CHAPTER IV

RESEARCH METHODOLOGY

4.1 Materials

4.1.1 Raw material

- *Caulerpa lentillifera*, green macroalga

4.1.2 Chemicals

- NaOH anhydrous
- NaCl reagent grade
- HNO_3 65% w/v
- Acetic acid (conc.)
- Hydrochloric acid (conc.)
- Commercial activated carbon (Union Chemical®)
- $NaNO_3$
- KBr pellet
- pH 4.01 and 7.01 Standard buffer solutions
- Methylene blue
- Astrazon® Blue FGRL (AB)
- Astrazon® Red GTLN (AR)

4.1.3 Instruments and equipments

- Spectrophotometer, Spectronic® UV/VIS Helios Alpha spectrophotometer with Vision32 software –v1.25
- Fourier Transform Infrared Spectrophotometer (FTIR), Perkin Elmer, Model 1760X
- Scanning Electron Microscopy (SEM), Jeol, JSM-5800LV
- Laser Particle size Analyser, Malvern, Mastersizer-S long bed Ver 2.19
- Surface area analyzer (Quantachrome, Autosorb-1)
- Zeta Meter electrophoresis, Zeiss/3.0^{+}
- Peristaltic pump, Watson-Marlow Sci-Q323
- Rotary shaker, GFL, 3017

- Fraction collector (Amersham® FRAC-100pH-meter, Hanna, HI 98240
- Oven, Memmert
- pH meter, HACH, SENSION 1
- Hot plate
- Analytical balance 4 digit, Sartorius
- Dessicator
- Blender
- Filter paper No. 93, Whatman
- Fraction collector, Amersham Biosciences FRAC-100
- Stopwatch, Casio
- Erlenmeyer flasks
- Volumetric flasks
- Test tubes
- Beakers
- Pipettes
- Cylinders
- Funnels
- Silicone tubing
- Laboratory sieves (sieve size no.20 and 140)

4.2 Methodology

4.2.1 *Caulerpa lentillifera* collection and preparation

(1) Collect the alga from Banchong Farm, Chachoengsao Province, Thailand

(2) Wash the alga with deionized water

(3) Dry the alga in an oven at 80 °C for 12 h

(4) Keep unground alga in the dessicator

(5) Grind the dried alga with blender

(6) Sieve to smaller size using sieve no. 20 and 140.

(7) Store the ground alga in the dessicator

4.2.2 Adsorbents characteristics

A. FTIR analysis

(1) Grind the dried alga (finely ground powder, particle size <20 μm)

(2) Mix the sample with 1000 mg KBr in the sample disk

(3) Study the spectra of the sample (compared with spectra library)

B. Particle size distribution

(1) Prepare 1 g of ground algal sorbent

(2) Measure its particle size using Laser Particle Size Analyser, Malvern, Mastersizer-S long bed Ver 2.19, wet method

C. BET specific surface area

(1) Prepare samples of ground algal sorbent (both ground and unground alga)

(2) Measure their surface area by Surface area analyzer (Quantachrome, Autosorb-1)

D. pH_{pzc}

(1) Add 50 mg of ground alga to 100 mL of 0.1, 0.01 and 0.001 mol l^{-1} $NaNO_{3(aq)}$

(2) Age the suspension for 24 h at its natural pH

(3) Adjust the pH of the suspension to 2, 4, 6, 8, and 10

(4) Measure the zeta potential of each suspension by zeta meter

4.2.3 Preparation of synthetic wastewater

(1) Dissolve 2.0 g of Astrazon® Blue FGRL in 1 l of deionized water as a stock solution

(2) Prepare standard solution for Astrazon® Red GTLN and methylene blue using the same procedure

(3) Keep the stock solution in refrigerator at 4°C

(4) Dilute the stock solution to the desired concentration before use

4.2.4 Basic dye properties

A. Determination of λ_{max}

(1) Prepare 100 mg l^{-1} of the Astrazon® Blue FGRL solution by diluting the stock solution with deionized water

(2) Scan for the wavelength of maximum light absorption using scan mode of the spectrophotometer

(3) Repeat with Astrazon® Red GTLN and methylene blue

B. Calibration curves

(1) Prepare 30 ml of Astrazon® Blue FGRL with the initial dye concentration 0, 20, 40, 60, 80 and 100 mg l^{-1}

(2) Measure the light absorbance of the solution by spectrophotometer at its λ_{max}

(3) Plot between the concentration (x-axis) and the light absorbance (y-axis)

(4) Repeat with Astrazon® Red GTLN and methylene blue

4.2.5 Adsorption in Batch mode

A. Adsorption efficiency

(1) Mix various mass of the dried alga (0.05-1.7 g) with 30 ml of 20-80 mg l^{-1} of Astrazon® Blue FGRL

(2) Mix the solutions slowly in a shaker operating at 130 rpm at the controlling temperature of 27°C for 60 min.

(3) Separate the solid phase with filter paper

(4) Measure the light absorbance in the filtrate

B Adsorption equilibrium

(1) Mix 0.5 g of dried alga with 30 ml of 100 mg l^{-1} of Astrazon® Blue FGRL

(2) Mix the solutions slowly in a shaker water bath operating at 130 rpm at the controlling temperature of 40, 50 and 70 °C for 60 min. (the temperature is controlled at 27°C in other experiments)

(3) Mix the solutions slowly in a shaker operating at 130 rpm for 60 min at the controlling temperature of 18 and 27°C (temperature is controlled by air conditioner)

(4) Separate the solid phase with filter paper

(5) Measure the light absorbance in the filtrate

(6) Change alga weight to 0.1, 0.2, 0.7, and 0.9 g, and then repeat all steps

(7) Triplicate the experiments

C. Adsorption kinetics

(1) Mix 0.5 g of dried alga in 30 ml of solution with 20 mg l^{-1} of AB

(2) Mix the solutions slowly in a rotary shaker operating at 130 rpm

(3) Separate the solid phase with filter paper for 1, 2, 5, 10, 20, 30, 60, 120, and 180 min (this contact time can be extended to ensure that the equilibrium is reached)

(4) Measure the light absorbance in the filtrate

(5) Repeat all experiment with various initial dye concentrations, i.e. 40, 80, 160, 320, 640, 1280 mg l^{-1}

(6) Triplicate the experiments

4.2.6 Desorption in batch mode

(1) Prepare 30 ml solution with the initial dye concentrations of 100 mg l^{-1} AB

(2) Add 0.5 g of dried alga into the solutions

(3) Mix the solutions slowly in a rotary shaker at a rate of 130 rpm for 60 min

(4) Separate solid phase with filter paper

(5) Rinse the used alga in order to remove the unadsorbed dye

(6) Shake the used alga in the 30 ml of deionized water (DI) in a rotary shaker operating at 130 rpm for 60 min

(7) Measure the light absorbance of the solutions

(8) Triplicate the experiments

(9) Change the desorption method in order to compare the desorption efficiency of each method (Table 4.1)

4.2.7 Comparison of adsorption performance with commercial activated carbon

A. Equilibrium capacity

(1) Prepare 6 sorption systems (detail in Table 4.2)

(2) Mix the sorbent (with the initial concentration ranged from 0.05 to 1.7 g) into 30 ml of synthetic dye solution for one hour (130 rpm on a rotary shaker).

(3) Measure the light absorbance of the solutions

(4) Triplicate the experiments

B. Sorption kinetics

(1) Prepare 6 sorption systems (detail in Table 4.2)

(2) Mix 0.5 g sorbent into 30 ml of synthetic dye solution (130 rpm on a rotary shaker).

(3) Collect the samples to analyze for the residue dye at 1, 3, 5, 10, 20, 30, 60, 120 min.

(3) Measure the light absorbance of the solutions

(4) Triplicate the experiments

4.2.8 Column experiments

A. Column configurations

The column configurations are shown in Fig. 4.1. The equipments used during the fixed-bed column study consisted of a one centimeter i.d. glass column. The glass wool and glass bead was introduced at the bottom of the column and at the top of the algal sorbent. The packing density was constant at 0.65 g cm^{-3} for all column experiments.

B. Effect of wastewater flow rate

(1) Feed the synthetic wastewater through the adsorption column with the flow rate of 1.2, 2.4, and 3.6 ml $min^{-1}cm^{-2}$ (the bed depth of 8 cm is used)

(2) Regulate the flow rate with peristaltic pump

(3) Collect the effluent at a specific time interval using the fraction collector

(4) Measure the light absorbance of the effluent

C. Effect of adsorbent bed depth

(1) Feed the synthetic wastewater through the adsorption column prepared at different bed depths of 4, 6, and 8 cm

(2) Regulate the flow rate with the peristaltic pump (the flow rate is controlled at 1.2 ml min cm^{-2})

(3) Collect the effluent at a specific time interval using the fraction collector

(4) Measure the light absorbance of the effluent

4.2.9 Binary dye mixture

A. Nature of wavelength shift

(1) Prepare 50 mg l^{-1} Astrazon® Blue FRRL (AB), Astrazon® Red GTLN (AR), and Astrazon® Golden Yellow GL-E (AY) solution

(2) Measure for their light absorbance in the wavelength range from 400 to 700 nm

(3) Mix the same amount of two dyes (from (1)) together, i.e., AB with AR, AB with AY and AR with AY

(4) Scan for their spectra following the same procedure as that for the single dye

(5) Prepare the binary dye mixtures of AB + AR in various mass ratios

(6) Scan for their light absorbance

(7) Repeat (5) and (6) using AB + AY and AR + AY

B. Sorption of binary dye mixture

(1) Prepare the synthetic binary dye mixtures (AB + AR, AB + AY, AR + AY) with the mass ratio of 1:1 (using 50 mg l^{-1} of AB, AR, and AY).

(2) Mix the 30 cm^3 of synthetic binary dye mixtures with 0.1, 0.5, and 0.9 g alga in the flask

(3) Place in the shaker operated at 130 rpm.

(4) Collect the residue dye mixtures

(5) Analyze for their light absorption spectra at the wavelength ranges from 400-700 nm

4.2.10 Quality control of the experiments

The pH during the experiments was controlled at 7±0.5 using 0.1 N HCl and 0.1 N NaOH. Temperature was controlled by air conditioner at 27±1°C. The effect of filter paper on dye uptake capacity was minimized with blank experiments by passing dye at various concentrations through the filter paper and recording the uptake capacity of the filter paper at each condition. No sorption of dyes by glassware was observed in this research.

Table 4.1 Desorption methods

Method	Solution	Temperature/additional condition
1	DI	Ambient temperature
2	DI	Preheated at 70°C but shake at ambient temperature
3	DI	Shake in shaker water bath at 70°C
4	0.1 N HCl	Ambient temperature
5	1.0 N HCl	Ambient temperature
6	0.1 N Acetic acid	Ambient temperature
7	1.0 N NaOH	Ambient temperature

Table 4.2 Detail and abbreviation of six sorption systems

Sorbate	Sorbent	Abbreviation
Astrazon® Blue FGRL	*C. lentillifera*	ALGA/AB
Astrazon® Blue FGRL	Activated carbon	CARBON/AB
Astrazon® Red GTLN	*C. lentillifera*	ALGA/AR
Astrazon® Red GTLN	Activated carbon	CARBON/AR
Methylene Blue	*C. lentillifera*	ALGA/MB
Methylene Blue	Activated carbon	CARBON/MB

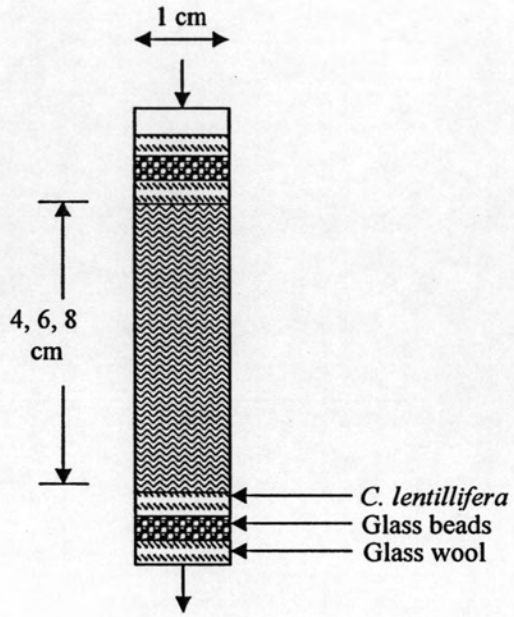

Fig. 4.1 Column configurations