CHAPTER III

EXPERIMENTAL

3.1 Algal collection and preparation

1) Collect the *Caulerpa lentillifera* from Banjong Farm, Chachoengsao province
2) Wash the alga with water until being fairly clean
3) Dry the alga at 80°C for 12 hours
4) Store the alga in dessicator.

3.2 Glassware preparation

1) Wash the glassware with water and immerse in 20% by volume HNO_3 overnight
2) Wash the glassware with water to make sure there is no acid deposited inside the glassware
3) Rinse the glassware with deionized water
4) Dry the glassware in the oven and store in the dessicator.

3.3 Effect of buffer solution on biosorption

1) Mix CH_3COONH_4 and CH_3COOH according to the quantities indicated in the following tabulation with the final volume of 250 mL (in the 250 mL volumetric flask). This results in a buffer of CH_3COONH_4 at the concentration of 10, 15, 50, 100, 200 mM accordingly.

	Buffer	
$[CH_3COONH_4]$ (mM)	CH_3COONH_4 (g)	CH_3COOH (ml)
10	0.1927	0.0720
15	0.2890	0.1073
50	0.9635	0.3581
100	1.9270	0.7155
200	3.8540	1.4310

2) Add 0.0058 of $Cu(NO_3)_2$ into the flask to make a Cu^{2+} buffer solution at 0.1 mM

(of Cu^{2+})

3) Take 30 mL of the solution from (2)

4) Add 0.5g of dry algae into solution

5) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 20°C

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

7) Measure heavy metal concentration in the filtrate by Flame & Graphite Furnace Atomic Absorption Spectrophotometer (AAS) (ZEEnit 700).

8) Repeat Steps 1-7 with 0.1744 g of $Cu(NO_3)_2$ which makes the solution with an initial concentration of 3 mM

9) Repeat Steps 1-8 with $Cd(NO_3)_2$ and $Pb(NO_3)_2$ at 0.1 and 3 mM, respectively.

To prepare 0.1 and 0.3 mM of $Cd(NO_3)_2$ and $Pb(NO_3)_2$, follow the instruction below:

Metal	Add this amount in Step 2	To obtain this initial concentration (mM)
Cd^{2+}	0.0077 g $Cd(NO_3)_2$	0.1
	0.2313 g $Cd(NO_3)_2$	3
Pb^{2+}	0.0083 g $Pb(NO_3)_2$	0.1
	0.2484 g $Pb(NO_3)_2$	3

3.4 Kinetic experiments

3.4.1 Experiment with variable heavy metal concentrations

1) Prepare 30 ml solution with heavy metal in following concentration: 0.1, 0.2, 0.3, 0.8, 1, 3, 5, 10mM at pH 5

2) Add 0.5g of dry algae into solution

3) Mix the solution slowly with a rotary shaker at a rate of 150 rpm for 30 minutes at 20°C

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

5) Measure heavy metal concentration in the filtrate by Flame & Graphite Furnace Atomic Absorption Spectrophotometer (AAS) (ZEEnit 700).

3.4.2 Experiment with variable biomass doses

1) Prepare 30 ml solution with heavy metal in the following concentration: 0.1 and 3 mM at pH5

2) Repeat Steps 2-5 in Section 3.4.1 with biomass dose varied from 0.1, 0.25, 0.5 and 1g.

3.4.3 Experiment with variable temperature

1) Prepare 30 ml solution with heavy metal in following concentration: 0.1 and 3mM at pH 5

2) Repeat Steps 2-5 in Section 3.4.1 with shaking temperature at 20, 30, 40°C.

3.4.4 Investigation for the rate limiting steps in the sorption process

1) Prepare 30 ml solution with heavy metal in the following concentration: 0.1 mM at pH 5

2) Repeat Steps 2-5 in Section 3.4.1 with the mixing speed of 0, 50, 150, 200 rpm.

3.5 Calculation

Determine each heavy metal removal uptake by using the following equation:

$$q = \frac{V(C_i - C_f)}{m} \qquad (3.1)$$

where q = amount of metal uptake per unit mass of biomass (mol kg^{-1})

C_i = initial concentration of heavy metal (mol m^{-3})

C_f = final concentration of heavy metal (mol m^{-3})

V = volume of the solution (m^3)

m = dry mass of the algae (kg).