CHAPTER 3

RESEARCH METHODOLOGY

3.1 Material

3.1.1 Equipment

- Rotary Shaker, GFL, 3017
- Magnetic stirer, Clifton UK, MSU-3
- Magnetic Bar
- pH-meter, HACH, SensIon 1 and SensIon 3
- Oven, WTB binder, FD115 (E2)
- Digital Balance (4 digits), Sartorius, BP2215
- Dessicator
- Water Purification System (For making DI water), ELGA, Ultraanalytic
- Filter paper No. 93, Whatman
- Filter paper GF/C, Toyo
- Vacuum Pump, KNF Neuberger, NO35AN. 18-IP20
- Refrigerator
- Microwave digester, ETHOL, 10S
- Inductively Couple Plasma (ICP) , Vista
- Fourier Transform Infared Spectrometer (FT-IR), Perkin Elmer, Model 1760X

3.1.2 Glassware

- Erlenmeyer flasks
- Volumetric flasks
- Filtering Flasks
- Bushner Flasks
- Three ways junction
- Glass watches
- Beakers
- Pipettes
- Cylinders
- Dropper
- Funnels

3.1.3 Chemical Reagents

- Analytical grade $Cu(NO_3)_2 * 2.5\ H_2O$
- Analytical grade $Cd(NO_3)_2 * 4\ H_2O$
- Analytical grade $Pb(NO_3)_2$
- Analytical grade $Zn(NO_3)_2 * 6\ H_2O$
- HNO_3 Conc. , 69%
- NaOH anhydrous
- Deionized water (DI water)
- pH buffer solution 4.00 ± 0.02, Scharlau chemie
- pH buffer solution 7.00 ± 0.02, HACH
- Mix metal standard solution for ICP, MERCK

3.2 Methodology

3.2.1 Algal collection and preparation

3.2.1.1 Collect the *Caulerpa lentillifera* from Banjong Farm , Chachoengsao province.

3.2.1.2 Wash the alga with deionized water.

3.2.1.3 Dry the alga at 80 °C for 12 hours.

3.2.1.4 Store the alga in dessicator

3.2.2 Glassware preparation

3.2.2.1 Wash the glassware with water

3.2.2.2 Immerse glassware in 20% HNO_3 overnight.

3.2.2.3 Wash the glassware with water to make sure there is no acid deposited inside the glassware.

3.2.2.4 Rinse the glassware with deionized water.

3.2.2.5 Dry the glassware on the shelf

3.2.3 Preparation for synthetic wastewater

3.2.3.1 Prepare stocks of heavy metal at 100 mg/l at a volume of 1 L for each heavy metal species, $Cu(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, and $Zn(NO_3)_2$

3.2.3.2 Keep stock solution in refrigerator at 4 °C

3.2.3.3 Prepare synthetic wastewater with a concentration of less than 100 mg/l by diluting the stock solution in 3.2.3.1 to the required concentration. The calculation is done by using a balance equation

$$N_1V_1 = N_2V_2 \qquad (3.1)$$

N_1 = heavy metal's concentration of stock solution.

V_1 = the required volume of stock solution.

N_2 = the required heavy metal's concentration.

V_2 = the required volume of solution

3.2.4 Determination for competitive biosorption behavior of multi-component heavy metal

3.2.4.1 Preliminary experiment

Determination of suitable pH for metal dissolution.

(1) Prepare synthetic wastewater with 100 mg/l of heavy metals.

(2) Control pH at 4, 5, 5.5, 6, 6.5, 7 using 0.1 N HNO_3 and 0.1 N NaOH

(3) Mix the solution with a rotary shaker at a rate of 150 rpm for 30 minutes.

(4) Separate solid phase with filter paper (Cellulose Nitrate 0.45 μm).

(5) Measure heavy metal concentrations in the filtrate by Inductively Couple Plasma (ICP).

The suitable pH is defined as the highest pH at which all four heavy metal ions can be dissolved but not precipitated.

Determination of the Component in pure algae

(1) Determine the metal composition in pure algae by extracting with microwave digestion and measure the extract by Inductively Couple Plasma (ICP).

(2) Measure functional group(s) in the alga by Fourier Transform Infrared Spectrometers (FT-IR).

3.2.4.2 Study effect of single heavy metal concentration

(1) Prepare 30 ml solution with heavy metal in following concentrations: 10, 25, 50, 75, 100 mg/l and 0.1, 0.2, 0.3, 0.4 mmol/l at suitable pH.

(2) Add 0.5 g of dry algae into the solution

(3) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 21± 1 °C.

(4) Separate solid phase with filter paper (Whatman No.93 and GF/C).

(5) Measure heavy metal concentrations in the filtrate by Inductively Couple Plasma (ICP).

(6) Determine each heavy metals's removal percentage by using the following equation:

$$\%\text{Removal} = \frac{C_i - C_f}{C_i} \times 100 \qquad (3.2)$$

where C_i = initial concentration of heavy metal

C_f = final (equilibrium) concentration of heavy metal

(7) Determine isotherm for each heavy metals and checking with Langmuir and Freundlich isotherm model.

3.2.4.3 Study the effect of two heavy metals in the same solution

(1) Select a pair of heavy metals from 4 main heavy metal compounds; Cd, Cu, Zn, and Pb, with the following combinations :

Cd-Cu , Cd-Zn , Cd-Pb

Cu-Cd , Cu-Zn , Cu-Pb

Zn-Cd , Zn-Cu , Zn-Pb

Pb-Cd , Pb-Cu , Pb-Zn

(2) Prepare 30 ml solution of mixed heavy metals in the same solution for each combination above with a primary heavy metal concentration fixed at 50 mg/l and the following secondary heavy metal concentrations: 10, 25, and 50 mg/l.

(3) Add 0.5 g of dry algae into the solution

(4) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 21± 1 °C.

(5) Separate solid phase with filter paper (Whatman No.93 and GF/C)

(6) Measure heavy metal concentrations in the filtrate by Inductively Couple Plasma (ICP).

(7) Determine the isotherm for binary sorption of each heavy metal.

3.2.4.4 Study effect of three heavy metal in the same solution

(1) Select 3 metals from 4 main heavy metal compounds; Cd, Cu, Zn, and Pb, with the following combinations :

Cd-Cu-Zn

Cd-Cu-Pb

Cd-Zn-Pb

Cu-Zn-Pb

(2) Prepare 30 ml solution of mix heavy metals at the same concentrations of 0.1, 0.2, 0.3 mmol/l.

(3) Add 0.5 g of dry algae into the solution

(4) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 21± 1 °C.

(5) Separate solid phase with filter paper (Whatman No.93 and GF/C)

(6) Measure heavy metal concentrations in the filtrate by Inductively Couple Plasma (ICP).

(7) Determine each heavy metal's removal percentage.

3.2.4.5 Study effect of four heavy metal in the same solution

(1) Prepare 30 ml solution of mixed 4 heavy metals Cu, Cd, Pb, Zn at the same concentration of 0.1, 0.2, 0.3 mmol/l.

(2) Add 0.5 g of dry algae into the solution

(3) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 21 ± 1 °C.

(4) Separate solid phase with filter paper (Whatman No.93 and GF/C)

(5) Measure heavy metal concentrations in the filtrate by Inductively Couple Plasma (ICP).

(6) Determine each heavy metal's removal percentage.

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 repetition was carried out.