag	Pseudo first order			Pseudo second order		
q	q_e	k_1	R^2	q_e	k_2	R^2
58.53 mg/l	2.1 mg/l	-0.155	0.71	48.78 mg/l	-0.2	0.9992
Cu	**Pseudo first order**			**Pseudo second order**		
q	q_e	k_1	R^2	q_e	k_2	R^2
25.12 mg/l	0.82 mg/l	-0.058	0.034	22.99 mg/l	-0.43	0.9992

Table 4.2 summarizes the rate constants of the pseudo first and second order equations for Ag (I) and Cu (II) uptake. From Figures 4.4 and 4.5, it is observed that pseudo second order rate equation fits the kinetic experiment data of Ag (I) and Cu (II) uptake results extremely well with high R^2 values. Pseudo second order rate equation can be applied in this case to describe the sorption kinetics of Ag (I) and Cu (II) from the multi metal solution.

For quick biosorption process, it is usually accepted that pseudo first order equation assumes that metal cation is binding with one sorption site on the sorbent surface, while the pseudo second order model assumes that metal binding takes place on two sorption sites (Mohan *et al.,* 2011). Results of the rate equation also manifest that the first order equation fits the experiment data at the beginning of the biosorption process, rather than the full length of the contact time. Described by the second order equation, the biosorption rate depends on both the sorbate amount on the sorbent's surface and maximum sorbate amount at equilibrium, which was directly proportional to the binding site amount on the sorbent surface (Mohan *et al.,* 2011).

The results of the current study demonstrate that uptake of Ag (I) on sorbent surface is faster than that of Cu (II). This is beneficial to the scale-up of the biosorption process for silver recovery.

4.3.3 Adsorption Isotherm

- **Batch Sorption Results**

The experimental results on the final concentration of Ag (I) and Cu (II) in the residual solution, the amount of metal ions adsorbed on the sorbent surface as well as the removal percentage of the two metal ions at various solution pHs are listed in Table 4.3.

Table 4.3 Experimental Data of Ag (I) and Cu (II) Biosorption

pH: 4.8

Ci(mg/l)	Ce_{Cu} (mg/l)	Ce_{Ag}(mg/l)	q_{eCu}(mg/g)	q_{eAg}(mg/g)	$Ad_{\% Cu}$	$Ad_{\% Ag}$
0.1	2.87	8.24	4.36	3.18	54.84%	23.61%
0.5	5.22	8.29	33.19	57.06	83.57%	84.63%
1	14.5	57.1	61.31	63.46	77.18%	47.07%
1.5	34.6	113.5	75.91	60.38	63.70%	29.85%
2.5	74.7	211.8	105.22	72.34	52.98%	21.46%
3.5	118.3	319.9	130.16	72.06	46.81%	15.27%
5	173.5	478.4	180.31	76.19	45.40%	11.30%

pH: 6.3

Ci(mg/l)	Ce_{Cu} (mg/l)	Ce_{Ag}(mg/l)	q_{eCu}(mg/g)	q_{eAg}(mg/g)	$Ad_{\% Cu}$	$Ad_{\% Ag}$
0.1	2.72	8.47	4.54	2.90	57.20%	21.48%
0.5	6.22	13.7	31.94	50.29	80.42%	74.60%
1	8.93	47.5	68.28	75.46	85.95%	55.97%
1.5	14.0	88.5	101.66	91.63	85.31%	45.30%
2.5	33.6	186.6	156.59	103.84	78.85%	30.81%
3.5	57.5	279.7	206.16	122.31	74.15%	25.92%
5	75.2	449.7	303.19	112.06	76.33%	16.62%

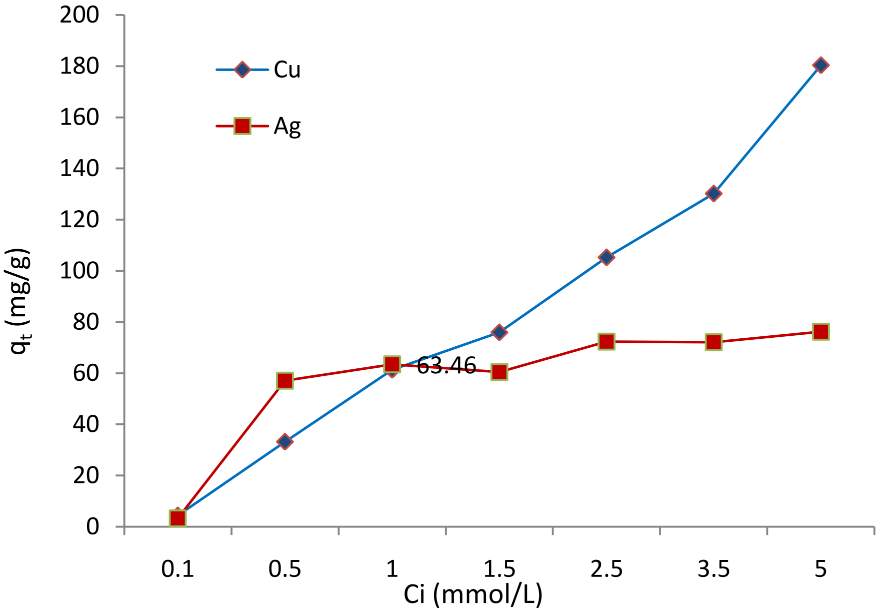

Figure 4.6 Measured Isotherm Data of Ag (I) and Cu (II) on the Acetone Washed *Fucus spiralis* at pH 4.8

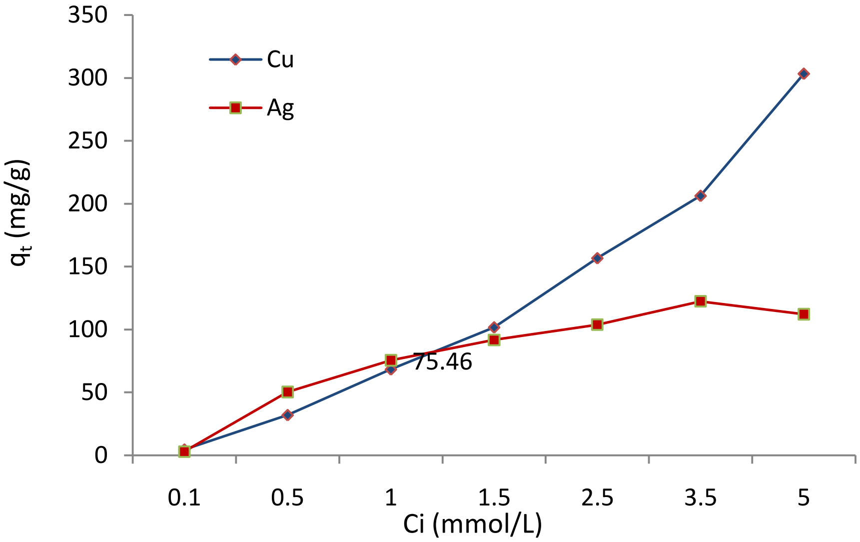

Figure 4.7 Measured Isotherm Data of Ag (I) and Cu (II) on the Acetone Washed *Fucus spiralis* at pH 6.3

As shown in Figures 4.6 and 4.7, the uptakes of Ag (I) onto the biosorbent at pH 4.8 and 6.3 are lower than those of Cu (II), when initial solution concentrations are lower than 1.0 mM and 1.25mM, respectively. But when solution concentration becomes higher, the uptake of Cu (II) increases significantly and exceeds the uptake of Ag (I). In addition, the isotherm patterns of Ag (I) and Cu (II) are different. Uptake of Ag (I) increased with increasing equilibrium Ag (I) concentration, and eventually reached a certain saturated value depending on the solution pH. The maximum uptakes of Ag (I) were estimated to be 61.3 and 68.2 mg/g at pH 4.8 and 6.3, respectively. However, the result of the isotherm of Cu (II) increased almost linearly with increasing equilibrium Cu (II) concentration. The main cause for this observation may be the existence of the strong chemical bonding between Cu (II) and the biosorbent.

- **Isotherm Model**

Very similar to molecular adsorption, the adsorption property of the metal ions can be analyzed using isotherm model. In this study, two widely used isotherm models, Langmuir and Freundlich equations were fitted to the metal-ion uptake on the sorbent (q_e) vs. equilibrium metal-ion concentration (c_e) data. The Langmuir and Freundlich equations and parameters to be determined are summarized as follows:

$$\mathrm{q}_{\mathrm{e,j}} = \frac{q_{\max} b C_{e,j}}{1 + b C_{e,j}} \qquad \text{Eq. (4-9)}$$

$$q_{e,j} = k_F C_{e,j}^{1/n} \qquad \text{Eq. (4-10)}$$

where b is the Langmuir constant; $q_{\max}$ is the maximum metal-ion uptake capacity of the biosorbent(mg/g sorbent); k_F is a constant indicative of the adsorption capacity of the sorbent (mg/g sorbent), and 1/*n* is a measure of adsorption intensity.

Both Langmuir and Freundlich equations can be transferred into linear forms as shown in Eq. (4-3) and Eq. (4-4) and the isotherm parameters can be determined by linear regression. Tables 4.4 and 4.5 listed the transformed experimental data of the isotherm study of Ag (I) and Cu (II).

Linear form of Langmuir isotherm can be expressed as:

$$\frac{c_{e,i}}{q_{e,i}} = \frac{c_{e,i}}{q_{\max}} + \frac{1}{bq_{\max}} \qquad \text{Eq. (4-11)}$$

Linear form of Freundlich isotherm can be expressed as:

$$\ln q_{e,j} = \ln k_F + \frac{\ln C_{e,j}}{n} \qquad \text{Eq. (4-12)}$$

Table 4.4 Transformed Experimental Data of Biosorption Isotherm of Ag (I)

pH of 4.8					pH of 6.3				
	Langmuir		Freundlich			Langmuir		Freundlich	
c_e mg/l	q_e mg/g	c_e/q_e	lnq_e	lnc_e	c_e mg/l	q_e mg/g	c_e/q_e	lnq_e	lnc_e
8.29	57.06	0.15	4.04	2.12	13.7	50.29	0.27	3.92	2.62
57.1	63.46	0.9	4.15	4.04	47.5	75.46	0.63	4.32	3.86
113.5	60.38	1.88	4.1	4.73	88.5	91.63	0.97	4.52	4.48
211.8	72.34	2.93	4.28	5.36	186.6	103.84	1.8	4.64	5.23
319.9	72.06	4.44	4.28	5.77	279.7	122.31	2.29	4.81	5.63
478.4	76.19	6.28	4.33	6.17	449.7	112.06	4.01	4.72	6.11

Table 4.5 Transformed Experimental Data of Biosorption Isotherm of Cu (II)

pH of 4.8					pH of 6.3				
	Langmuir		Freundlich			Langmuir		Freundlich	
c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$	c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$
2.87	4.36	0.66	1.47	1.05	2.72	4.54	0.6	1.51	1
5.22	33.19	0.16	3.5	1.65	6.22	31.94	0.19	3.46	1.83
14.5	61.31	0.24	4.12	2.67	8.93	68.28	0.13	4.22	2.19
34.6	75.91	0.46	4.33	3.54	14	101.66	0.14	4.62	2.64
74.7	105.22	0.71	4.66	4.31	33.6	156.59	0.21	5.05	3.51
118.3	130.16	0.91	4.87	4.77	97.5	156.16	0.62	5.05	4.58
173.5	180.31	0.96	5.19	5.16	75.2	303.19	0.25	5.71	4.32

Comparison of the linearized experimental data and model predictions for the biosorption of Ag (I) and Cu (II) are illustrated in Figures 4.8-4.11. The determined Langmuir and Freundlich isotherm equations are listed in Table 4.6 and equation parameters can be found in Table 4.7.

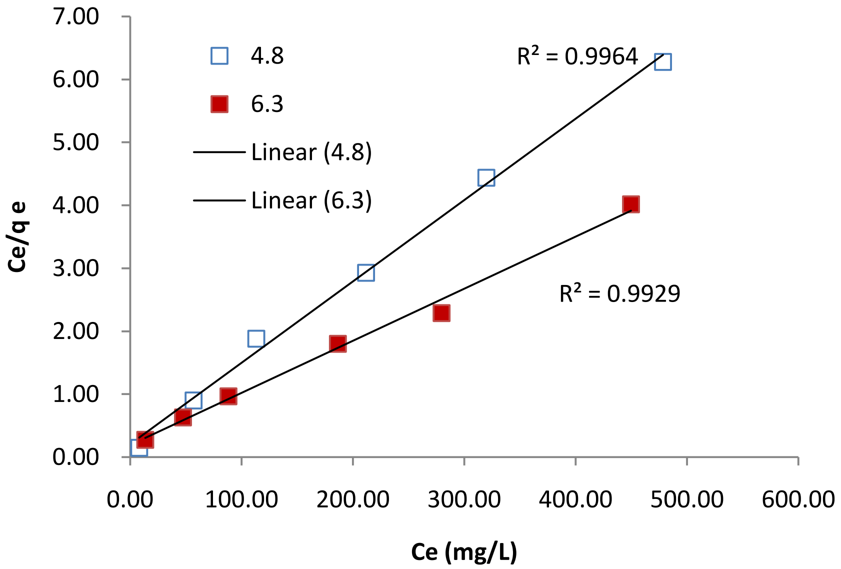

Figure 4.8 The Linear Regression Plot of Langmuir Isotherm Model for Ag (I) Uptake at Two pH Values

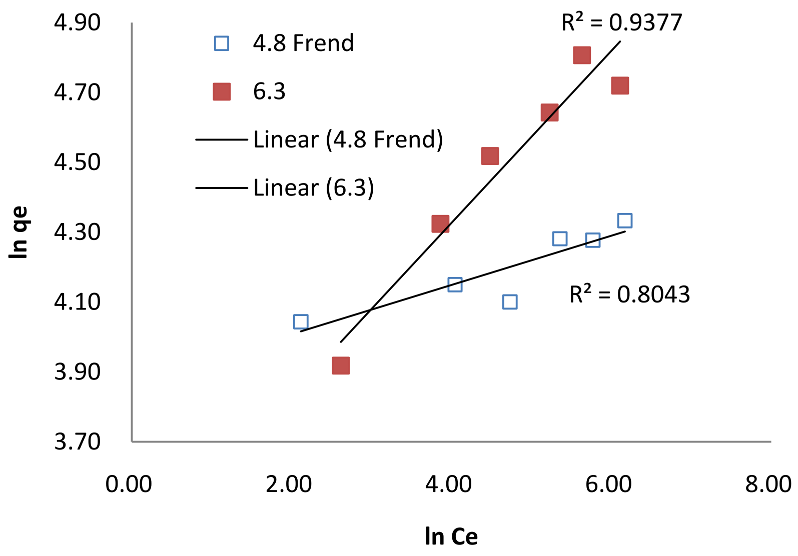

Figure 4.9 The Linear Regression Plot of Freundlich Isotherm Model for Ag (I) Uptake at Two pH Values

Two linear regression plots of Ag (I) on two models in Figure.4.8 and 4.9 fit experimental data with high R^2, well describing the relationship between initial metal concentration and equilibrium metal uptake. However, Langmuir model have a higher R^2 under both pH conditions than Freundlich model.

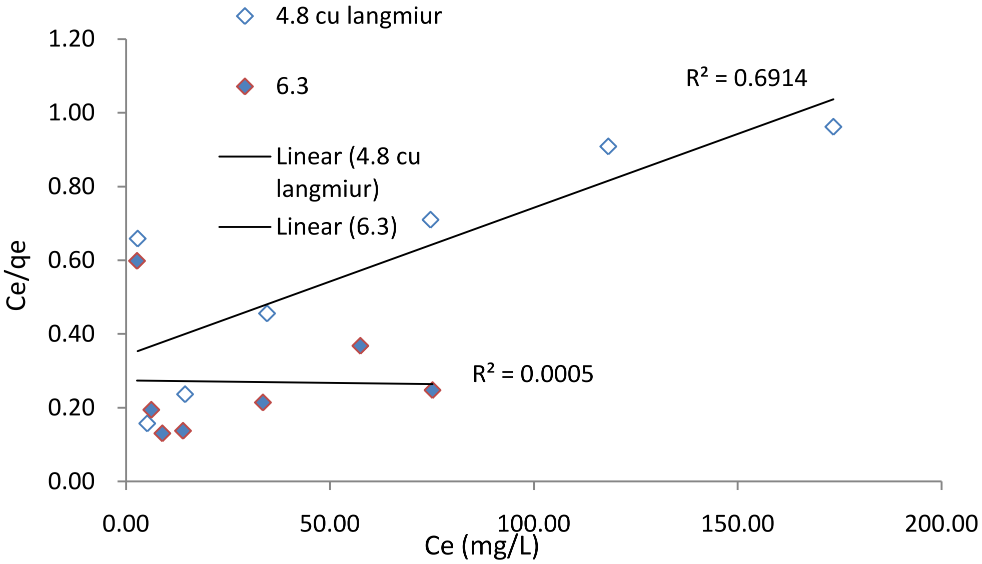

Figure 4.10 The Linear Regression Plot of Langmuir Isotherm Model for Cu (II) Uptake at Two pH Values

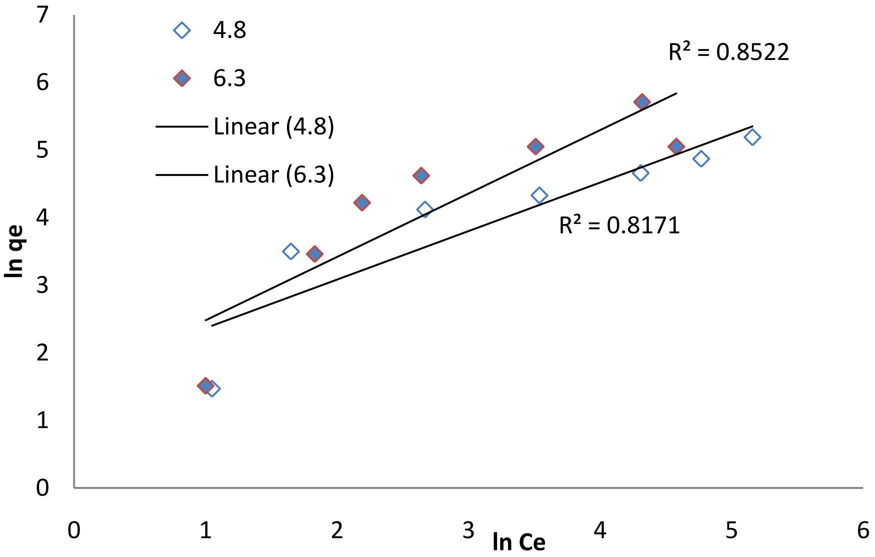

Figure 4.11 The Linear Regression Plot of Freundlich Isotherm Model for Cu (II) Uptake at Two pH Values

Langmuir linear regression plots of Cu (II) in Figure.4.10 did not fit experimental data. However, Freundlich model have a higher R^2 under both pH conditions than Langmuir model on Cu (II) uptake trend fitting in this case.

Table 4.6 Linear regression results for Ag (I) and Cu (II) biosorption Isotherm at pH 4.8 and 6.3

Ion	pH of 4.8		pH of 6.3	
	Langmuir	Freundlich	Langmuir	Freundlich
Ag (I)	$\frac{C_{e,i}}{q_{e,i}} = 0.0129\,C_{e,i} + 0.1986$, R^2 =0.9964	$\ln q_{e,i} = 0.2464 \ln C_{e,i} + 3.3408$, R^2 =0.9377	$\frac{C_{e,i}}{q_{e,i}} = 0.0083 C_{e,i} + 0.1894$, R^2 =0.9929	$\ln q_{e,i} = 0.0705 \ln C_{e,i} + 3.8668$, R^2 =0.8043
Cu (II)	$\frac{C_{e,i}}{q_{e,i}} = 0.004\,C_{e,i} + 0.2421$, R^2 =0.6914	$\ln q_{e,i} = 1.0531 \ln C_{e,i} + 1.2944$, R^2 =0.8522	$\frac{C_{e,i}}{q_{e,i}} = -0.0001 C_{e,i} + 0.274$, R^2 =0.0005	$\ln q_{e,i} = 0.7182 \ln C_{e,i} + 1.6427$, R^2 =0. 8171

Based on R^2 value, Langmuir model is determined to describe Ag (I) uptake trend with initial metal concentration change under pH of 4.8 and 6.3, while Freundlich model is determined for competing ion Cu (II) uptake trend in the same solution.

Table 4.7 Isotherm Parameters for the Biosorption of Ag (I) and Cu (II)

Metal	pH of 4.8				pH of 6.3			
	Langmuir		Freundlich		Langmuir		Freundlich	
	q_{max} mg/g	b	k_f mg/g	n	q_{max} mg/g	b	k_f mg/g	n
Ag^+	77.52	0.065	28.24	4.06	120.48	0.044	47.79	14.18
Cu^{2+}	250	0.012	3.65	0.95	-	-	5.17	1.39

As shown from Figures 4.8-4.11, the Langmuir model fits the experimental data better than the Freundlich model for Ag (I) biosorption, while copper biosorption is better described by the Freundlich isotherm model. The Langmuir model is used to estimate maximum uptake values, where these could not be obtained experimentally. The maximum Ag (I) uptake by pretreated *Fucus spiralis* is around 78 mg/g at pH of 4.8 and is 120 mg/g at pH of 6.3. For Cu (II) biosorption, single component Langmuir isotherm provides poor prediction of the experimental data. Competitive Langmuir isotherm model is more suitable for Cu (II) biosorption under this scenario. The reproducibility of the experiment on duplicated sample data gave a confidence interval of 6.24% on 95% confidence level.

4.4 Conclusion

The pH effect in a single ion system study showed that the sorbent made from *Fucus spiralis* has a higher silver uptake capacity than pretreated *Laminaria Japonica* within the whole range of pH levels. The uptake capacity of Ag (I) by acetone washed *Fucus spiralis* soars from less than 20mg/g at pH 2.0, to about 70 mg/g at a pH 4.0, and then increases slowly with the increasing solution pH.

The kinetic study indicates that selective biosorption of Ag (I) is a fast process. Compared with the pseudo first order equation, the pseudo second order equation describes this procedure much better. This means that this biosorption system can facilitate shorter sorption columns for practical application for better efficiency and economic benefits.

Both Langmuir and Freundlich isotherm models were applied to describe the biosorption of Ag (I) and Cu (II) from a tri-metallic environment. The Langmuir model interprets Ag (I) uptake data very well, while the Freundlich model fits copper uptake data better than Langmuir. Experimental data from isotherm study confirmed that higher solution pH enabled higher metal capacity for both Ag (I) and Cu (II) on the pretreated *Fucus spiralis*.

Chapter 5 Identification of Sorption Mechanism

5.1 Introduction

Mechanisms of the biosorption have been reported to be complicated and could dominate the process with more than two theories simultaneously. Most reported mechanisms can be divided into chemical and physical interactions between metal ions and binding sites present on the surface of the biosorbents, such as metal ion chelation, complextion, microprecipitation, electrostatic attraction, ion exchange, and adsorption. (Das, 2010; Volesky and Holan, 1995; Wang and Chen, 2009).

It is vital to study the mechanisms of silver biosorption in this case to reveal the optimal biosorption processe conditions from aqueous solutions. With well understanding of the dominant biosorption mechanisms, the recovery rate, silver uptake capacity, and desired selectivity can be designed and further improved by controlling key parameters during the processes (Park *et al*., 2010).

According to the literature review, seaweed contains lots of dietary and insoluble fibers, minerals and proteins. Sulfated polysaccharides are rich in marine algae's cell wall of agars. The complex of alga structure provides more functional sites associating with silver uptake.

Studies on heavy metal biosorption mechanism have been reported from open literature, many characteristic technologies have been applied for the biosorption mechanism study, such as XPS, EDS, NMR, FTIR, Raman spectroscopy and XRD (Park *et al*., 2010; Volesky, 2007)

Most of characteristic studies have been conducted on the single metal biosorption and the efforts on silver uptake study are limited. Although some researches focused on the numerical models of the biosorption using natural *Fucus vesiculosus* (Asmathunisha and Kathiresan, 2012; Mata *et al.*, 2009), there is no biosorption study on silver uptake with coexisting copper ions by using pretreated *Fucus spiralis*.

This study aims to investigate the mechanisms of silver biosorption at the presence of competing metal ions from aqueous solution by modified *Fucus spiralis* sorbents. FTIR and Raman spectroscopy was adopt complementally to study the change of functional groups of sorbents and the interaction between them and metal ions. Moreover, XRD was applied to characterize and identify the conformation of metal ions bound to the sorbent surface.

5.2 Materials and Methods

5.2.1 Materials

- Dry *Fucus spiralis* seaweed
- Biosorbents pretreated by NaOH, HNO_3, methanol and acetone
- FSA (*Fucus spiralis* treated by acetone washing) powders after Ag (I) biosorption
- FSA powders after ternary metal (Ag^+, Cu^{2+} and Fe^{3+}) biosorption

5.2.2 Methods

FTIR

FTIR was used to determine the vibration frequency changes in the functional groups in the biosorbents. The spectra were collected by an ALPHA FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) with a diamond ATR accessory (Golden Gate, Specac) within the range of wave-number of 360-4000 cm^{-1}. The image of the ALPHA FTIR spectrometer is illustrated in Figure 5.1. Specimens of various biosorbents were pressed onto sample plates using the attached clamp. 24 scans and 4 cm^{-1} resolution were applied in recording the spectra. Background spectra of a clean plate surface were required prior to each sample measurement under same setting. All the spectra were analyzed by using the OPUS 6.0 software suite (Bruker).

Figure 5.1 ALPHA FTIR Spectrometer

Raman spectroscopy

Raman spectra were obtained by using a confocal LabRAM® Raman spectrometer from Horiba Scientific (Figure 5.2) excited by laser with a resolution of 0.5 cm^{-1} between 4000 and 95 cm^{-1}. For the Raman analysis, biosorbent powders were pressed into a compact pellet to enhance heat transfer and then the pellet was closely adhered to a copper disk with Apiezon grease. A liquid helium-flow cooling system was used to control the sample temperature. In order to avoid the laser heating effect, a low laser power of 3 mW were used with a prolonged accumulation time (2.0s) to ensure enough signal–noise ratio.

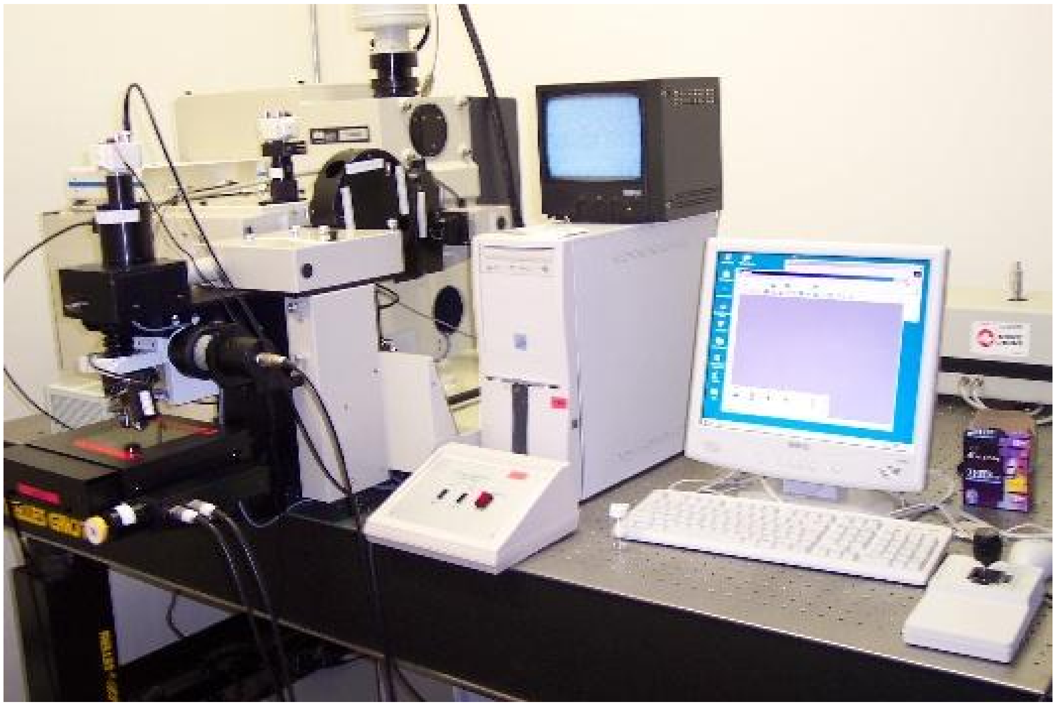

Figure 5.2 Confocal LabRAM® Raman Spectrometer

X-Ray Diffraction

Powder XRD patterns of biosorebnts before and after Ag (I) and Cu (II) uptake were obtained with a Rigaku Ultima IV X-Ray diffractometer (Figure 5.3) using Cu Kα radiation (40kV, 44mA). The sorbent powders were placed on an Al sample holder and scanned from a diffraction angle (2θ) of 30° to 95° with a step width of 0.02° and a counting time of 1.0s for each step. The methods were validated for accuracy and precision. Data was analyzed according to the International Centre for Diffraction Data (ICDD) PDF2 powder diffraction database.

Figure 5.3 Rigaku Ultima IV X-Ray Diffractometer

5.3 Results and Discussion

Due to the complicated structure and compositions of the cell wall of brown algae, the mechanism of Ag (I) sorption on the modified *Fucus spiralis* from monometallic and tri-metallic systems are quite complex. Here we only presented some of the available results which might be useful in partially manifesting such complex sorption mechanism.

5.3.1 FTIR Analysis for Chemical Modification of Brown Seaweeds

Different chemical reagents have been applied in chapter 3 to find the optimal chemical modification conditions of brown seaweeds so that modified sorbent with both high capacity and good selectivity of Ag (I) can be obtained. To further study the effect of functional groups on Ag (I) biosorption by modified brown seaweeds, FTIR was applied to analyze the effect of chemical washing on the sorbent performance. Figures 5.4-5.7 show the FTIR spectra of dry *Fucus spiralis* and its modifications by acid, alkaline and organic solvent washing.

The major peaks corresponding to various functional groups of seaweed *Fucus spiralis* are numbered from 1 to 6 absorption bands in Table 5.1 within the wavelength range of 400 cm^{-1} to $4000cm^{-1}$. All the major peaks had absorption band shifts after four different treatments. Sorbents (d and e) treated by organic solvents showed band shift different from those (b and c) treated by acid and alkaline. The bands at 1608.3 cm^{-1} and 1415.58 cm^{-1} represent carbonyl and carboxyl groups. Significant band shifts for sorbent A were observed at banks 1608.3 cm^{-1} and 1415.58 cm^{-1}. Major band shift for sorbent B took place at 1216.10 cm^{-1}, corresponding to the C-N group. From the experimental results in Chapter 3, *Fucus spiralis* treated by acid and alkaline washing have higher Cu (II) uptake and relatively lower Ag (I) uptake. From the FTIR analysis, pretreatment of acid and alkaline washing make the sorbent to bind more copper ions through amid and carboxyl groups.

For sorbents D and E, bands corresponding to C=O and C-N groups shifted noticeably. Moreover, the bands at 2855.52, 1755.33 cm^{-1} for sorbent E disappeared, probably suggesting the treatment with acetone might change the characteristics of free CH, C=O, or amid groups. These bands also have been found responsible for metal binding in many heavy metal biosorption studies (Bai *et al*., 2002; Li *et al*., 2008; Zhou *et al*., 2005), which benefited to Cu (II) binding. In this study, we observed that FSA sorbent shows slight preference to Ag (I) sorption.

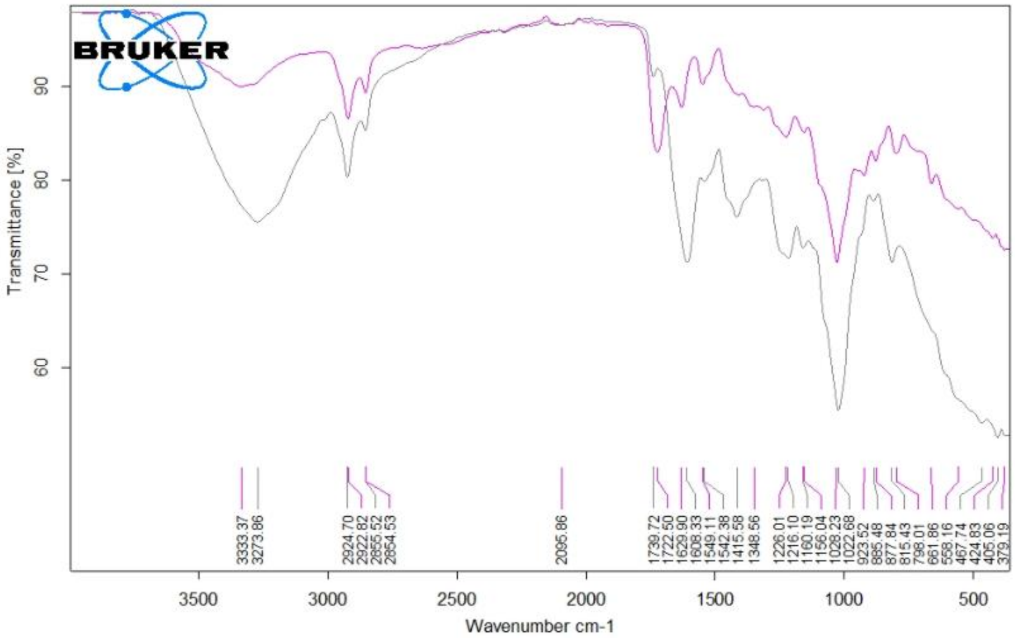

Figure 5.4 FTIR Spectra of *Fucus spiralis* and Its Modification by Acid Washing (Up curve: *Fucus spiralis* after HNO_3 washing; Low curve: dry *Fucus spiralis*)

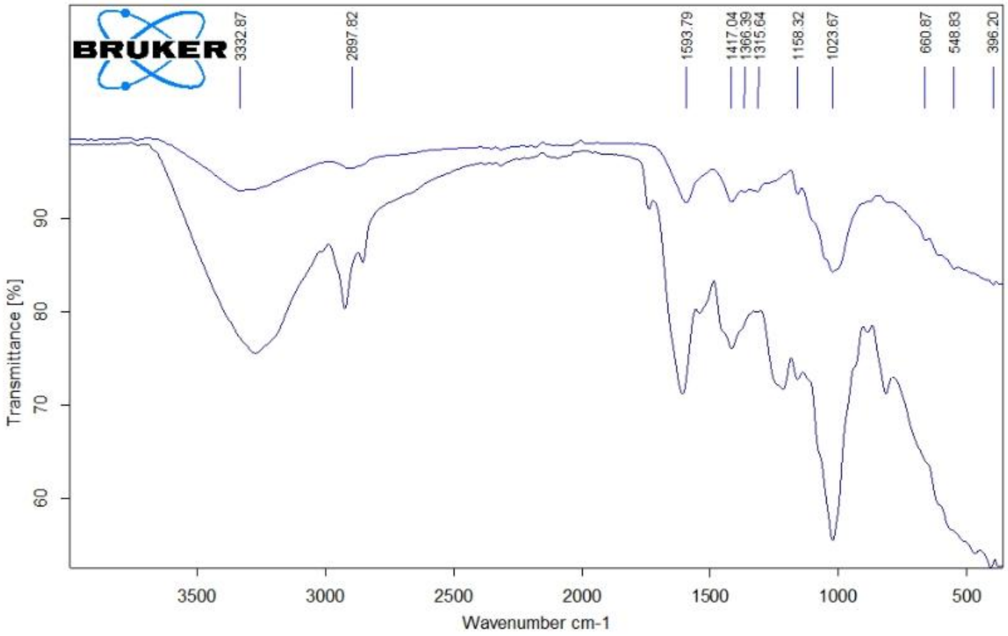

Figure 5.5 FTIR Spectra of *Fucus spiralis* and Its Modification by Alkaline Washing (Up curve: *Fucus spiralis* after NaOH solution washing; Low curve: dry *Fucus spiralis*)

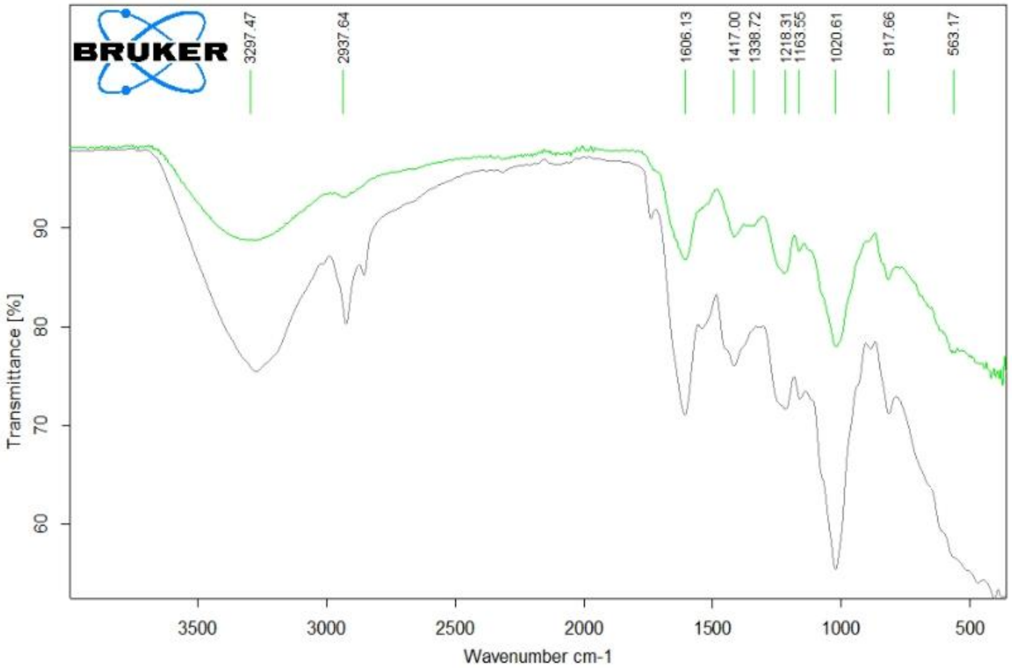

Figure 5.6 FTIR Spectra of *Fucus spiralis* and Its Modification by Methanol Washing (Up curve: *Fucus spiralis* after Methanol washing; Low curve: dry *Fucus spiralis*)

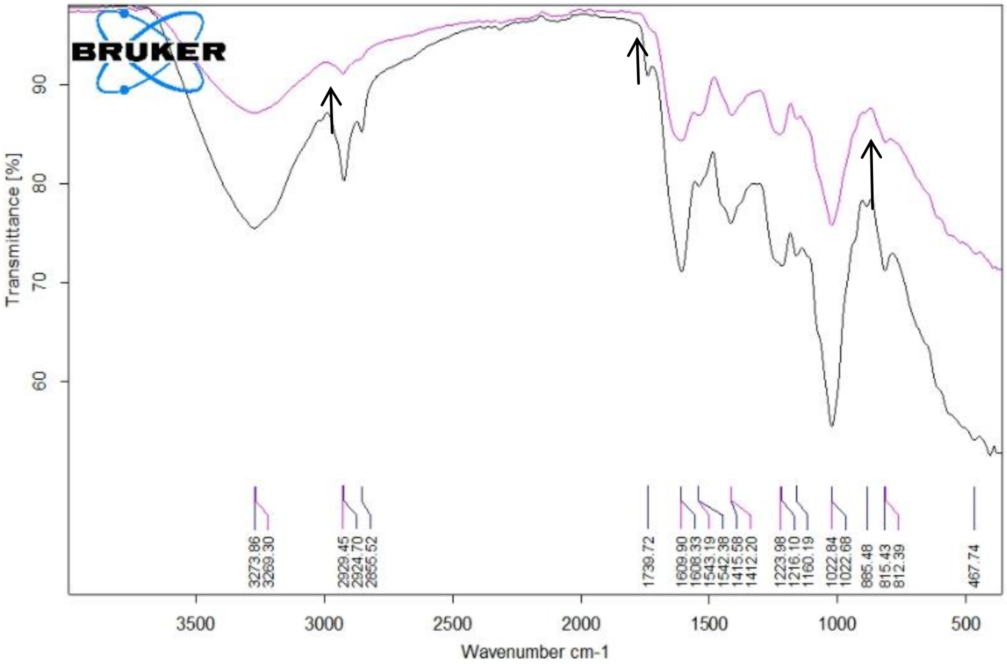

Figure 5.7 FTIR Spectra of *Fucus spiralis* and Its Modification by Acetone Washing (Up curve: *Fucus spiralis* after acetone washing; Low curve: dry *Fucus spiralis*)

Table 5.1 Specific FTIR Spectral Bands from FTIR Results

Spectral bands	Fucus s., A, cm^{-1}	HNO_3, B cm^{-1}	NaOH, C cm^{-1}	Methanol, D cm^{-1}	Acetone, E cm^{-1}
1γO-H/ N-H	3273.96	3333.37	3333.32	3297.43	3274.02
2 γC-H	2924.70	2922.82	2898.08	2937.64	2930.36
3 γHOC=O	1608.33	1629.90	1593.65	1606.13	1603.22
4 γOC-OH	1415.58	Gone	1417.16	1417.00	1412.57
5 γC-N	1216.10	1226.01	Gone	1218.31	1224.70
6 γC-OH	1022.68	1028.23	1023.34	1020.61	1021.59
Additional bands shifted		1722.50, 1549.11, 800-1260 appear	1315.58 appear		2855.52, 1755.33 (gone)

5.3.2 Sorption Mechanism of Ag (I) from Monometallic and Tri-metallic Systems

- **FTIR Analysis**

Figure 5.8 shows the FTIR spectra of the virgin and Ag (I) bound FSA sorbents. The increase in intensity as well as broadening of the band at 1224.7 cm^{-1} indicates changes in the C-N group during the Ag (I) sorption. Carbonyl and carboxyl groups exhibit bands at 1608.3 cm^{-1} and 1417.6 cm^{-1} for the virgin FSA. Both bands observed shift to different extents after the Ag (I) biosorption to 1603.2 and 1412.6 cm^{-1}. This shift can be explained by the associations of the carbonyl/carboxyl groups with metal ions. In addition, the band changes at 813.05 cm^{-1}, 521.44 cm^{-1} and 421.11 cm^{-1} suggests that the potential silver binding through –SO_3 or C-N-C groups.

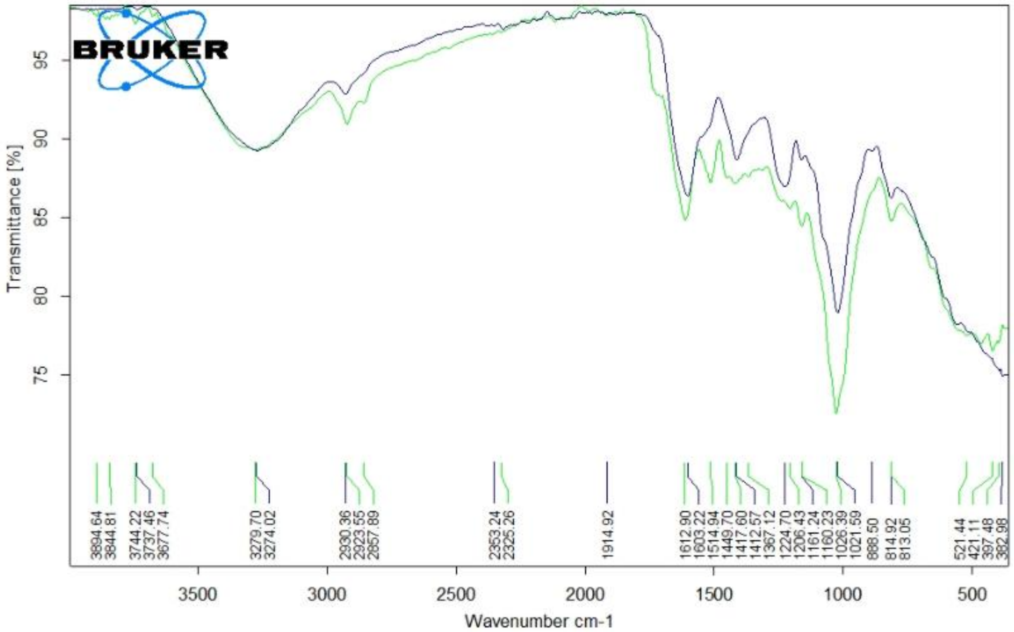

Figure 5.8 FTIR Spectra on FSA Before and After Ag (I) Biosorption
(Up curve: FSA; Low curve: FSA after Ag (I) uptake)

Besides of involving in metal binding, carboxyl group, carboxylate, amid and other groups might influence the pH of the medium where metal biosorption happened (Ashkenazy, 1997), which further affects the sorbent capacity and selectivity of metal ions.

- **Raman Spectroscopy Analysis**

Raman spectra of FSA before and after metal-ion sorption are shown in Figure5.9. Compared with Raman spectrum of dry FSA (spectrum 1), noticeable changes in the fundamental modes of FSA associated with metal ions can be observed in three frequency ranges, 300- 800 cm^{-1}, 1400-2600 cm^{-1} and 2900-3800 cm^{-1}.

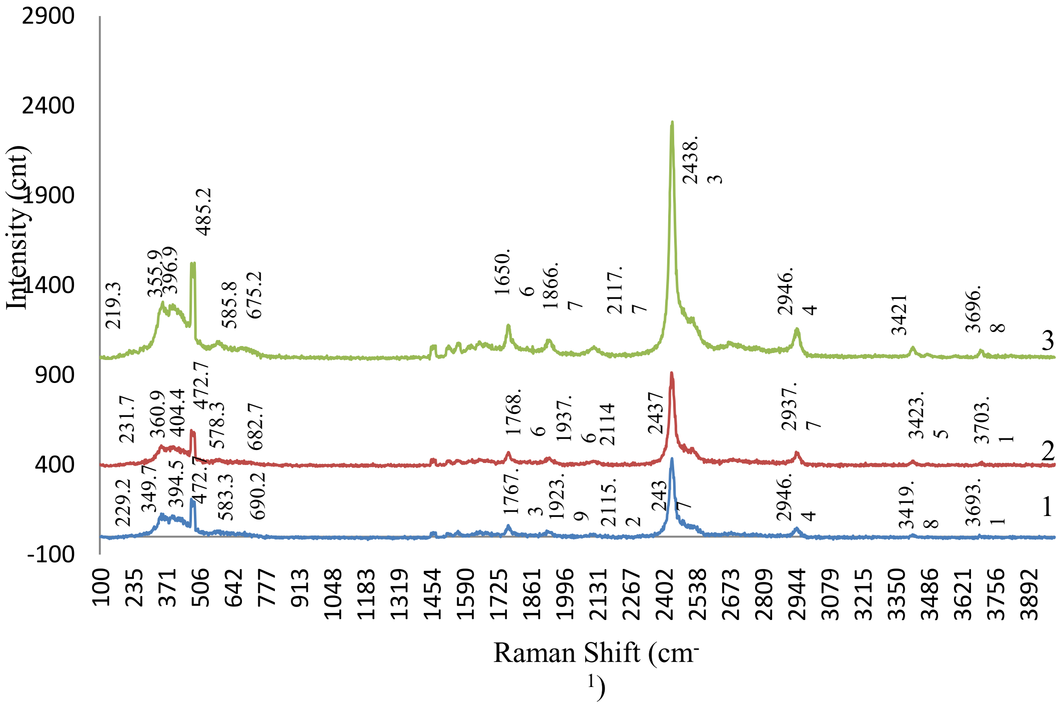

Figure 5.9 Raman Spectra of Three Sorbent Samples

(Curve 1: FSA; 2: FSA after Ag (I) sorption; 3: FSA after Ag (I) and Cu (II) sorption)

Major changes of Raman spectrum of FSA associated with Ag (I) include: (1) an increase of the O-H stretch mode intensity at 3693 cm^{-1}; (2) peak shift observed at wave number 349.7 cm^{-1} and 394.5 cm^{-1} before metal binding to 360.9 cm^{-1} and 404.4 cm^{-1} after silver uptake implies that new binding group of metal-O has been formed in the biosorbents after silver uptake.

By comparison the spectrum of FSA after Ag (I) sorption (Spectrum 2), the effect of copper ions competing with silver ion for the sorption sites can be seen from spectrum 3. After tri-metallic biosorption, intensities of the major bands on Spectrum 3 increased significantly. Most of them laid on the frequency ranges of bands like -S, C-C, C=C-, O-metal, C-N, -OH, C-H, halogen (-I, -Cl, -Br), alkenes, and aromatic rings. Compared with spectra of FSA and FSA after monometallic sorption, the peak shift on FSA after tri-metallic sorption shows that the existence of competitive ions did affect the binding of Ag (I) on the sorbent surface.

- **XRD Analysis**

The XRD patterns of FSA after Ag (I) sorption and ternary metal sorption were illustrated in Figure 5.10 and Figure 5.11, respectively. Binding of Ag (I) on the surface of FSA has been proved by the presence of AgCl crystals on the sorbent XRD pattern, suggesting that Ag (I) was first adsorbed on the sorbent and then precipitated in the form of AgCl on the sorption sites. Weak peaks of elemental silver were also present in the XRD pattern shown in Figure 5.10, which indicates that minor silver reduction might take place during Ag (I) biosorption. XRD pattern of FSA after tri-metallic (Ag (I), Cu (II) and Fe(III)) sorption didn't show much difference to that of FSA after Ag (I) sorption. And no evidence of copper ions and iron precipitation could be found on the FSA sorbent surface.

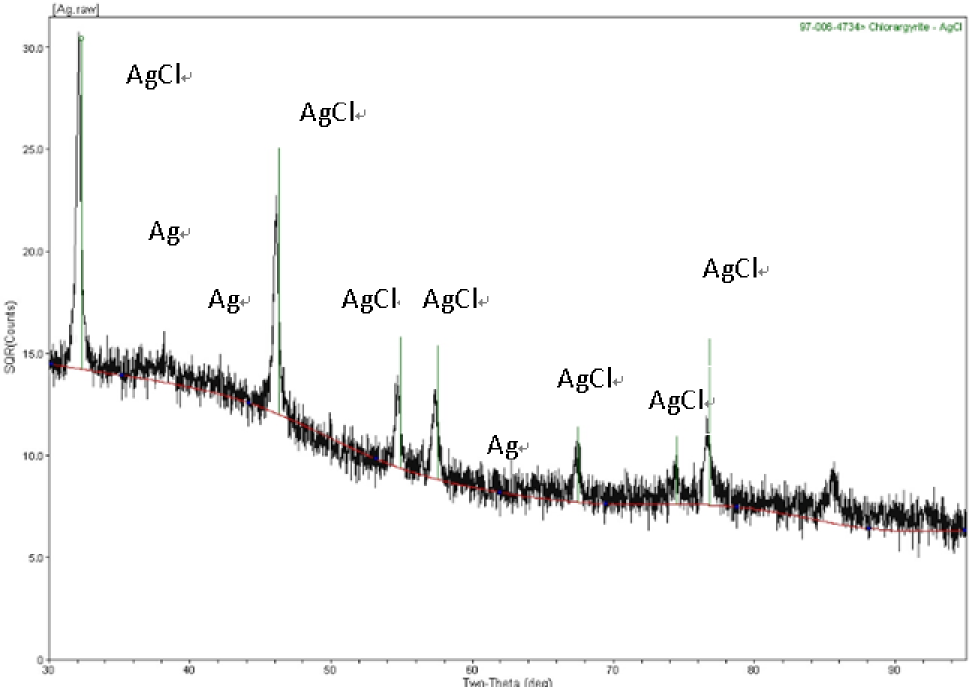

Figure 5.10 XRD Pattern of FSA after Ag (I) Biosorption

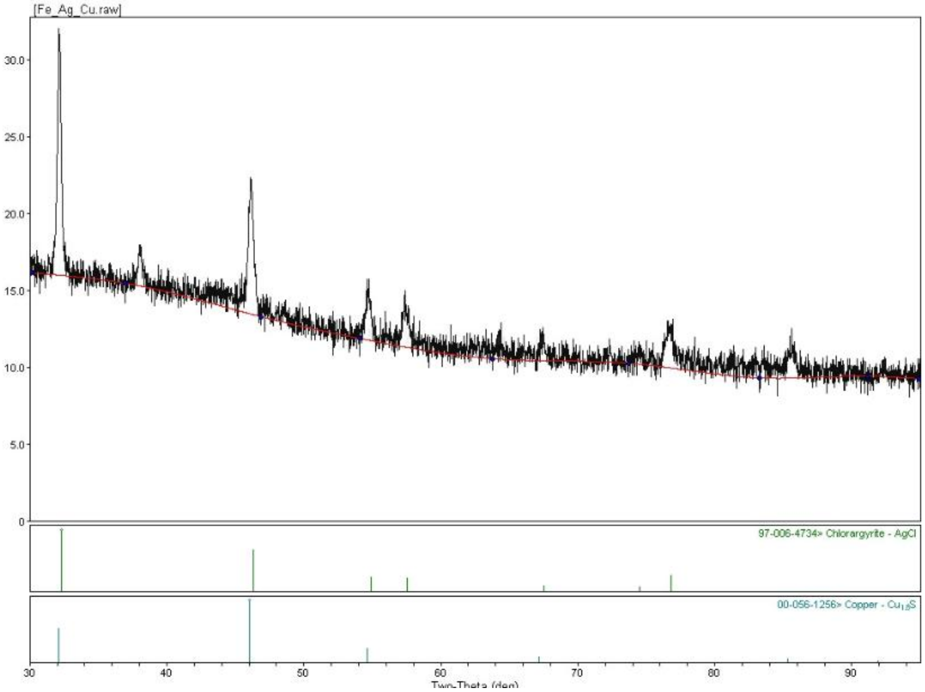

Figure 5.11 XRD Pattern on FSA Solids After Ternary Biosorption

5.3.3 Effect of Fe (III) on the Selective Sorption of Ag (I)

The presence of Fe (III) plays an important role in the selective sorption of Ag (I) from multi-metal environment. From the experimental results of bi-metallic system (Ag^+ and Cu^{2+}) and tri-metallic system (Ag^+, Cu^{2+} and Fe^{3+}) biosorption, FSA sorbent showed higher selectivity of Cu (II) for binary-metal sorption, while the same sorbent showed a relatively high selectivity of Ag (I) with the presence of Fe(III) in the solution mixture. Previous research on the heavy metal biosorption proved that *Fucus spiralis* is very selective in the sorption of copper versus other heavy metals (González *et al*., 2011). The relatively high selectivity of this biomass to Ag (I) observed from tri-metallic system in this study might be explained from the hard-soft acid-base (HSAB) theory (Pearson, 1968). According to the Pearson's concept of HSAB theory, Fe(III) is a hard acid, Ag (I) is a soft acid while Cu (II) is a borderline acid. In the HSAB theory, hard ions tend to bind well together and soft ions tend to bind well together. This helps to explain that Fe(III) competes with Cu (II) on the binding sites (hard base) of sorbent while Fe(III) doesn't affect the binding of Ag (I) on the sorption sites too much, which leads to a lower uptake of Cu (II) on the sorbent surface and an increase in the selectivity of Ag (I).

Strong base groups on the sorbent surface reacts with Fe(III) very fast and ion precipitate can be observed during the biosorption of tri-metallic system in this study. The formation of iron precipitate has been confirmed from the results of ICP-MS analysis of the filtered residual solution and the XRD analysis of the sorbent particles. ICP-MS results show that over 95% of the Fe(III) has been removed from solution; while no evidence of iron compound bound to FSA sorbent has been detected by X-Ray Diffractometer.

5.4 Conclusion

Raman spectroscopy suggests the copper and silver ions all have interactions with most of functional groups on the surface of sorbents, and copper binding indeed competes with silver binding on functional groups, such as -S, C-C, C=C-, O-metal, C-N, -OH, C-H, halogen, alkenes, and aromatic rings.

FTIR analysis suggests silver ions were sequestered on more binding sites on *Fucus spiralis* after pretreatment by alkaline, but copper uptake was greater than silver uptake in this competing biosorption. Pretreatment with acetone gave brown seaweed *Fucus spiralis* greater silver uptake capacity than copper uptake in the binary metal biosorption. Free CH, C=O, or amid groups may have more binding affinity with silver ions.

XRD results provide a strong evidence of the presence of AgCl precipitation on the sorbent, but no crystalline form of copper ions. It suggests some part of silver ions was captured in the form of precipitation, but there is no strong proof of reduction of Ag (I) happened during biosorption.

The residual chloride on the surface and inner the sorbents play very important role on microprecipitation of AgCl in this process. Otherwise, bands appearing after silver biosorption in Figure 5. 9 suggested there might be some halogen bands on the surface of sorbents releasing to act with silver ions during the biosorption. More characterizations are needed for confirmed mechanism such as NMR, EDS, or SEM on sorbents and those solid after biosorption.

Chapter 6 Conclusion and Recommendations

6. 1 Summery of Study

This study investigates biosorption behavior on silver uptake by using pretreated seaweed. By applying design of experiment method and statistic analyses, proper seaweed type and optimal sorbent preparation process were screened out for later tests with aims to gain maximum silver ion uptake capacity when competing ions exist in the solution. Kinetic and Isotherm studies on prepared sorbents described the metal ion uptake capacities change under varied time and conditions (pH and initial metal ion concentrations) with determined mathematic models. By comparing sorbents before and after biosorption, several characteristic analysis technologies have been conducted to reveal dominant biosorption mechanisms.

6.2 Research Achievement

Silver biosorption by brown algae is a green, economical and environment friendly approach to recover silver from industrial wastes. The maximal silver uptake by pretreated *Fucus spiralis* with acetone is around 78 mg/g at pH of 4.8 and is 120 mg/g at pH of 6.3, in a competing environment with copper ions. It is a very promising result compared with other reported sorbents in silver biosorption.

In the monometallic system, factors of seaweed species and treatment are the two significant factors for sorbent preparation design on purpose of the optimal silver uptake capacity. *Fucus spiralis* treated by NaOH has a maximum silver uptake capacity around

82 mg/g of biosorbent at pH of 4.8. In binary metallic solution, five methods applied to two species of *Fucus* algae have been studied in a binary metallic solution of Cu (II) and Ag (I). Based on ANOVA result, the factor of treatment is the only significant factor. For silver selectivity test, the optimal method is acetone treatment on S (*Fucus spiralis*) with particle size of 250-500μm in 3-hour modification. That biosorbent has metal uptake capacity of copper at 23.04 mg/ g and silver at 52.59 mg/ g. In monometallic solutions, sorbents made from *Fucus spiralis* has a higher silver uptake capacity than others. The silver uptake capacity is 70 mg/g at pH of 4, keeping climbing up along with increasing pH value.

The Kinetic study shows silver biosorption on seaweed based sorbent is a fast process, which can be described well by the Pseudo second order. This means that this biosorption system can facilitate shorter sorption columns for practical application for better efficiency and economic benefits.

Freundlich model fits copper uptake data during the binary Ag-Cu biosorption system study and Langmuir model has a better interpreting on silver uptake data. Pretreated *Fucus spiralis* by acetone shows a preference of silver biosorption than copper uptake in weight unit, from initial metal concentration range of 0.1- 1 mmol/L. Biosorption performance was compared at two different pH values. Higher pH environment enables higher metal capacity, for both of copper and silver ions.

In characterization studies, Raman spectroscopy result suggests the most of functional groups on the surface of biosorbents would involve in both of the copper and silver biosorption, and spectra after copper biosorption shows more intense results than silver uptake. Functional groups, such as -S, C-C, C=C-, O-metal, C-N, -OH, C-H, halogen, alkenes, and aromatic rings are the main concerned binding sites.

FTIR analysis suggests silver ions are sequestered on more binding sites on *Fucus spiralis* after pretreatment by alkaline in monometallic environment, while copper uptake is greater than silver uptake in this base pretreatment. Pretreatment with acetone enables brown seaweed *Fucus spiralis* greater silver uptake capacity than copper uptake in the binary metal biosorption in certain initial concentration. Free CH, C=O, or amid groups may attribute to the binding affinity with Ag (I).

A strong evidence of AgCl precipitation was found on the sorbent by XRD tests, but no crystalline form of copper ions. It suggests some part of silver ions was captured in the form of precipitation. For copper uptake, main mechanisms would be ion exchange or metal binding with functional groups. More characterizations, such as NMR, EDS, or SEM, are needed to confirm silver biosorption mechanism in bi-metallic system.

6. 3 Recommended Future Works

In this study, only batch sorption test was conducted for all the study of the project. For further study, the continuous column test is essential scale for numerical study in biosorption application. A laboratorial column study needs to be designed and planned according to other related literature records. During this continuous study, CCD or BBD method in DOE is recommended for experiment design. For sorbent preparation, an immobilization on biosorbent is a necessary process to maintain some physical properties in practical operation.

Due to the limitation of time, the simulation in batch study has been simplified. The more modified mathematical models should be applied in the kinetic and isotherm study in continuous biosorption study to simulate the real life application with all kinds of factors.

Last but not least, more oceanic species and treatment methods could be considered in sorbent screening on silver uptake capacity test. More complicated ion competing situation could be considered for practical scale-up. More analytical techniques are needed to explore the sorption mechanism of Ag (I) under competitive sorption environment.

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Appendix

Table3.8 Copper and Silver Uptake Results of Modified Sorbents
(Selective Sorption of Ag^+)

Sample	Seaweed type	Treatment	q_{Cu} (mg/g)	q_{Ag} (mg/g)
1	S	85ºC NaOH	25.42	22.92
2	V	85ºC NaOH	26.21	25.62
3	S	NaOH	27.80	26.97
4	V	NaOH	28.60	25.62
5	S	HNO_3	10.33	18.88
6	V	HNO_3	13.50	17.53
7	S	Methanol	23.83	40.45
8	V	Methanol	26.21	37.75
9	S	Acetone	23.04	52.59
10	V	Acetone	24.63	44.50

Table 4.3 Experimental Data of Ag (I) and Cu (II) Biosorption

pH: 4.8

Ci(mg/l)	Ce_{Cu} (mg/l)	Ce_{Ag}(mg/l)	q_{eCu}(mg/g)	q_{eAg}(mg/g)	$Ad_{\% Cu}$	$Ad_{\% Ag}$
0.1	2.87	8.24	4.36	3.18	54.84%	23.61%
0.5	5.22	8.29	33.19	57.06	83.57%	84.63%
1	14.5	57.1	61.31	63.46	77.18%	47.07%
1.5	34.6	113.5	75.91	60.38	63.70%	29.85%
2.5	74.7	211.8	105.22	72.34	52.98%	21.46%
3.5	118.3	319.9	130.16	72.06	46.81%	15.27%
5	173.5	478.4	180.31	76.19	45.40%	11.30%

pH: 6.3

Ci(mg/l)	Ce_{Cu} (mg/l)	Ce_{Ag}(mg/l)	q_{eCu}(mg/g)	q_{eAg}(mg/g)	$Ad_{\% Cu}$	$Ad_{\% Ag}$
0.1	2.72	8.47	4.54	2.90	57.20%	21.48%
0.5	6.22	13.7	31.94	50.29	80.42%	74.60%
1	8.93	47.5	68.28	75.46	85.95%	55.97%
1.5	14.0	88.5	101.66	91.63	85.31%	45.30%
2.5	33.6	186.6	156.59	103.84	78.85%	30.81%
3.5	57.5	279.7	206.16	122.31	74.15%	25.92%
5	75.2	449.7	303.19	112.06	76.33%	16.62%

Table 4.4 Transformed Experimental Data of Biosorption Isotherm of Ag (I)

pH of 4.8					pH of 6.3				
	Langmuir		Freundlich			Langmuir		Freundlich	
c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$	c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$
8.29	57.06	0.15	4.04	2.12	13.7	50.29	0.27	3.92	2.62
57.1	63.46	0.9	4.15	4.04	47.5	75.46	0.63	4.32	3.86
113.5	60.38	1.88	4.1	4.73	88.5	91.63	0.97	4.52	4.48
211.8	72.34	2.93	4.28	5.36	186.6	103.84	1.8	4.64	5.23
319.9	72.06	4.44	4.28	5.77	279.7	122.31	2.29	4.81	5.63
478.4	76.19	6.28	4.33	6.17	449.7	112.06	4.01	4.72	6.11

Table 4.5 Transformed Experimental Data of Biosorption Isotherm of Cu (II)

pH of 4.8					pH of 6.3				
	Langmuir		Freundlich			Langmuir		Freundlich	
c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$	c_e mg/l	q_e mg/g	c_e/q_e	$\ln q_e$	$\ln c_e$
2.87	4.36	0.66	1.47	1.05	2.72	4.54	0.6	1.51	1
5.22	33.19	0.16	3.5	1.65	6.22	31.94	0.19	3.46	1.83
14.5	61.31	0.24	4.12	2.67	8.93	68.28	0.13	4.22	2.19
34.6	75.91	0.46	4.33	3.54	14	101.66	0.14	4.62	2.64
74.7	105.22	0.71	4.66	4.31	33.6	156.59	0.21	5.05	3.51
118.3	130.16	0.91	4.87	4.77	97.5	156.16	0.62	5.05	4.58
173.5	180.31	0.96	5.19	5.16	75.2	303.19	0.25	5.71	4.32