Heavy Metals in Wastewater: Their Removal through Algae Adsorption and Their
Roles in Microwave Assisted Pyrolysis of Algae

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Last but not least, I would like to thank my parents, who always give me unfailing support and encouragement.

Dedication

This thesis/project is dedicated to my parents Chi Zhao and Ran Ren.

Abstract

Chlorella vulgaris was found as a good biosorbent for copper, zinc and aluminum. pH value, reaction time, initial metal and algal sorbents concentrations were considered as parameters affecting metal removal efficiency. In appropriate conditions, 85% of copper(II), 70% of zinc(II) and 99% of aluminum(III) could be removed from solutions by tested microalgae within 20 minutes. In following pyrolysis of the algae, metals were further concentrated in the charcoal. 96.17% of Copper and 97.34% of Zinc stayed in the char portion. Metals in the algal feedstock improved the bioenergy production during microwave assisted pyrolysis of the algae by reducing heating time to about half of before. The presence of metals also significantly decreased the nitrogen containing compounds and the carbon dioxide output and increased the aromatics generation.

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Chapter 1 Introduction

1.1 Background

Heavy metal contamination from industrial wastewater is a disturbing problem. Heavy metals have direct physical, chemical, and neurological impacts on humans, their bioaccumulation also brings some chronic issues (Mehta and Gaur, 2005). Conventional treatments including ion-exchange, coagulation, adsorption, membrane separation, ultrafiltration, were limited by their disadvantages, such as inefficiency, high cost and high energy needed, especially at very low concentrations of heavy metal (Davis et al., 2003). As a relatively new technology, bio-sorption could overcome some of the limitations of conventional technologies (Ahluwalia and Goyal, 2007). Many researchers tested various low-cost biomass as bio-sorbents for heavy metal. This research provides another way of thinking: combining the bio-sorption with other technology to create more valuable bio-products.

There is a growing demand for energy. It is essential to find an alternative source to conventional fossil fuels to meet the growing consumption of energy. For long-term consideration, renewable energy will probably replace fossil fuel, and bio-energy is remarkable since the derivative products have similar performance and properties with petroleum oil, natural gas or coal.

Microalgae have great potential to produce bio-energy (Brennan & Owende, 2010). They are also great bio-sorbents for heavy metals. In this research, we took *Chlorella vulgaris* to adsorb metal ions, then to produce bio-energy by microwave assisted

pyrolysis. Besides being able to recover most metal and produce bio-oil efficiently by the tested microalgae, we also expect the adsorbed metals can be further concentrated by bio-oil production and work as catalysts to improve the oil production.

1.2 Hypothesis

In this project, it was hypothesized that

- *Chlorella vulgaris* had capability to bind metal ions in the industrial wastewater, especially when metals are in relatively low concentration (up to 10mg/L);

- The adsorbed metal could be further concentrated in the charcoal by microwave assisted pyrolysis of the algae;

- It was also hypothesized that presence of metals can improve bio-oil production in both quantity and quality.

1.3 Objectives

The objectives of this thesis project were:

- To determine the optimized conditions for copper, zinc and aluminum uptake by *Chlorella vulgaris*;

- To further concentrate metal ions from algal sorbents by microwave-assisted pyrolysis;

- To determine if the adsorbed metals can improve the bio-oil production during microwave-assisted pyrolysis.

Chapter 2 Literature review

2.1 Heavy metal issues

There are various harmful metal ions in industrial waste water, most of which are heavy metals such as mercury, cadmium, lead, copper, zinc, chromium, etc. Discharge without treatment causes serious problems such as environmental pollution, threatening plants, animals and even human health. Many harmful metals can accumulate in living beings and cause long-term issues. Present methods for removal or recovery of metal ions in industrial waste water include ion-exchange, electrolysis, coagulation, adsorption, chelation, membrane separation, ultrafiltration, etc. However, these methods have some disadvantages, such as ineffectiveness, high cost of equipment or chemicals, high energy required, generation of other toxic waste, etc. Besides, they are exceptionally expensive and very inefficient when the concentration of heavy metal in the waste water is very low (10 - 100mg/L). (Volesky, 2001)

Ion exchange is a stoichiometrical reaction in an electrolyte solution where some exchangeable cations or anions are exchanged for other ions of the same sign. Ion exchange is a reversible process and the ion exchanger can be regenerated or loaded with desirable ions by washing with an excess of these ions. Ion exchangers used for removal of metal ions as cation exchangers or amphoteric exchangers, include ion exchange resins, zeolites, montmorillonites, clay, and soil humus. Some ion exchangers are selective, and very ineffective for some ions (Helfferich, 1962). Besides, ion exchange is relatively expensive compared to other methods (Ahluwalia and Goyal, 2007).

Electrolysis needs few chemicals, but it is extremely expensive in large scale treatment and in low concentration of metals. In this process, large amount of sludge is generated and treatment of the sludge in the following processes is required. (Vanýsek, 2007)

Coagulation and precipitation are very common processes for the removal of harmful metal ions. But they are costly due to the large amount of required chemicals, which leads to the generation of a high water content sludge, the disposal of which is cost intensive (Gary, 1999). Furthermore, they are very ineffective when metal ions are in very low concentrations in the effluents.

Ultrafiltration is a kind of membrane filtration (Hank and Wyckoff, 2010). Since metal ions are small molecules, membrane technology is usually jointed with other methods, such as coagulation and chelation. Chelation is using large molecular weight chelating agents, such as proteins and polysaccharides, to capture metal ions in the waste water. The chelating agents, or chelants, are polydentate ligands for metal ions. But a large amount of chelants are needed since many chelation reactions are irreversible. Though chelation has great performance in lab scale experiments, large scale treatment of chelation for metal ions is very costly.

Throughout the above chemical or physical methods of metal ions removal, they have more or less disadvantages related to cost or effectiveness. Researchers cast their hope on biological methods. Using biomass like microalgae as bio-sorbents provides an

effective pathway to break through some of the limitations. Following the adsorption process, some biomass can also provide valuable products, such as biodiesel.

However, few reports cover post-treatments of biosorption of heavy metals. Some researchers tried to regenerate or reuse biosorbents by acidic eluents to desorb metals. For instance, Roy, Greenlaw, and Shane (1993) found about 90% desorption of Pb and Zn with *Chlorella minutissima* by lowering the pH of the suspension to 1.55. However, the biosorbents were damaged and inactivated at low pH and future adsorption capacity significantly declined. In this project, desorption of metals and regeneration of biosorbents were not considered. Microalgae adsorbed metals were pyrolyzed to biofuels and charcoal, which could be burned for energy recovery. The adsorbed metals would be further concentrated in the solid residues. This process significantly reduces the use of eluents.

2.2 Targeted metals

In this research, the targeted metal ions were copper(II), zinc(II) and aluminum(III). Copper(II) ions commonly exist in metallurgical industry waste water and mining industrial effluents. In sufficient amounts, they are poisonous to higher organisms; at lower concentrations it is an essential trace nutrient for all higher plant and animal life. The U.S. EPA's Maximum Contaminate Level (MCL) in drinking water is 1.3mg/L. Free cupric ions generate reactive oxygen species such as superoxide, hydrogen peroxide, the hydroxyl radical. These damage proteins, lipids and DNA. (Li, Trush and Yager, 1994) Acute ingestion of copper(II) ions causes vomiting, vomiting of blood,

low blood pressure, coma, gastrointestinal distress, etc. Chronic copper toxicity does not normally occur in humans because of transport systems that regulate absorption and excretion. However, at a high enough level, chronic overexposure to copper can damage the liver and kidneys; and autosomal recessive mutations in copper transport proteins can disable the transport systems, leading to Wilson's disease with copper accumulation and cirrhosis of the liver in persons who have inherited two defective genes. (NHDES, 2005; Brewer, 2010)

Since being smelted with copper for alloys like brass and bronze, zinc is also present in metallurgical industry waste water. Even though zinc is an essential element for a healthy body, excess zinc can be harmful. The USDA Recommended Dietary Allowance is 11 and 8 mg Zn per day for men and women, respectively. Excessive absorption of zinc can suppress copper and iron absorption, or adversely affect cholesterol. The free zinc ion is a powerful Lewis acid up to the point of being corrosive. Copper and Zinc are known to bind to amyloid beta proteins in Alzheimer's disease, which is thought to mediate the production of reactive oxygen species in the brain.

Aluminum is the most abundant metal in the Earth's crust. It's found in over 270 different minerals. It's hard to avoid aluminum in most metals and mining industry. Even though aluminum is almost non-toxic, some toxicity can be traced to deposition in bone and the central nervous system, which is particularly increased in patients with reduced renal function. Because aluminum competes with calcium for absorption, increased amounts of dietary aluminum may contribute to the reduced skeletal

mineralization observed in preterm infants and infants with growth retardation. Some researchers have expressed concerns that the aluminum in antiperspirants may increase the risk of breast cancer (Exley et al., 2007), and aluminum has controversially been implicated as a factor in Alzheimer's disease (Ferreira et al., 2008).

2.3 Microalgae as biosorbents

Many different species of algae were reported as effective biosorbents for metal ions, reported algal sorbents include *Scenedesmus obliquus* (Donmez et al., 1999), *Pilayella littoralis* (Carrilho & Gilbert, 2000), *Spirulina sp.* (Chojnacka et al., 2004), *Sargassum sp.* (Cruz et al., 2004), *Chlorella vulgaris* (Mehta and Gaur, 2001), *Nannochloropsis oculata* (Zhou et al., 1998), *Palmaria palmate* (Prasher et al., 2004), *Laminaria japonica* (Sandau et al., 1996), *Ecklonia radiate* (Matheickal and Yu, 1996), *Microcystis sp.* (Singh, Pradhan, and Rai, 1998), *Gracilaria fisheri* (Chaisuksant, 2003), *Oscillatoria angustissima* (Mohapatra and Gupta, 2005), *Ascophyllum nodosum* (De Carvalho, Chong, and Volesky, 1995), *Durvillaea potatorum* (Yu and Kaewsarn, 1999), *Lyngbya taylorii* (Klimmek et al., 2001), etc. In this project, *Chlorella vulgaris* was applied to be the biosorbent to metals.

Chlorella vulgaris is a genus of single-cell green algae. It is spherical in shape, about 2 to 10 μm in diameter, and is without flagella. *Chlorella* was selected since it multiplies rapidly, requiring only carbon dioxide, water, light, and a small amount of minerals to reproduce. The photosynthetic efficiency of *Chlorella vulgaris* can reach 8% (Zelitch, 1971).

Table 2.1 Removal efficiency of metal ion uptake by *Chlorella vulgaris*			
Metal ions	q_{max}*(mmol g−1)	Metal ions	q_{max} (mmol g−1)
Ni	0.205~1.017	Cd	0.30
Pb	0.47	Au	0.13
Zn	0.37	Fe(III)	0.439
Cr(VI)	0.534~1.525	Cu	0.254~0.758

q_{max}*: maximum removal efficiency of metal ion uptake

2.4 Mechanisms of algae adsorption
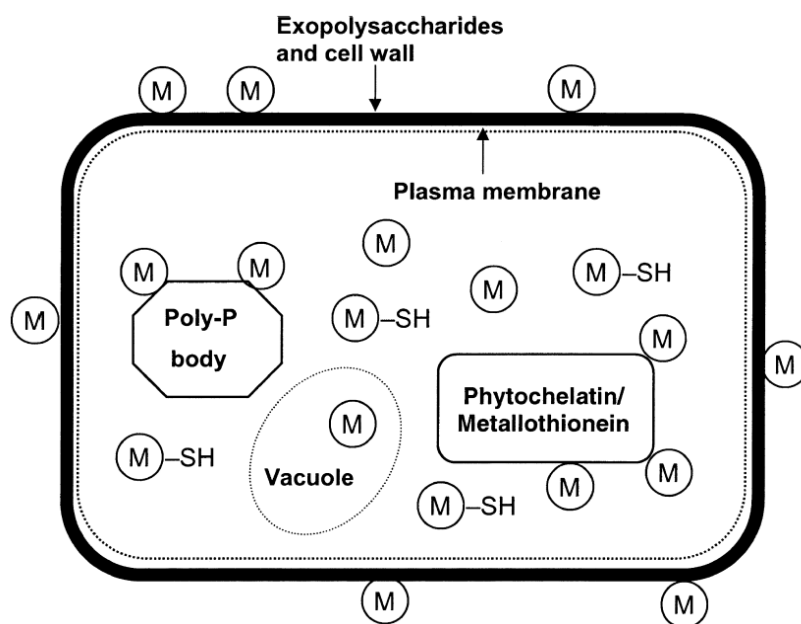
Figure 2.1 Metal binding sites of a typical algal cell. (M represents the metal species)

Microalgae biosorption has two modes: intracellular and extracellular. Intracellular

sorption is called accumulation, which is an active, metabolism-dependent, but very

slow uptake process. Some metals (Cu, Fe, Co, Mo, Zn, etc.) can be accepted as essential elements during algae culture. Compared to the extracellular process, intracellular sorption is much slower so that the efficiency is too low to treat industrial effluents, even though the adsorbed metals are very stable in the algae. While extracellular is passive but extremely rapid process (Bates et al., 1982). In this process, metal ions are adsorbed onto the cell surface within a relatively short span of time (a few seconds to minutes), and the process is metabolism-independent. Figure 2.1 shows the sites of a typical algal cell for binding of metal ions. (Mehta and Gaur, 2005)
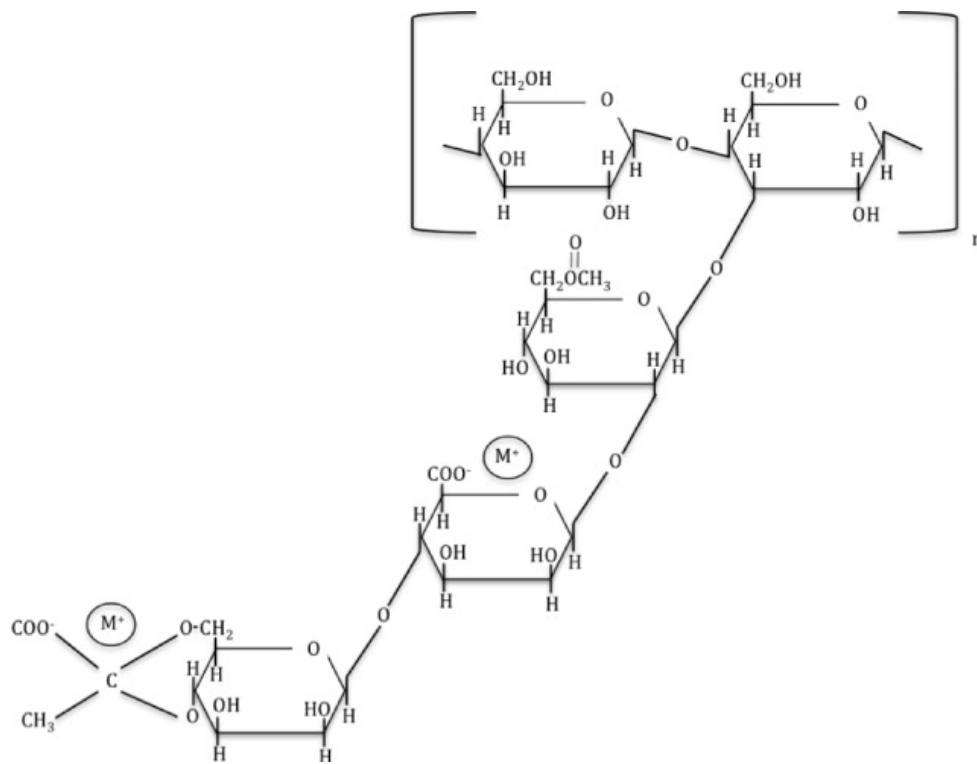
Figure 2.2 Metal ions (M+) uptake by carboxyl groups.

The mechanisms of extracellular adsorption include ion exchange and functional groups effect. It should be pointed out that the term ion exchange does not exactly identify the mechanism, rather it is used here as an umbrella term to describe the experimental observations. The precise mechanisms include physical and chemical binding, such as electrostatic, London–van der Waals forces, covalent etc. Electrostatic binding is considered as a main effect since microalgae perform negatively charged in algae suspension, on the contrary, metal ions are positively charged. They are naturally attracted to each other. The sources of the algal surface negative charge are: ionization of ionogenic functional groups at the algal cell wall (Golueke & Oswald 1970) and selective adsorption of ions from the culture medium (Shaw1969). Cell walls of microalgae offer hydroxyl (-OH), phosphoryl ($-PO_3O_2$), amino($-NH_2$), carboxyl (-COOH), sulphydryl (-SH) groups (Lee 1980). They perform as adsorption sites during metal uptake. Figure 2.2 shows metal uptake by carboxyl groups. (Donot, 2012)

2.5 Biofuels from microalgae

As we all know, the global energy demand is growing very fast, but reserves are limited. World proved oil reserves in 2010 were sufficient to meet only 46.2 years of global production (BP Statistical review, 2011). An alternative renewable energy is in urgent need to replace conventional fossil fuels. Biofuels from thermodynamical processes generate three phases of products: bio-oil, syngas, and bio-char, which have similar compositions and performances with conventional fossil fuels: petroleum oil, natural gas, and coal. Especially the liquid fuels from biomass have good higher heating values (HHV), and they can be burned in current engines with few modifications due to their

good physical properties. Also according to life cycle assessment, biofuels create 0 additional CO_2. The greenhouse effect can be greatly alleviated by applying bio-energy for sustainable development.

In this research, we believe that microalgae are excellent feedstock due to their rich oil content and great potential values for biofuel production. Present feedstocks of biofuels are mainly oil rich crops such as corn, soybean, palm, etc. Compared to other oil crops, microalgae have outstanding oil yield per unit area (Table 2.2). (Chisti Y, 2007) Without occupying exciting cropping land, microalgae have tremendous potential for alternative liquid fuel production to petroleum oil.

Table 2.2 Comparison of some sources of biofuel			
Crop	Oil yield (Gal/Acre)	Land area needed (M Acre)[a]	Present of US cropping area (%)[b]
Corn	18.4	3804	1208
Soybean	47.7	1468	466
Canola	127	551	175
Jatropha	202	346	110
Coconut	287	244	78
Oil Palm	636	110	35
Microalgae[c]	14636	4.8	1.5
Microalgae[d]	6275	11.2	3.6

[a] For meeting 50% of all transport fuel needs of the United States.

<superscript>b</superscript> The cropping area of the U.S. in 2011 is 315 million acres.

<superscript>c</superscript> 70% oil (by wt) in biomass.

<superscript>d</superscript> 30% oil (by wt) in biomass

2.6 Microwave-assisted pyrolysis

Pyrolysis is thermal degradation in the absence of oxygen, which results in the production of charcoal (solid), bio-oil (liquid), and gas products. Lower process temperature and longer vapor residence times favor the production of charcoal. High temperature and longer residence time increase the biomass conversion to gas and moderate temperature and short vapor residence time are optimum for producing liquids. The product distribution obtained from different modes of pyrolysis process is summarized in the table 2.3. Fast pyrolysis for liquids production is of particular interest currently since the liquids are very convenient for transportation and storage.

Table 2.3 Typical product yields obtained by different modes of pyrolysis of lignocellulose				
Mode	Conditions	Char	Liquid	Gas
Fast	~500˚C, short vapor residue time ~1s	12%	75%	13%
Intermediate	~500˚C, vapor residue time ~10~30s	25%	50%	25%
Slow	~400˚C, long vapor residue time ~hours	35%	30%	35%
Gasification	~800˚C	10%	5%	85%

During fast pyrolysis, biomass decomposes to generate mostly volatile matters and some charcoal. After cooling and condensation, a dark brown liquid is formed which

has a heating value about half that of conventional fuel oil. Fast pyrolysis is an advanced process with carefully controlled parameters to give high yields of liquid. The essential features of a fast pyrolysis process for producing liquids are:

- very high heating and heat transfer rates at the reaction interface, which usually requires finely ground biomass feedstock,
- carefully controlled pyrolysis reaction temperature of around 500˚C and vapor phase temperature of 400-450˚C,
- short vapor residence times of typically less than 2 seconds,
- rapid cooling of the pyrolysis vapors to give the bio-oil product.

Microwave heating technology has been widely used in many fields due to its effectiveness, low cost and energy saving. Because the heterogeneous materials contain microwave receptive components, little energy is wasted during biomass pyrolysis. Water is a good microwave receptor, helps microwave energy be dispersed into the sample. It's known that moisture content is a very important essential for microwave heating. (Shang H. et al., 2005) However, evaporation of water engages some energy and mixes non-heating valuable water into the liquid product. Therefore, presence of extra water in the feedstock is avoided.

Microalgae have several advantages as feedstock for fast pyrolysis for producing bio-oil: first, microalgae have much higher lipid content (some species reach 80%) than most lignocellulose materials (Metting F.B., 1996), which means they have good potential for bio-oil production. Second, microalgae are sufficiently small particles to ensure very

high heating transfer rates and rapid reaction, so extra fine grinding is unnecessary.

Third, microalgae can be cultivated rapidly, and occupy less forest or cropping lands.

Some species of metal such as zinc, aluminum and cobalt, were reported as catalysts for microwave-assisted pyrolysis. They may function as microwave absorbents to speed up heating or participate in *in-situ* upgrading of pyrolytic vapors during the microwave assisted pyrolysis of biomass (Wan et al., 2009). We tested microwave assisted pyrolysis of microalgae that adsorbed metals for two objectives, besides testing whether adsorbed metals can perform as catalysts, we also expected that after pyrolysis, adsorbed metals can be further concentrated in charcoal.

Chapter 3 Material and Methods

In this project, we mixed dewatered microalgae with prepared metal solutions for the adsorption experiments. pH value, adsorption time, initial metal and algae concentrations were considered as factors in adsorption experiments. After adsorption, we measured final metal concentrations in the supernatant fractions by spectrophotometer. Microalgae adsorbed metals were oven-dried for microwave assisted pyrolysis. Py-GC-MS was also used to test whether the compositions of volatile products showed any changes when metals were present in the feedstock for pyrolysis.

3.1 Microalgae culture

Microalgae were cultivated in TAP-Medium (Tris-Acetate-Phosphate), which consisted with: NH_4Cl, 0.4 g/L; $MgSO_4 \cdot 7H_2O$, 0.1 g/L; $CaCl_2$, 0.05 g/L; K_2HPO_4, 0.108 g/L; KH_2PO_4, 0.056 g/L; Tris (hydroxymethyl aminomethane), 2.42 g/L. Liquid chemicals included 1 mL/L glacial acetic acid and 1 mL/L trace elements solution. The trace elements solution prepared by Hutner's methods. For a 1 liter final mix, each compound was dissolved in the volume of water indicated: EDTA disodium salt, 50g in 25ml water; $ZnSO_4 \cdot 7H_2O$, 22g in 100ml water; H_3BO_3, 11.4g in 200 ml water; $MnCl_2 \cdot 4H_2O$, 5.06g in 50ml water; $FeSO_4 \cdot 7H_2O$, 4.99g in 50ml water; $CoCl_2 \cdot 6H_2O$, 1.61g in 50ml water; $CuSO_4 \cdot 5H_2O$, 1.57g in 50ml water; and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.10g in 50ml water. The EDTA was dissolved in boiling water, and the $FeSO_4$ was prepared last to avoid oxidation. All solutions except EDTA were mixed. They were brought to a boil, and then EDTA solution was added. The mixture should turn green. When everything was dissolved, they were cooled to 70˚C. Keeping temperature at 70,

add 85 ml hot 20% KOH solution (20 g / 100 ml final volume). Bring the final solution to 1 liter total volume. Stopper the flask with a cotton plug and let it stand for 1-2 weeks, shaking it once a day. (Hutner et al., 1950)

A screened local microalgae strain 10B (*Chlorella vulgaris*) was cultured in 3 paralleled 2L flasks and 3 paralleled 250ml flasks (used as concentrated seed) for adsorption experiments use, and in 500L greenhouse for pyrolysis experiments use. The algae inoculation amount is 100ml concentrated algal seed to 900ml new tap nutrient solution. Culturing environmental temperature is 20˚C, with an illumination intensity at 80 $\mu mol/(m^2/s)$, and magnetic stirrer (Corning® PC-353 Stirrer) was used to mix the algal particles uniformly suspended in the nutrient for flask culture, and the flasks of seed were positioned on Lab-Line® orbit shaker, with an illumination intensity at 80 $\mu mol/(m^2/s)$ also. A discontinuous pump is used for greenhouse microalgae mixing. The culture solution could not be used until the density of algae increased to above 1g dry weight per liter which is close to the common concentration in industrial PBR culture. The concentration of algal solution was measured with a standard curve that x axis is algae dry mass concentration (g/l), and y axis is OD (Å) displayed by using spectrophotometer (Spectronic Instruments Genesys 5 USA) at 550nm.

3.2 Metal solution preparation

In this research project, targeted metals are copper(II), zinc(II) and aluminum(III). Copper solutions are diluted from 1g/L cupric sulfate standard solution, zinc solutions are diluted from 1g/L zinc chloride standard solution, and aluminum solutions are

diluted from 1g/L aluminum chloride standard solution. In the metal interaction experiments, a mix solution of cupric sulfate (1g/L), zinc chloride (1g/L) and aluminum chloride (1g/L) was prepared. Chemicals are from Fisher Scientific. Solvent is deionized water.

3.3 Adsorption experiments

For accurate concentrations of microalgae and metal ions, microalgae are dewatered by centrifugation before mixing with metal solutions. Each centrifuge tube (50ml) contains 30 ml microalgae suspension. The centrifuge is operated in 1400 r/min for 20 minutes. The microalgae are flung to the bottom of the centrifuge tube. After removal of the supernatant water from the tube, prepared metal solution is poured into the tube. The sample of metal solution and algal sorbent are transferred to a 50 ml conical flask. A magnetic bar was put into the flask to stir the solutions with a magnetic stirrer with a rotation speed of 250r/min. After a certain time (20 min except time-efficiency experiments), the solution was transferred into the centrifuge tube and the microalgae are dewatered by centrifugation as in the former process. The supernatant is separated from the algal sorbents. The algal sorbents are collected and oven dried ($105°C$, 12 hours in Precision Scientific[®] Model 28 Oven) for the following pyrolysis experiments, while the concentrations of metals in the supernatant are tested by UV-Vis spectrophotometer (Hach[®] DR 5000™). They are compared to the initial concentration, and the difference reflects the amount of removed metal ions.

Control and blank samples include the microalgae sample (microalgae suspension only),

metal solution (only) sample and deionized water sample.

3.4 Spectrophotometer methodology

3.4.1 Copper Concentration Measurement

Cupric ions concentration is measured by Bathocuproine Method. Copper (I) ions form

an orange-colored complex with the disodium salt of bathocuproine disulphonic acid.

(Figure 3.1) Any copper (II) ions present in the water sample are reduced to copper (I)

ions by ascorbic acid before the complex is formed. Test results are measured at 478 nm.

This method is suitable to measure copper ions concentration from 0.1 to 8.0 mg/L.

Highest accuracy is on 4.0 mg/L. For precision, each sample has prospect concentration

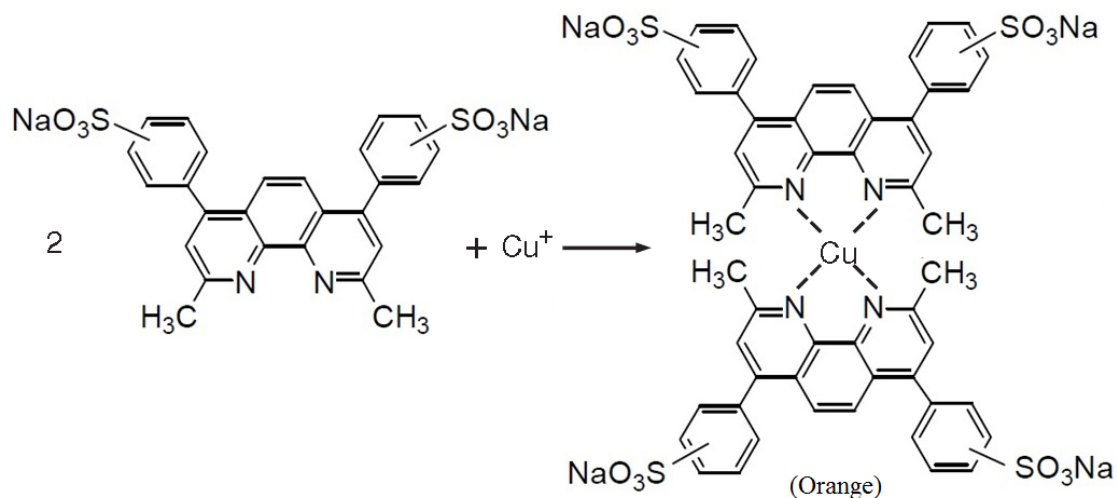over 6.0 mg/L (6.0 to 10.0 mg/L) is diluted to half of original.

Figure 3.1 Reaction of Bathocuproine with copper(I)
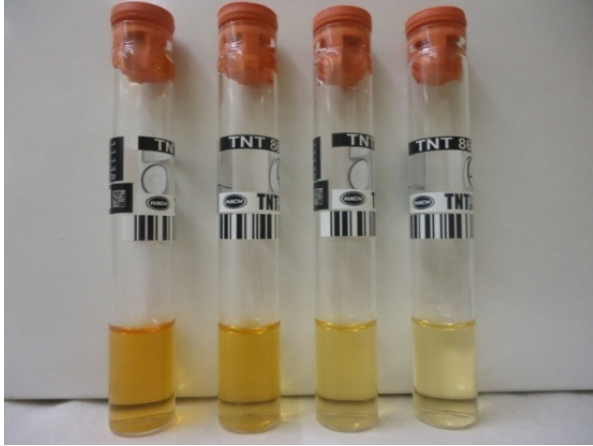
Figure 3.2 Color differences of samples show concentration of copper ion.

Figure 3.2 shows color differences of supernatant samples of copper (5mg/L) adsorbed by *Chlorella vulgaris* (in 0mg/L, 25mg/L, 100mg/L and 250mg/L, from left to right respectively), the concentrations are (from left to right): 5.00mg/L, 4.51mg/L, 2.30mg/L and 0.85mg/L.

3.4.2 Zinc Concentration Measurement

Zinc ions concentration is measured by USEPA Zincon Method. Zincon is dry powder form of 2-carboxy-2'hydroxy-5'sulfoformazyl benzene indicator. In the analysis, zinc and other metals in the sample are complexed with cyanide. Adding cyclohexanone causes a selective release of zinc. The zinc reacts with zincon indicator to form a blue-colored complex forms in direct proportion to the amount of zinc in the sample (Figure 3.3). The blue color is masked by the brown color from the excess indicator (Figure 3.4). Test results are measured at 620 nm.

Figure 3.3 Reaction of zincon with zinc with color change

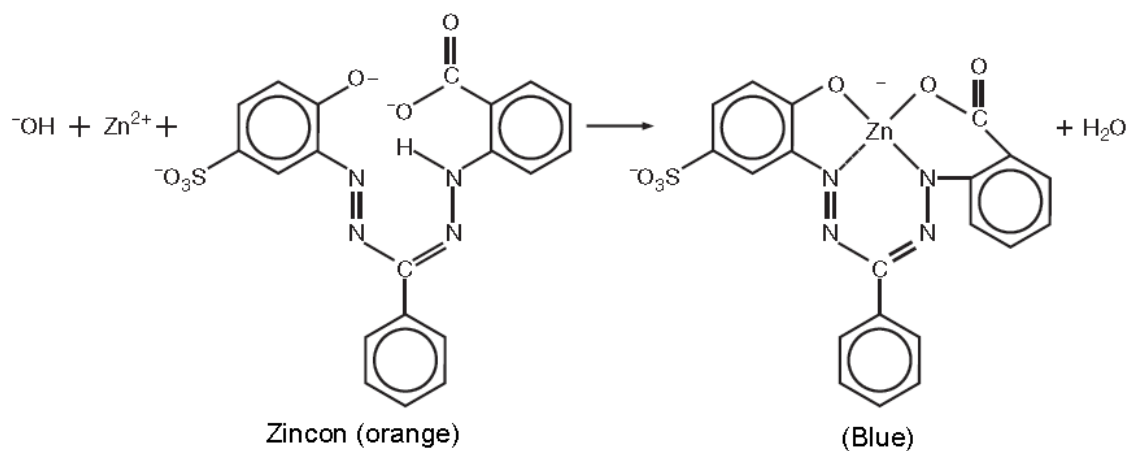This method is suitable to measure zinc ions concentration from 0.01 to 3.00 mg/L. Highest accuracy is on 4.0 mg/L. For precision, samples are diluted from 20.0mg/L to 2.00mg/L. The instrument shown records multiply 10 to acquire actual data.

Figure 3.4 The sample (left) and the blank (right) of zinc ion concentration measurement

3.4.3 Aluminum Concentration Measurement

Aluminum ions concentration is measured by Aluminon Method. Aluminon indicator combines with aluminum in the sample to form a red-orange color. The intensity of color is proportional to the aluminum concentration. Ascorbic acid is added before the AluVer 3 reagent to remove iron interference. To establish a reagent blank, the sample is split after the addition of the AluVer 3. Bleaching Reagent is then added to one-half of the split sample to bleach out the color of the aluminum aluminon complex. The AluVer 3 Aluminum reagent, packaged in powder form, shows exceptional stability and is applicable for fresh water applications. Test results are measured at 522 nm.

3.5 Microwave assisted pyrolysis experiments

Oven dried microalgae are put into a round quartz flask. Each sample contains 40 g algae (dry weight). A Panasonic NN-SD797S stainless steel microwave oven (1.6 Cubic Foot/1250W) is used to provide microwave energy. After an elbow glass tube (for violent products observation), there is a three stage condensation system. Each stage has a liquid collector. End gas is inhaled and treated by an extractor hood and end tail gas filter.

Before the pyrolysis process, cooling water was turned on and let the condensation system work. The reaction needs several minutes, depends on the composition and amount of the feedstock, to accumulate enough microwave energy to achieve the temperature needed for pyrolysis. Violent products can be seen through the elbow glass tube. Mist emerges at the beginning and condensed water droplets can be observed on

the tube wall inside during the pre-heat period (about 1-2 minutes). Then white smog emerges at the heat up period (around 2-5 minutes). Later, yellow-brown smog moves rapidly in the tube, which is the sign that pyrolysis has started. Dark brown droplets appear from the elbow glass tube to the condensation system. When they reach the third stage of the condensation system, end gas should be fired to reduce pollution and the flame is the signal for the end pyrolysis. Since the dark brown droplets on the inside of elbow glass tube, it's hard to observe smog when reaction is close to end. Turn off the microwave when the flame at the end tube is disappearing.

After the pyrolysis process, the cooling water supply was turned off and another flask with the appropriate amount ethanol (around 200 ml) was put in place. The microwave was turned on and the ethanol will dissolve the dark brown droplets attached on the inside of the whole system, and flow into the three collectors. After the first cleaning process, all liquid in the three collectors was collected and poured into the ethanol flask. Repeat the cleaning process with the liquid (ethanol and bio-oil) two or three times until all bio-oil on the glass wall is clean. Then distill the ethanol by rotary evaporator (Buchi® Rotavapor RII) to gain bio-oil.

Figure 3.5 The microwave-assisted pyrolysis system

Chapter 4 Results and Discussion

4.1 Factors that affect metal ion adsorption by *Chlorella vulgaris*

Biosorption by microalgae can be affected by several factors, such as initial

concentration of metal and microalgae, pH value, time, presence of other ions, etc.

4.1.1 Effect of pH value on metal removal efficiency

Many studies have shown that pH value affect the efficiency of metal removal,

especially at the range of pH>5.50, due to chemical precipitation. But at the initial pH

range (4.00-5.00), few differences have been observed. The experiments conditions are

copper sulfate solution, 5mg/L; and *Chlorella vulgaris*, 50mg/L. It's shown that

pH<2.50 increase the metal adsorption efficiency. However, pH control raises the cost

and brings other issues such as equipment corrosion and acid pollution. According to

our experiments, high enough efficiency has been found without pH control. Therefore,

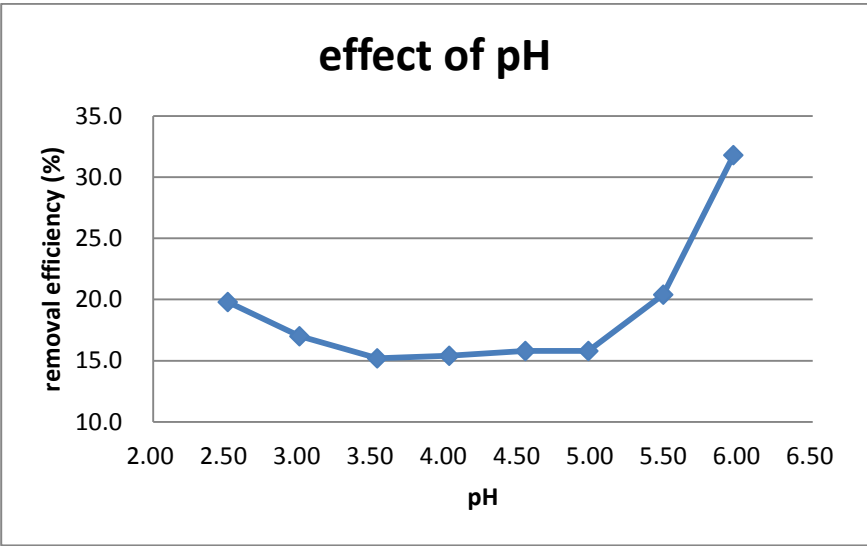pH value adjusting is not recommended for practical applications.

Figure 4.1 Effects of pH value for copper removal on metal removal efficiency

4.1.2 Effect of adsorption time on metal removal efficiency

5mg/l copper sulfate solution and 250mg/L *Chlorella vulgaris* are applied for these experiments. Ion exchange effect happens rapidly, within 5 minutes, most cupric ions (78%) have been removed from the solution. But the adsorption through functional group continue works and helps the removal efficiency reach around 85% in 20 minutes. After then, little obvious change of efficiency is observed. Hence, 20 minute was taken as a standard adsorption time for following experiments.
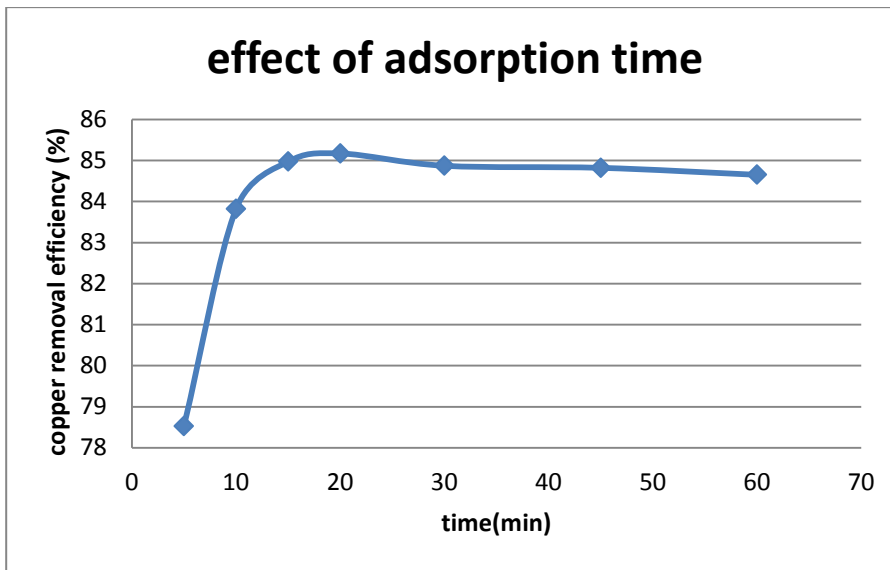
Figure 4.2 Effects of adsorption time on metal removal efficiency

4.1.3 Effect of initial concentration of metal ions on metal removal efficiency

Metal removal efficiency closely depends on initial concentration of metal ions. The concentration factor varies in different metal ions, but the removal efficiency increases as the initial concentration of metal decreases. (Kelly, 1988) Figure 4.1 shows the effect of initial concentration of copper(II). 2, 5 and 10 mg/L copper sulfate solution are

treated by 100mg/L *Chlorella vulgaris*. 68% of copper(II) is adsorbed in the initial

concentration of 2mg/L, but only 42% for 10mg/L copper(II) solution.
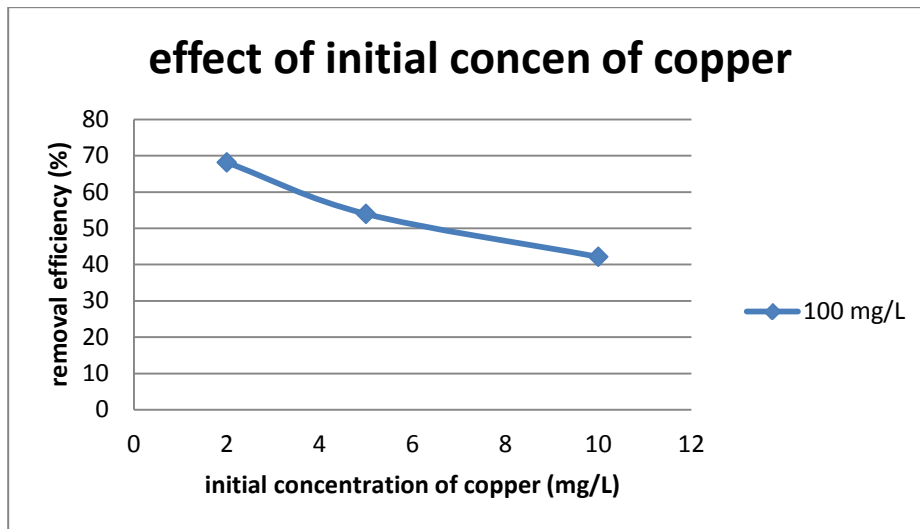
Figure 4.3 Effects of initial concentration of copper(II) on metal removal efficiency

Effect of initial concentration of aluminum and zinc also reflect above finding.

Wastewater of concentration under 10mg/L of metal ions is very hard to treat due to

extremely low effectiveness and very high cost by conventional methods, but this is the

best range of biosorbents. It is the reason why we suggest applying biosorption to later

stage of a multi-stage wastewater treatment system.
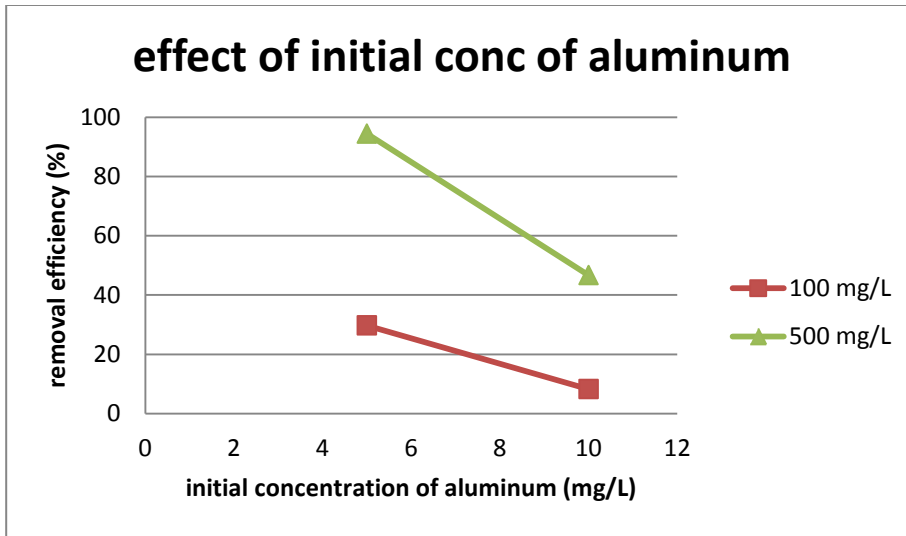
Figure 4.4 Effects of initial concentration of aluminum(III) on metal removal efficiency

Figure 4.5 Effects of initial concentration of zinc(II) on metal removal efficiency

4.1.4 Effect of concentration of microalgae on metal removal efficiency

Obviously, more algal sorbents provide more sites to capture metal ions, but in most cases, there is a limitation. Besides, dewatering of microalgae is a costly process, which should be avoided if possible. It's impractical to apply too much biosorbents for metal

adsorption. This section is trying to find out the most reasonable amount of sorbents for use.

Figure 4.4 shows the effect of concentration of copper(II) and *Chlorella vulgaris*. X axis is algae-copper ratio, and y axis is copper removal efficiency. Blue, red and green curves are reflects initial copper concentration in 2mg/L, 5mg/L and 10mg/L, respectively. It shows that around 84% of cupric ions are removed from solution by no more than 500mg/L *Chlorella vulgaris* at all different copper concentrations. The remaining copper is hard to adsorb even through more algae are added to the solution. Actually even at the highest initial copper concentration in this research, the final concentration of copper(II) has been reduced to 1.5mg/L, almost meeting the EPA's drinking water level (1.3mg/L).
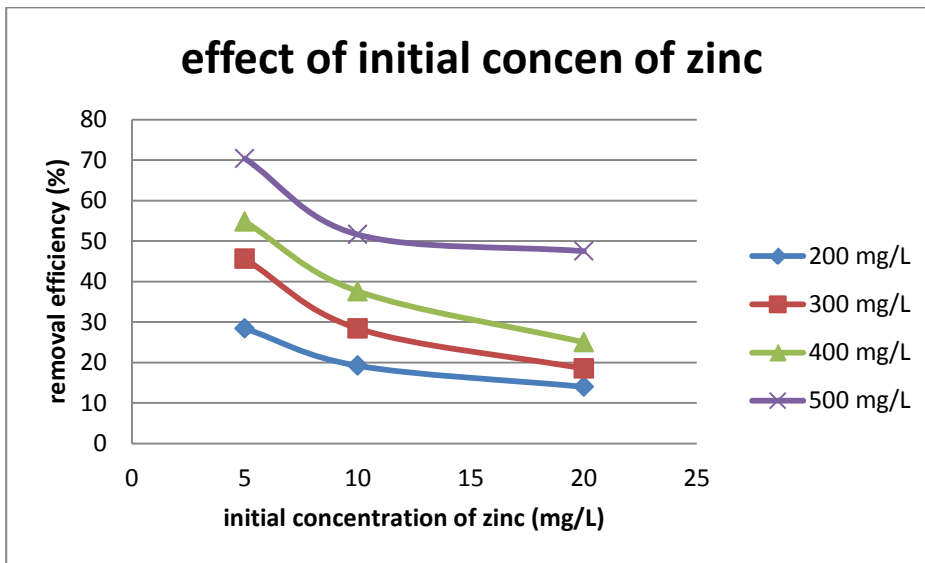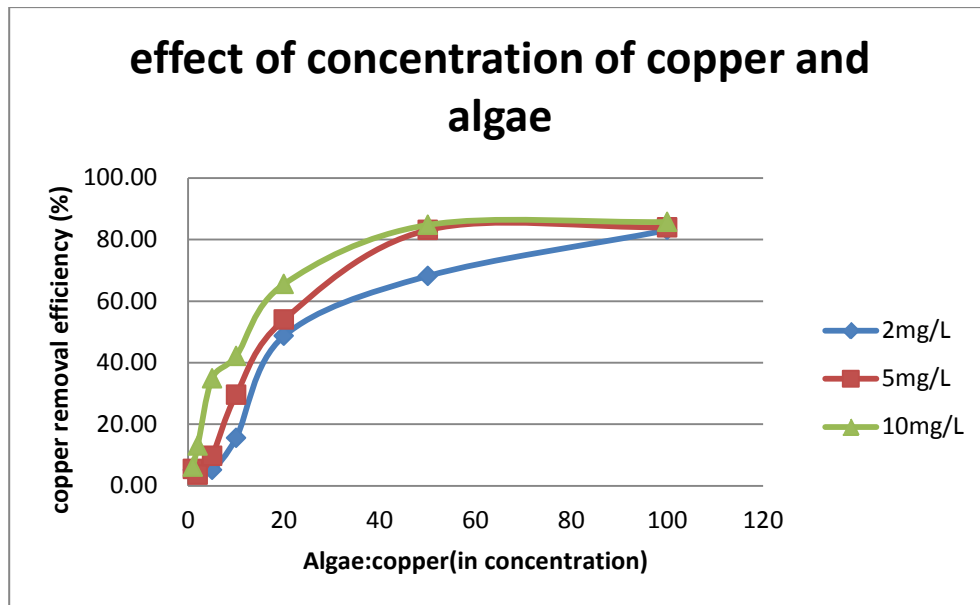

Figure 4.6 Effects of concentration of copper(II) and algae on metal removal efficiency
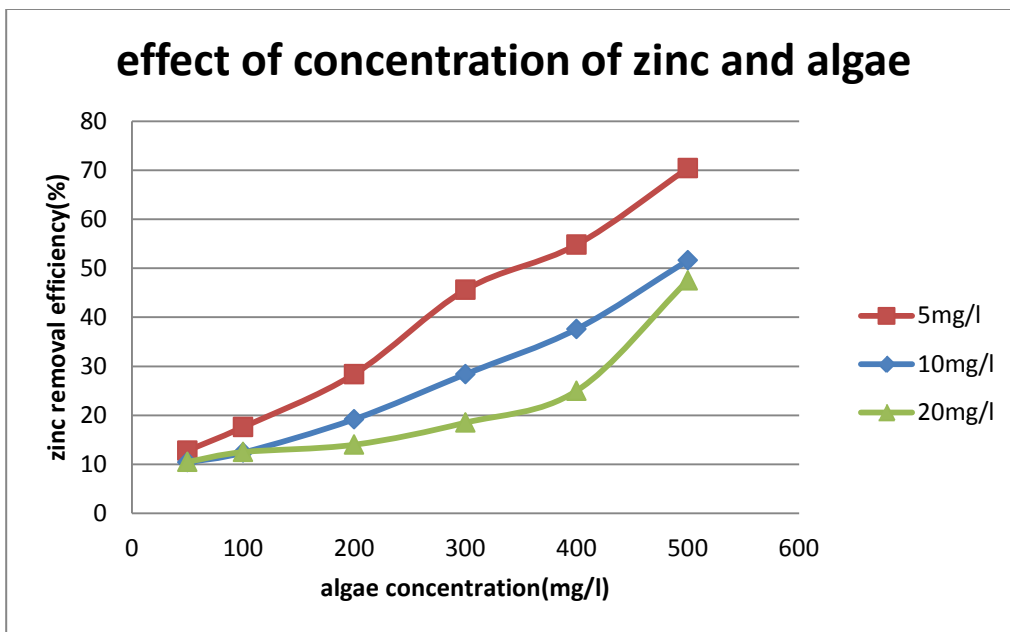
Figure 4.7 Effects of concentration of zinc(II) and algae on metal removal efficiency
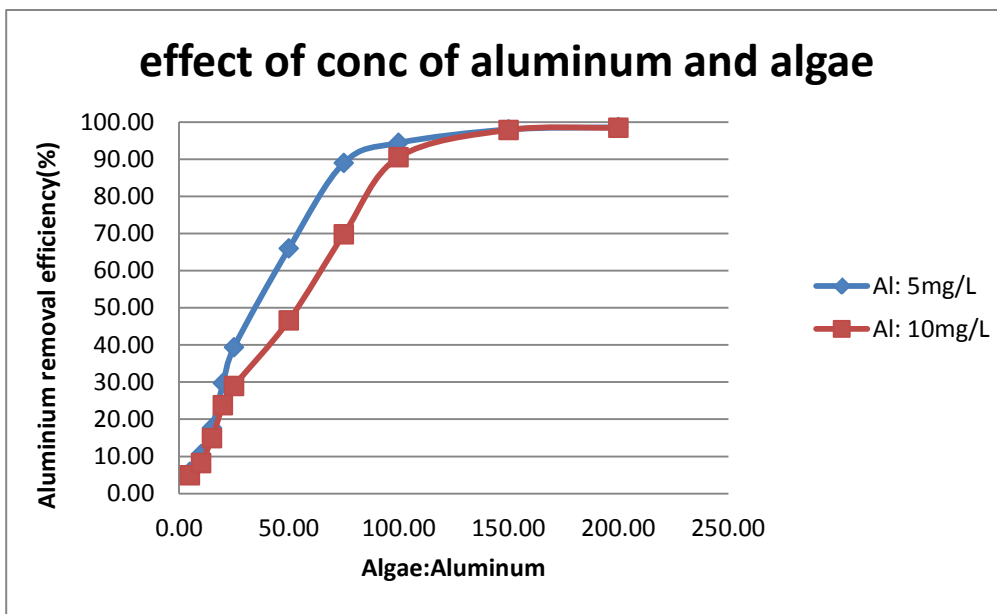
Figure 4.8 Effects of concentration of aluminum(III) and algae on metal removal

efficiency

4.1.5 Effect of metal ions interactions on metal removal efficiency

Few studies have covered ion interactions during biosorption of metal ions. In these experiments, efficiency in mixed solutions of 5mg/L copper(II), 5mg/L zinc(II) and 5mg/L aluminum(III) are compared to those in single metal solutions. Figure 4.9, 4.10 and 4.11 shows effects of ions interactions on copper, zinc and aluminum removal efficiency, respectively. From the figure, copper and zinc removal efficiency is reduced when algal sorbents are insufficient, but aluminum is not affected by copper and zinc ions. An explanation goes with formation of AlO_2- and $Al(OH)_4-$, which compete to negative charged algal sorbents and block the ion exchange mechanism for copper and zinc removal. Another signal is that after most aluminum (95%) has been adsorbed by microalgae, copper and zinc removal efficiency finally reaches the highest value obtained from the former single metal experiments.

Figure 4.9 Effects of other ions on copper removal efficiency

Figure 4.10 Effects of other ions on zinc removal efficiency

Figure 4.11 Effects of other ions on aluminum removal efficiency
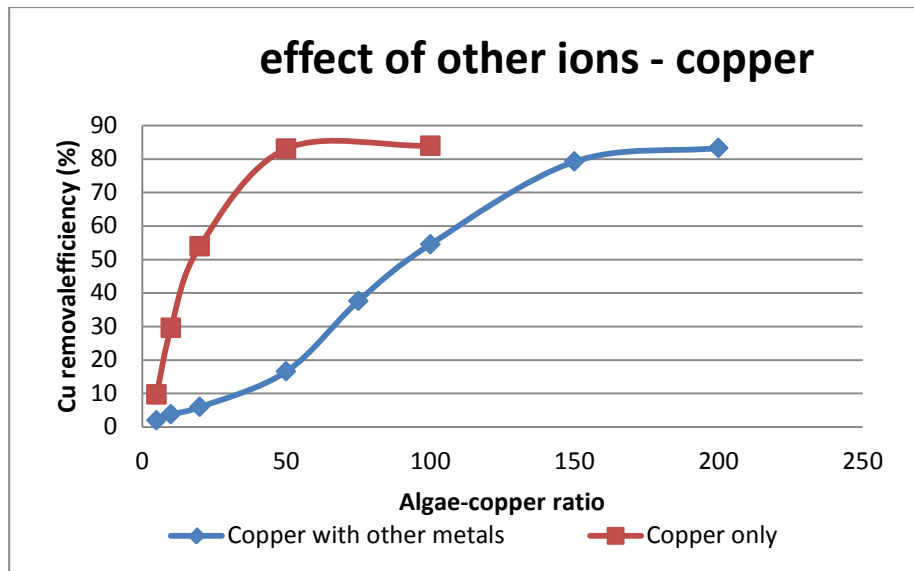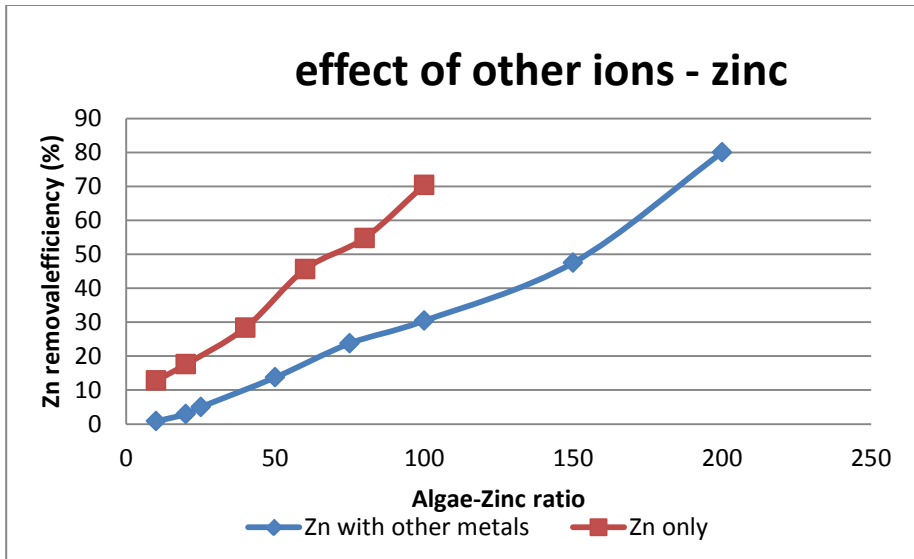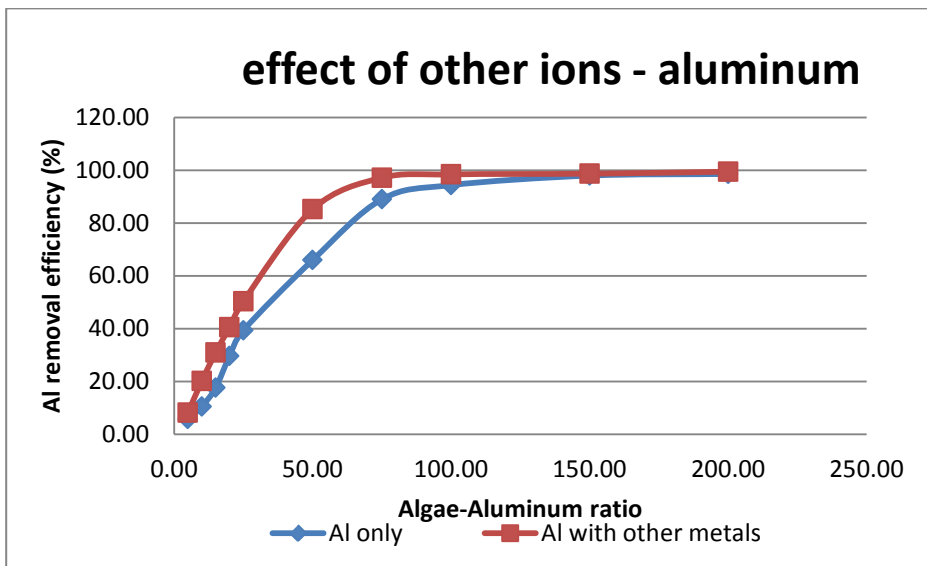
4.2 Microwave-assisted pyrolysis results

According to the previous adsorption experiments, we found that in an effective

adsorption model, metal content in *Chlorella vulgaris* is 1% - 5% (in dry weight). So

we determined we needed to have three groups of samples: non-metal, 1% metal and 5% metal.

Table 4.1 Microwave-assisted pyrolysis experiment results			
Samples	#1	#2	#3
Metal content (% in dry w/t)			
Copper	0	1	5
Zinc	0	1	5
Aluminum	0	1	5
Heating up time (min)	10	5	4-5
Condensable bio-oil yield (%)	14.4%	15.5%	15.6%
Charcoal yield (%)	35.2%	33.3%	33.4%

We expected to see differences in oil-yield after adding metals in algal feedstock, but 1% improvement is not an exciting result. Besides, due to the variability of the microalgae samples and instability of the microwave-assisted pyrolysis system, we could not reject the possibility that oil yield increase is actually system error. However, another unexpected discovery was found to be quite valuable. Before the pyrolysis reaction starts, the non-metal samples need 10 minutes heating time, which includes 2 minutes pre-heat time to see the steam appears, heating level of microwave oven is on 2; 6 minutes heat-up time when white smog emerges, heating level on 6; and 2 minutes full heat on level 10 to start the pyrolysis reaction. While metals are present in the feedstock samples, about 5 minutes total heating time is sufficient. 1 minute pre-heat time, 3-4 minutes heat-up time is needed, then the yellow-brown smog is observed following

white smog appearing. Total heating time is almost halved by adding metals in the microalgae.

4.3 Results from Py-GC-MS

After adsorption experiments, the microalgae and adsorbed metals were dried in an oven at around 105 ˚C. Then they are pyrolyzed and analyzed by Pyrolysis-Gas Chromatography–Mass Spectrometry (Py-GC-MS). The Py-GC/MS pyrograms at 500˚C indicate the presence of a range of ketones, pentosans, nitrogen containing compounds and phenols.
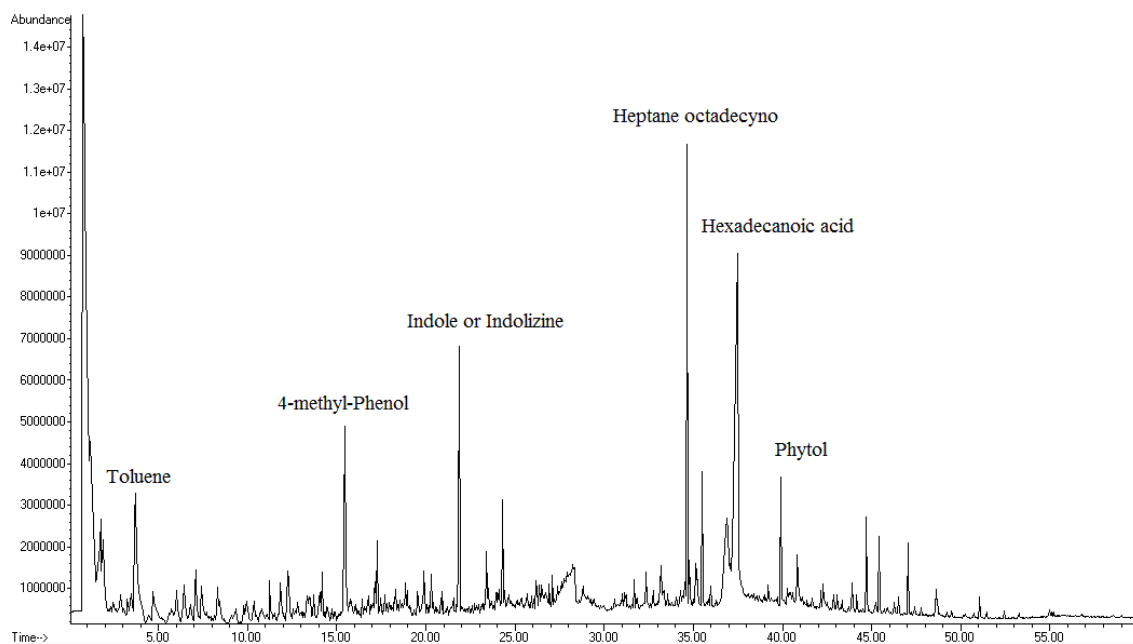
Figure 4.12 Py-GC-MS analysis result of *Chlorella vulgaris*
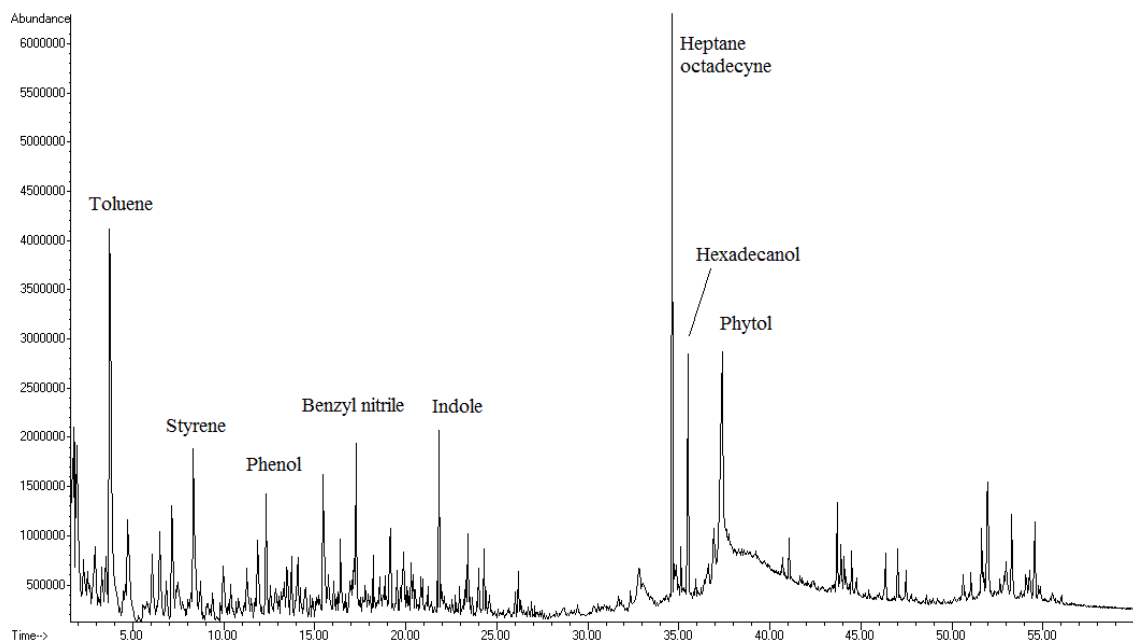
Figure 4.13 Py-GC-MS analysis result of *Chlorella vulgaris* and 5% metal

Figure 4.12 shows Py-GC-MS analysis result of *Chlorella vulgaris*, while figure 4.13

shows that of microalgae with adsorbed metal (5% each). The results reflect that when

metals exist, more phenols are generated and the volatile products are more variable,

also, less nitrogen containing compounds are produced and much less CO_2 output.

4.4 Results from ICP

Char samples (0.50g) were microwave digested with HNO3 then analyzed by ICP. The

results (Table 4.2) show metal compositions of char from different feedstock on

microwave-assisted pyrolysis. The results reflect that the percentages of metals

remained in the char to those in algae. 96.17% Cu and 97.34% Zn remain in the solid

products after pyrolysis.

Table 4.2 ICP results of composition of char from different feedstock *			
Metal	Char from Algae	Char from Algae + Cu	Char from Algae +Zn
Al	**319.87**	**361.58**	**355.81**
B	28.76	26.42	25.88
Ca	20009.00	21399.00	21074.00
Cd	< 0.01	< 0.01	0.21
Cr	6.08	8.88	4.71
Cu	**733.79**	**50851.00**	**777.12**
Fe	5530.00	6583.40	7616.10
K	19408.00	19795.00	18693.00
Mg	9117.10	9535.60	9237.40
Mn	1547.30	1694.00	1613.30
Na	314.25	317.60	335.43
Ni	9.92	14.27	10.25
P	53916.00	58333.00	55241.00
Pb	< 0.18	62.73	< 0.18
Zn	**2998.20**	**3539.60**	**51666.00**

*: All data in this table are in mg metal/kg sample

Chapter 5 Conclusions and Future Work

In this research project, *Chlorella vulgaris* was used as a biosorbent for copper(II), zinc(II) and aluminum(III) when the metals are in relatively low concentrations. Compared to other technologies, biosorption is a cost-effective method. Up to 85% of copper(II), 70% of zinc(II) and 99% of aluminum(III) could be removed from solution within a very short time (20 minutes), and only a small amount of extra energy was needed since the algal sorbents have extremely high potential for bioenergy production. Besides, feedstocks containing metals need just about half of the heating time to reach a high enough temperature for microwave assisted pyrolysis, which significantly reduced the energy use for running the microwave ovens. After pyrolysis, most Cu and Zn (96.17% and 97.34%, respectively) remained in the bio-char portion. Last but not least, the presence of metals in the pyrolysis feedstock also improved the composition of the volatile products. The products analysis showed that more aromatic compounds and less nitrogen containing products as well as CO_2 were obtained. Aromatic compounds have various and useful values in the chemical industry, and also higher heating values. Nitrogen containing products are unwelcome compounds in fuels due to the burden of the tail gas cleaning systems required. Less CO2 output also improved the syngas heating value.

The project results suggested great potentials for future study. First, a variety of microalgae dewatering methods are being applied currently. Some chemicals such as flocculating agents are used for microalgae harvesting. But little research has been undertaken to measure the effects of these chemicals on metal biosorption. Second, we

expect that a combination of biosorption and microwave-assisted pyrolysis can be applied to other species of microalgae or other microorganisms such as fungi, yeast and bacteria. A systematic study on this will be very valuable. Third, all experiments are applied under simulative conditions. Real industrial effluents represent a more complicated situation. The residue from previous decontamination by other methods and presence of other chemicals in the wastewater may have effects on biosorption. Nevertheless, although some insufficiencies exist in the research, the thought of integration of wastewater treatment and renewable energy production has a profound significance.

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