PRETREATMENT OF GREEN MACROALGA, *Caulerpa lentillifera*, FOR HEAVY METAL REMOVAL

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Environmental Management (Inter-Department)
Graduate School
Chulalongkorn University
Academic Year 2003

ISBN: 974-17-4167-7

การปรับปรุงคุณภาพสาหร่ายมหภาคสีเขียวชนิดช่อพริกไทยเพื่อกำจัดโลหะหนัก

นายประทีป สุทธิปริญญานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการจัดการสิ่งแวดล้อม (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2546

ISBN: 974-17-4167-7

Thesis Title	PRETREATMENT OF GREEN MACROALGA, *Caulerpa lentillifera*, FOR HEAVY METAL REMOVAL
By	Mr. Prateep Suthiparinyanont
Field of study	Environmental Management
Thesis Advisor	Assistant Professor Prasert Pavasant, Ph.D.

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ประทีป สุทธิปริญญานนท์: การปรับปรุงคุณภาพสาหร่ายมหภาคสีเขียวชนิดช่อพริกไทยเพื่อกำจัดโลหะหนัก (Pretreatment of green macroalga, *Caulerpa lentillifera*, for heavy metal removal) อ. ที่ปรึกษา: ผศ.ดร. ประเสริฐ ภวสันต์, 95 หน้า ISBN 974-17-4167-7

งานวิจัยนี้เกี่ยวข้องกับการศึกษาการดูดซับโลหะหนัก (ทองแดง, แคดเมียม, สังกะสี และ ตะกั่ว) โดยใช้สาหร่ายช่อพริกไทยที่ถูกปรับปรุงคุณภาพแล้ว จากการทดสอบความสามารถในการดูดซับโลหะหนักของสาหร่ายที่ผ่านการปรับปรุงคุณภาพทั้งหมด 24 วิธี พบว่า มี 2 วิธี ที่มีผลของความสามารถในการดูดซับโลหะหนักสูงสุดเพิ่มขึ้น เมื่อเทียบกับสาหร่ายที่ยังไม่ได้รับการปรับปรุงคุณภาพ คือ วิธีการปรับปรุงด้วย $CaCO_3$ 24 ชั่วโมง เพิ่มขึ้น 29 และ 78 เปอร์เซ็นต์สำหรับทองแดง และแคดเมียมตามลำดับ และวิธีการปรับปรุงด้วย NaOH 1 ชั่วโมง เพิ่มขึ้น 16 และ 67 เปอร์เซ็นต์สำหรับทองแดง และตะกั่วตามลำดับ ทั้งนี้ พบว่าไม่มีวิธีใดที่เหมาะสำหรับสังกะสี การทดสอบความสามารถในการดูดซับโลหะทองแดง โดยสาหร่ายที่ผ่านการปรับปรุงคุณภาพเป็นระยะเวลานานต่างกัน พบว่าระยะเวลาที่เหมาะสมอยู่ในช่วงเวลาระหว่าง 0.5 ถึง 1 ชั่วโมง และ น้อยกว่า 5 นาที สำหรับวิธีการปรับปรุงด้วย 0.5N NaOH และ การให้ความร้อนใน 0.5N NaOH ตามลำดับ สำหรับการทดสอบหาความเป็นไปได้ของหมู่ฟังก์ชั่นที่น่าจะเกี่ยวข้องกับการดูดซับโลหะหนัก สรุปลักษณะของการกระทำกับหมู่ฟังก์ชั่น ได้ดังนี้ เกิดพันธะโคเวเลนต์ กับ C-O, เกิดแรงดึงดูดทางไฟฟ้า หรือพันธะไอออนิกกับไฮดรอกไซด์ O-H และเกิดพันธะโคเวเลนต์ หรือพันธะไอออนิกกับเอมีน (N-H) , อะมิโน (N-H) และซัลโฟเนต (S-O) และยังพบอีกว่า วิธีที่เหมาะสมสำหรับการปรับปรุงคุณภาพของสาหร่ายชนิด จะมีผลต่อการเปลี่ยนแปลงของหมู่ฟังก์ชั่นที่น่าจะเกี่ยวข้องต่อการดูดซับโลหะหนักน้อยที่สุด

	ลายมือชื่อนิสิต..
สาขาวิชาการจัดการสิ่งแวดล้อม	ลายมือชื่ออาจารย์ที่ปรึกษา...............................
ปีการศึกษา 2546	

##4589439220 : MAJOR ENVIRONMENTAL MANAGEMENT
KEY WORD: SORPTION / ALGAE / CAULERPA / PRETREATMENT

PRATEEP SUTHIPARINYANONT: PRETREATMENT OF GREEN MACROALGA, *Caulerpa lentillifera,* FOR HEAVY METAL REMOVAL. THESIS ADVISOR: ASST. PROF. PRASERT PAVASANT, Ph.D. 95 pp. ISBN: 974-17-4167-7

This work investigated the adsorption of heavy metals (Cu, Cd, Zn and Pb) using the pretreated biosorbent derived from the biomass of marine algae, *Caulerpa lentillifera*. Twenty four pretreatment methods were examined based on their adsorption capacities. Only a few methods were found to have higher adsorption capacity than the untreated algae. These included: $CaCO_3$ 24 hours (29% enhancement in maximum adsorption capacity) and NaOH 1 hour (16%) for Cu, $CaCO_3$ 24 hours (78%) for Cd, and NaOH 1 hour (67%) for Pb. No improvement for Zn adsorption was found from all 24 methods. The optimal exposure time for the pretreatment by 0.5N NaOH was found with in the range of 0.5 to 1.0 hours. For the pretreatment by heating in 0.5N NaOH, the optimal heating time should be shorter than 5 minutes. The evaluation of functional group suggested that the possible functional groups for the adsorption of these heavy metals were a covalent bond with C-O, an electrostatic or ionic bond with a hydroxide (O-H), and a covalent or ionic bond with amine (N-H), amino, (N-H) and sulfonate (S-O) groups. Most of the pretreatment methods were found to decrease the quantity of potential functional groups. Appropriate pretreatment methods were those with low potential to change the appropriate functional groups.

Field of study Environmental Management	Student’s signature……………………..
Academic year 2003	Advisor’s signature……………………..

ACKNOWLEDGEMENTS

Especially, I would like to express my sincere gratitude to my advisor, Assistant Professor Dr. Prasert Pavasant, for his encouragement, invaluable support, and kind guidance throughout my work. This thesis would not have been accomplished without his excellent supervision. I am grateful to Dr. sorawit Powtongsook, Dr. Pairat Kaewsarn and Dr. Ekawan Luepromchai for their helpful recommendations throughout the course of my M.Sc. time. Many thanks should also be directed towards Miss Ramnaree Netvichien, Miss Chantana Intim, Mr. Vorapot Kanokkantaphong, Miss Chalatip Ratsuk and Mr. Ronbanchob apiratikul for many available helps and comments.

Special thanks are also for all students and staff at the National Research Center for Environmental and Hazardous Waste Management (NRC-EHWM) Program. Special gratitude to Banjong farm at Chachoengsao province for supporting algal material and to the Scientific and Technological Research Equipment Center, Chulalongkorn University, for supporting lab instrument. Moreover, I would like to express gratitude to Miss Vimonrat Sungkhum for her nice support and imperative helps.

Most of all, grateful thanks to my parents and my sister and brothers for their love and inspiration throughout my study.

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ABBREVIATIONS AND NOTATIONS

b = constant value of Langmuir isotherm
C_i = initial heavy metal concentrations
C_e = equilibrium heavy metal concentrations
^oC = degree celcius
C=O = Carbonyl
COOH = Carboxyl
FTIR = Fourier Tranform Infrared Spectrometer
g = gram
g/l = gram per litre
ICP = Inductively Coupled Plasma
K_f = constant value of Freundlich isotherm
KBr = potassium bromide
l = litre
mg = milligram
mg/g = milligram per gram
mg/l = milligram per litre
ml = millilitre
mmol/g = millimol per gram
mmol/l = millimol per litre
No. = number
NH_2 = amino
$1/n$ = intensity indicator
p = probability (t-test)
ppm = part per million
q_{max} = the maximum adsorption capacity
R^2 = linear line
rpm = round per minute
S=O = sulfonyl
x/m = solute (metal) concentration in the sorbent (algae)
V = volume of the heavy metal concentration
W = dry weight of the biomass-added

CHAPTER 1

INTRODUCTION

1.1 Motivations

Electroplating processes have been known as significant sources of extremely toxic wastewater. The most harmful toxic contaminants are acids and heavy metals e.g. copper, cadmium, zinc, lead, chromium, nickel and tin. These contaminants if allowed to enter natural watercourses will affect the health and welfare of human beings. Therefore it is necessary that heavy metals in industrial wastewater be appropriately removed before being discharged into the environment. Heavy metal bearing effluents can be treated effectively by several methods. Conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes [Kojima and Lee, 2001]. The aim of these technologies is to reduce the heavy metal content in the effluent to below permissible discharge limits. The selection of a particular treatment technique depends notably on a number of factors including waste type and concentration, other constituents, the level of clean-up required and the economics of the process. One similar characteristic of these technologies is that all of them are usually practical and cost-effective only when the wastewater contains large amounts of heavy metals. On the other hand, these techniques are often ineffective when applied to dilute wastes with heavy metal concentrations less than 100 ppm [Volesky,1990].

In recent years, biosorption has emerged as a cost effective and efficient alternative for the removal of low concentration heavy metals from wastewaters. Biosorption is the uptake of heavy metals and radionuclides from aqueous solutions by biological materials. Microorganisms including algae, bacteria, yeast, fungi, plant leaves and root tissues can be applied as bioadsorbents for the detoxification and recovery of toxic or valuable metals from industrial discharged water. One of the most promising bioadsorbents is algae.

Caulerpa lentillifera is a marine green macroalga cultivated as food for animals and humans, and also commonly used to treat wastewater from shrimp farms. Due to its rapid growth rate, farmers often have trouble with the over populated algae.

Turning excess *Caulerpa lentillifera* into bio-adsorbent might present a viable answer to this problem.

Previous work [Sungkhum, 2002] illustrated that this algae exhibited adsorption potential for four types of heavy metals, namely Cu, Cd, Zn and Pb. However, the adsorption capacities for these metals are still not comparable with other types of biosorbent reported in literatures. This work was therefore proposed to investigate the efficiency of various treatment techniques on the enhancement of the adsorption capacities of *Caulerpa lentillifera*.

1.2 Objectives

1.2.1 Investigate the adsorption characteristics of *Caulerpa lentillifera* which underwent various types of pretreatment for the adsorption of Cu, Cd, Zn and Pb.

1.2.2 Determine the appropriate conditions (i.e. pH and equilibrium time) for pretreating algae in order to enhance the heavy metals adsorption capacity of the algae.

1.3 Scopes of this work

1.3.1 Only the adsorptions of Cu, Cd, Zn and Pb were investigated.

1.3.2 The experiment was conducted with synthetic wastewater with concentrations of the heavy metal from 0-100 mg/l.

1.3.3 The pretreatment of *Caulerpa lentillifera* included:

- An acid treatment with 0.5N HCl.
- An alkaline treatment with 0.5N. NaOH.
- A salt treatment with 0.5N of $CaCO_3$, $NaNO_3$, $CaSO_4$-$2H_2O$, Na_2SO_4, NH_4Cl, $Fe(NO_3)_3$-$9H_2O$, Na_2CO_3 and NaCl .

1.3.4 The range of the contact time was 0-60 minutes.

1.4 Benefits of this work

1.4.1 Establishment of basic knowledge on the use of algae in the adsorption of heavy metals.

1.4.2 Alternative technology for the removal or recovery of heavy metals.

1.4.3 Potential development of local technology for the management of wastewater from the electroplating industry.

CHAPTER 2

BACKGROUNDS AND LITERATURE REVIEW

2.1 Microorganisms in heavy metal removal

Microorganisms can accumulate heavy metals and radionuclides from their external environment. Amount of accumulation can be large and variety of physical, chemical and biological mechanisms including adsorption, precipitation, complexation and inter/intracellular transports, may involve. Living and dead cells, as well as products exerted by, or derived from microbial cells, such as cell wall constituents, pigments, polysaccharides and metal-binding proteins, are capable of metal or radionuclide removal from a solution. The removal of radionuclide, metal or metalloid species, compounds and particulates from solution by biological material, particularly by non-directed physico-chemical interactions, is now termed "biosorption". Biosorption is important because the removal of potentially toxic and/or valuable metals and radionuclides from aqueous effluents can result in the detoxification and therefore their safe environmental discharge. Moreover, an appropriate treatment of loaded biomass can enable recovery of valuable elements for recycling or further containment.

The removal mechanisms employed by microorganisms can be quite diversified. Essential metals such as K, Ca, Mg, Cu, Zn Fe, Co, and Mn and those with no essential biological functions such as Cs, Cd, Pb, Al, Sn and Hg can be accumulated by microorganism. The precipitation, complexation, and crystallization of heavy metals and radionuclide exterior to cells can result in detoxification. Moreover, metal ions may be compartmentalized and/or converted to less toxic forms by microorganisms. Most living cells systems exploited to date have been used for the decontamination of effluents containing metals at concentration below toxic levels. These systems may employ a mixture of microorganisms as well as higher plants for the same purpose. For instance, alga and cyanobacterial blooms were used to adsorb Cu, Cd, Zn, Hg and Fe in mining effluents [Fry et al., 1992].

Microorganisms regulate and influence trace element uptake, accumulation, transformation and release, which are direct and indirect mechanisms in natural aquatic systems. Many inorganics compounds are involved in the oxidative and reductive reactions that comprise cellular metabolism. Thus, a participation of toxic substances in biological processes can lead to the chemical transformation of a compound into a form quite different from its origin. Many cationic elements (e.g. Ag, Cd, Hg, and Zn) are sorbed rapidly. There are differences in the accumulation abilities of the between different phytoplankton species; however, that may be driven by physiological or biochemical differences between their cells [Langston and Bebianno, 1998].

2.2 *Caulerpa sp.*

Caulerpa is a macroalga classified in Division *Chlorophyta* (green algae), Class *Caulerpaceae*, Order *Caulerpales*, Family *Caulerpacese*. It grows in various shades of bright green colors, as well as different forms and shapes – some growing tall, others growing as mats. *Caulerpa* spreads almost entirely by asexual growth via fragmentation. A spore producing *Caulerpa* can often release such large numbers of spores that it turns the aquarium water into a milkly-green color. Most species grow well under brilliant lighting in a nutrient rich tank environment. There are over a hundred species of *Caulerpa* found worldwide [HDL-Technical Section, 2001]. Some of the species of *Caulerpa* are edible and are traditionally utilized as food in the form of fresh vegetable or salad. *Caulerpa lentillifera* is one of the favorite edible species due to its soft and succulent texture.

2.3 Mechanisms of biosorption

The kinetics of metal uptake by green algae is a two-step process.

1. The first step involves the passive transport mechanism, which is fast (less than 5-10 minutes), reversible and involves a metabolic-independent surface reaction called "biosorption", an example of this includes the physical sorption or ion exchange of the adsorbate species at the cell surface of cells.
2. The second step involves the active transport mechanism, which involves an uptake of the heavy metals into the living cell. This is a slow metal bonding process (up to 600 hours in some algae), irreversible and metabolism-dependent called "bioaccumulation" [Kojima and Lee, 2001].

Bacteria, cyanobacteria, algae, fungi and yeasts are able to remove metal ions from their surrounding by both mechanisms. Metabolic-independent adsorption of metal ions to cell wall (polysaccharides or other materials) occurs in living and non-viable cells and is generally rapid. Metabolic-dependent intracellular uptake or transport occurs in living cells and is usually a slower process than adsorption, although greater amounts of metal may be accumulated by this mechanism in some organisms. Although both living and non-viable cells are able to accumulate, there may be a difference in the mechanisms involved in either case. The choice of living or non-viable biomass for metal ions removal depends on each particular case because both options have advantages and disadvantages.

2.4 Equilibrium behavior and sorption isotherms

The constant-temperature equilibrium relationship between the quality of adsorbate per unit of adsorbent, *q*, and the equilibrium are the well-known Langmuir and Freundlich sorption isotherms which can be expressed as follows:

Langmuir sorption isotherm:

$$q = \frac{x}{m} = \left[\frac{bC_e\left(\frac{x}{m}\right)_{\max}}{1 + bC_e} \right] \tag{2.1}$$

Freundlich sorption isotherm:

$$q = \frac{x}{m} = K\left(C_e\right)^{\frac{1}{n}} \tag{2.2}$$

where x/m is the solute (metal) concentration in the sorbent (algae); C_e, the equilibrium concentration of solute in the solution; K, a constant, $(x/m)_{max}$, the maximum solute concentration in the sorbent; and b and n are constants related to the energy of sorption.

The Langmuir isotherm was originally formulated based on the following theoretical assumptions:

1) the sorption reaction can be represented as a coordination reaction with 1:1 stoichiometry (i.e. monolayer sorption).

2) the activities of the surface sites are proportional to heavy metal concentration.

3) the number of sorption sites is fixed.

The Freundlich isotherm was based on sorption on a heterogeneous surface, which has generally been considered an empirical relationship and has been used widely to fit experimental data [Kojima and Lee, 2001].

In general, conformity to the Freundlich model suggests adsorption in multilayers. This indicates that the untreated biomass, having the relatively low sorption ability is almost completely saturated and the metal ions are adsorbed on to the surface in a multilayered pattern. The Langmuir model explains a monolayer type of sorption in which the surface of the biomass is not fully saturated. In contrast to the Fruendlich model, the sorption data for the higher concentration range (200-500mg/l) could be fitted to the Langmuir model. Most of study revealed that both the Langmuir and the Freundlich adsorption models were sutiable for describing the biosorption [Ahuja *et al*., 1999; Donmez *et al*., 1999; Kapoor *et al*., 1999; Ozer *et al*., 1999; Bai et al., 2002], whereas some studies reported that sorption fitted to the Langmuir isotherm [Ariff *et al*. 1999; Schneider *et al*. 1999; Schmitt *et al*., 2001; Nuhoglu *et al*., 2002; Pagnanelli et al. 2002; Rangsayatorn *et al*. 2002; Satiroglu *et al*., 2002]. This was also a case where Freundlich was found to be used to fit the experiment [Tien, 2002].

Factors controlling biosorptions on Table 2.1 summarizes experimental findings regarding the effects of various parameters such as pH, metal chemistry, heavy metal concentration, size effect of algae, contact time, temperature, interaction of co-ions, and regeneration and re-use on the biosorption of heavy metals. For a more detailed description of these experiments, the full reviews by Sungkhum [2002] and Davis [2003] should be consulted.

2.5 Pretreatment of biosorbent

There are quite a number of research papers regarding the treatment of bioadsorbents in metal adsorption. These are summarized in Table 2.2. However, the treatment methods can easily be categorized into a few classes and these are summarized as follow:

2.5.1 The Effect of heat treatment

The effect of heat treatment may lead to a better or worse metal adsorption capacity than untreated cell.

1. The improvement effect.

Puranik and Paknikar (1997) observed that certain boiling water pretreatment given to the *Streptoverticillium cinnamoneum* biomass could increase the metal sorption capacity. Kapoor *et al.* (1998) pretreated *Aspergillus niger* (fungi) by boiling it in 0.1N NaOH and showed that a 2.6-3.5 times higher metal's capacity could be achieved. Yin et al. (1999) observed the pretreating fungal biomass by increased their heavy metal uptake capacities.

Heat treatment, such as treatment with boiling water possibly serves as a good cleansing method and thereby exposes the metal binding sites, increasing the loading capacity of the algae or allowing for more complex actions between the algae and metal. These complex actions include the formation of electrostatic bonds, changes in the overall surface charge, a modification of binding sites and the masking/unmasking of binding sites.

2. The inhibition effect.

Heat treatment was also reported to give an adverse effect on adsorption. Yan and Viraraghavan (2000) observed the reduction of a biosorption capacity of autoclaved *Mucor rouxii* biomass (Fungi), when compared with living biomass. This was thought to be attributed to the loss of intracellular uptake. In addition, Whistler and Daniel (1985) reported that heat treatment could cause a loss of amino-functional groups on the fungal. Amino-functional groups were reported to be among the functional groups in the composition of polysaccharides, which were responsible for the binding of heavy metals.

2.5.2 Effect of alkaline treatment

1. The improvement effect.

Most studies found that the alkaline treatment exhibited higher removal capacities of metals for algae and fungi sorption. For instance, Kapoor and Viraraghavan (1998) found that sodium hydroxide, formaldehyde, dimethyl sulphoxide and detergent each of pretreatment significantly improved the biosorption of lead, cadmium and copper. Huang et al. (1996) suggested that an increase in metal biosorption after pretreating the biomass could be due to the removal of surface impurities and the exposure of available binding sites for metal biosorption.

Mameri, et al. (1999) observed that a sodium hydroxide pretreatment on biosorption capacity of the Zn(II) resulted in an increase of about 250% in the amount of Zn(II) biosorbed. Puranik and Paknikar (1999) found that the specific metal uptakes of pretreated-biomass were the highest for the NaOH treatment (higher than physical and other chemical treatments). Sar et al. (1999) reported that the NaOH and NH_4OH treated *Pseudomonas aeruginosa* (bacteria) enhanced the metal loading capacity. Yan and Viraraghavan (2000) observed that the biosorption capacity of alkali treatment was significantly enhanced in comparison with autocalving. Dow and Rubery (1977) found that cell walls of *Mucor rouxii* could be ruptured using the NaOH treatment. Besides, the pretreatment could release polymers such as polysaccharides that possess a high affinity towards certain metal ions.

The clarification of alkaline treatment may be concluded as follows:

(1) The removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal biosorption after pretreatment may be the reason for the increase in metal biosorption (Yan and Viraraghavan, 2000).

(2) The alkali treatment of biomass may destroy autolytic enzymes that caused putrefaction of biomass and removed lipids and proteins that masked reactive sites. (McGahren et al., 1984; Brierleyet et al.,1985; and Muraleedharan and Venkobachar, 1990).

(3) The pretreatment could be due to an exposure of active metal-binding sites embedded in the cell wall or chemical modifications of the cell wall components.

2. No significant effect

There was also evidence indicating that alkaline treatment had no particular advantage to the biosorption. For instance, Ahuja et al. (1999) observed that the pretreatment of *Oscillatoria anguistissima* (Cyanobacteria) did not significantly affect the biosorption of Zn. However, they did not explain any reason of this result.

2.5.3 Effect of acid treatment

The acid treatment was usually found to inhibit metal biosorption. Yan and Viraraghavan (2000) observed that acid pretreatment (HCl and H_2SO_4) significantly decreased the biosorption of heavy metals, which was in good agreement with the observation of Kapoor and Viraraghavan (1998) in the case of *Aspergillus Niger*.

The reasons for the above observations may be that the H^+ ions bound to the biomass after acid treatment, which reduced the available binding sites for metals (Yan and Viraraghavan, 2000). The polymeric structure of the biomass surface exhibits a negative charge due to the ionization of organic groups and inorganic groups. Greater biomass electronegativity leads to the attraction and easy adsorption of heavy metal cations. The remaining H^+ ions on the acidic pretreated biosorbent may change the biomass electronegativity, resulting in a reduction in its bioadsorption capacity (Kasan, 1994).

2.5.4 Effect of salt compound treatment

1. Improvement effect.

Matheickal and Yu (1999) observed that both of Pb and Cu's uptake capacities for the marine algae pretreated by $CaCl_2$ far exceeded the uptake capacities of activated carbon, zeolite and other biosorbents, and were comparable with those of the commercial ion exchange resin. Yin et al. (1999) reported that the pretreated fungal biomass by calcium solution led to an increase in its heavy metal uptake capacities.

Kaewsarn and Yu (2000) observed that the biomass of marine alga *Padina* sp. by $CaCl_2$, the maximum removal capacity for Cd of 0.53 mmol/g could be obtained by pretreating. This study demonstrated that the pre-treated biomass could be used as an efficient biosorbent for the treatment of cadmium(II)-bearing wastewater streams. Therefore, Kaewsarn (2002) observed the pre-treated biomass of marine alga *Padina* sp. by $CaCl_2$ and reported that the maximum removal capacity of the biomass for Cu was 0.80 mmol/g.

2. No significant effect.

Some researchers found that pretreatment with salt did not lead to appreciable improvement in the adsorption capacity of the algae. Puranik and Paknikar (1999) observed the Zn uptake of a *Citrobacter* strain, MCM B-181 after pretreatment by both sodium carbonate and ammonium sulfate and reported that these treatments had no effect on its sorption capacity. Yan and Viraraghavan (2000) found that pretreatment using $CaCl_2$ and NaCl for *M. rouxii* (fungi) slightly reduced its bioadsorption capacity. However, no explanation on the detailed mechanisms of this pretreatment was given.

Clearly there is a potential for the development of biosorption technology for the removal of heavy metal. Lately, Sungkhum (2002) evaluated the biosorption of heavy metals using *Caulerpa lentillifera* and found that this marine algae could be employed as a biosorbent. This work intends to further investigate the adsorption performance of this algae with various pretreatment methods.

Table 2.1 Adsorption of heavy metals by bioadsorbents: Examples

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
Sungkhum (2002)	*Caulerpa lentillifera*, marine macroalga / Cu, Cd, Zn and Pb	pH effect In most cases, the removal efficiency increased steadily with pH value. But Pb presented different a character as the removal efficiency increased with pH only at a very low pH range afterwhich, the removal reached a max. of 80 – 90%. The removal efficiency decrease when pH > 5.5. Kinetics of biosorption In all cases, the metals uptake was rapid with the system reaching 80-90% of the equilibrium within 20 min. After the optimal time, the removal efficiencies became quite independent with adsorption time with a very slow increasing rate.	Particle size effect Most of percent removals are independent of particle size. However, q_{max} of Pb for both cases (normal and ground size) similar but the ground algae occurred at a significantly faster rate. Desorption study 1. The adsorption on the surface of the algae should not involve Van Der Waal's forces. 2. Bonding between heavy metals and the algae must have involved the formation of strong molecule bonds such as ionic or covalent bonds.
Matheick-al and Yu (1999)	*Durvillaea potatorum* and *Ecklonia radiata*, Australian	pH effect q_{max} increased with the solution pH, which sharply increased between pH levels of 2	Homogeneity of biomass samples The biosorbent samples were rather homogeneous.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
	marine algae / Pb and Cu	and 3 and reached its plateau at a pH of 4.5. Kinetics of biosorption The uptake rate was rather fast and 90 % of the total uptake occurred in 30 min. However, after 30 min., there was a slower rate of uptake until about 1 h, afterwhich no further significant adsorption was observed.	Effect of light metal ions on biosorption The biosorbents have much higher relative affinities for heavy metal ions than for the light metal ions studied. Effect of anionic ligands on heavy metal adsorption It is possible for EDTA to be used as an effective recovering agent for Cu ions from the loaded biosorbent. Organic leaching from biomass Organic leaching was high from the raw biomass in all cases. However, the modified biomass showed a negligible leaching of organics even at low pH values, indicating the stabilisation of the biomass attained through the modification process.
Yin et al. (1999)	*Rhizopus oryzae*, *Rhizopus oligosporus*, *Aspergillus*	pH effect q_{max} of the pretreated biomass are the sharpest increase between pH values of 4.0 and 5.5.	

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
	oryzae, and *Rhizopus arrhizus*, Fungi / Cd	<u>Biosorption kinetics</u> About 90 % of the Cd uptake occurred within 10 min. and an equilibrium was reached in 30-40 min.	
Kaewsarn and Yu (2000)	*Padina* sp. marine alga / Cd	<u>Effect of pH</u> 1. Cd adsorption capacity reached a maximum at a pH of 5. 2. The pH effect may be from the competition effect between the H_3O^+ ions and Cd(II) ions. Metal ions that could associate with the cell wall ligands would have to compete with the H_3O^+ ions for the active sites. <u>Kinetics</u> 90 % of the total soluble Cd was removed within 35 min. Afterwards, a small decrease in the soluble Cd concentration was still noticed for the next 5 h.	

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
Yu et al. (1999)	Macroalga 9 spicies / Cd, Cu, and Pb	Adsorption isotherms The equilibrium isotherms were analyzed by the Langmuir equation and the general affinity sequence was Pb > Cu > Cd.	
Kaewsarn (2002)	*Padina* sp., marine alga / Cu	pH effect The sharpest uptake increase was between pH values of 3 and 4 and the highest occurred at a pH of 5. Adsorption isotherms The Langmuir model was used to correlate the isotherm data obtained at constant solution pH values. The adsorption capacity of *Padina* sp. (0.80 mmol/g) was relatively high when compared with other adsorbents. Adsorption kinetics 90% of the total soluble Cu was removed within 15 min. Afterwards, slower rates of uptake continued for about 30 min and no further	Effect of light metal ions The effect of Na^{+} on Cu uptake was negligible at a concentration of 10 mM, and K^{+},Mg^{2+}, and Ca^{2+} reduced the removal efficiency by 4%, 11 %, and 13%, respectively. Therefore, the presence of Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} did not significantly interfere with the binding of copper(II) ions. Fixed-bed breakthrough curves The adsorption capacities of column for various initial concentrations ranged between 0.78 and 0.83 mmol/g, which agreed well with the maximum value of 0.80 obtained from batch experiments.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		significant adsorption was observed beyond this period.	
Mameri et al. (1999)	*Streptomyces rimosus*, Antibiotic product / Zinc	Adsorption kinetics 1. The biosorption capacity was fixed the limiting value of Zn (II), was reached after 240 min. 2. The chemical treatment increased considerably the kinetic constant, *K*.	Effect of particle size The biosorption capacity depended on the particle size of the biomaterial. The best performances were obtained with the smaller particle sizes (22-140 and 140-250 μm). the 140-250 μm class was selected to avoid the floating phenomenon of the biomass observed with the 22-140 μm class. Temperature effect The increase in temperature led to a reduction of the biosorption capacity of the biomass. Influence of the stirring speed on the biosorption capacity Moderate stirring speed gave a better homogeneity to the mixture solution-biomass granulates. At stirring speeds lower than 250 rpm, the biomass

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
			granulates agglomerated and may take much more time to reach the equilibrium. The biosorption mechanism The ion exchange between Zn(II) ions and hydronium ions of some compounds of the biomaterial was believed to be involved in the biosorption mechanism. The carboxylic and amino groups were mainly involved in this ion exchange. The infrared analysis of the biomass performed before and after the Zn(II) biosorption showed that the amino and carboxylic bands disappeared after the saturation of the active sites of the biomass.
Gardea-Torresdey et al. (1998)	*Larrea tridentata*, Creosote bush / Pb, Cd, Zn, Cr (III), Cr(VI),	Effect of pH Maximum binding occurred at pH values of 5 and 6. Kinetic adsorption Rapid binding occurs	

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
	and Ni	within 15 min.	
Haluk Ceribasi and Yetis, (2001)	*Phanerochaete chrysosporium* , White-rot fungus / Ni(II) and Pb (II)	Adsorption kinetics Rapid surface adsorption occurred within the first 30 min, where take place a slow intracellular diffusion till the end of the 3 h contact time. In the first minute of contact solution pH decreased sharply parallel to the fast metal uptake, probably because of the protons released by the biosorbent. Adsorption isotherms q_{max} was 109.5 mg/g and was reached at a pH of 5. Under these circumstances Ni(II) and Pb(II) uptake capacities were 55.9 mg Ni/g and 53.6 mgPb/g, respectively.	Initial metal concentration effect Metal biosorpiton capacities increased as the initial metal concentrations increased, independent of initial pH, and generally the metals with higher C_i had a higher uptake capacity.
Yan and Viraraghavan (2001)	*Mucor rouxii*, Fungi / Pb, Cd, Ni, and Zn	Adsorption isotherms 1. For single-component metal solutions, the metal removal capaicties of the beads for Pb, Cd, Ni , and Zn were 4.06,	Desorption study The adsorbed metal ions were easily desorbed from the beads with 0.05N HNO_3 solution. After acid desorption and regeneration with

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		3.76, 0.36, and 1.36 mg/g, respectively. 2. or multi-component metal solutions containing Zn, Cd, and Ni, the capacities were 0.40, 0.36, and 0.31 mg/g, respectively.	deionized water, the beads could be reused to adsorb metal ions at a comparable capacity.
Yakup et al. (2001)	*Trametes versicolor* , White-rot fungus / Cd	<u>Adsorption isotherms</u> The q_{max} for entrapped dead live fungal mycelia of T. versicolor were found as 102.3±3.2 mg Cd(II)/g and 120.6± 3.8mg Cd(II)/g, respectively. <u>Biosorption equilibrium</u> It was established in about 1 h. and biosorption was well described by both Langmuir and Freundlich biosorption isotherms.	<u>Desorption study</u> The biosorbents were reused in three consecutive adsorption/desorption cycles without a significant loss in their biosorption capacity.
Say et al. (2001)	*Phanerochaet chrysosporium*, The filamentous fungi / Cd (II), Pb(II), and Cu(II)	<u>Effect of pH</u> The maximum adsorption of different heavy metal ions on the fungal biomass was obtained at a pH of 6.0.	

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		<u>Kinetic adsorption</u> The biosorpiton equilibrium was established after about 6 h. The data were in good agreement with those calculated by the Langmuir model.	
Kapoor et al. (1999)	*A. niger*, Fungi / Cd, Cu Pb, and Ni	<u>Effect of pH</u> 1. The biosorption of metal ions was inhibited at a pH of 3.0, and sharply increased when the pH of the solution was increased to 4.0. 2. Biosorption on the pretreated biomass followed the Fruendlich and Langmuir adsorption models, except at the pH value of 4.0 for Pb, Cd,and Cu and at a pH value of 5.0 for Ni. <u>Kinetic adsorption</u> The biosorption of Pb and Cd reached equilibrium in 5 h., and equilibrium was reached	<u>Desorption study</u> 1. The biosorbed metal ions were effectively eluted by a 0.05 N HNO_3 solution 2. The pretreated biomass could be used for five cycles of biosorption – elution of biosorbed ion – regeneration of biomass.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		in 6 and 8 h. for Cu, and Ni, respectively.	
Puranik, and Paknikar (1999)	Strain MCM B-181, *Citrobacter* sp. / Pb, Cd, and Zn	<u>Effect of pH</u> When the pH increased, specific metal uptake was enhanced. However, the optimal pH for maximal specific uptake for each of the three metals differed (4.5, 6.0, and 6.5 for Pb, Cd, and Zn, respectively). Further increase in the solution pH decreased the uptake of metals. <u>Kinetic adsorption</u> 1. The metal uptake was rapid in the first 5 min of contact, accounting for about 85% of the sorption of Pb and 70% of both Cd and Zn. Time required for attaining an equilibrium was < 30 min. 2. The rate of uptake during the entire course of biosorption was independent of an initial metal	<u>Effect of initial metal concentration</u> The specific metal uptake increased with an increase in the initial metal concentration due to an increase in the electrostatic interactions (relative to covalent interactions), involving sites of progressively lower affinity for metal ions. <u>Effect of biomass concentrations</u> There was a decrease in the specific metal uptake with an increasing biomass concentration. This could be attributed to interference between the binding sites. <u>Effect of competing cations</u> The biomass exhibited a preferential order of metal sorption as Pb > Zn > Cu > Cd > Ni > Co

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		concentrations used (0.5, 1.0, and 1.5 mM).	Desorption study 1. At elution with 0.1M HCl, desorption efficiency was higher than that eluted by EDTA but the sorption capacity of the beads decreased in subsequent cycles. This could be due to a deleterious effect of that acid treatment on biomass as was observed while studying the effects of pretreatment. 2. Elution with 0.1M EDTA, the sorption capacity of beads was found to be afffected in subsequent cycles but desorption capacity decreased considerably.
Sar et al. (1999)	Lyophilized *Pseudomonas aeruginosa*, Bacterium and IRA 400 cation exchanger /	Effect of pH The optimum pH for Cu adsorption was 7.0 and 8.0 for Ni. Isotherm adsorption Baterial biomass showed significant sorption of	Bimetallic effect Na, K or Ca increased the sorption of Ni as well as Cu in contrast to Cd or Pb. Desorption study 1. Mineral acid (HCl, , H_2SO_4, and HNO_3)

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		both Ni or Cu, and was superior over the cation exchanger, IRA 400. Kinetic adsorption Biosorption occurred within the first 10 min. followed by an almost negligible increase after 20 min.	and chelating agent , NTA, could recover more than 75% of Ni or Cu adsorbed on the biomass. 2. Calcium carbonate (10mM) was efficient in Ni desorption (71%) compared to Cu (57%). 3. Sodium carbonate remained specific for Cu remobilization (88%) than Ni (21%).
Jalali et al. (2002)	Brown algae (*Sargassum hystrix*, *S. natans*, and *Padina pavonia*); Green algae (*Ulva lactuca* and *Cladophora glomerata*); and Red algae (*Gracilaria corticata, G.canaliculata, and*	Effect of pH An increased uptake of metal with increasing pH was demonstrated. Kinetic adsorption The biosorption of lead was rapidly occurred and most of the sorbed metal was bound in less than 30 min of contact. Isotherm adsorption *S. hystrix* > *S. natans* > *P. pavonia* removed lead most efficiently from an aqueous solution, respectively.	Desorption study 1. Desorption was conducted by decreasing the pH values to lower than 1.0. 2. Removal of lead from *Sargassum* was efficiently performed by 0.1M HNO3 for 15 min. and a high degree of metal recovery was observed (95%). 3. For optimum operation in the subsequent metal uptake cycle,

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
	Polysiphonia violacea) / Pb		regeneration of the *Sargassum* was efficiently performed by 0.1 M $CaCl_2$ for 15 min; it was total and reversible. In the repeated use of biomass, the lead uptake capacity of *Sargassum* was remained constant (98%) and no significant biomass damage took place after 10 sorption-desorption cycles.
Yin et al. (2001)	*Laminaria japonica*, Marine alga / Cd	<u>Effect of pH</u> The maximum uptake capacity was observed at a pH of 6. <u>Kinetic adsorption</u> Less than 90% of the adsorption occurred within 20 min. and equilibrium was reached within 1 h. <u>Isotherm adsorption</u> The equilibrium isotherm can be described well with the Langmuir adsorption equation.	<u>Desorption study</u> The adsorbed Cd cannot be desorbed by distilled water, but it can be effectively recovered by using acidic or EDTA solutions.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
Kratochvil (1997)	*Sargassum Fluitans*, Brown marine alga / Cu		Desorption study HCl is more effective eluent of Cu than $CaCl_2$. Other The pH would have to be adjusted with $Ca(OH)_2$, since the addition of NaOH would cause the leaching of aliginate which is considered to be the key Cu-binding component in the marine algal biomass.
Satiroglu (2002)	*Polyporus versicolor*, White-rot basidiomycete / Zn, Cd, and Hg	Effect of pH The q_{max} of metal ions on the three different forms of *P. versicolor* was observed at a pH of 6.0. Kinetic adsorption 1. The adsorption rate was high at the beginning but plateau values were reached in 60 min. 2. Explained two stages; an initial rapid uptake due to surface adsorption on the cell walls and a subsequent slow uptake due to	Desorption study The biosorbent could be regenerated using 10 mM HCl solution, with up to a 98% recovery and it could be reused in five biosorpton - desorption cycles without any considerable loss in its biosorption capacity.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		membrane transport of the metal ions to the cytoplasm of the cells. The effect of temperature Temperature change between 15 and 45°C did not affect the biosorption capacity.	
Ahuja et al. (1999)	*Oscillatoria anguistissima*, Cyanobacterium / Zn	The effect of pH Bisorption of Zn was pH dependent and the pH optima was 5.0. The value declined by about 30% at a pH of 6. The effect of temperature Temperature in the range 25-45°C did not produce any significant difference in Zn adsorption at a pH of 5.0.	Effect of biomass concentration The specific uptake of metal decreased with an increase in biomass concentration. Desorption study The zinc bound to the biomass could be effectively stripped using EDTA (10 mM) and the biomass was effectively used for multiple sorption-desorption cycles with in between charging of the biomass with tap water washing.
Puranik, and Paknikar (1997)	Streptovertilcillium. *cinnamoneum*, Waste biomass generated by	The effect of pH The optimum pH range for lead uptake was 3.4-4.5 while it was 5.0-6.0 for zinc.	Desorption study Treatment with 0.1 M Na_2CO_3 permitted the reuse of the desorbed biomass although the metal loading capacity in

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
	an aureofungin fermentation plant / Pb and Zn	Kinetic adsorption Greater than a 90% uptake of lead and zinc by *S. cinnamoneum* was achieved in 15 and 5 min, respectively.	the subsequent cycles decreased by 14-37%. *Other* Lead and zinc adsorption data when applied to Fruendlich and Langmuir isotherm equations showed a good correlation (R^2 = 0.97) and hence equal conformity to both models.
Nuhoglu et al. (2002)	Ulothrix *zonata*, Green alga / Cu	The effect of pH The optimum pH was determined to be 4.5 Adsorption isotherm The data were fitted to the Langmuir model (R^2 = 0.97). The high fitness of the Langmuir model for this biosorption system indicates the monolayer coverage of Cu^{2+} on the outer surface of the biosorbent. The negative values of free energy change (ΔG^0 = -12.60 kJ/mol) indicate the spontaneous nature of biosorption.	Biomass concentration When the dried alga concentration was increased from 0.1 to 1.0 g/l, the amount adsorbed decreased approximately from 160 to 38 mg/l.

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
Ariff et al. (1999)	*Rhizopus. oligosporus*, Fungus / Pb	1. The optimum biomass concentration and initial solution pH for lead sorption at initial lead concentrations ranging from 50-200 mg/l was obtained at 0.5 g/l and a pH of 5, respectively. 2. The ratio of initial lead concentration to biomass concentration ratio, the highest lead adsorption was obtained at 750 mg/g which gave a q_{max} of 126 mg/g. 3. Data of lead sorption by *R. oligosporus* fitted well into the Langmuir sorption isotherm model, indicating that the sorption was similar to that for an ion-exchange resin. This means that the sorption is a single layer metal	1. The cell wall of microorganisms, consisting mainly of polysaccharides, proteins and lipids, offered many functional groups, which could bind metal ions e.g. carboxylate, hydroxyl, sulphate, phosphate and amino groups. 2. The resulting spectra of X-ray analysis for native and lead exposed cells did not indicate any crystalline structure, implying the incorporation of lead within the cells did not cause any changes in the amorphous structure of *R. oligosporus*. 3. TEM and EDX analyses, it was concluded that the exposure of powderized *R. oligosporus* to solution containing lead caused

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		layer metal adsorption that occurred as a molecular surface coverage. 4. The increase in lead adsorption with the increasing of C_0/x has two possible explanations (Fourest and Roux 1992). Firstly, the reduction of the biosorbent concentration decreased the electrostatic interaction between cells, which in turn, increased the lead adsorption rate. Secondly, an increased ratio of initial lead concentration to the biosorbent conc. caused an increased number of lead ions available around the cells, environment, which were more easily bound to the active sites of the	the lead ions to adsorb to the surface of the cell wall and very little or none was absorbed into the cells. This meant that the biosorption of lead ion by *R. oligosporus* cells was mainly an adsorption phenomenon. 4. Adsorption on the external cell surface was a biomass defence system against toxic heavy metals, the microorganism producing an external polymeric layer to avoid metal penetration through the cell wall (Scott & Palmer 1990). This was known as a “microprecipitation mechanism” in which ions were removed from solution through the precipitation process, enhanced by the compounds produced by the active

Author	Biosorbent / Metals	pH effects / Kinetic / Isotherm	Others results
		active sites of the cells as long as the active sites were not saturated.	defense system of the cells.

Table 2.2 Adsorption of heavy metals by pretreated biosorbents: Examples

Author	Biosorbent / Metals	Pretreatment Results
Yan and Viraraghavan (2000)	*Mucor rouxii*, Fungi / Pb, Cd, Ni, andZn	1. Alkali pretreatment (NaOH, and detergent) improved their biosorption capacity. 2. Pretreatment using $CaCl_2$ and NaCl slightly reduced the biosorption capacity. 3. Acid pretreatment (HCl, H_2SO_4, and $C_2H_4O_2$) resulted in a significant reduction in the biosorption capacity.
Matheickal and Yu, (1999)	*Durvillaea potatorum* and *Ecklonia radiata*, Australian marine algae / Pb and Cu	The pre-treated biomass had much higher the maximum adsorption capacities of Pb and Cu than commercial ion exchange resins.
Kapoor and Viraraghavan (1998)	*Aspergillus Niger*, Fungi / Pb, Cd, and Cu	Pretreatment Results 1. Pb 1.1 Pretreated by NaOH, detergent, formaldehyde, and dimethyl sulphoxide increased the biosorption of lead by 3 times incomparison with live biomass. 1.2 Pretreated by CH_3COOH, ammonium persulphate, ether, and ethanol also increased the biosorption of lead by about 1.5 – 2.0 times. 1.3 Pretreated by autoclaving, Ơ-phosphoric acid, and H_2O_2 marginally decreased lead biosorption. 2. Cd 2.1 Pretreated by NaOH, detergent, formaldehyde, and dimethyl sulphoxide increased biosorption of Cd by more than 2.4 times in comparison to live biomass. 2.2 Pretreated by ammonium persulfate, ether,

Author	Biosorbent / Metals	Pretreatment Results
		and ethanol also resulted about 1.5 times increased. 2.3 CH_3COOH significantly reduced biosorption. 2.4 H_2O_2 and Ơ-phosphoric acid inhibited biosorption. 3. Cu 3.1 Pretreated by NaOH, detergent, formaldehyde, and dimethyl sulphoxide increased biosorption of Cd by more than 3.75 times in comparison with live biomass. 3.2 Pretreated by ammonium persulphate, Ơ-phosphoric acid, CH_3COOH, ether, and ethanol also resulted in improvement. 3.3 Autoclaved wasn't improved. 3.4 H_2O_2 inhibited biosorption. 4. Ni 4.1 NaOH, detergent, formaldehyde, and dimethyl sulphoxide resulted in lower biosorption. 4.2 H_2O_2 and Ơ-phosphoric acid resulted in inhibition. Explaination of pretreatment results 1. An exposure of active metal-binding sites embedded in the cell wall or chemical modifications of the cell wall components. 2. Removal of surface impurities.
Yin et al. (1999)	*Rhizopus oryzae*, *Rhizopus oligosporus*, *Aspergillus oryzae*, and *Rhizopus*	The maximum adsorption capacities of biomass from high to low were *R. arrhizus* > *A. oryzae* > *R. oligosporus* > *R. oryzae.*

Author	Biosorbent / Metals	Pretreatment Results
	arrhizus, Fungi / Cd	
Mameri et al. (1999)	*Streptomyces rimosus*, Antibiotic product / Zinc	<u>The influence of a sodium hydroxide pretreatment on Zn(II) biosorption capacity</u> 1. About 35% of the biomass was lost after this chemical treatment. 2. The infrared spectrum showed that the amino and carboxylic bands disappeared after the NaOH treatment suggesting again a H^+ / Na^+ inter conversion. 3. The chemical treatment resulted in an increase of about 250% in the amount of Zn (II) biosorbed.
Gardea-Torresdey et al. (1998)	Alfafa biomass, Plant tissue / Cu, Ni and Pb	1. Acetylation of amino ligands on the alfafa biomass resulted in a small reduction of metal binding for Cu and Ni, while no significant change was observed for Pb at a pH value of 5.0. 2. Succination increased the binding of Cu, Ni, and Pb. 3. Sulfydryl ligands were chemically blocked resulting in no significant change in metal binding for Cu, Ni, and Pb.
Kapoor et al. (1999)	*Aspergillus niger*, Fungi / Cd, Cu Pb, and Ni	1. The biosorption of Cu > Pb > Cd on pretreated A. niger was approxiamately 350%, 320%, and 260% times higher than the comparable biosorption by live biomass. 2. Ni biosorpton by the pretreated biomass was lower than that of live biomass.

Author	Biosorbent / Metals	Pretreatment Results
Puranik and Paknikar (1999)	*Citrobacter* Strain MCM B-181 / Pb, Cd, and Zn	The specific metal uptakes of pretreated-biomass (mg metal/g biomass) were from high to low as: NaOH > triron X-100 > untreated > ethanol > $Na_2(CO_3)$ > $(NH_4)_2SO_4$ > acetone > methanol > HCl > H_2SO_4 > autocalving > boiling water
Sar et al., (1999)	Lyophilized *Pseudomonas aeruginosa*, Bacterium and IRA 400, cation exchanger / Cu and Ni	Pretreatment details 1. Inorganic (0.1N of HCl, H_2SO_4, and HNO_3; 0.1 mM of NaOH and NH_4OH) 2. Organic (ethanol, toluene, acetone and an methanol-chloroform mixture (3:1)) 3. Surfactant (0.1% SDS, Sigma, USA) 4. Enzyme (Lysozyme, 1mg/ml, Sigma), along with heat treatment (oven drying, 80 º C, 2h) as well as autoclaving (15 lbs, 120 º C, 15 min.) Pretreatment results 1. NaOH, NH_4OH or toluene enhanced the metal loading capacity. 2. Oven heating (80 ºC), autoclaving (120ºC, 15lb/in^2), acid, detergent and acetone treatments were inhibitory.
Yin et al. (2001)	*Laminaria japonica*, Marine alga / Cd	Biomass pre-treated with calcium solution exhibited a higher (about 30%) uptake capacity and can be settled from aqueous solutions.
Kratochvil et al. (1997)	*Sargassum Fluitans*, Brown marine alga / Cu	1. The Ca-cycle applied to the biomass in a packed bed led to a higher degree of a column utilization but did not allow an effective Cu recovery. 2. The H-cycle permitted 100% Cu recovery but also shortened the sorption column service time.

Author	Biosorbent / Metals	Pretreatment Results
		3. The Ca-cycle applied to the biomass in a packed bed led to a higher degree of a column utilization but did not allow an effective Cu recovery. 4. The H-cycle permitted 100% Cu recovery but also shortened the sorption column service time. 5. The combined Ca/H-cycle was shown to be inefficient due to the time consuming regeneration of biomass from the H-form to the Ca-form. 6. Pretreatment with 1% (w) of $CaCl_2$ and with 0.1M of HCl resulted in the same Cu uptake of 75 mg/g. 7. The Ca-pretreated biomass lost 30% of its Cu capacity with subsequent acidic wash.
Satiroglu et al. (2002)	*Polyporus versicolor*, White-rot basidiomycete / Zn, Cd, and Hg	1. The order of affinity of the biosorbents was found as: NaOH treated > heat-inactivated > active. 2. The order of the amount of metal ions adsorbed was Hg(II) > Cd(II) > Zn(II).
Ahuja et al., (1999)	*Oscillatoria anguistissima*, Cyanobacterium / Zn	1. Pretreatment of biomass at a high temperature and with alkali did not significantly affect the biosorption of Zn. 2. HCl conditioned biomass showed a significant reduction in zinc adsorption compared with the native biomass.
Puranik and Paknikar, (1997)	*Streptovertilcillium cinnamoneum*, Waste biomass generated by an aureofungin	1. Boiling water was the most effective pretreatment for both metals. 2. H_2SO_4 pretreatment adversely affected the loading capacity of both metals.

Author	Biosorbent / Metals	Pretreatment	Results
	fermentation plant / Pb and Zn		

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Equipment and chemicals

Equipment:

- Rotary shaker, GFL
- Laboratory refrigerator, Puffer Hubboard
- Fume cupboard, Newlab
- Shaking water bath, GFL
- Suction pump, UNF Neuberger
- Hot plate, Stuart Scientific
- Magnetic stirrer, Clifton
- pH-meter, HACH
- Oven, Binder
- Analytical balance, Sartorius
- Dessicator
- Filter paper No. 93, Whatman
- Filter paper GC50, Toyo Roshi Kaisha, Ltd.
- Inductively Coupled Plasma (ICP) , Vista-MPX, CCD Simultaneous ICP-OES

  Wavelength (λ) for Cu: 327.395 nm
  Wavelength (λ) for Cd: 214.439 nm
  Wavelength (λ) for Zn: 213.857 nm
  Wavelength (λ) for Pb: 220.353 nm
- Fourier Transform Infrared Spectrometer (FTIR), Perkin Elmer, Model 1760X
- Chemical protective mask, NIOSH 3M/6003

Glassware:

- Erlenmeyer flasks
- Volumetric flasks

- Test tubes
- Beakers
- Pipettes
- Auto Pipettes, Eppendorf
- Cylinders
- Funnel

Chemical reagents:

- $Cu(NO_3)_2$, Analytical Univar Reagent
- $Cd(NO_3)_2$, Laboratory Unilab Reagent
- $Zn(NO_3)_2$, APS Ajax Finechem
- $Pb(NO_3)_2$, Laboratory Unilab Reagent
- HNO_3, AnalaR
- NaOH, Carlo Erba Reagent
- HCl, BDH Analar
- $CaCO_3$, Fisher Chemicals
- $NaNO_3$, Analytical Univar Reagent
- $CaSO_4\text{-}2H_2O$, Carlo Erba Reagent
- Na_2SO_4, Carlo Erba Reagent
- NH_4Cl, Carlo Erba Reagent
- $Fe(NO_3)_3\text{-}9H_2O$, Analytical Univar Reagent
- Na_2CO_3, Scharlau
- NaCl, Analytical Univar Reagent
- Deionized water
- pH 7 and 4 buffer solution, Scharlau
- ICP multi-element standard solution, MERCK

3.2 Methodology

3.2.1 Algal preparation

Caulerpa lentillifera was collected from Banjong farm in Chachoengsao province.

3.2.2 Glassware preparation

- Wash the glassware with water.
- Immerse glassware in 10% HNO_3 overnight.
- Wash the glassware with water to make sure that there is no acid deposited inside the glassware.
- Rinse the glassware with deionized water.
- Dry the glassware in a 100 ^{O}C oven.

3.2.3 Preparation of the algae

- Wash the algae with deionized water.
- Dry the algae at 80^{O}C for 12 hours.
- Store the algae in a dessicator.

3.2.4 Preparation of the synthetic wastewater

- Dissolve calculated metal nitrate for 100 mg/l with deionized water and preserve it in an acidic condition (i.e. below a pH value of 2.0), and used as a stock solution.
- Dilute the stock solution to 10, 25, 50 and 75 mg/l of metal concentration.
- Store the stock solution in a refrigerator at 4^{O}C.

3.2.5 Preparation of pretreated algae

- Submerge 12 g of dry algae in 100 ml of various chemicals, i.e. HCl, $CaCO_3$, $NaNO_3$, $CaSO_4$-$2H_2O$, Na_2SO_4, NH_4Cl, $Fe(NO_3)_3$-$9H_2O$, Na_2CO_3, and NaCl, each at 0.5N.
- Shake the containers slowly using a rotary shaker for 24 hours.
- Wash the pretreated algae with plenty of water.
- Dry the pretreated algae at 80^{o}C for 12 hours.
- Record the weight before and after the pretreatment to calculate the percent of algal weight loss.
- Store the algae in the dessicator.

For the case of pretreating with 0.5N NaOH, follow the preparation steps below:

- Submerge 12 g of dry algae in 1 litre of selected chemicals at 0.5N.

- Slowly stir for 0.5, 2.0 and 24.0 hours.
- Follow preparation procedure starting from the second step described above.

For the case of pretreatment with hot 0.5N NaOH, the preparation steps are as follows:

- Submerge 12 g of dry algae in 1 litre of selected chemicals at 0.5N.
- Heat the solution to 60°C for 5 and 10 min.
- Follow the preparation procedure starting from the second step described above.

The summary table of the pretreatment is provided in Table 3.1.

3.2.6 Preparation of pretreated algae for the isotherm test

This preparation was only applied to some of the selected pretreated algae as discussed in Chapter 4. Also this preparation was employed for the experiment with 0.5N NaOH when it was evaluated for the effects of exposing time.

- Submerge 12 g of dry algae in 1 litre of selected chemicals at 0.5N.
- Shake the containers slowly using a rotary shaker.
- Wash the pretreated algae with plenty of tap water.
- Dry the pretreated algae at 80°C for 12 hours.
- Record weight before and after the pretreatment to calculate the percentage of algae weight loss.
- Store the algae in the dessicator.

3.2.7 Solubility test

- Prepare the metal solution at a concentration of 100 mg/l.
- Adjust the pH in each solution using 0.03N of nitric acid and sodium hydroxide, and vary the pH to a range of 4-7.
- Shake the solution in a rotary shaker at 150 rpm.
- Measure the heavy metal concentration by Inductively Coupled Plasma (ICP).
- Compare the concentrations obtained from the experiment with calculation.

3.2.8 Determination of heavy metal removal

- Submerge 30 mg of prepared algae in 30 ml of solution containing 100 mg/l of metal.
- Shake the solution with a shaker at a rate of 150-200 rpm for 60 minutes.
- Isolate solid phase with filter paper.
- Preserve the sample with HNO_3 solution.
- Measure the heavy metal concentrations of the filtrated solution.
- To investigate the adsorption isotherm, repeat the experiment with the metal concentration range from 0-100 mg/l.

Experiments were repeated at least three times to ensure the accuracy of the experimental results. In the case that the results were not clear, further repetitions were carried out.

3.3 Calculation

Cu, Cd, Zn and Pb adsorption capacity is calculated from:

$$q = \frac{(C_i - C_e)V}{W} \tag{3.1}$$

where		
	q	is the metal adsorption (mmol/g dry weight)
	C_i	is the initial metal concentrations (mmol/l)
	C_e	is the equilibrium metal concentrations (mmol/g)
	V	is the volume of the metal concentration (ml)
	W	is the dry weight of the biomass-added (g)

3.4 Analytical experiment

3.4.1 Determination of the heavy metal concentrations

The quality of heavy metal in the solution was determined using Inductively Coupled Plasma (ICP) where the quantity of heavy metals accumulated in the algae was subsequently calculated from a simple mass balance equation of the heavy metal.

3.4.2 Determination of functional groups of cell walls of algae

The functional groups of dried algal mass was determined using FT-IR (Fourier Transform Infrared Spectrometer) at the Scientific and Technological

Research Equipment Center, Chulalongkorn University. FTIR spectra were obtained for the biomass samples. Samples of 100 mg KBR disks containing 1% finely ground powder (<20 μm) of each biomass sample were prepared for this study.

Table 3.1 Description of various pretreatment for *Caulerpa lentillifera*

No.	Treating chemical	Concentration, Normality	Time, hours	Temp., ^{o}C
1	Unpretreated			
2	NaOH	0.5	1	Room*
3	NaCl	0.5	24	Room*
4	Boiling in NaOH	0.5	1	100
5	NaOH	0.5	24	Room*
6	$CaCO_3$	0.5	24	Room*
7	Boiling in $CaCO_3$	0.5	1	100
8	$NaNO_3$	0.5	24	Room*
9	Boiling in $NaNO_3$	0.5	1	100
10	$CaSO_4$-$2H_2O$	0.5	24	Room*
11	Boiling in $CaSO_4$-$2H_2O$	0.5	1	100
12	Na_2SO_4	0.5	24	Room*
13	Boiling in Na_2SO_4	0.5	1	100
14	NH_4Cl	0.5	24	Room*
15	Boiling in NH_4Cl	0.5	1	100
16	$Fe(NO_3)_3$-$9H_2O$	0.5	24	Room*
17	Boiling in $Fe(NO_3)_3$-$9H_2O$	0.5	1	100
18	Na_2CO_3	0.5	24	Room*
19	Boiling in Na_2CO_3	0.5	1	100
20	Boiling in NaCl	0.5	1	100
21	HCl	0.5	24	Room*
22	NaOH	0.5	0.5	Room*
23	NaOH	0.5	2.0	Room*
24	NaOH	0.5	5 min.	Room*
25	NaOH	0.5	10 min.	Room*

* = Room temperature

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Solubility tests

The solubilities of metal compounds depend on the pH level and on the complex form of metal such as hydroxide, carbonate, sulfite. It is important that the information on solubility of metals is being aware of as metal precipitation can easily lead to a misperception of the adsorption process. To date, experimental data on the solubility of metal nitrates are still not available. It was therefore imperative to find the relation between pH and solubility of metal nitrates prior to the adsorption experiment so that an appropriate level of pH in subsequent experiments could then be selected to eliminate the effect of metal precipitation. Figure 4.1.1 demonstrates that all of the metal species employed in this work could dissolve well at low pH and, as expected, low solubility was observed at high pH. Noted that these solubility tests were conducted at metal concentration of 100 mg/l, which was the target range for biosorption.

Copper, cadmium, zinc and lead ions were found to precipitate at a relatively high pH (as metal hydroxide) where their solubilities were lower than 50 mg/L at pH higher than 6.5. $Cu(NO_3)_2$ and $Cd(NO_3)_2$ were completely dissolved (100% solubility) at pH lower than 6. $Zn(NO_3)_2$ showed high solubility at a wide range of pH, as it was still totally dissolved (at 100 mg/l) at pH as high as 7.76. On the other hand, $Pb(NO_3)_2$ was insoluble at a relatively low pH, and precipitation was detected at pH of 5.64.

Previous work also suggested that the suitable pH for the heavy metals adsorption experiments should be conducted at pH lower than 5.5 [Sungkhum, 2002]. Low pH was shown to be ineffective for the biosorption of metals and the selected pH range should be as high as possible. In other words, the selected pH for adsorption should avoid the precipitation and the ineffectiveness range, and for this experiment, the pH was always controlled between 4.5 – 5.5.

4.2 Effect of initial metal concentration

The initial concentrations of heavy metals employed in this experiment were 0, 10, 25, 50, 75 and 100 mg/L. Figures 4.2.1 and 4.2.2 demonstrate the adsorption capacities for the four heavy metals of interest which reveals that the adsorption capacity for each metal increased with the increase in initial metal concentration. This result was similar to others' finding and it was reported that this could be the result from the increase in electrostatic interactions between the metal and the binding site in biomass (relative to covalent interactions), [Puranik and Paknikar, 1999 and Ceribasi, 2001]. There was one exception for Zn adsorption where the increase in initial concentration from 1 to 1.5 mmol/L reduced the adsorption capacity. The exact reason for this finding still could not be drawn from this work, but it was expected that there existed an inhibition adsorption characteristics where high concentration of Zn seemed to impose adverse effect on adsorption capacity.

4.3 Adsorption isotherms of untreated *Caulerpa lentillifera*

The isotherms were constructed from the equilibrium data illustrated in Figure 4.2.2. A linear regression of these experimental results to both Langmuir's and Freundlich's isotherms as shown in Figures 4.3.1 and 4.3.2 gave an R^2 of close to one for all cases, indicating that the adsorptions of these metals could be monolayer or multilayer on the surface of the algal biomass.

Firstly, with considering the results of the Langmuir's isotherm, it is reminded that a good adsorbent is the one with high capacity (q_{max}) and high affinity (b). The associated Langmuir's isotherm parameters obtained from these experiments are tabulated below:

Parameter	Metal						
q_{max}	Zn	=~	Cu	>	Cd	=~	Pb
mmol/g alga	0.0842		0.0819		0.0367		0.0363
b	Pb	>	Cd	>	Cu	>	Zn
g alga/mmol	62.921		17.862		12.874		2.248

The results revealed that the adsorption capacity (q_{max} in mmol-metal per g-dried algal biomass) was highest for Zn which was only slightly higher than that of Cu. q_{max} of Cd and Pb were in a similar range with about half of the capacity of Zn and Cu. Pb was the lowest adsorbed metal among the four. The b value decreased in order from Pb, Cd, Cu and Zn, respectively. The high b value indicated a high affinity of the heavy metal onto the binding site of the algae. This meant that the binding site in the algae was most active with the adsorption of Pb and least active with the adsorption of Zn.

The following observations could be drawn from this experiment. Pb exhibited the lowest q_{max}, but the highest b value among the four metals. Cu had the highest of q_{max}, about 2 times higher than Pb, whereas its b value was as much as 5 times lower than Pb. It should therefore be taken into consideration that a high capacity does not necessarily mean high affinity. The comparison between the heavy metal adsorption should consider both of these two parameters.

As stated earlier, the experimental results also follow Freundlich's isotherm where the isotherm parameters are summarized in the following tabulation. Generally, a good adsorbent is the one with high sorption capacity (K) and high sorption intensity indicator ($1/n$). In this experiment, the maximum capacity (K) was in the order from high to low as follows: lead, copper, cadmium and zinc, respectively. It should be remarked that the maximum capacity (K) of cadmium was only one third of those for lead and copper, and the lowest adsorbed metal among of the four was zinc. The $1/n$ value, although not significantly different from each other, decreased in order from copper, zinc, lead and cadmium, respectively.

Parameter	Metal						
K	Pb	>	Cu	>	Cd	>	Zn
	0.1981		0.1932		0.0570		0.0261
$1/n$	Cu	>	Zn	>	Pb	>	Cd
	0.659		0.605		0.595		0.455

Interestingly, the conclusions from Langmuir and Freundlich isotherms were not consistent. Whilst Zn was found to be the most adsorbed component with Langmuir's, it was the lowest with Freundlich's. The conclusion for Pb was the opposite. This was considered to be the consequence of the scope of the experiment where the range of the metal concentration under the investigation was quite limited, and may not be adequate for constructing complete isotherms, particularly for Pb and Cu. Figure 4.2.2 demonstrates that the isotherm curves for Pb and Cu had not reached their maximums. This information might be misleading when it was used to construct both Langmuir and Freundlich isotherms as high R^2 might be obtained with low accuracy. However, this range of concentration was considered adequate for this study which had a special emphasis only on a low strength solution. Hence, the isotherms proposed above should be used with extra care and should be strictly limited to the range of concentration employed in this work only.

It should also be mentioned that this specific strain of algae could contain a high level of Zn in their molecular structure. Figure 4.3.3 shows the results from the adsorption experiment with Cu. At the end of the adsorption, Zn was detected in the solution. This indicated that Zn was released from the cell of the alga. The amount of Zn released from the alga seemed to vary with the equilibrium concentration of Cu. This specific point about the leaching of Zn is not in the scope of this study and mentioned here for potential further investigation.

4.4 Various pretreatments of *Caulerpa lentillifera*

Twenty-four pretreatment methods for this algae were tested to identify appropriate sorption methods with metal solution of 100 mg/l. All twenty-four methods covered both physical and chemical (acid, base and salt chemicals) treatments and the summary of these pretreatment methods is given in Chapter 3. This experiment was designed as a screening test for the most practical methods. Each of the methods was investigated for its effectiveness by comparing its adsorption capacity with that of untreated algae at the pH range between 4.5-5.5. Effective treatment methods would be evaluated based on their percent metal removal and the loss of weight after pretreatment and only these selected methods would undergo subsequent investigation.

The results on the average adsorption capacities (and their associated standard error) for each pretreatment method were shown in Figures 4.4.1 – 4.4.4. The two different shade bars in these figures illustrate the adsorption capacities of the pretreated algae, one without and one with algal weight loss consideration. The weight loss would provide information on the amount of the alga after the treatment. A summary of the findings from this experiment could be concluded as follows:

1. Significant improvement results (with weight loss consideration) were found for two metal species, i.e. copper and cadmium.

Copper: The methods with an improved adsorption capacities could be summarized as:

- 0.5N NaOH for 1 hour: 24% increase in q (with a probability, p, of 0.035)

- 0.5N $CaCO_3$ for 24 hours: 26% increase in q ($p = 0.043$)
- 0.5N Na_2SO_4 for 24 hour: 14% increase in q ($p = 0.110$)
- 0.5N Na_2CO_3 for 24 hours: 12% increase in q ($p = 0.118$)

Cadmium: The methods with an improved adsorption capacities could be summarized as:

- 0.5N NaOH for 24 hours: 29% increase in q ($p = 0.345$)
- 0.5N $CaCO_3$ for 24 hours: 19% increase in q ($p = 0.284$)
- 0.5N $NaNO_3$ for 24 hours: 24% increase in q ($p = 0.204$)
- 0.5N $CaSO_4$ for 24 hours: 17% increase in q ($p = 0.305$)
- 0.5N Na_2CO_3 for 24 hours: 17% increase in q ($p = 0.314$)

Among all of the pretreated methods that provided an enhancement in metal adsorption capacity, there were only two methods that were beneficial for both Cu and Cd. These were the treatment with 0.5N $CaCO_3$ for 24 hours and with 0.5N Na_2CO_3 for 24 hours.

2. No pretreatment methods were found to be beneficial for the adsorption of Zn and Pb. However, the previous section illustrated of Pb was the fastest at low concentration range (high affinity). It is therefore surprising that the adsorption of Pb by the pretreated algal showed no signs of improvement. The examination of the adsorption capacity without the consideration of algal weight loss demonstrated that there was actually a capacity enhancement with the pretreatment with 0.5N NaOH. This method was therefore selected for further evaluation in this work.

In summary, this part revealed that most of the pretreated methods did not lead to an enhancement in metal adsorption capacity of the algae. Only those methods with positive results (higher adsorption capacity) were selected for further investigation in this work. It should be noted that the pretreatment by boiling would be also excluded from further study. This was because this technique hardly presented an improvement to the metal adsorption process. In addition, the pretreatment by boiling did not provide an economical solution to the actual treatment.

4.5 Adsorption isotherms of various pretreated *Caulerpa lentillifera*

4.5.1 Copper adsorption

The selected pretreatment techniques for the copper adsorption experiments included the treatments with 0.5N NaOH for 1 hour, 0.5N $CaCO_3$ for 24 hours, 0.5N Na_2SO_4 for 24 hours and 0.5N Na_2CO_3 for 24 hours. The experiments were performed with various concentrations of metal and the adsorption isotherms were developed. The resulting isotherm parameters are summarized as follows:

Method/ Period	Untreated	0.5N NaOH 1 h.	0.5N $CaCO_3$ 24 h.	0.5N Na_2SO_4 24 h.	0.5N Na_2CO_3 24 h.
Langmuir's model					
$q_{max,}$, mmol/g	0.0819	0.0946	0.1059	0.0796	0.0727
b, g/mmol	12.874	34.718	18.812	23.049	40.407
R^2	0.9264	0.9627	0.9082	0.9772	0.9553
Freundlich's model					
K	0.1932	0.1434	0.6563	0.1136	0.0948
1/n	0.659	0.389	0.886	0.424	0.327
R^2	0.9223	0.9009	0.9251	0.9351	0.9021

The results from this experiment revealed that the best pretreatment technique for copper adsorption was the conditioning with 0.5N $CaCO_3$. This was obvious both from Langmuir or Freundlich isotherms points of view. In the fitting with Langmuir isotherm, the maximum adsorption capacity or q_{max} for the case with $CaCO_3$ was as much as 30% higher than the untreated case whereas the *b* value was also significantly higher than the untreated case. This meant that Cu could be adsorbed in much higher quantity and in much faster rate (see Figure 4.5.2).

The other pretreatment method that yielded a better adsorption capacity for copper was the treatment with 0.5N NaOH (see also Figure 4.5.2). In fact, the maximum adsorption capacity from this method was rather close to the treatment with $CaCO_3$, and the *b* value was in a much greater extent. The *b* value of as high as 34.7 g/mmol was almost twice the level from the $CaCO_3$ treatment case. This meant that

NaOH provided a better treatment for the surface of the algae as copper could adsorb more easily but the number of binding sites might not be as great as that obtained from $CaCO_3$ treatment.

The other two methods, i.e. Na_2SO_4 and Na_2CO_3, were also found to be beneficial for the surface treatment of the algae as high b value indicated that copper could adsorb to the surface more easily than the untreated case. However, both of these methods did not show improvement in the adsorption capacity of the algae.

The evaluation using Freundlich isotherm (Figure 4.5.3) was much more complicated than the Langmuir as both Freundlich parameters were not as independent from each other as the case of Langmuir. K and $1/n$ do not have specific meanings, unlike q_{max} and b in Langmuir isotherm. However, the results from Freundlich isotherm were consistent with the Langmuir in that $CaCO_3$ was found to yield the greatest improvement for the adsorption of copper.

It is worth noting that Freundlich isotherm always resulted in a curve that did not seem to reach plateau. This was because the range of concentration employed in this work was quite narrow and did not cover the range where the adsorption reached the maximum capacity. The usage of data from this work should then be confined strictly to the range of concentration of heavy metals from 0-100 mg/l.

4.5.2 Cadmium adsorption

The selected pretreatment techniques for the cadmium adsorption experiments included the treatments with 0.5N $CaCO_3$ for 24 hours, 0.5N $NaCO_3$ for 24 hours, 0.5N Na_2NO_3 for 24 hours and 0.5N $CaSO_4$-$2H_2O$ for 24 hours. Similar experimental technique to the Cu adsorption case above was conducted here for Cd and the resulting isotherm parameters are summarized as follows:

Method/ Period	Untreated	0.5N $CaCO_3$ 24 h.	0.5N Na_2CO_3 24 h.	0.5N $NaNO_3$ 24 h.
Langmuir's model				
$q_{max,}$, mmol/g	0.0367	0.0654	0.0420	0.0301
b, g/mmol	17.862	9.123	1.693	66.595
R^2	0.9324	0.9077	0.9348	0.9254

Method/ Period	Untreated	0.5N $CaCO_3$ 24 h.	0.5N Na_2CO_3 24 h.	0.5N $NaNO_3$ 24 h.
Freundlich's model				
K	0.0570	0.1110	0.0261	0.0884
$1/n$	0.455	0.668	0.605	0.879
R^2	0.9196	0.9284	0.8969	0.9092

The results from this experiment revealed that the best pretreatment technique for cadmium adsorption was the pretreatment with 0.5N $CaCO_3$. This was obvious both from Langmuir or Freundlich isotherms points of view (excepted for b). In the fitting with Langmuir isotherm, the maximum adsorption capacity or q_{max} for the case with $CaCO_3$ was as much as 80% higher than the untreated case. However, a slower adsorption response became obvious as the b value was significantly lower than the untreated case (50% decreased). This meant that Cd could be adsorbed in a much higher quantity, but with a lower affinity (Figure 4.5.4). Again, as the two Freundlich parameters are not independent quantities, it is always complicated to explain the meaning of the changes in their values. In this case, increases in both parameters were observed and the trend of isotherm is shown in Figure 4.5.5.

The other pretreatment method that yielded a better adsorption capacity for cadmium was the treatment with 0.5N Na_2CO_3 (about 15% increased as compared to the untreated algae). The vertical dotted line in Figure 4.5.4 was the equilibrium liquid concentration for the case where the initial concentration of Cd was set at 100 mg/l. This line was actually for the untreated algae but the results revealed that the line for all other cases were not far apart from this line (results not shown in this figure). This illustrated that the maximum adsorption capacity summarized in the above tabulation was obtained from the extrapolation of the experimental data as the isotherm results did not reach the maximum at this concentration. Therefore the results on the maximum adsorption capacity for Cd (for the pretreatment with Na_2CO_3) might not be accurate and should be considered not applicable for further reference. In addition, the b value from this method was significantly lower than the untreated case. This indicated that Cd adsorption at the concentration range employed in this work was quite slow and was not applicable for the large scale system.

The treatment with 0.5N $NaNO_3$ was rather interesting. Although the maximum adsorption capacity was found to be slightly lower than that from the untreated algae, the rate of adsorption process (affinity) was much faster as reflected in the *b* value. This implied that the adsorption of low strength wastewater containing Cd would take place much more rapidly than the originally untreated algae.

4.5.3 Lead adsorption

There was only one selected pretreatment technique for the lead adsorption experiment, which was the pretreatment with 0.5N NaOH for 1 hour. The resulting isotherm parameters are summarized as follows:

Method/Period	Untreated	0.5N NaOH 1 hr.
Langmuir's model		
$q_{max,}$, mmol/g	0.0363	0.0607
b, g/mmol	62.921	48.246
R^2	0.9116	0.9491
Freundlich's model		
K	0.1981	0.9296
$1/n$	0.595	0.883
R^2	0.9072	0.9241

The results demonstrated that this pretreatment method provided better values of q_{max}, K and $1/n$ than the untreated algae. Although the Langmuir parameter b was observed to be lower for the treated algae than that from the untreated case, Figure 4.5.8 (and Figure 4.5.9) demonstrated clearly that the adsorption curve for the treated algae always had a higher slope at low concentration range. This indicated that the treated algae had higher affinity for Pb than the original algae.

4.6 Effect of time period for 0.5N NaOH treatment

In the previous section, the observed effect of treatment was only limited to that of fixed exposure time. However, the different exposure time scale might impose different or the same effect with varying intensity. This section intended to investigate on this point. Only the treatment with 0.5N NaOH was considered here as a case study, and the investigation would also include the effect of the simultaneous heating during the pretreatment. NaOH was believed to clean up the surface of the algae and resulted in a better contact between the binding site and the heavy metal ions. However, the long exposure of algal surface with NaOH might lead to the destruction of the binding sites.

In short, there may exist an optimal contacting time during the pretreatment that yielded the best results for metal adsorption. The heat treatment of the algae was also reported to have positive effect on the adsorption (see Section 2.5.1). The heating time was therefore one of the parameters whose effect would be discussed herein.

Figure 4.6.1 demonstrates the effect of the contacting time on the adsorption of Cu in the case of 0.5N NaOH (on the first four columns). The comparison between adsorption capacities of untreated alga and of the treated alga with NaOH at various treatment periods was presented below:

- 0.5N NaOH for 0.5 hour: 38% increase in q ($p = 0.006$)
- 0.5N NaOH for 1.0 hour: 41% increase in q ($p = 0.008$)
- 0.5N NaOH for 2.0 hours: 23% increase in q ($p = 0.029$)

Interestingly, the adsorption for Cu was improved by pretreating the algae with 0.5N NaOH. This effect might be a result from the cleaning mechanism as mentioned above. Increasing the contacting time from 0.5 to 1.0 hour led to an increase in the total adsorption capacity as presented in Figure 4.6.1. The longer time might lead to a much cleaner algal surface which resulted in a better adsorption. However, a much too long pretreatment time lowered this positive effect and a lower adsorption capacity of Cu was observed (the adsorption capacity in mmol/g was 0.0959 with 1 hour treatment but was 0.0837 with 2 hour treatment). This negative effect from a long exposure time was, possibly, due to the destruction effect caused by the attack of NaOH on the clean surface of the algal, which destroyed the binding site.

Note that the above conclusion was only based on the trend observed from the results depicted in Figure 4.6.1. However, statistics (t-test) illustrates that the differences between the adsorption capacities of various pretreated alga were insignificant (with p greater than 0.05). This was illustrated below:

- Adsorption capacity of alga pretreated with 0.5N NaOH for 1 hour was about 2% greater than the pretreated alga with the same solution for 0.5 hour. However, the probability was only 0.782.

- Adsorption capacity of alga pretreated with 0.5N NaOH for 2 hour was about 11% lower than the pretreated alga with the same solution for 0.5 hour. The probability, on the other hand, was only 0.094.
- The probability between the comparison between adsorption capacities of alga pretreated with 0.5N NaOH at 1 and 2 hours was only 0.092 (with an increase in adsorption capacity of 13% when the treatment time was reduced from 2 to 1 hour).

Therefore further investigation using the results reported in this document should be carried out with extreme care and, where possible, a more detailed experiment is strongly recommended, for example, with a wider range of metal concentrations, or with more data on the treatment time.

The same figure also demonstrates the effect of heating time. The adsorption capacities of the untreated alga (the first column) and the pretreated algae (the last three columns are illustrated in Figure 4.6.1. The heating was found to be possibly beneficial for the treatment of algal surface (for the adsorption of Cu), and the adsorption capacity of the treated alga was improved with a short heating time. However, statistics revealed that there was no significant difference between experimental results on adsorption capacities of the untreated and the treated alga (with p greater than 0.05). The results from the t-test were summarized as:

- $p = 0.101$ when examined the difference between the results from the pretreated alga with 0.5N NaOH for 5 and for 10 min.
- $p = 0.192$ when examined the difference between the results from the pretreated alga with 0.5N NaOH for 5 min (at 60^{o}C) and for 60 min (at boiling point).
- $p = 0.143$ when examined the difference between the results from the pretreated alga with 0.5N NaOH for 10 min (at 60^{o}C) and for 60 min (at boiling point).

Therefore, these results indicated that a much longer time and a higher treating temperature did not always lead to a significantly positive influence on the adsorption capacity of the algae. This figure did not include the weight loss of the algae into consideration. Taking into account the algal weight loss would lower the adsorption capacity to the unattractive level (lower than the untreated case). In summary, this section proved that there must exist an optimal contacting time for the treatment with 0.5N NaOH that may rendered a better adsorption capacity of the algae.

4.7 Identification of functional groups in algae

Sungkhum (2002) reported that the adsorption of heavy metals onto the surface of *Caulerpa lentillifera* was in fact not through a physical means as the metal could not simply be desorbed by applying heat to the spent algae. EDTA was found to be a good eluent for the metal from this algae and therefore there should not be a strong reaction between the metals and the binding site. Hence, it was postulated that this type of adsorption occurred due to some weak bonding between metal ions and some functional groups in the algal surface. The determination of functional groups in the algae was, in this regard, significant for a better understanding of metal biosorption.

Literature revealed some relationships between metal species and the associated functional groups on the surface of microorganisms, which could possibly act as an active site for metal binding. These are summarized as follows.

Biosorbent, Type	Functional group	Metal	Reference
Scenedesmus obliquus, Algae	Carboxyl groups	Cd	Crist et al., 1981
Cladophora, *Rhizoconium* and *Polysiphonia*	Amino, carboxyl, sulfonate groups	Pd, Al, Cd	Crist et al., 1990; Crist et al., 1992
Maitenus truncata, Dried leaves	C=C, OH and CH_2	Cu	Carvalho et al., 2003
Chlamydomonas reinhardtii, Algae	Carboxyl groups	Cd	Adhiya et al., 2002
Various leaves	C-C and C=O	Cu	Carvalho et al., 2001

A recent review on the biochemistry of heavy metal biosorption by brown algae [Davis et al., 2003] suggested that the carboxylic groups were generally the most abundant acidic functional group in brown algae. The second most abundant acidic functional group was the sulfonic acid of fucoidan [Davis et al., 2003]. These groups will have different affinities for different metal sorption species.

It can be seen from the above review that various types of biosorbents will possess different types of functional groups in variable quantities, and the suitability of each biosorbents on various species of heavy metals must be determined individually. This study only examined the functional groups of nine algal samples using FTIR (Fourier Transform Infrared). It was intended to observe the alteration of possible functional groups during the biosorption and the pretreatment processes. The results are discussed below.

4.7.1 FTIR results from untreated algae

In this section, the resulting functional groups from the untreated algae both before and after the heavy metal adsorption were analyzed. The results indicated that the shifts or changes in the functional groups of the algae were similar for all adsorption experiments. In other words, the four heavy metals of interest (Cu, Cd, Zn and Pb) showed similar characteristics in altering the functional groups in algae (the raw data FTIR results were given in (Appendix B: Figure B.1-B.5). The results for all experiments were then combined and present in the following form.

Functional group	Standard Wavenumber (cm^{-1}) [Pranaom, 1996]	Wavenumber (cm-1) from the results	Change after adsorption
Carboxylic Acid;			
C-O stretching	1,210-1,320	1,242-1,244	Reduced
O-H banding	1,395-1,440	1,414	Disappeared
O-H	2,500-3,300	2,922	Reduced
Amine;			
N-H banding	1,580-1,650	1,648-1,650	Reduced
C-N stretching	1,020-1,342	1,324	Disappeared
Amide;			
C-O stretching	1,640-1,670	1,648-1,650	Reduced

Functional group	Standard Wavenumber (cm^{-1}) [Pranaom, 1996]	Wavenumber (cm-1) from the results	Change after adsorption
Amino;			
N-H stretching	2,600-3,300	2,922-2,924	Reduced
N-H banding	1,500-1,610	1,534-1544	Reduced
Sulfonyl;			
S=O stretching	1,380-1,410	1,384	Occurred
Sulfonate;			
S=O	1,335-1,372	1,366	Disappeared

Six main functional groups, i.e. carboxylic acid, amine, amide, amino, sulfonyl and sulfonate were found to be altered by the adsorption of heavy metals by *Caulerpa lentillifera*. FTIR interpretation also revealed that there was also probable alteration of other main functional groups; i.e. alkyl and aliphatic alcohol. In the case of Cu adsorption, aliphatic alcohol group appeared after a batch treatment. No explanation of this finding can be given from this work. In case of zinc sorption, the comparison between algae before and after the batch adsorption experiment indicated that there was a possibility of a disappearance of alkyl and/or hydroxyl and/or amino groups. These functional groups might involve with the adsorption of Zn and the disappearance of this functional group might not be a desired consequence from this pretreatment. For the adsorption of Cd and Pb, alkyl group was found to disappear which could lead to the conclusion that alkyl group might play a significant role in the adsorption of these two metal species.

To further verify this point, the chemistry of the heavy metals was considered. Pearson (1976) classified metallic ions according to their hardness scale. The definition of hard or soft ions was given using the thermodynamic stability of the complex forms. Hard ions are usually required as nutrients by microorganisms whereas the borderline and soft ions are toxic heavy metals. The hard ions form very strong with hard cations, also the soft ions form strong bond with soft anions [Volesky, 1990; Shriver and Atkins, 1999]. From the reason given above, the various possible couplings between the heavy metals and functional groups in the algae could be formulated as follows:

Heavy metal	Class	Possible coupling functional group	Associated bonding type
Cu	Intermediate	carboxylic acid, amide, amino, sulfonyl, sulfonate, alkyl and aliphatic alcohol	Electrostatic and/or Covalent
Cd	Soft cation	carboxylic acid, amide, amino, sulfonyl, sulfonate and alkyl	Electrostatic and/or Covalent
Zn	Intermediate	carboxylic acid, amide, amino, sulfonyl, sulfonate and alkyl	Electrostatic and/or Covalent
Pb	Intermediate	carboxylic acid, amide, amino, sulfonyl, sulfonate and alkyl	Electrostatic and/or Covalent

According to the discussion by Sungkhum (2002), it was possible that in this work, Cu, Cd, Zn and Pb formed a covalent bond with the soft C-O, or they could form an electrostatic or ionic bond with a hard hydroxide (O-H) or form covalent or ionic bonds with the intermediate groups; amine (N-H), amino, (N-H) and sulfonate (S-O) group.

4.7.2 FTIR results for treated algae

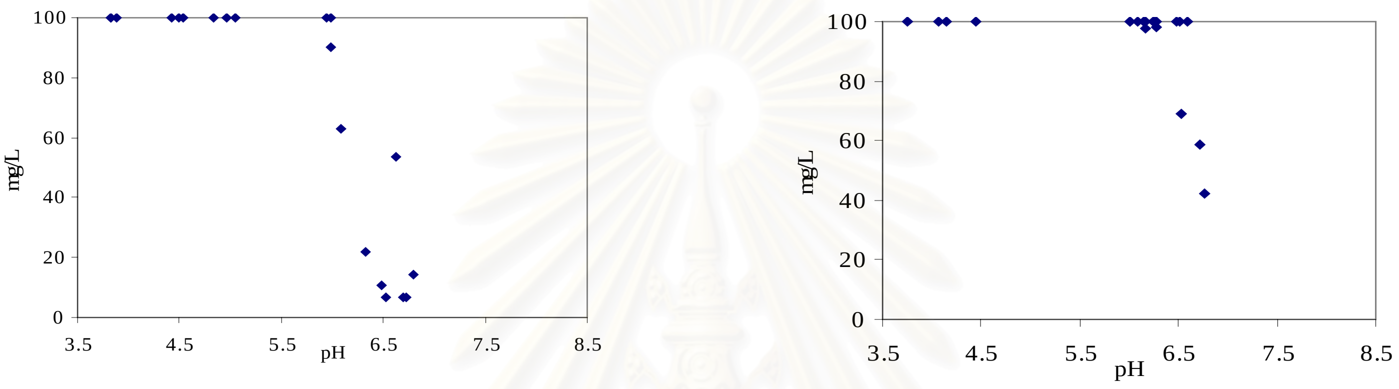
This investigation aimed to consider alteration of possible functional groups between the untreated algae and the pretreated algae by several methods, i.e. NaOH for 1 hour, HCl for 1 hour, NaCl for 24 hour, Na_2CO_3 for 24 hours $CaCO_3$ for 24 hours. Not all pretreatments were investigated in this work as there were limitations particularly on the time scale of experiments. The resulting FTIR are shown in Appendix B: Figure B.1 and B.5-B.10. As was noted on the previous section the line spectrum of each cases could only be used to analyze the alteration of the peak of possible functional groups. The changes of FTIR patterns, e.g. disappearing, shifting, provided qualitative evaluation of the possible functional groups involved with the metal species of interest, are summarized as:

Functional group	Standard wavenumber * (cm^{-1})	Wavenumber (cm^{-1}) from the results	Pretreatment Method				
			NaOH	HCl	NaCl	Na_2CO_3	CaCO3
Carboxylic Acid; C-O Stretching	1,210-1,320	1,210	-	O	-	O	-
Carboxylic Acid; O-H	2,500-3,300	2,922-2,928	R	R	R	R	-
Amine; N-H Banding	1,580-1,650	1,650	D	D	D	D	D
Amine; C-N Stretching	1,020-1,342	1,064	-	-	D	-	-
		1,210	-	O	-	O	-
		1,246	-	R	-	-	-
		1,324	D	D	D	D	-
Amide; N-H Stretching	3,100-3,500	3,408	S	S	S	S	-
Amino; C-O	1,405-1,580	1,544	S	S	S	S	I
Amino; N-H Banding	1,500-1,610	1,544	-	S	S	S	D

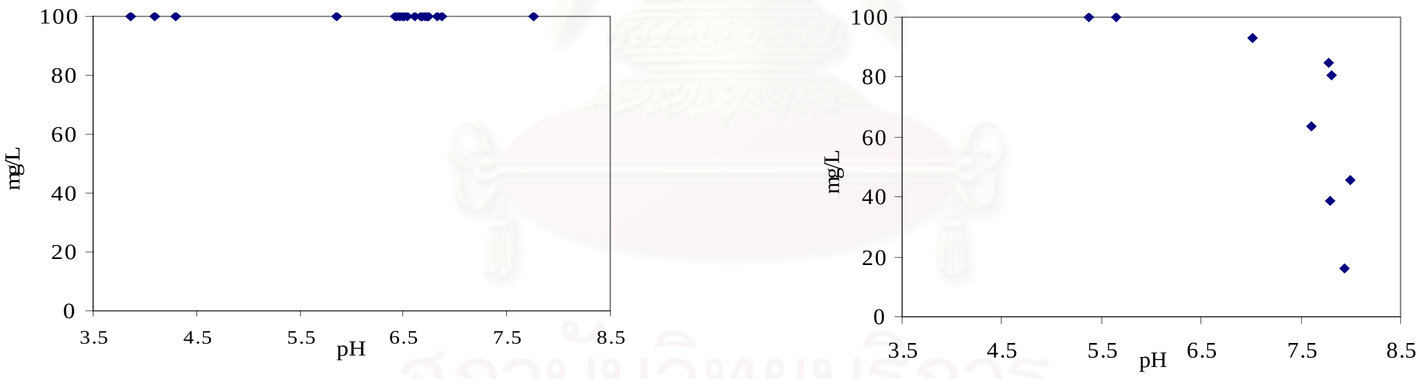
D = Disappeared, S = Shifted, O = Occurred, R = Reduced,
I = Increased and - = No change
* Pranaom, 1996

The amine, N-H banding was observed to disappear on the biosorbent after all pretreatments. It was likely that this amine could be easily destroyed by chemical reaction on the cell surface of algae. From the discussion in the previous section, this N-H banding was found to be one of the potential functional groups that attracts the four heavy metals. Hence, the disappearance of this group might not be beneficial for the metal adsorption.

Interestingly, most of the pretreatment techniques were found to reduce or even destroy the function groups useful for metal adsorption. Unfortunately, the FTIR results did not provide accurate quantitative evaluation of the changes in the functional groups on the algae surface which rendered the analysis difficult. In this work, the changes in percent transmission were employed as a rough estimate on the changes in quantity of the functional group. Specifically, a change of more than 10% transmission would be considered as significant. $CaCO_3$ and NaOH were the two pretreatment techniques found to have lesser influence on the functional groups. $CaCO_3$ was only found to decrease N-H banding in both amine and amino groups whereas NaOH decreased amine (N-H banding) and amin (C-N stretching) groups. Other techniques seemed to be more effective in reducing useful functional groups for metal sorption. This was perhaps the reason why these two techniques always provided a better adsorption characteristics for the four heavy metals examined in this work.



(A) $Cu(NO_3)_2$ (B) $Cd(NO_3)_2$



(C) $Zn(NO_3)_2$ (D) $Pb(NO_3)_2$

Figure 4.1.1 Solubility of metal nitrates (contact time 60 min, initial conc. = 100 mg metal/l)

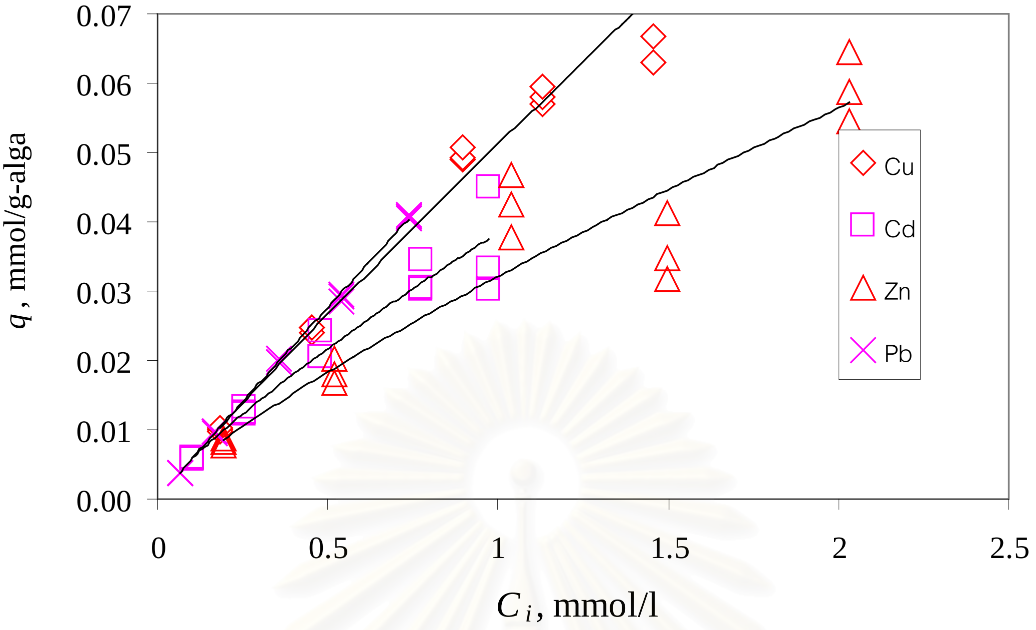


Figure 4.2.1 Relationship between metal capacity and initial metal concentration

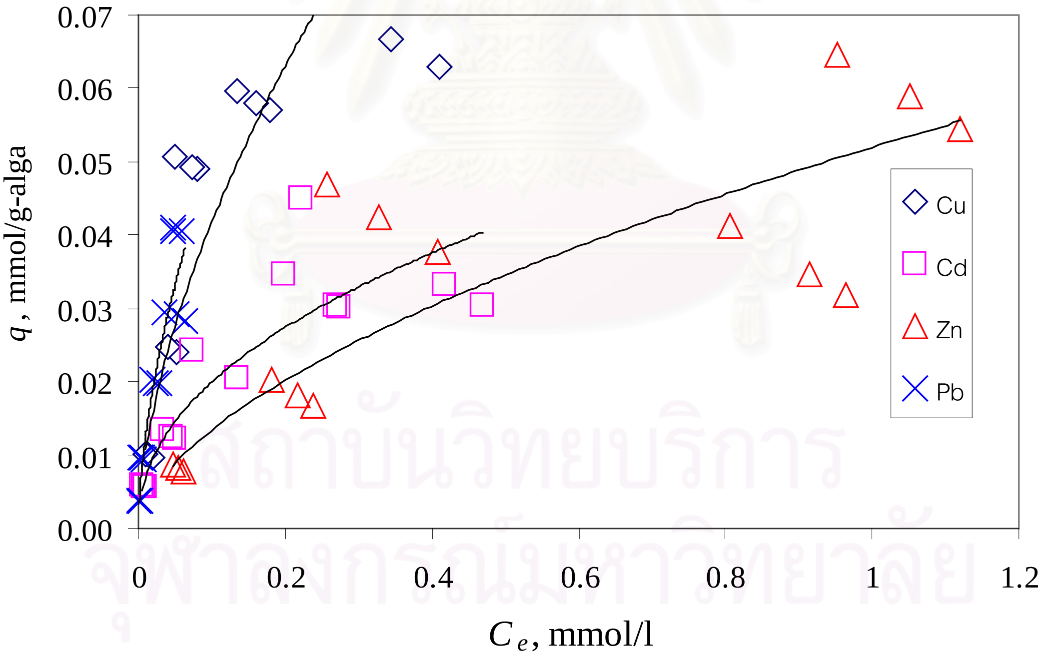
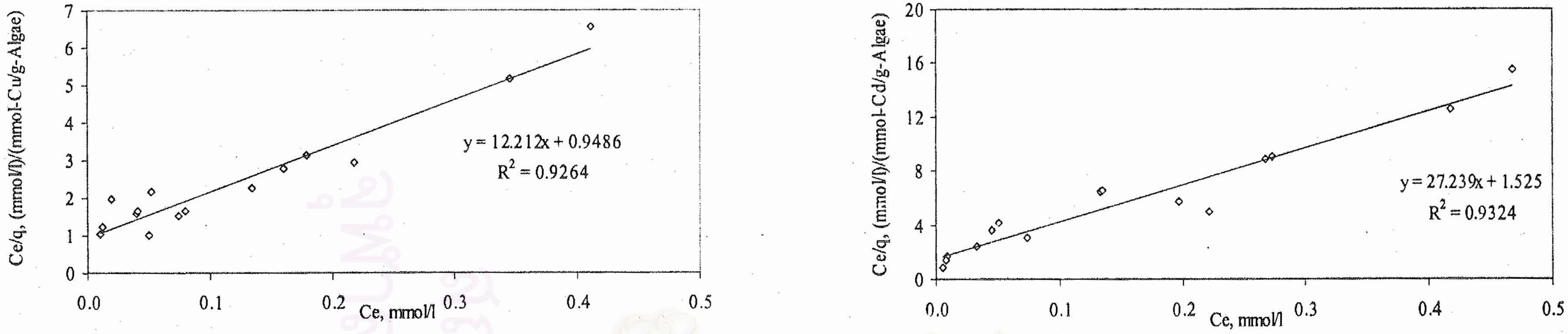
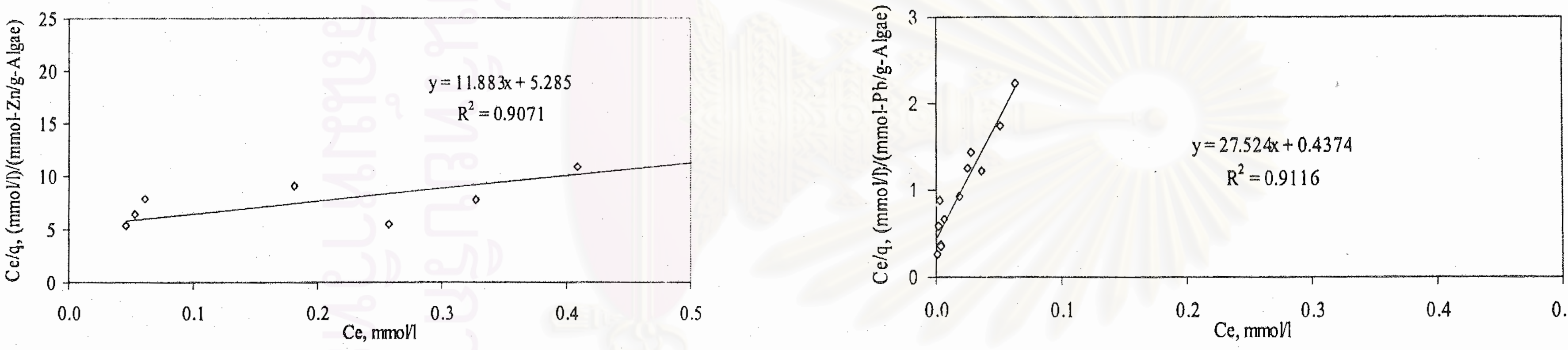


Figure 4.2.2 Relationship between metal capacity and equilibrium metal concentration



(A) Cu

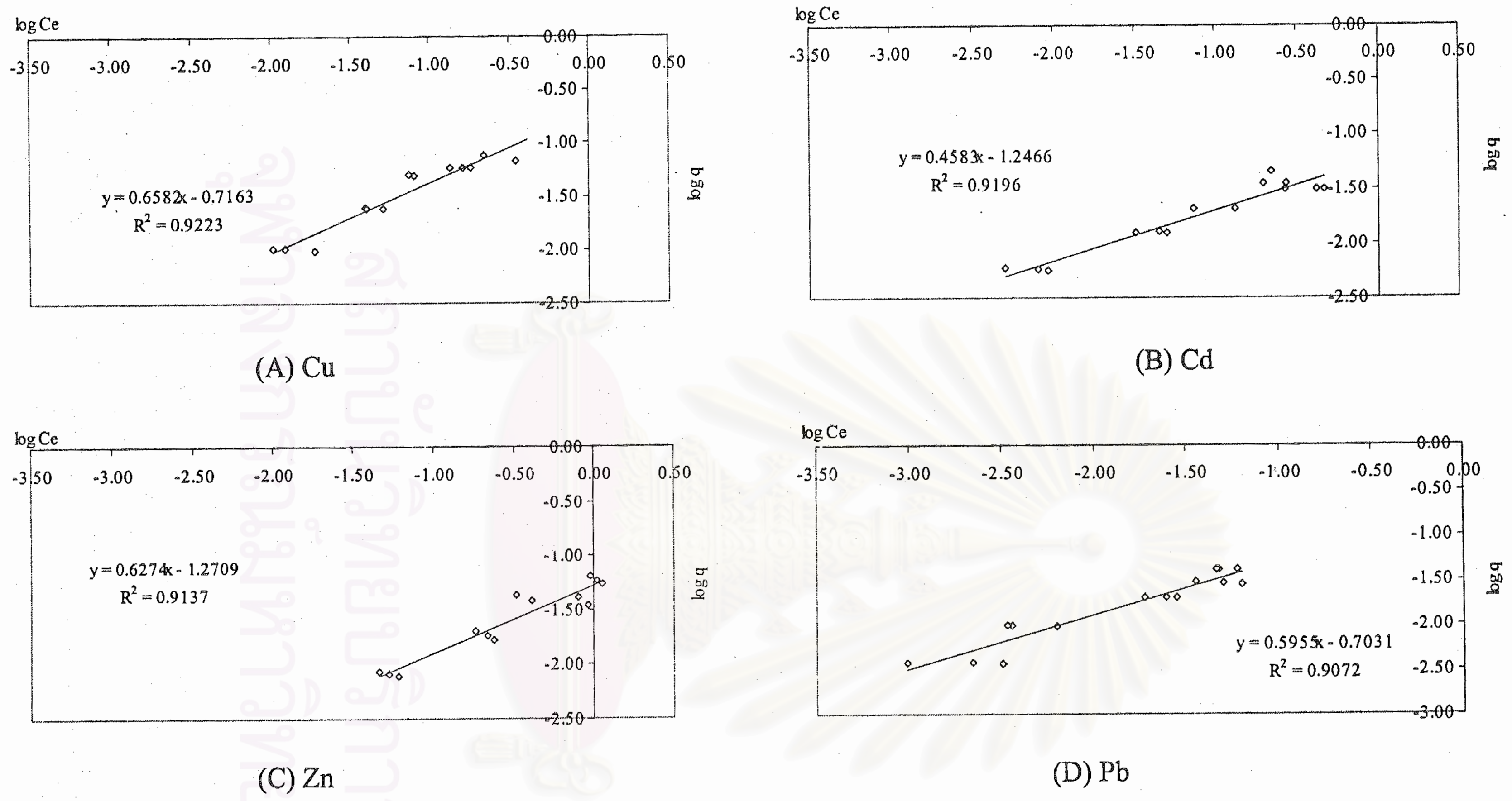
(B) Cd



(C) Zn

(D) Pb

Figure 4.3.1 Langmuir's isotherm of heavy metals by *Caulerpa lentillifera* (algal mass = 0.5 g/ 30 ml, initial con. = 10 - 100 mg metal/l, contact time 1 hour, pH 4.5 - 5.5)



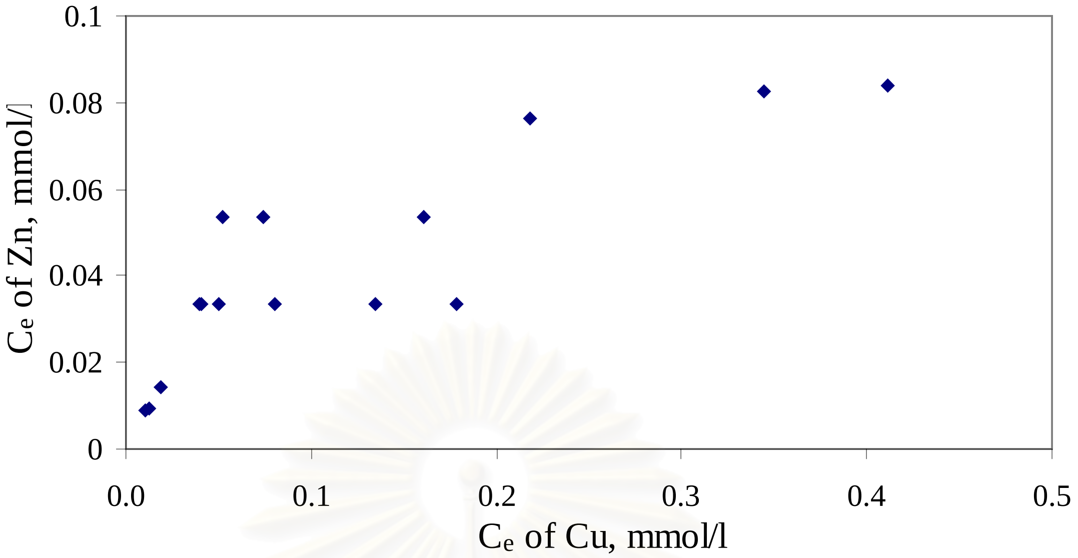
(A) Cu

(B) Cd

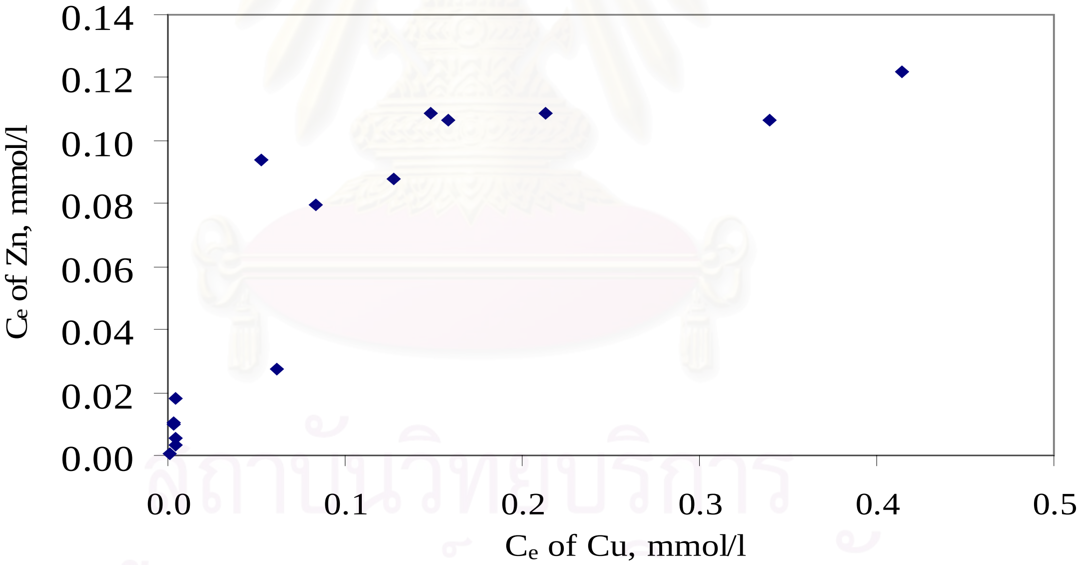
(C) Zn

(D) Pb

Figure 4.3.2 Freundlich's isotherm of heavy metals by *Caulerpa lentillifera* (algal mass = 0.5 g/ 30 ml, initial con. = 10 - 100 mg metal/l, contact time 1 hour, pH 4.5 - 5.5)



(A) The untreated algae



(B) The pretreated algae by 0.5N NaOH 1 hour

Figure 4.3.3 Zn leaching concentration from the cell biomass during batch experiment of Cu removal

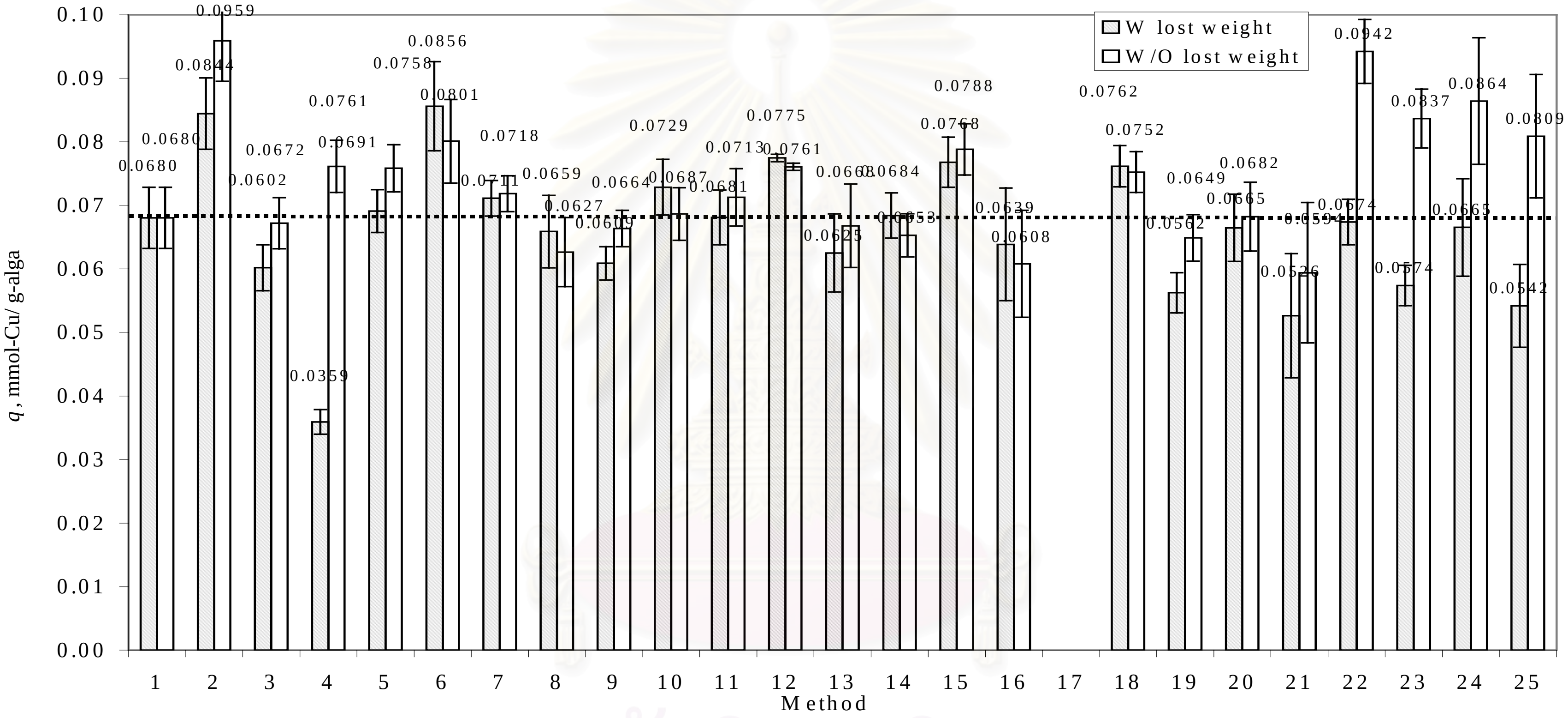


Figure 4.4.1 Biosorption of Cu by various pretreated algae (algal mass = 0.5 g, initial conc. = 100 mg/l, pH = 4.5-5.5)

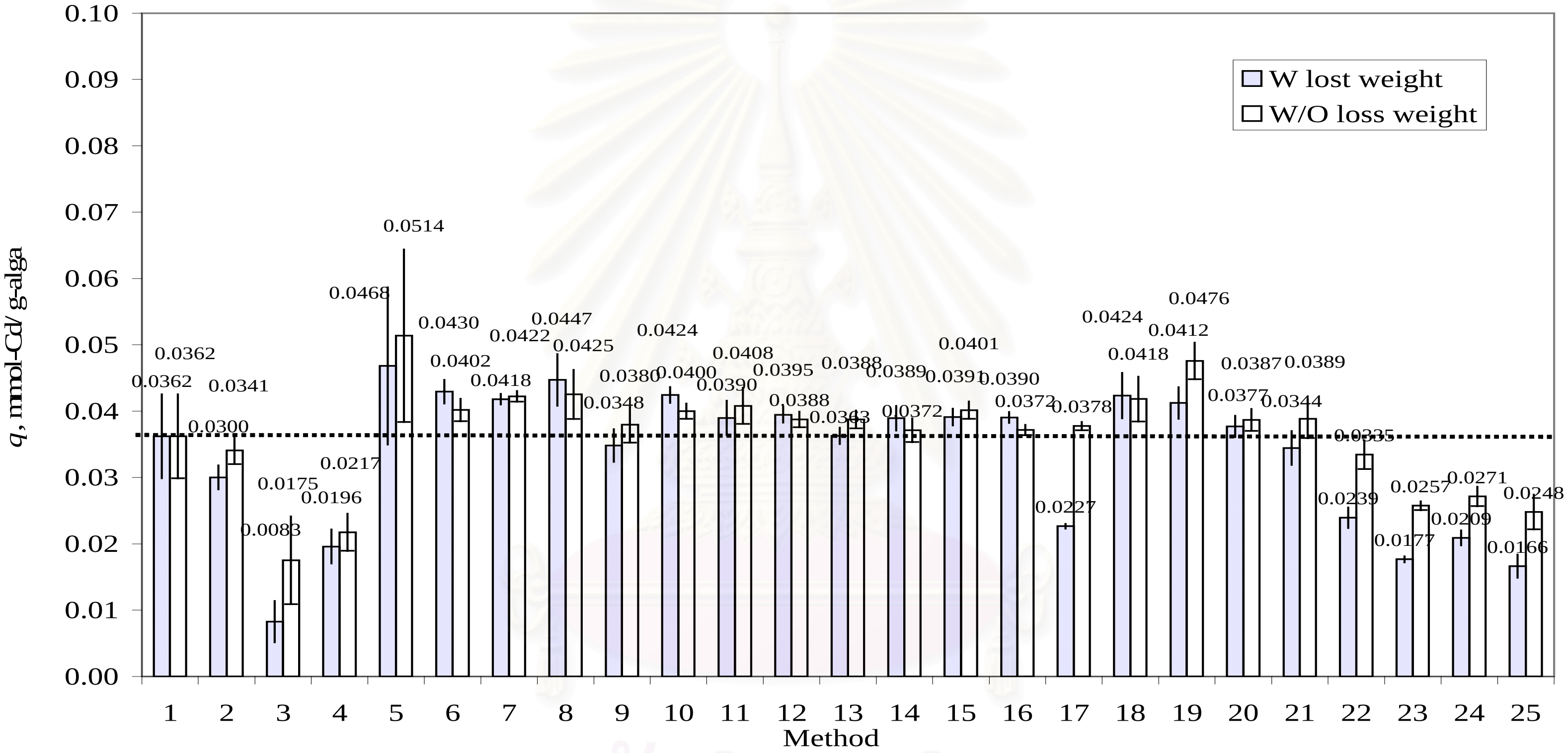


Figure 4.4.2 Biosorption Cd by various pretreated algae (algal mass = 0.5 g, initial conc. = 100 mg/l, pH = 4.5-5.5)

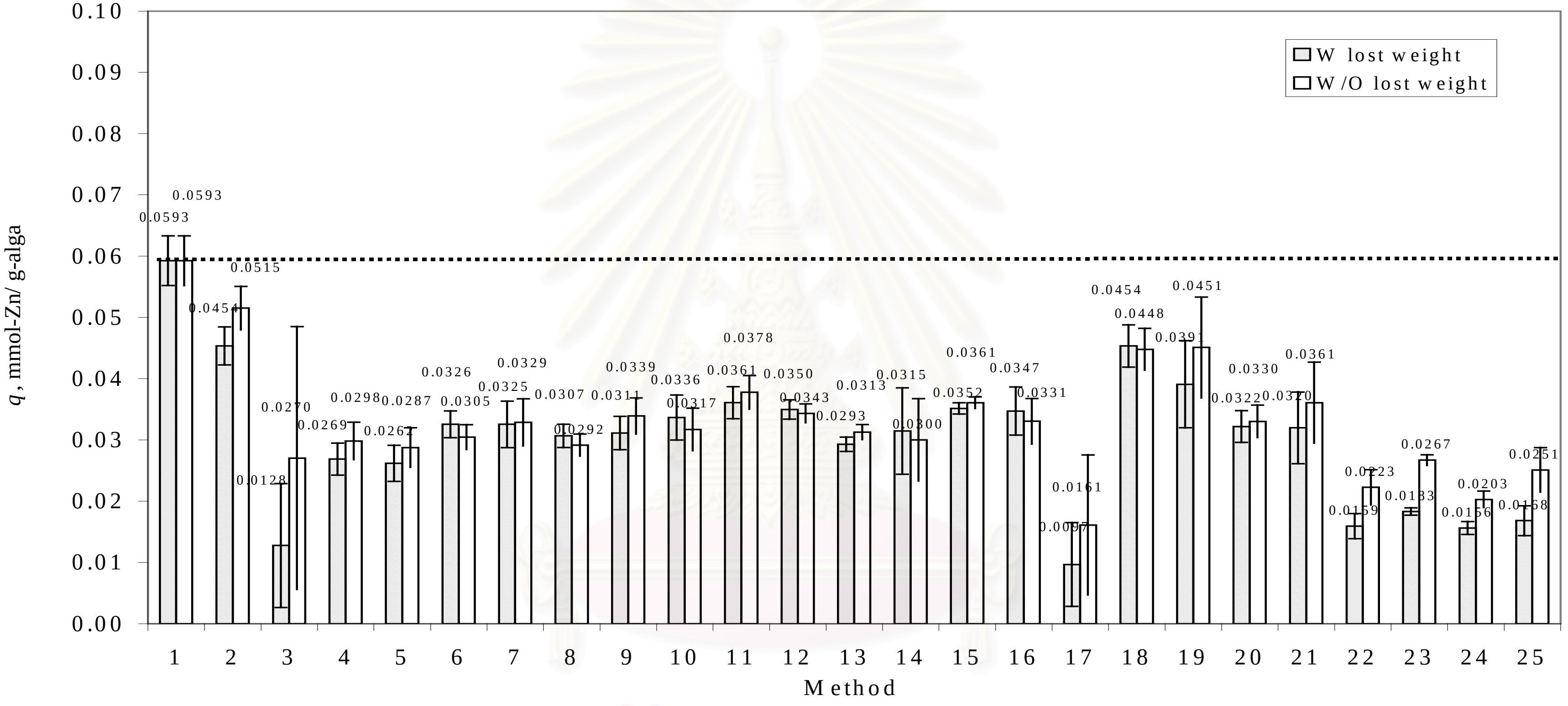


Figure 4.4.3 Biosorption of Zn by various pretreated algae (algal mass = 0.5 g, initial conc. = 100 mg/l, pH = 4.5-5.5)

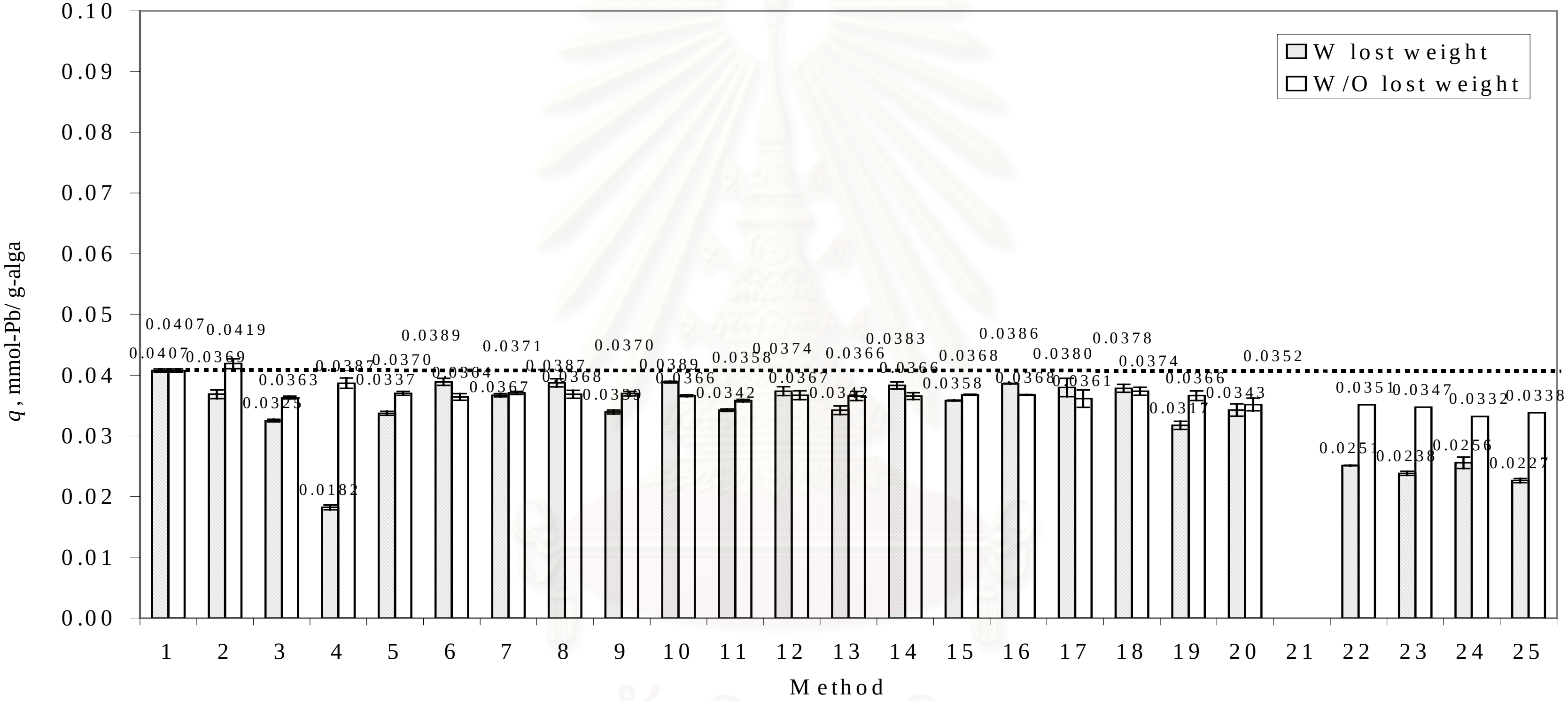


Figure 4.4.4 Biosorption of Pb by various pretreated algae (algal mass = 0.5 g, initial conc. = 100 mg/l, pH = 4.5-5.5)

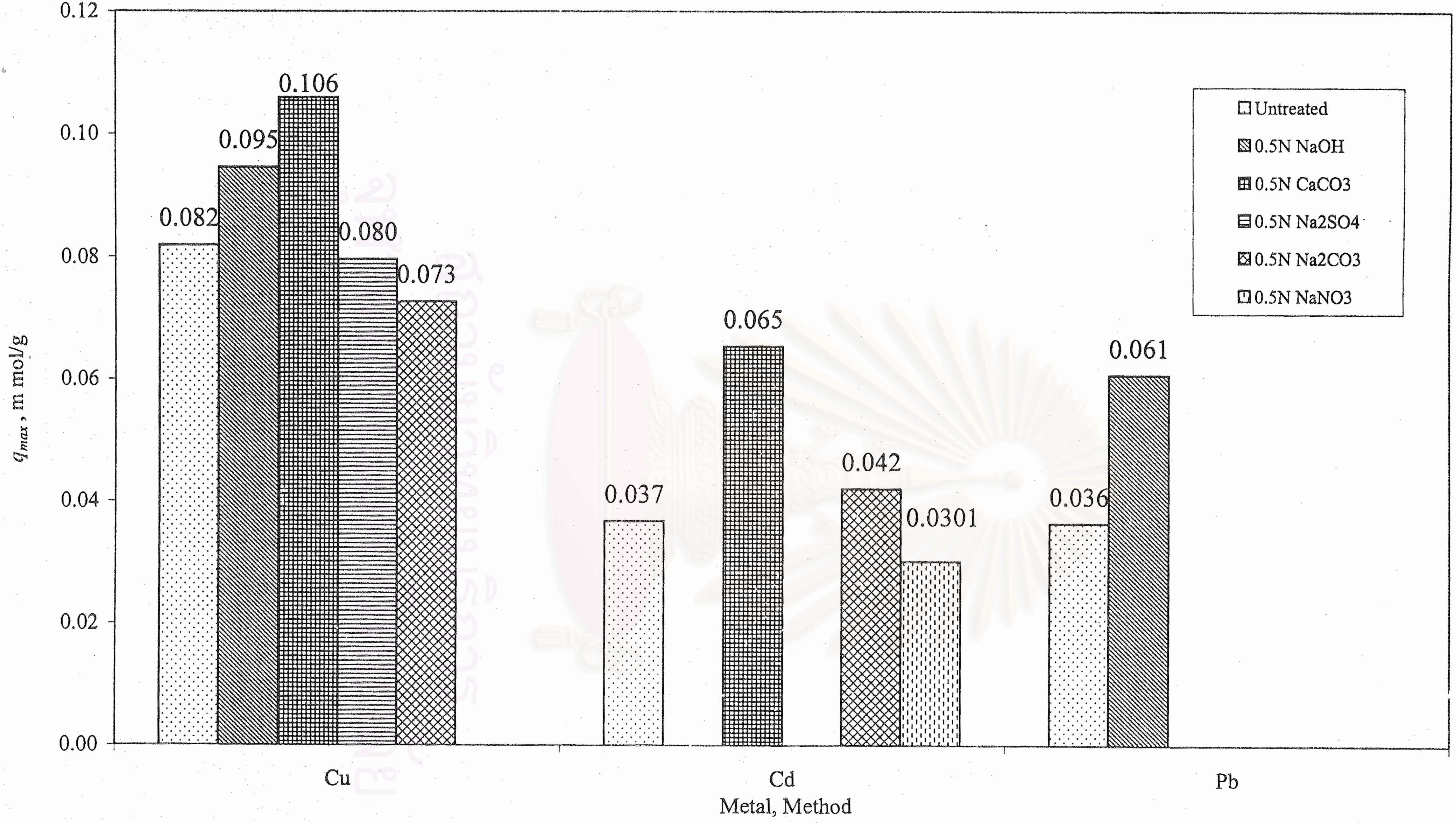


Figure 4.5.1 Adsorption isotherm of various pretreated *Caulerpa lentillifera* alga, q_{max} mmol-metal/g-alga, Langmuir isotherm

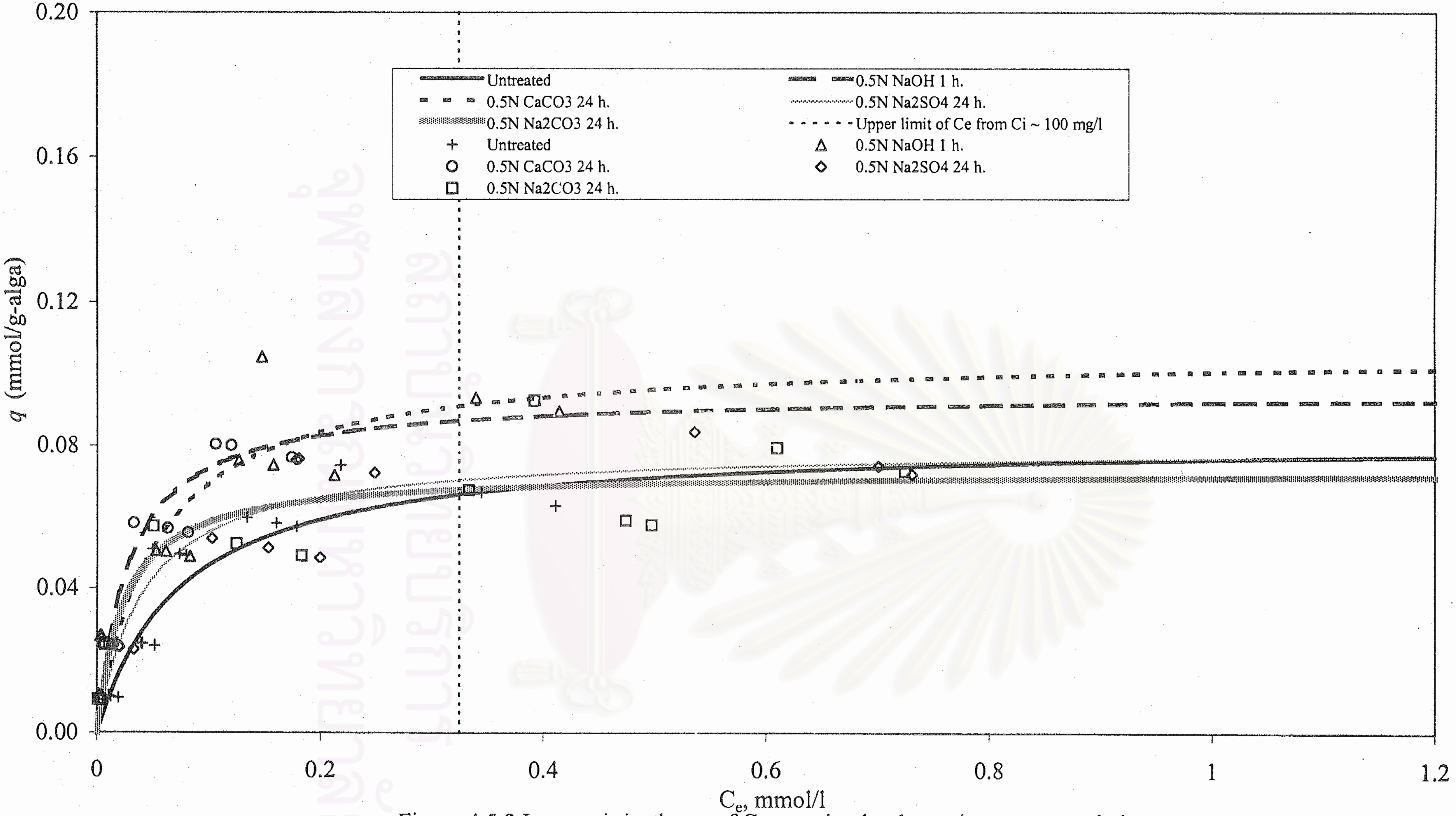


Figure 4.5.2 Langmuir isotherm of Cu sorption by the various pretreated alga

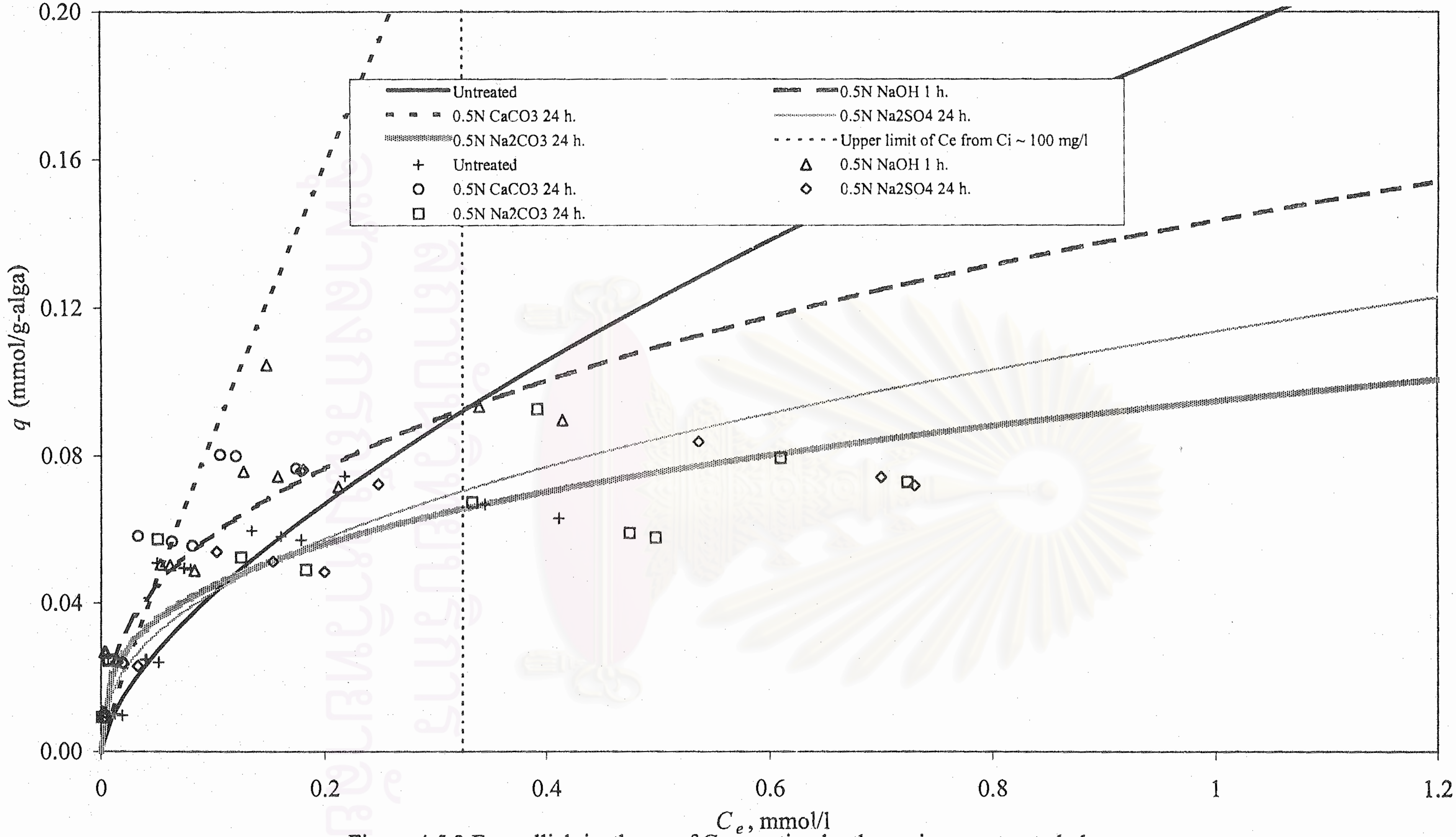


Figure 4.5.3 Freundlich isotherm of Cu sorption by the various pretreated alga

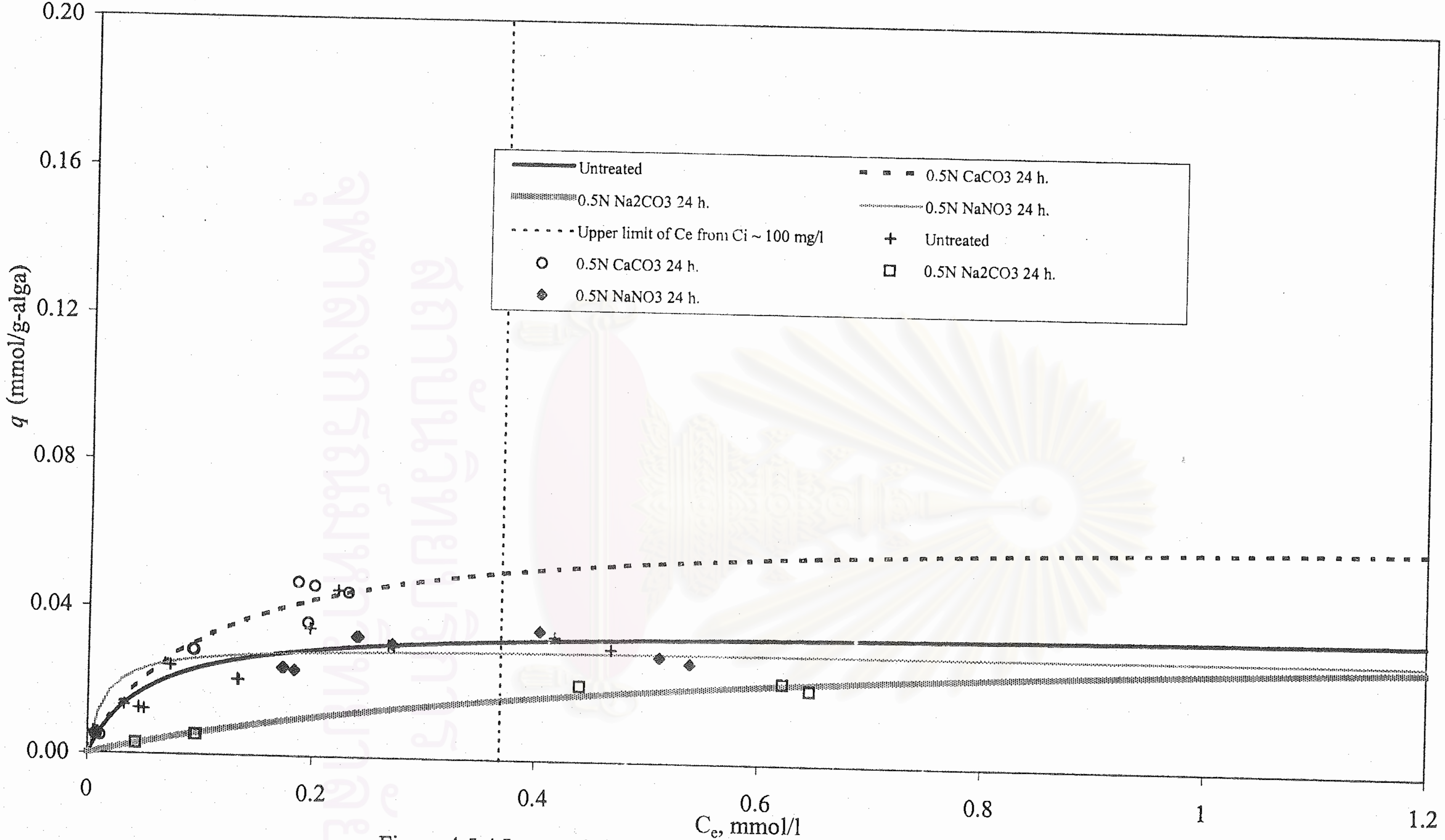


Figure 4.5.4 Langmuir isotherm of Cd sorption by the various pretreated alga

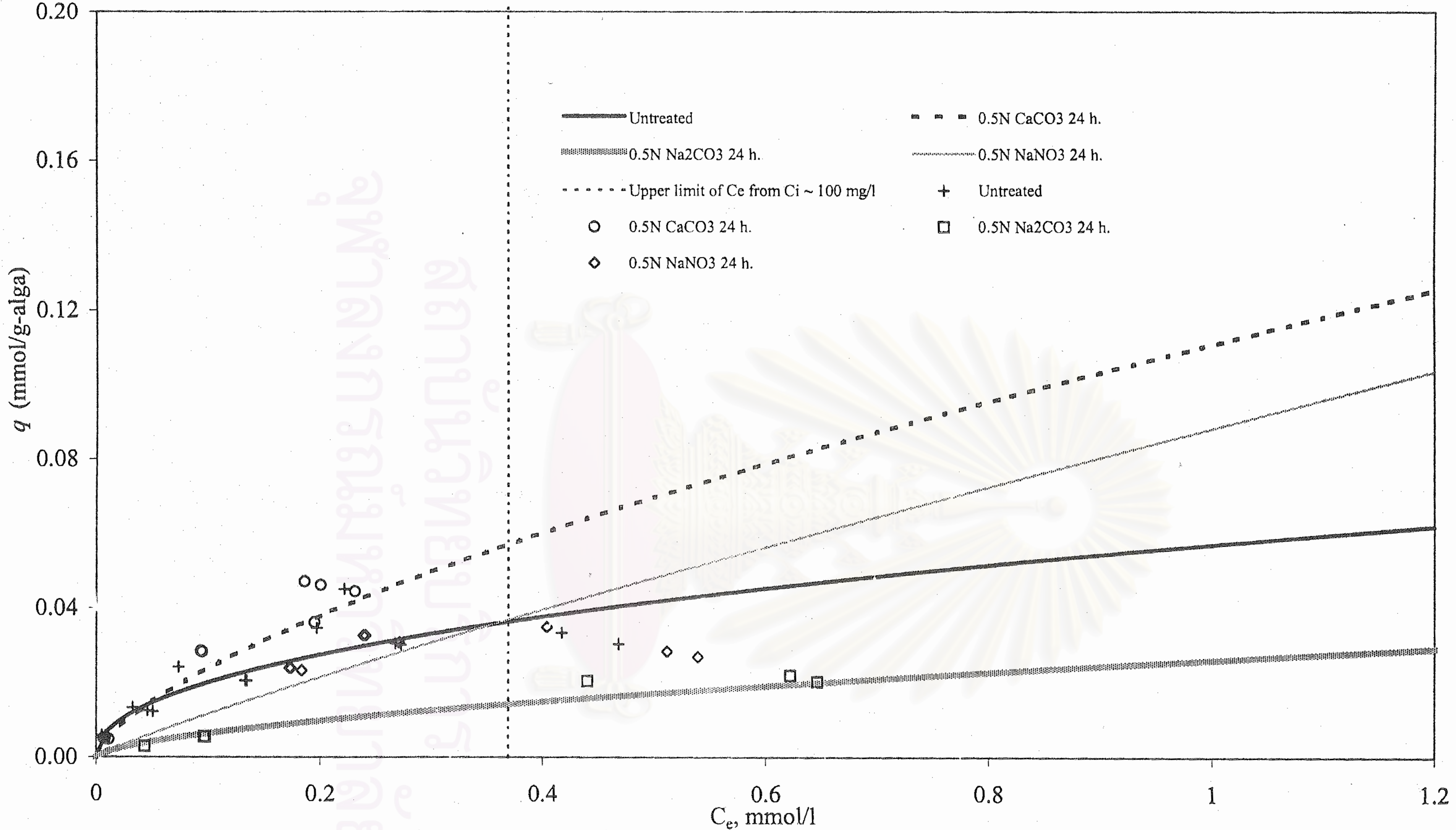


Figure 4.5.5 Freundlich isotherm of Cd sorption by the various pretreated alga

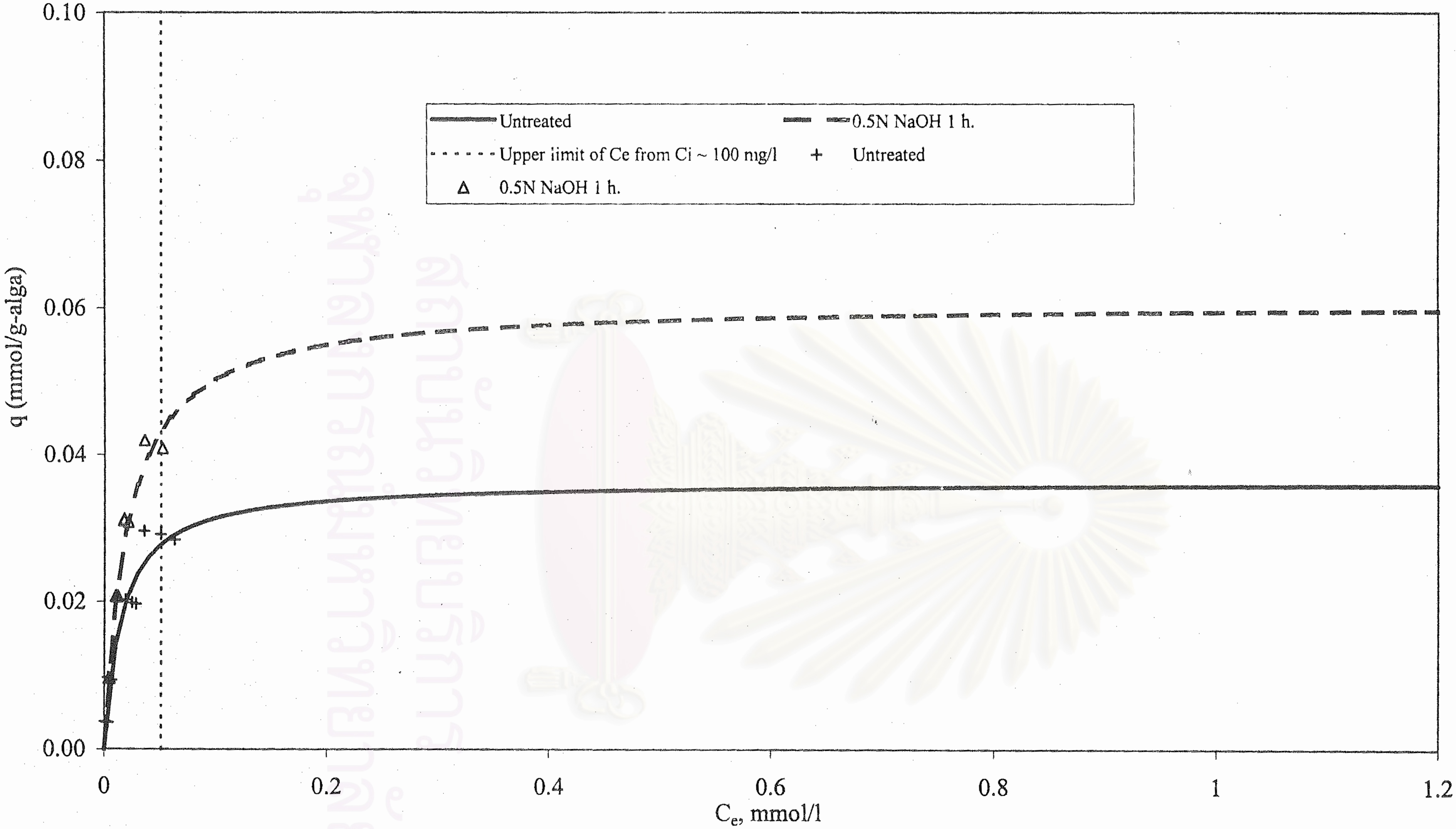


Figure 4.5.6 Langmuir isotherm of Pb sorption by the pretreated alga

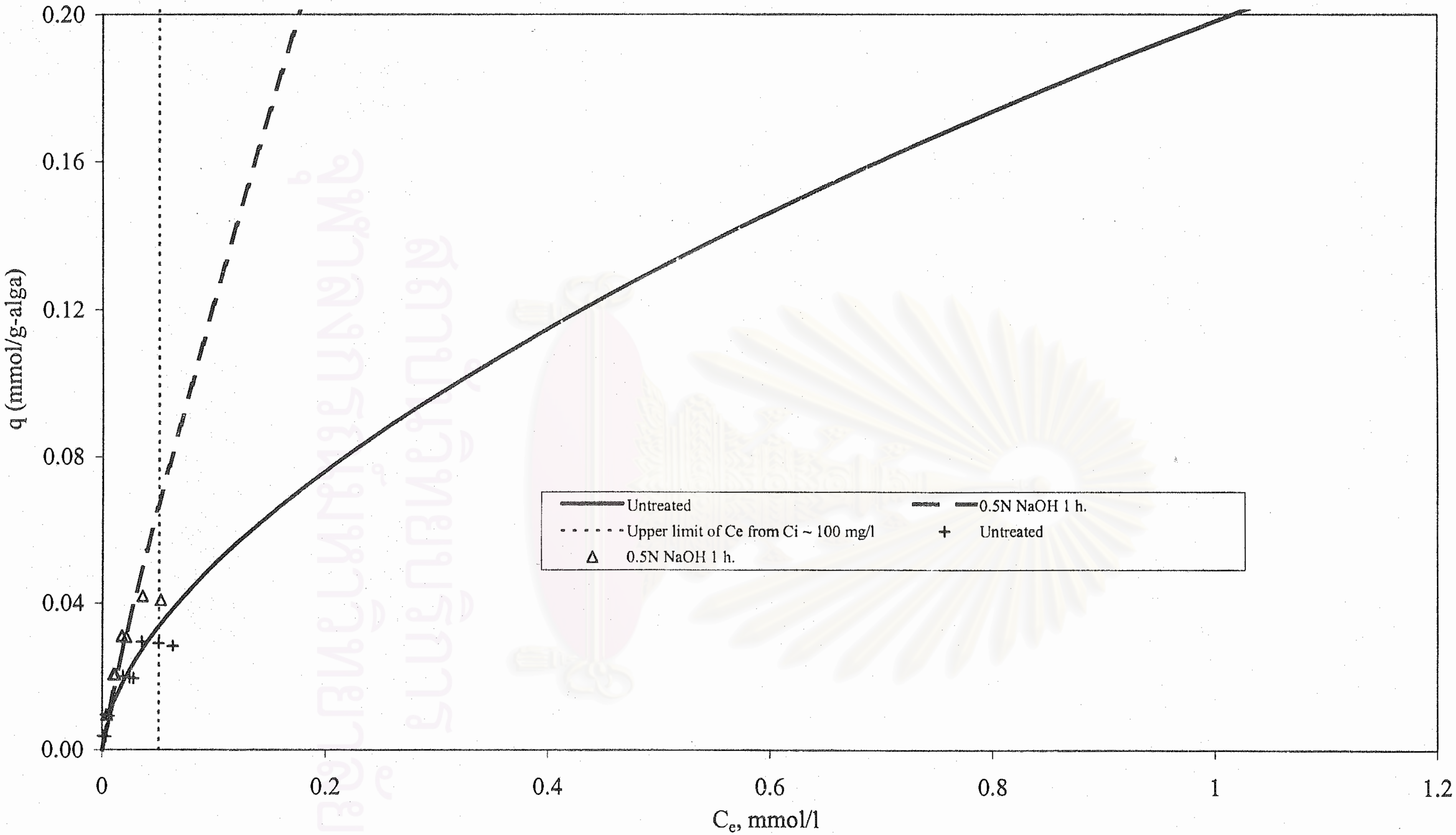


Figure 4.5.7 Freundlich isotherm of Pb sorption by the pretreated alga

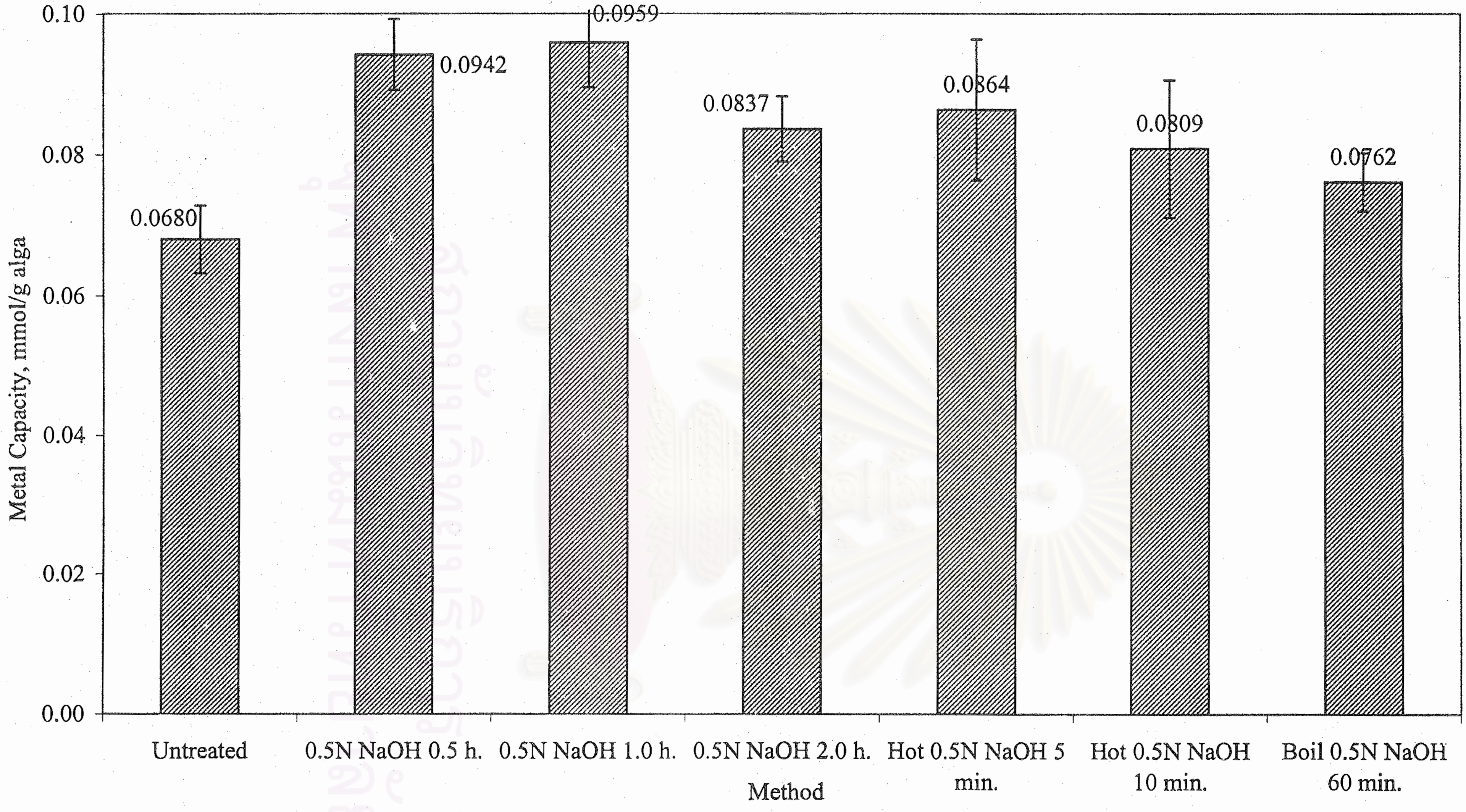


Figure 4.6.1 Effect of different exposure period for Cu adsorption by 0.5N NaOH pretreated alga

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of experimental results

This work provided fundamentals required for the biosorption of Cu, Cd, Zn and Pb. The appropriate pH for the adsorption for each heavy metal was reported along with its associate adsorption isotherm. Specific conclusions on the pretreatment of the algae can be summarized as follows:

1. Among the twenty four pretreatment techniques, only a few were found to increase the adsorption capacities of the metals. These techniques included NaOH 1 hour, $CaCO_3$ 24 hours, Na_2SO_4 24 hours and Na_2CO_3 24 hours for Cu; NaOH 24 hours, $CaCO_3$ 24 hours, $NaNO_3$ 24 hours, $CaSO_4$-$2H_2O$ 24 hours and Na_2CO_3 24 hours for Cd; and 0.5N NaOH 1 hour for Pb.
2. There seemed to be an optimal pretreatment period by 0.5N NaOH suitable for the biosorption. A short treatment time was considered not adequate for the conditioning of algae whilst the too long treatment time seemed to have negative effect on the adsorption capacity.
3. Possible functional groups for the heavy metal sorption were carboxylic acid, amine, amide, amino, sulfonyl and sulfonate. Most pretreatment techniques were found to potentially reduce the quantity of these functional groups. The appropriate treatment techniques were those methods that had slight consequence on the existence of the functional groups in the algae. These included the treatment with $CaCO_3$ and NaOH.

5.2 Contributions of this work

A number of new findings are presented in this work. The effects of various pretreatment techniques were scrutinized in detail with the limited experimental time period. The model evaluation technique for analyzing the effect of pretreatment technique on the possible functional groups for metal sorption was proposed. This is considered more useful than the actual findings as it can be further utilized in this research area to establish the knowledge platform required for the work on the metal adsorption.

However, when compared the heavy metal adsorption capacities of *Caulerpa lentillifera* with those reported for other biosorbents, it was still clear that the capacities of this alga were still not comparable scale (see Table 5.1). Hence, the application of this alga in terms of heavy metal removal still needs more research work particularly on the conditioning of the algal properties. Nevertheless, this work emphasizes the possibility of converting the unwanted agricultural material into a more useful product which should be beneficial for the overall shrimp industry in Thailand.

5.3 Recommendations

Although large research areas were covered in this work, there are still many points that could not be fully examined. It has to be admitted that the scope of this work was quite large and detailed investigation on each particular subject, e.g. effect of pretreating time, FTIR work, etc. could not be completed with the time required for the Master degree work. In order to ensure the completeness of this study, further work or more detail work should be carried out. Some recommendations are inherited during the course of this work and these are summarized as follows:

1. Detailed investigation on the functional groups including both quantitative and qualitative analyses of *Caulerpa lentillifera* should be carried out to further identify the target functional groups for each of the metals.
2. Experiments should be conducted to examine the effect of concentration of the treating media and perhaps the times and other condition required during the pretreatment of the algae.
3. Further work on the actual adsorption unit is urgently needed to ensure the applicability of this alga. In addition, tests with actual wastewater containing heavy metals are extremely important.

Table 5.1 Comparison of Cu, Cd, Zn and Pb uptake capacities (q_{max}, mmol/g) of various adsorbents.

Adsorbent	Type	Copper	Cadnium	Zinc	Lead	Reference
Caulerpa lentillifera	Marine alga	0.107	0.037	0.037	0.053	This work.
0.5N NaOH *Caulerpa lentillifera*	Marine alga	0.095	0.034	0.051	0.058	This work.
Boiled with 0.5N NaOH *Caulerpa lentillifera*	Marine alga	0.067	0.019	0.029	0.040	This work.
0.5N $CaCO_3$ *Caulerpa lentillifera*	Marine alga	0.1059	.0654			This work.
Durvillaea. potatorum treated by 0.2M $CaCl_2$	Marine alga	1.30			1.55	Matheickal and Yu (1999)
Ecklonia radiata treated by 0.2M $CaCl_2$	Marine alga	1.11			1.26	Matheickal and Yu (1999)
Padina sp. treated by 0.2M $CaCl_2$	Marine alga	0.80				Kaewsarn (2002)
Ascophyllum nodosum 1	Marine alga	1.19	1.03			Yu et al. (1999)
Ascophyllum nodosum 2	Marine alga	1.09	0.93			Yu et al. (1999)
Padina sp. treated by 0.2M $CaCl_2$	Marine alga		0.53			Kaewsarn and Yu, 2001.

Adsorbent	Type	Copper	Cadnium	Zinc	Lead	Reference
Laminaria japonica	Marine alga		1.30			Yin et al. (2001)
Scenedesmus subspicatus	Green microalga			1.1023	0.1868	Schmitt et al. (2001)
Porphyridium purpureum	Seawater red alga			0.0307	0.0015	Schmitt et al. (2001)
Phaeodactylum tricornutum	Seawater diatom			0.2221	0.0072	Schmitt et al. (2001)
Cyclotella crytica	Brackish water diatom			3.7158	0.1770	Schmitt et al. (2001)
Siliceous earth				0.0031	0.0004	Schmitt et al. (2001)
Gracilaria canaliculata	Red marine alga				0.2017	Jalia et al. (2002)
Sargassum hytrix	Brown marine alga				1.3755	Jalia et al. (2002)

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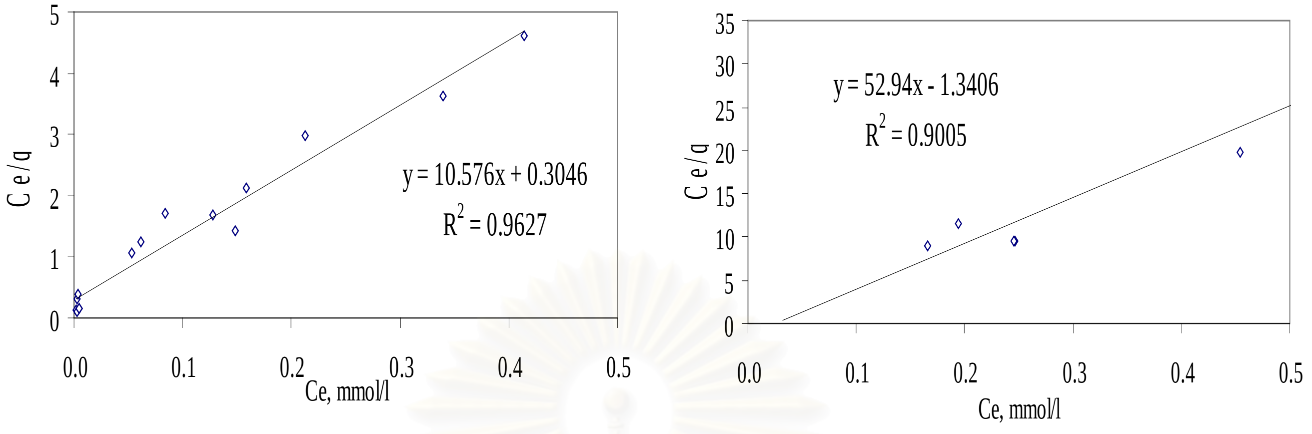
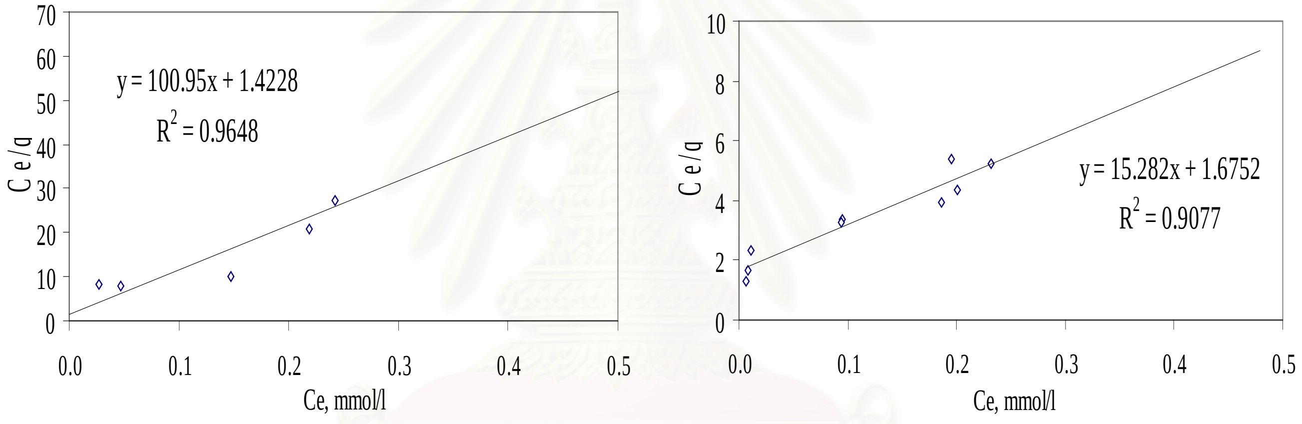
APPENDICES

APPENDIX A
The isotherm relation of Langmuir and Freundlich



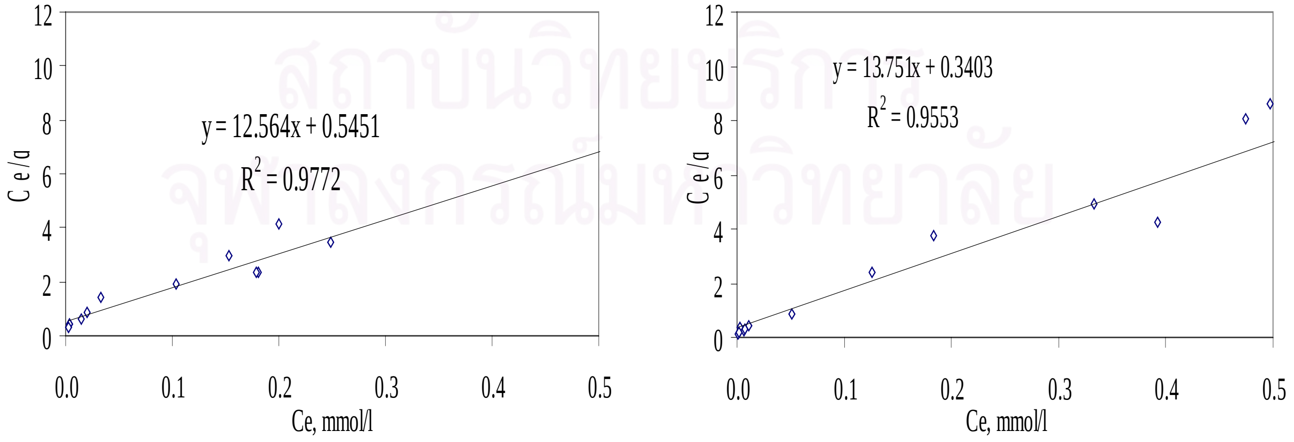
(A) 0.5N NaOH 24 hours

(B) boiling in 0.5N NaOH 1 hour



(C) 0.5N NaCl 24 hours

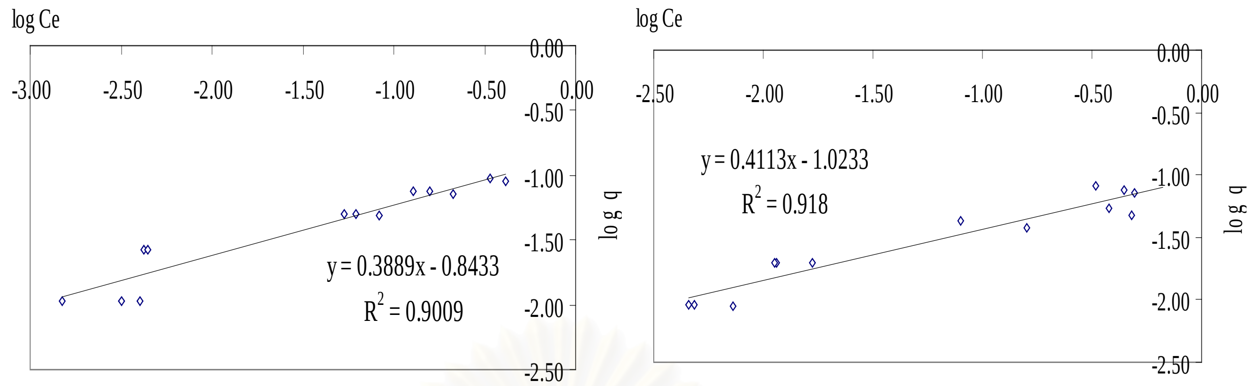
(D) 0.5N $CaCO_3$ 24 hours



(E) 0.5N Na_2SO_4 24 hours

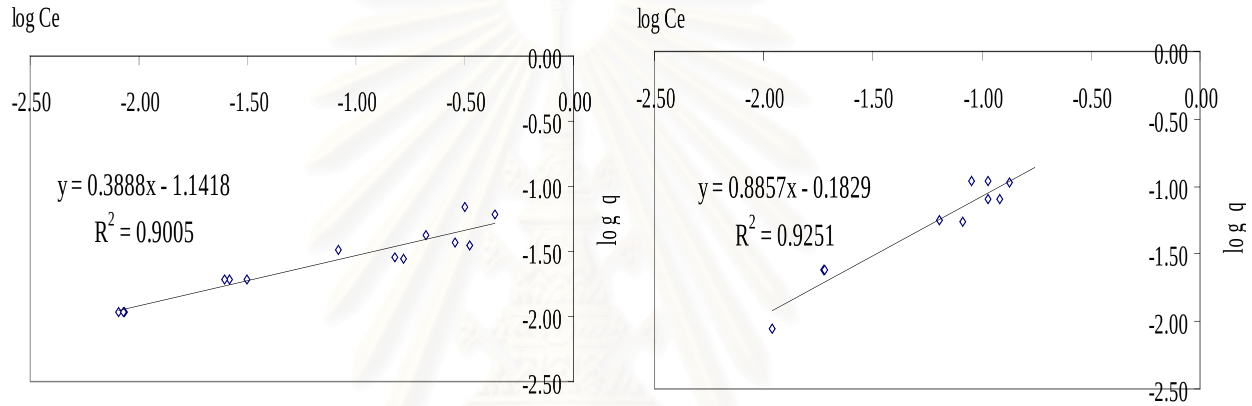
(F) 0.5N Na_2CO_3 24 hours

Figure A.1 Langmuir's isotherm of Cu for the pretreated algae



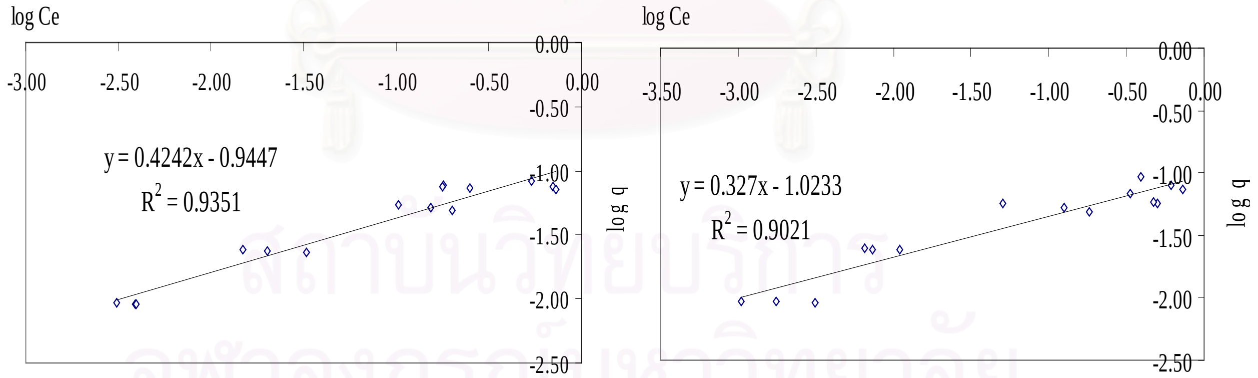
(A) 0.5N NaOH 1 hours

(B) boiling in 0.5N NaOH 1 hour



(C) 0.5N NaCl 24 hours

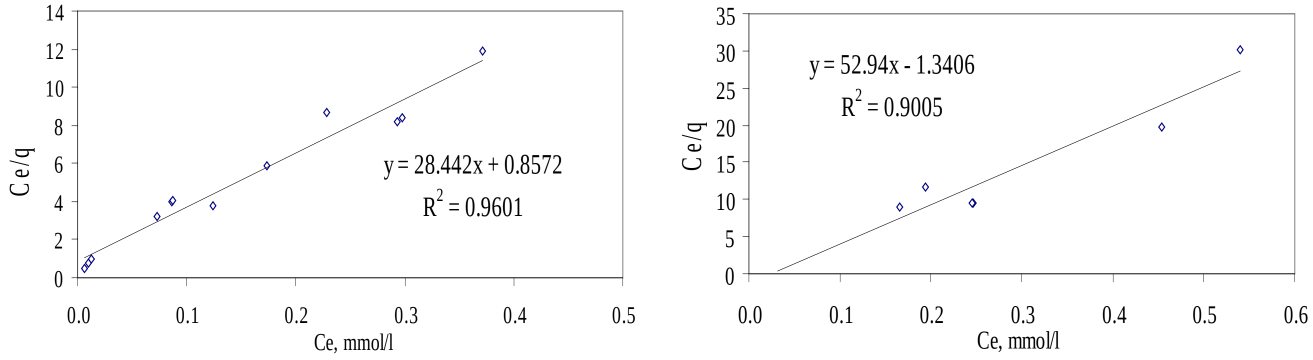
(D) 0.5N $CaCO_3$ 24 hours



(E) 0.5N Na_2SO_4 24 hours

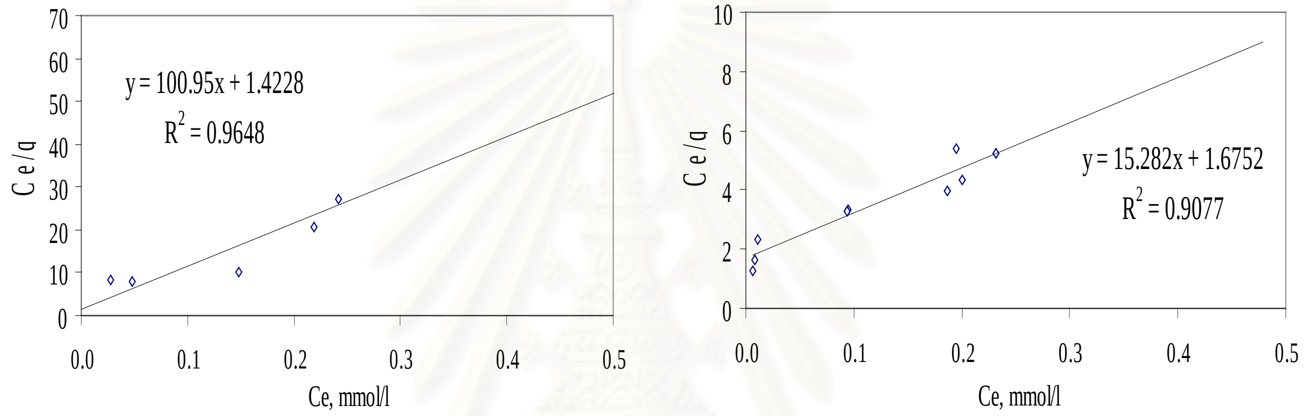
(F) 0.5N Na_2CO_3 24 hours

Figure A.2 Freundlich’s isotherm of Cu for the pretreated algae



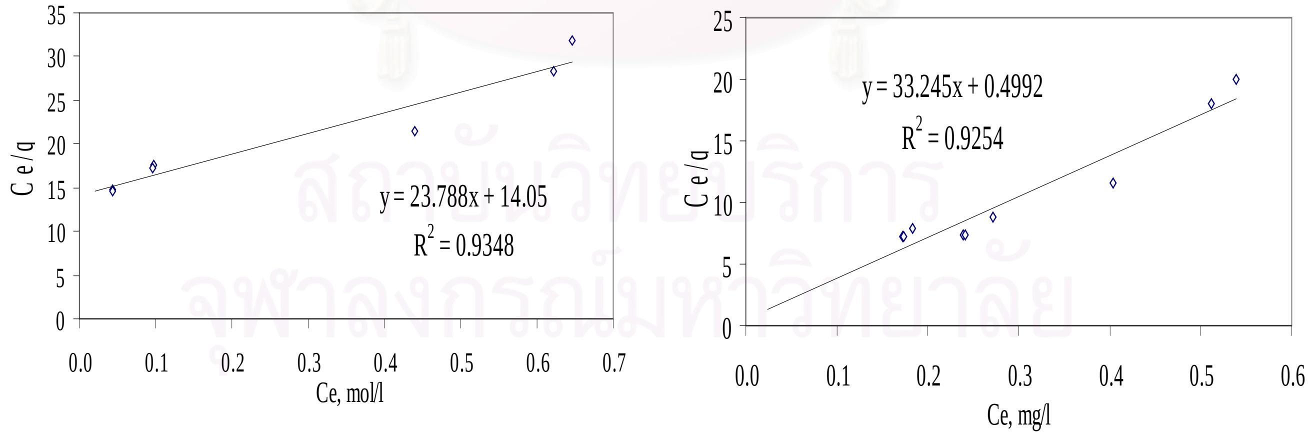
(A) 0.5N NaOH 24 hours

(B) Boiling in 0.5N NaOH 1 hour



(C) 0.5N NaCl 24 hours

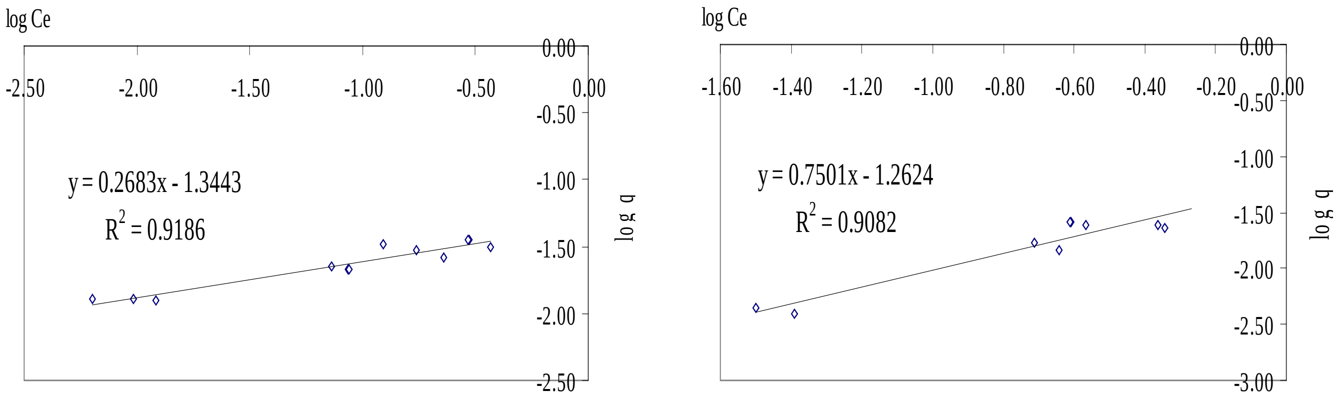
(D) 0.5N $CaCO_3$ 24 hours



(E) 0.5N Na_2CO_3 24 hours

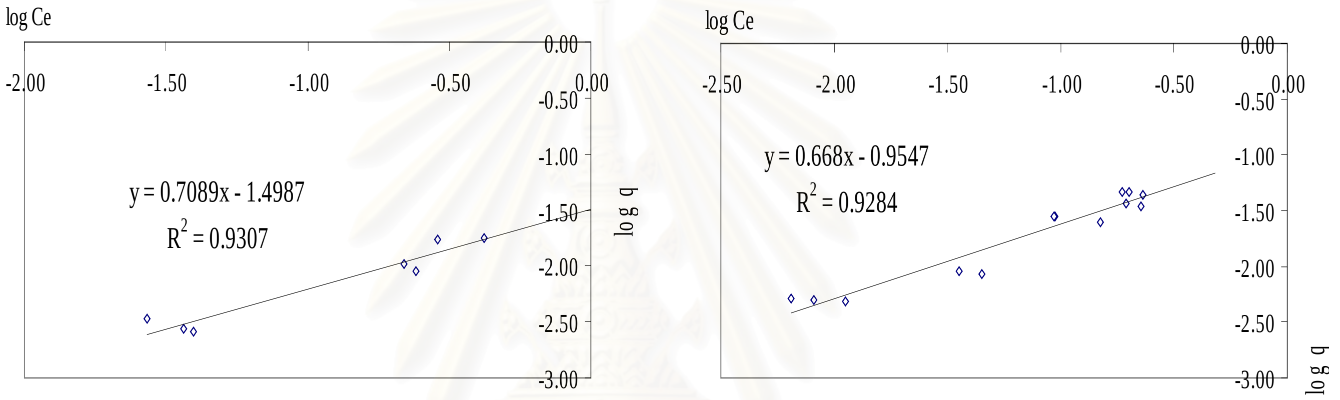
(F) 0.5N $NaNO_3$ 24 hours

Figure A.3 Langmuir's isotherm of Cd for the pretreated algae



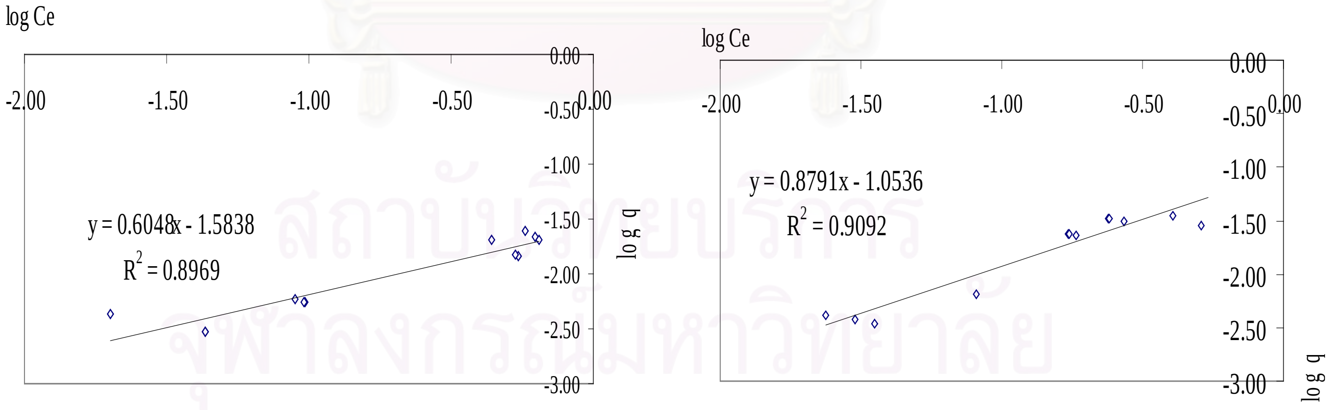
(A) 0.5N NaOH 24 hours

(B) Boiling in 0.5N NaOH 1 hour



(C) 0.5N NaCl 24 hours

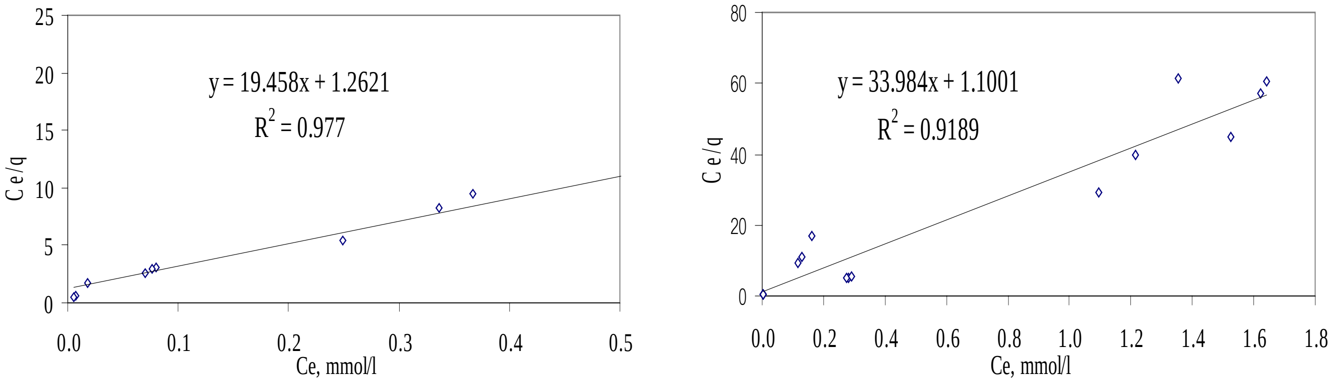
(D) 0.5N $CaCO_3$ 24 hours



(E) 0.5N Na_2CO_3 24 hours

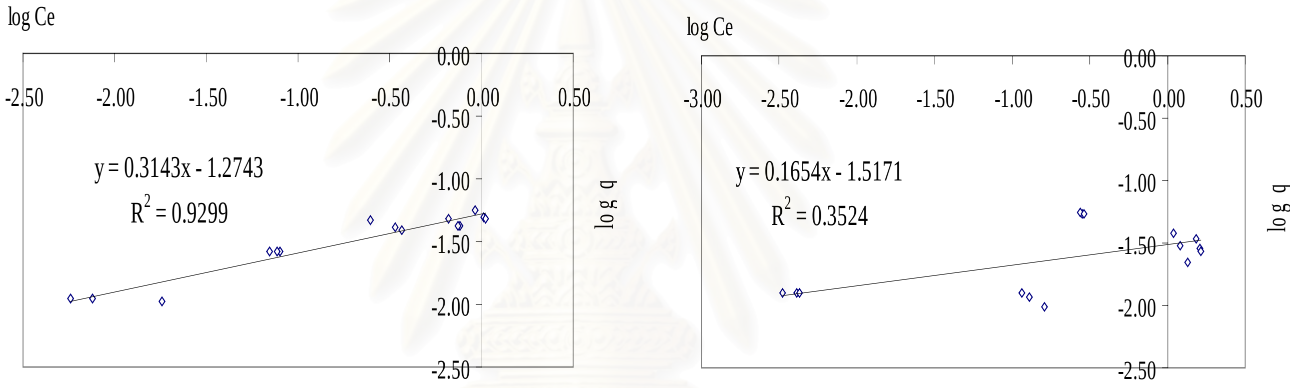
(F) 0.5N $NaNO_3$ 24 hours

Figure A.4 Freundlich's isotherm of Cd for the pretreated algae



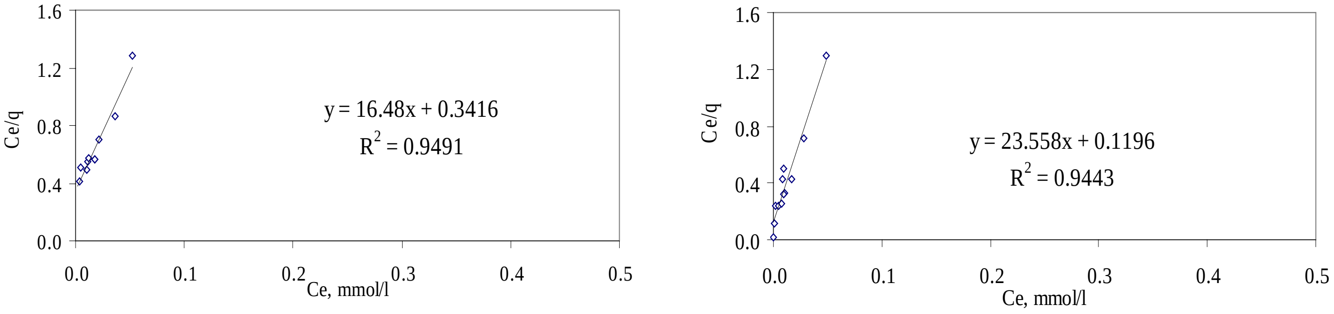
(A) 0.5N NaOH 24 hours (B) Boiling in 0.5N NaOH 1 hour

Figure A.5 Langmuir's isotherm of Zn for the pretreated algae



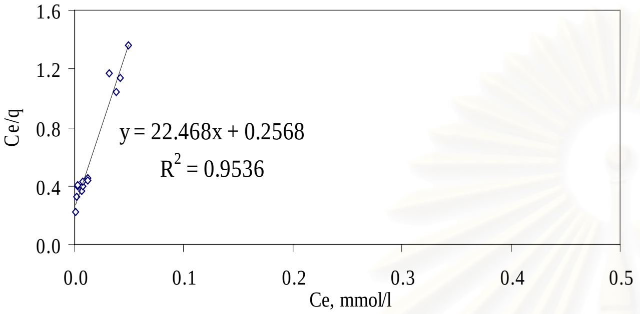
(A) 0.5N NaOH 24 hours (B) Boiling in 0.5N NaOH 1 hour

Figure A.6 Freundlich's isotherm of Zn for the pretreated algae



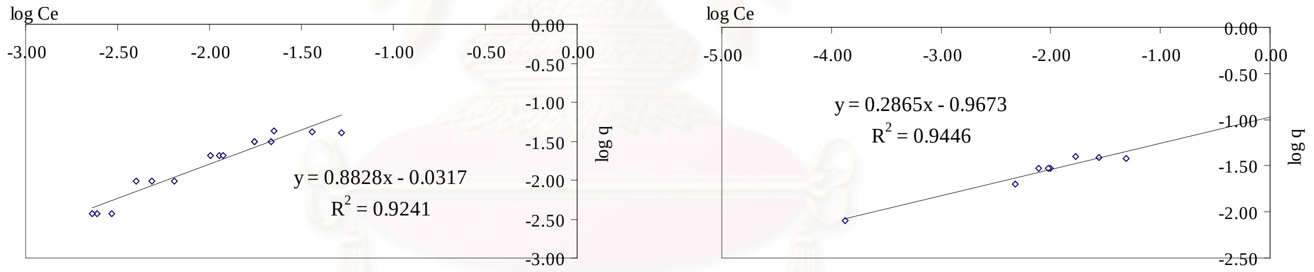
(A) 0.5N NaOH 24 hours

(B) Boiling in 0.5N NaOH 1 hour



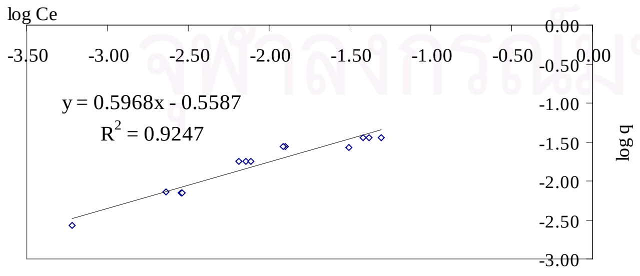
(C) 0.5N NaCl 24 hours

Figure A.7 Langmuir’s isotherm of Pb for the pretreated algae



(A) 0.5N NaOH 24 hours

(B) Boiling in 0.5N NaOH 1 hour



(C) 0.5N NaCl 24 hours

Figure A.8 Freundlich’s isotherm of Pb for the pretreated algae

APPENDIX B
FTIR line spectrum of the biosorbents

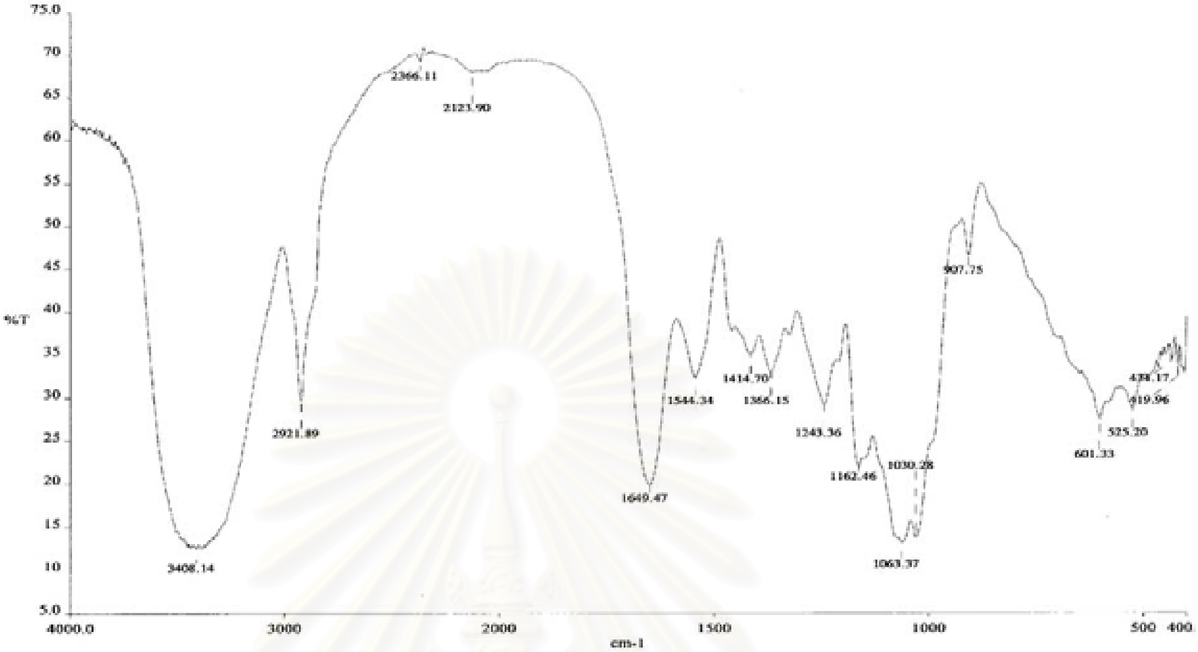


Figure B.1 FTIR results: Untreated *C. lentillifera*

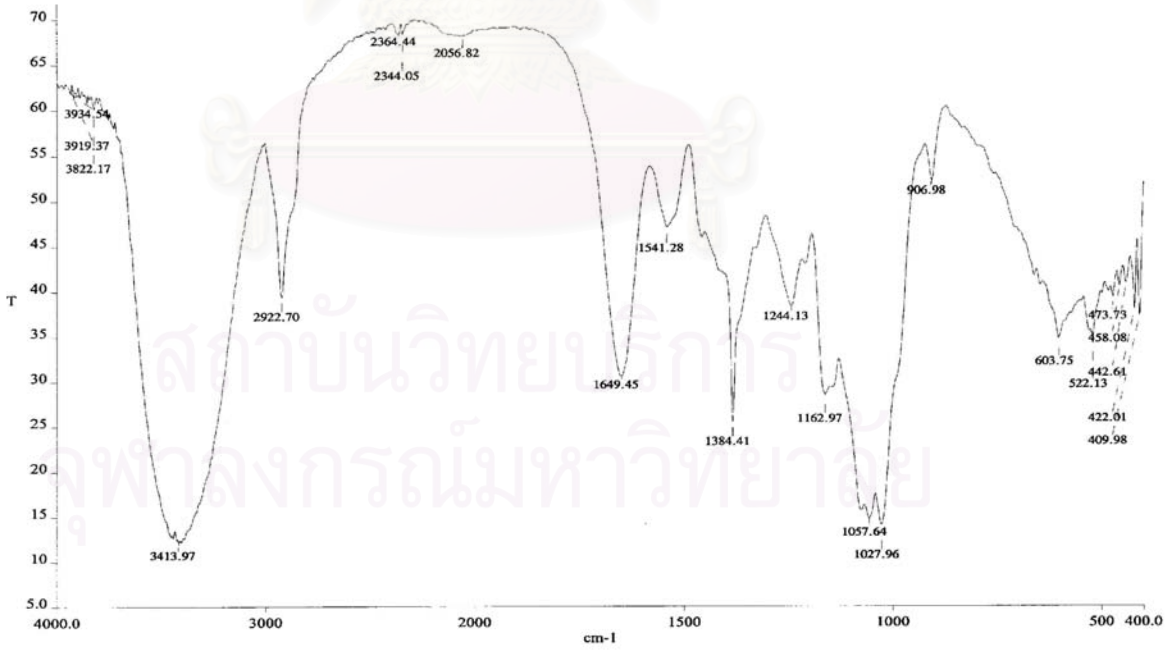


Figure B.2 FTIR results: Untreated *C. lentillifera* after Cu adsorption

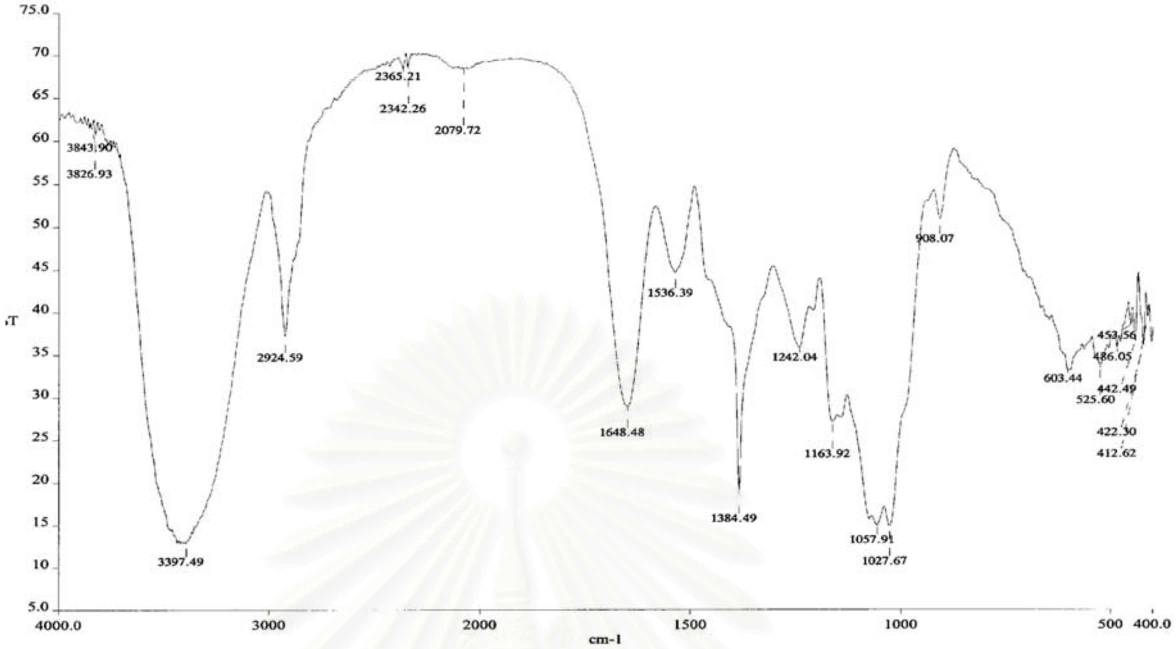


Figure B.3 FTIR results: Untreated *C. lentillifera* after Cd adsorption

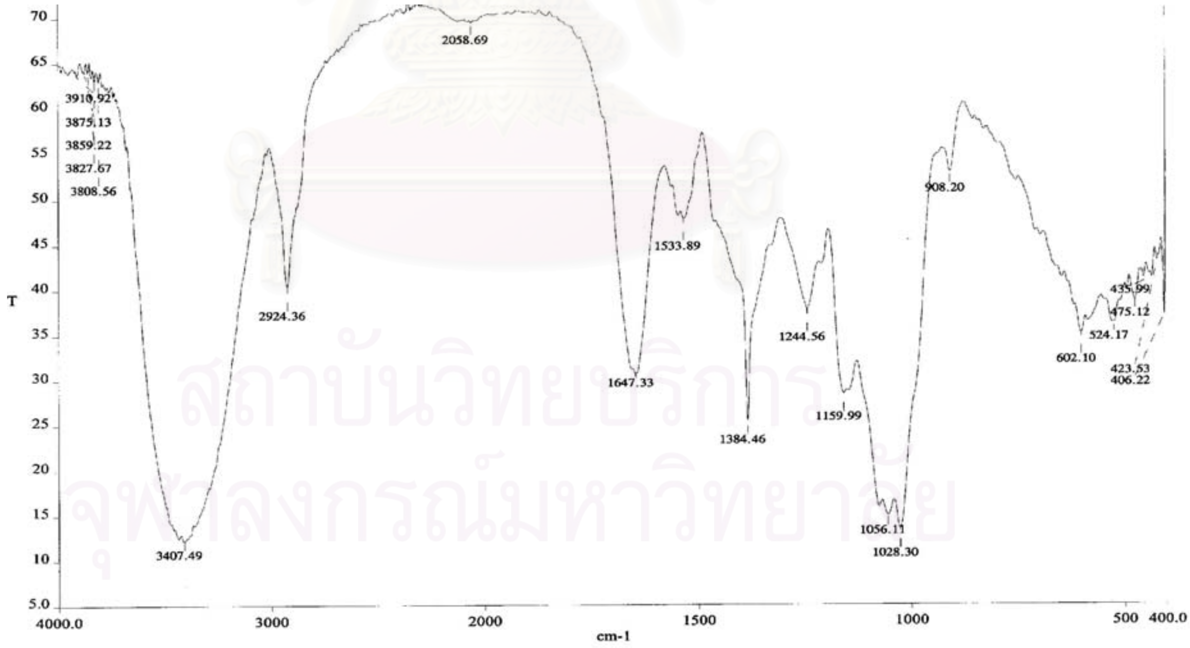


Figure B.4 FTIR results: Untreated *C. lentillifera* after Zn adsorption

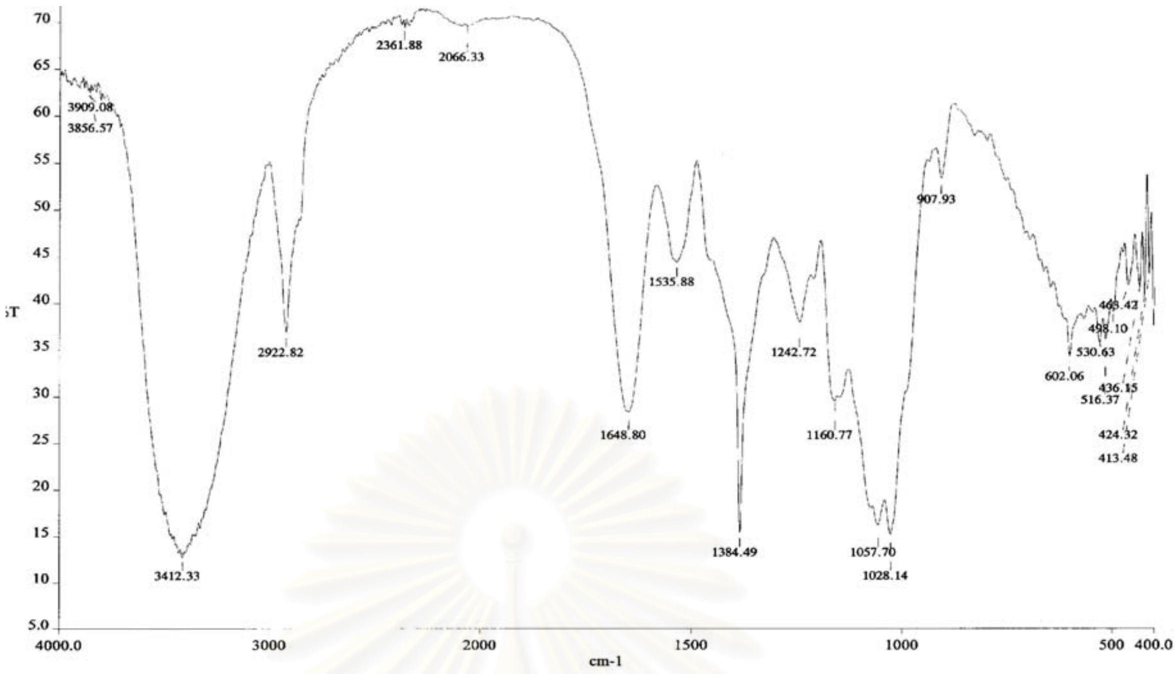
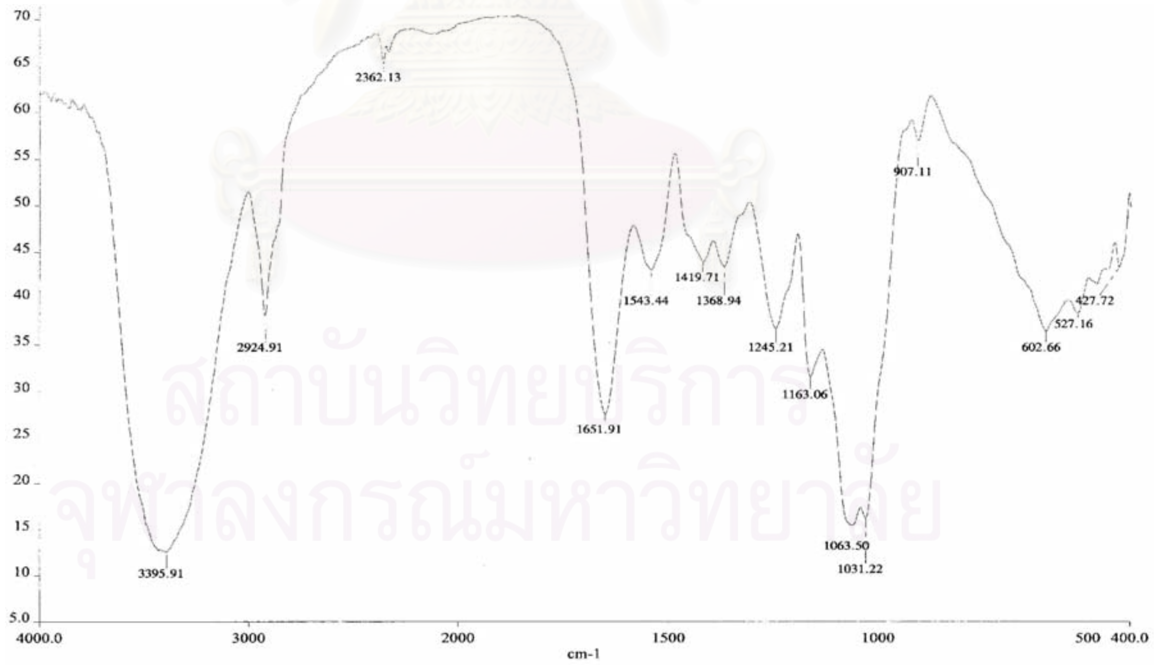


Figure B.5 FTIR results: Untreated *C. lentillifera* after Pb adsorption



FigureB.6 FTIR results: Pretreated *C. lentillifera* by 0.5N NaOH 1 hour

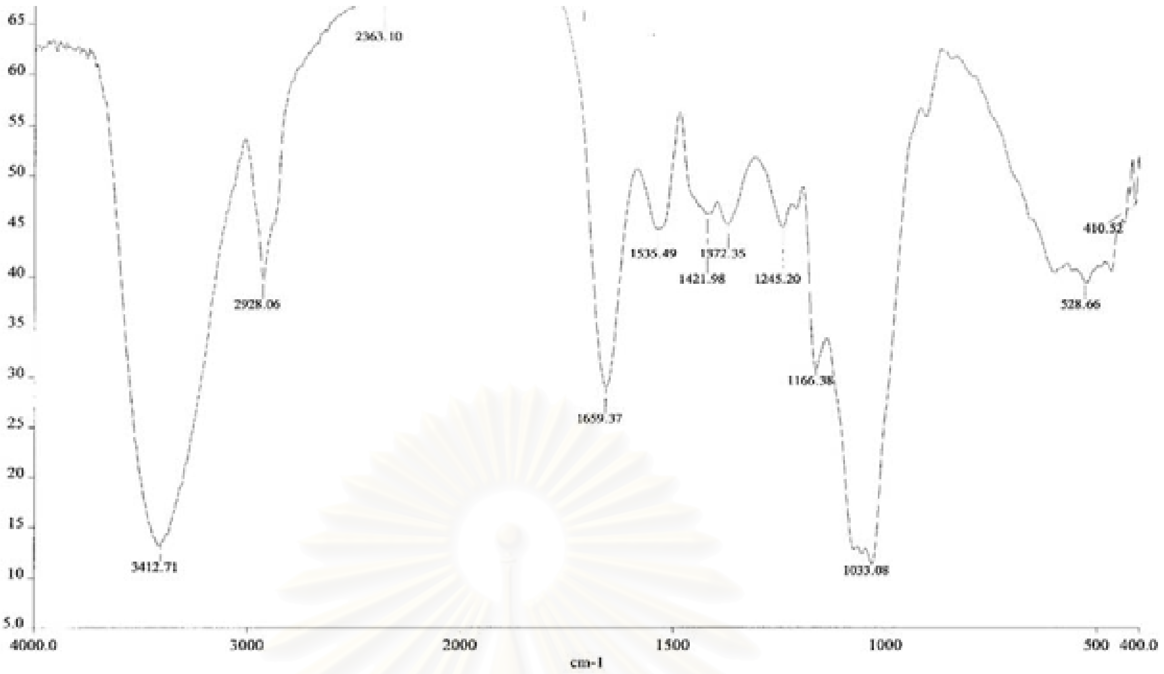


Figure B.7 FTIR results: Pretreated *C. lentillifera* by 0.5N NaCl 24 hours

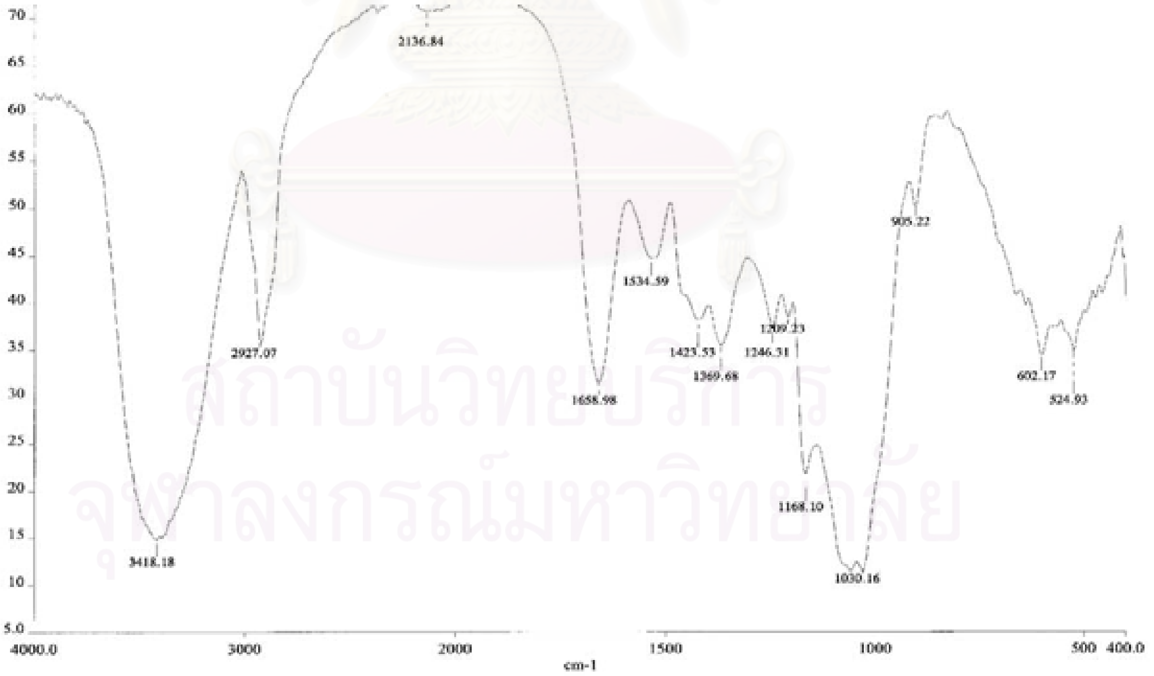


Figure B.8 FTIR results: Pretreated *C. lentillifera* by 0.5N Na_2CO_3 24 hours

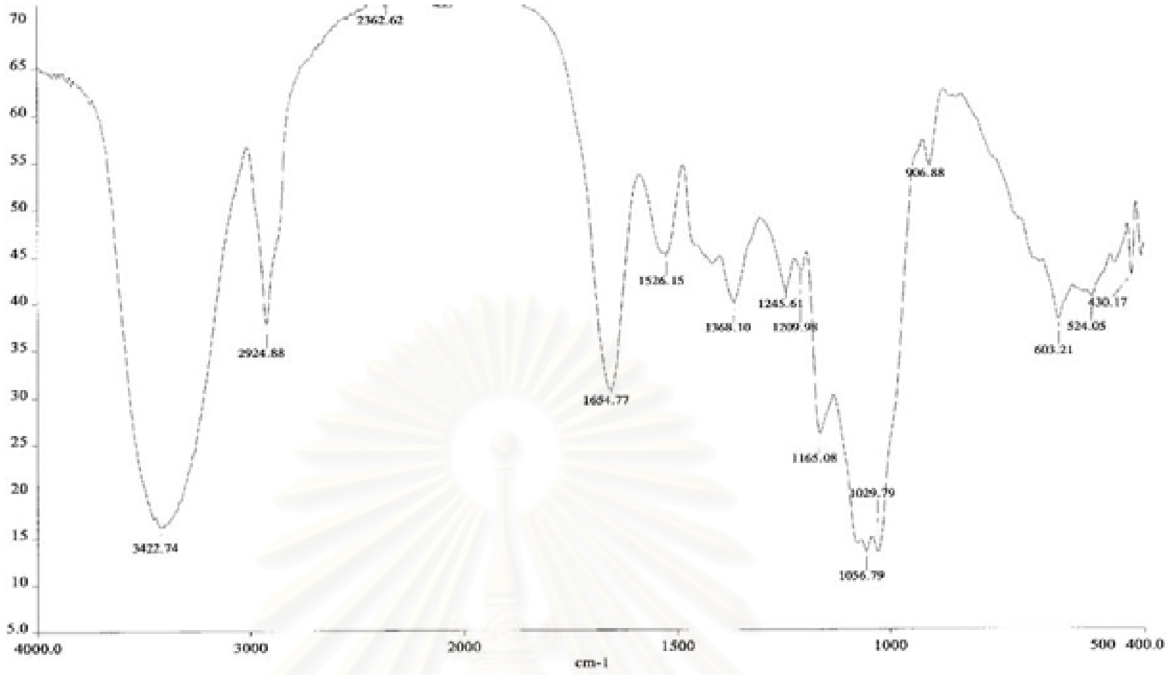


Figure B.9 FTIR results: Pretreated *C. lentillifera* by 0.5N HCl 24 hours

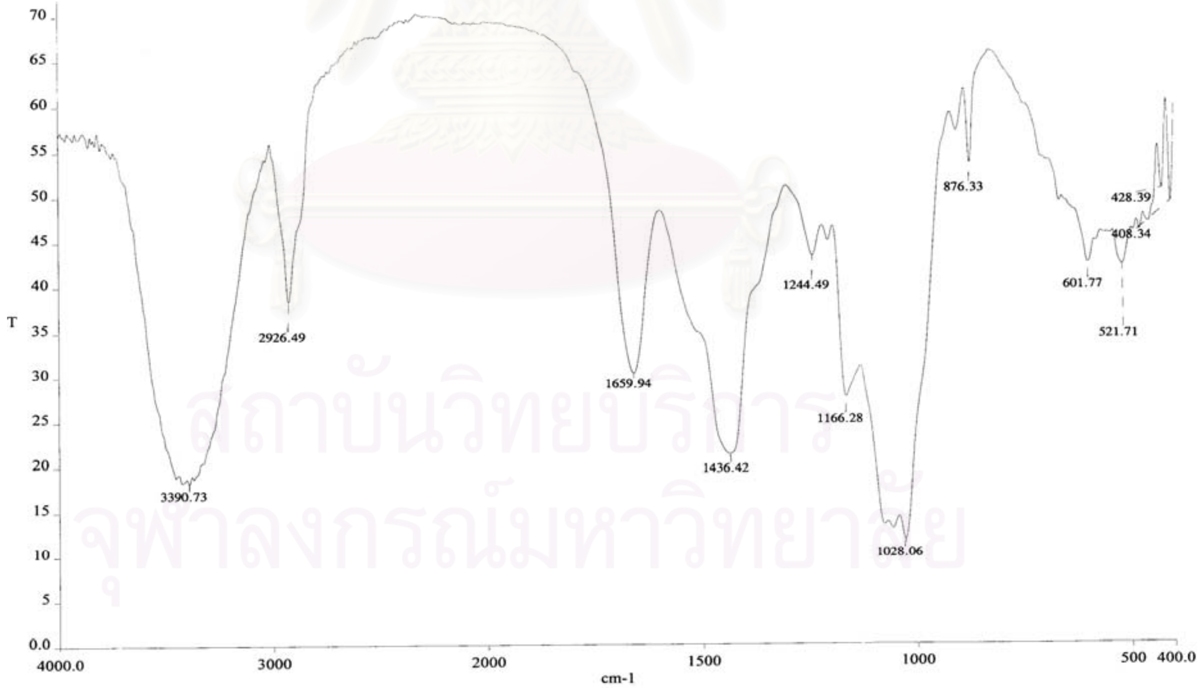


Figure B.10 FTIR results: Pretreated *C. lentillifera* by 0.5N $CaCO_3$ 24 hours

BIOGRAPHY

Mr. Prateep Suthiparinyanont was born on 27th September, 1976 in Bangkok. He was graduated bachelor's degree in the major of Environment in Faculty of Engineering from Chulalongkorn University. He worked as a process engineer of water and effluent department in Advance Agro Public Co., Ltd., integrated pulp and paper industry for 4 years. Afer that he continued his further study for Master's degree in International Environment Science at Chulalongkorn University and achieved his Master's degree in April, 2004. Recently, he presented a part of this thesis entitled "Pretreatment of *Caulerpa lentillifera* with NaOH for biosorption of Cu, Cd, Pb and Zn" in the 3rd national environmental conference, 2004 at Songkhla, Thailand.