CHAPTER III

METHODOLOGY

3.1 Studied Site

3.1.1 Ping River

Ping River, the main river of Chiangmai basin in northern Thailand, is one of the main contributaries of Chao Phraya River. It originates at Doi Chiang Dao in Chiang Dao district, Chiangmai Province. The Ping Basin is one of the largest drainage basins of the Chao Phraya Watershed, draining 33,896 km^2 of land area. This river contains high concentration of solids (> 80 NTU), which is a majority raw water for water supplied production in urban and local area. As shown in Figure 3.1 and Figure 3.2, the selected sampling point situated at N 18°51'7", E 98°58'57.9" corresponds to the 10 kilometers-upstream far from Chiangmai municipal area

Figure 3.1 Sampling point in Ping River, Chiangmai Province

Figure 3.2 Sampling point in Ping River, Chiangmai Province

3.1.2 Ang Keaw Reservoir

Ang Keaw Reservoir is located in Chiangmai University, Chiangmai province. Water from Ang Keaw Reservoir is utilized as raw water for producing the water supply (500-800m^3/day) to the communities, faculties, offices and dormitories that are located in the area of the Chiang Mai University . Water form Ang Keaw Reservoir was sampled from the sampling at the intake of raw water supply to water supply plant as shown in Figure 3.3.

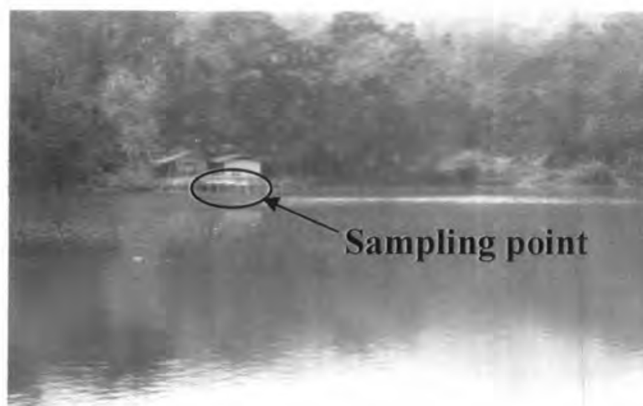

Figure 3.3 Sampling point in Ang Keaw Reservoir, Chiangmai Province

3.2 Experimental Procedure

Forty liters of grab sample from Ping River and Ang Keaw Reservoir were collected, transported in polyethylene tanks and stored at 4°C before analyzed within 24 hours. The water collected times were shown in Table 3.1.

Table 3.1 Schedules of water collection from Ping River and Ang Keaw Reservoir

Water sources		**Ping River**			**Ang Keaw Reservoir**		
Date of water collection		8-Oct-08	18-Nov-08	10-Dec-08	30-Nov-08	27-Dec-08	25-Jan-09
For Jar Test experiment		X			X		
For ceramic membrane filtration	1.0 μm		X			X	
	MF			X		X	
	UF			X			X

3.2.1 Removal of DOMs

The experimental procedures are shown in the following steps and conclusively described in the diagram in Figure 3.4.

1. All of raw water samples were measured for pH, temperature, turbidity, alkalinity, and conductivity.

2. Raw waters were divided into 3 portions

   1st portion: For Jar-Test experiment with varied PACl dose (1.5, 2.0, 2.5, and 3.0 mg/l as Al)

   2nd portion: For 1.0 μm, MF(0.1 μm), UF(0.01 μm) ceramic membrane filtration experiment

   3rd portion: For in-line coagulation combined with 1.0μm, MF, UF ceramic membrane filtration experiment

3. Water samples from three portions were measured for pH, EC, and turbidity.

4. Water samples from three portions were filtered through 0.7μm GF/F filter paper and kept in amber glass bottles with TFE-lined screw caps.
5. Characteristic parameters of water samples from three portions were UV-254, DOC, SUVA, FEEM, Cl_2 demand, and THMFP.

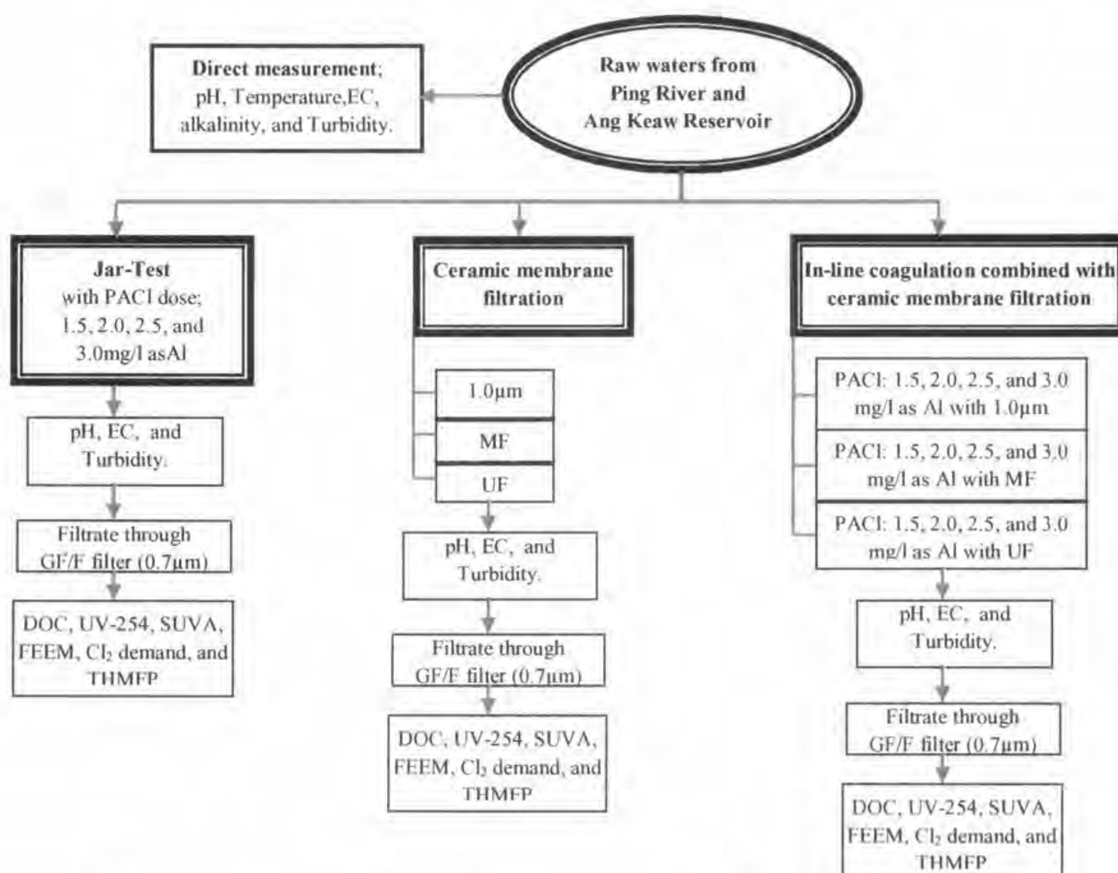

Figure 3.4 Diagram of overall experimental procedure for DOMs removal

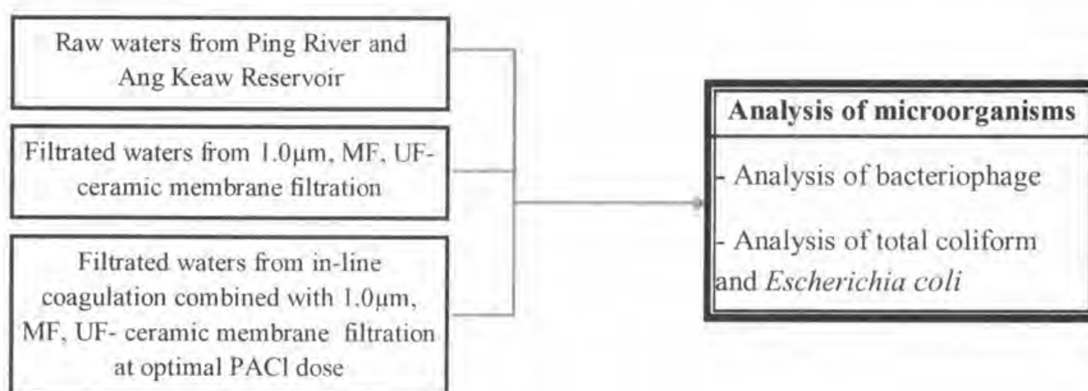

Figure 3.5 Diagram of experimental procedure for bacteriophages, total coliform and *Escherichia coli* removal

3.2.1 Removal of Microorganisms

The experimental procedure is conclusively described in the diagram in Figure 3.5.

3.3 Jar Test Experiment

Polyaluminium chloride or PACl in powder form (High purity grade PACl from DR. EXCELLENT Chemical Co., Ltd.) containing approximately 30 percent of Al_2O_3 was used for preparing the 1% PACl stock solution (10mg PACl/ml or equal 1.5 mgAl/ml) in the experiments.

The lab-scale of conventional coagulation was performed in standard Jar Test experiments (PHIPPS&BIRD Jar-Tester, Model 7790-902) as shown in Figure 3.6. The variable coagulant dosages (PACl) are following; 1.5, 2.0, 2.5, and 3.0 mg/l as Al. The mixing protocol employed was a rapid mixing for 60 seconds at 150 rpm (G = 320 s^{-1}) followed by a slow mixing for 20 minutes at 30 rpm (G = 30s^{-1}), and the suspension was left undisturbed for 30 minutes. After settling, the supernatant was collected for further analysis.

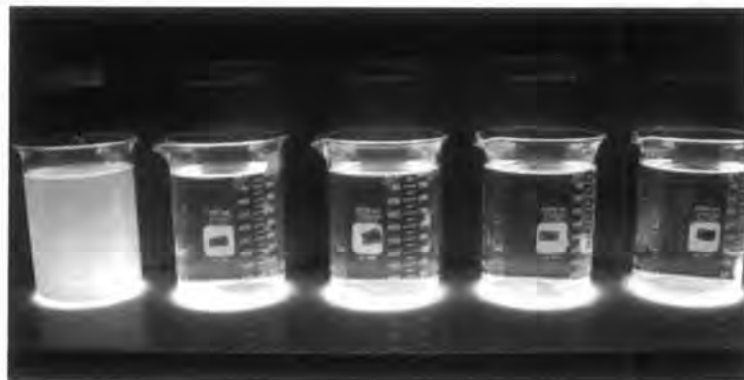

Figure 3.6 Jar Test apparatus (PHIPPS&BIRD Jar Tester, Model 7790-902)

3.4 In-line coagulation combined with ceramic membrane filtration experiment

3.4.1 Ceramic membrane module preparation

The ceramic membranes used in this study (provided by Metawater Co., Ltd., Japan) were the lab-scale ceramic membrane modules, which consist of nominal pore size 1.0µm, 0.1 µm (MF), and 0.01µm (UF). The dimension of each ceramic membrane module is 3 centimeters in diameter, 10 centimeters height and 55 tubular channels, as shown in Figure 3.7.

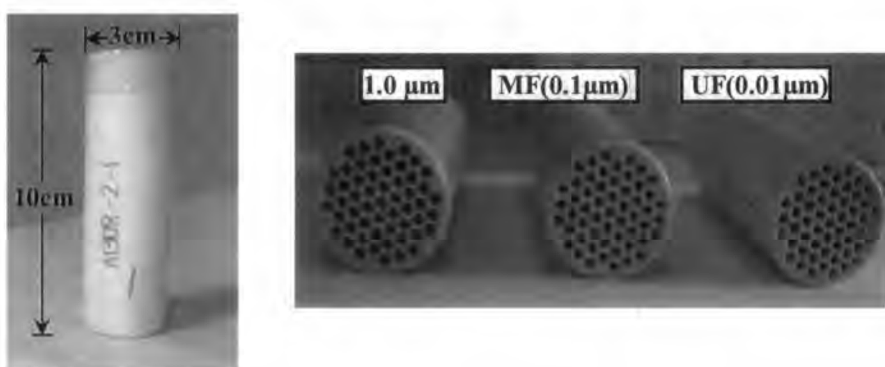

Figure 3.7 Ceramic membrane modules

All membrane modules used in filtration tests were boiled for 10 minutes before using. After feed water processing, in order to remove the organic and inorganic fouling from the membrane surfaces, the cleaning procedure was performed by submerging ceramic membrane module into the acid and base solutions in the following order: 1% nitric acid solution and 0.3%(as available chlorine) sodium hypochlorite solution, each for one hour.

3.4.2 The operation of In-line coagulation combined with ceramic membrane filtration

Ceramic membrane modules, which installed in a stainless steel vessel vertically, were operated in the cross flow filtration and dead end mode, as shown in Figure 3.8 and Figure 3.9. The purged pressure was controlled by the adjustment from the pressure regulator of nitrogen gas.

Water samples were mixed with PACl (the variable PACl dosages are following; 1.5, 2.0, 2.5, and 3.0 mg/L as Al) in jar test with rapid mixing for 60seconds at 150 rpm. After that, the coagulated water was poured to pressurized tank immediately. By the controlled pressure at 0.2 MPa, the coagulated waters in pressurized tank were allowed to 7-meters-nylon tube prior flowing to the bottom end of the ceramic membrane module and the filtrate was allowed through membrane pore perpendicularly. Filtrates were measured sample flux and collected for further measurement.

3.4.3 Flux measurement

Initial flux was measured by measuring the filtration time of 1 liter of filtrate from RO water (average value from 2-3 times) by the controlled pressure at 0.2 MPa. The initial flux measurement was performed before sample filtration of every batch experiment in order to check membrane fouling after the chemical cleaning process (not less than 90% recovery). Similarly, water sample flux was measured as the same procedure as the initial flux measurement. The water sample fluxes of all experiment were summarized in Appendix A.

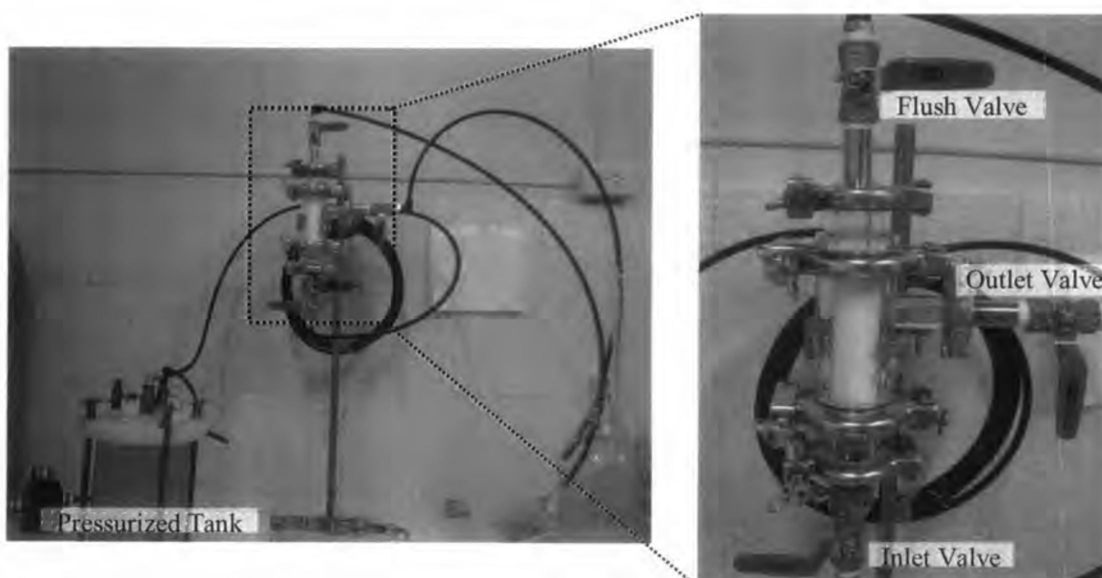

Figure 3.8 The pictorial of experimental set-up of in-line coagulation combined with ceramic membrane

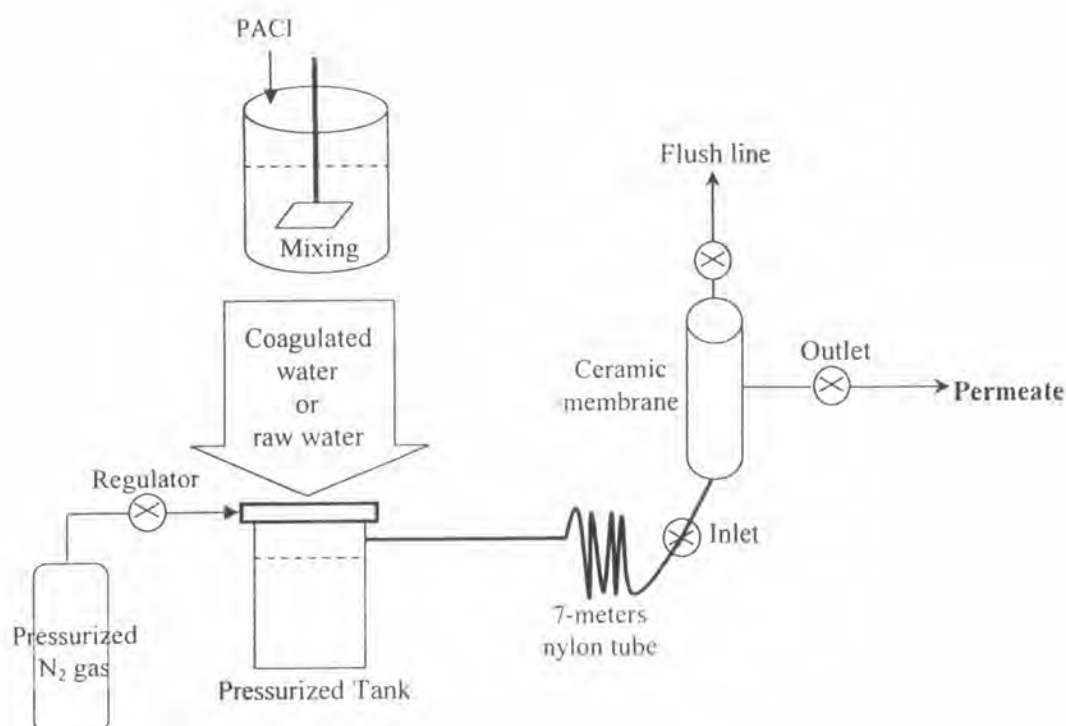

Figure 3.9 Schematic of in-line coagulation combined with ceramic membrane

3.5 Analytical Methods

3.5.1 Physico-chemical parameters

The water samples were analyzed for pH, turbidity, alkalinity, TOC, UV-254, DOC, SUVA, FEEM, Cl_2 demand, and THMFP. The summary of analytical methods and standards used for analyzing the mentioned parameters demonstrated in Table 3.3. These parameters are described below.

The analyzed parameters were done by duplicate samples. The results of these analyses should be within ±5%, or corrective action is necessary

3.5.1.1 pH

pH was directly measured by a Model F-21 Horibra pH-meter with an accuracy of ± 0.01 pH unit. The unit was daily calibrated with buffer solutions at pH 4.00, 7.00 and 9.00.

3.5.1.2 Temperature

Temperature was directly measured by Horiba Thermometer, Model D-13E.

3.5.1.3 Turbidity

The HACH Turbidity meter Model 2100 was used to measure turbidity.

3.5.1.4 Alkalinity

Alkalinity was measured in accordance with Standard Method 2320 B.

3.5.1.5 Electro Conductivity

Electro conductivity was directly measured by WTW Conductivity meter, Model cond.330i

3.5.2 DOM Parameters

3.5.2.1 DOC

The samples were filtered through a combusted 0.7 μm GF/F filter paper prior to measurement by O.I. analytical 1010 TOC Analyzer. DOC of water samples were measured in accordance with standard method 5310 Total Organic Carbon (TOC); section 5310 C Persulfate-Ultraviolet Oxidation Method

3.5.2.2 UV-254 nm

UV-254 of water samples were measured in accordance with standard method 5910 B Ultraviolet Absorption Method. The samples were filtered through a 0.7 μm GF/F filter paper prior to measurement by Perkin-Elmer Model Lambda 25, UV/VIS spectrophotometer.

3.5.2.3 THMFP

The four Trihalomethanes (THMs) species detected during the experiment were Chloroform, Bromodichloroform, Chlorodibromoform and Bromoform. In addition to analyzing THMs, three analytical methods were used to analyze the water samples. The details are briefly described below:

THMs

THMs were measured in accordance with standard method 5710, formation of Trihalomethanes and Other Disinfection By-Products. Gas Chromatography was used (Agilent 6890 Series Gas Chromatographic with ECD detector) under the following conditions:

Inlet Condition

Mode: Split, Initial temp: 225°C, Pressure: 31.33 psi, Split ratio: 10:1 Split flow 15.9 mL/min, Gas Type: Helium and Total flow: 20.5 mL/min

Oven Condition

The temperature programs of oven adjusted for analyzing THMs are shown in Table 3.2.

Detector Condition

Temperature: 300 °C, Mode: Constant make up flow, Makeup flow: 60 mL/min, Makeup Gas Type: Nitrogen

Table 3.2 Temperature programs for analyzing THMs

Ramp	Rate (°C/min)	Final temperature (°C)	Holding time of final temperature (min)
1	15	180	1.00*
2	15	130	1.00
3	15	180	1.00

* Initial temperature: 75°C, Initial temperature holding time: 1.00 min

Free Chlorine Residual

Free chlorine residual was measured in accordance with Standard method 4500-Cl G. DPD Colorimetric Method. Due to THMFP analysis, the chlorinated water samples must have 3 mg/L to 5 mg/L free chlorine residual.

Liquid-Liquid Extraction

Water samples were extracted in accordance with standard method 6232 B Liquid-Liquid Extraction Gas Chromatography Method.

3.5.2.5 Fluorescence Excitation-Emission Matrix (FEEM)

FEEM is the total sum of emission spectra of a sample at different excitation wavelengths, recorded as a matrix of fluorescent intensity in coordinates of Excitation (Ex) and Emission (Em) wavelengths, in a definite spectral window. FEEM represent in physical signatures by JASCO FP-6200 Spectrofluorometer.

Quinine Sulfate Standard

The quinine sulfate $[(C_{20}H_{24}N_2O_2)_2H_2SO_42H_2O]$ solution was used to check the stability of spectrofluorometry. The calibration curve was regularly established using 5 points of quinine sulfate in 0.1 M H_2SO_4. 10 quinine sulfate units (QSU) are equivalent to the fluorescent spectra of 10 μg/L of quinine sulfate solution at 450 nm with an excitation wavelength of 345 nm (Kasuga *et al.* 2003).

Spectrofluorometor Operating Conditions

The operating conditions used to measure the FEEM of all water samples in this study are following:

Measurement Mode: Emission

Band with excitation: 5 nm

Band with emission: 5 nm

Response: Medium

Sensitivity: High

Scanning speed: 2000 nm/min

Excitation wavelength: Start at 220 nm, end at 730 nm

Emission wavelength: Start at 220 nm, end at 730 nm

Excitation wavelength interval: 5 nm

Emission wavelength interval: 1 nm

FEEM Measurement Procedure

- Check the Raman Test Photometric Stability. The value should be less than ±1%/ hour.
- Measure the fluorescent intensity of the quinine sulfate solution of 10 QSU at 450 nm with an excitation wavelength of 345 nm.
- Measure the FEEM of the Milli-Q water.
- Measure the FEEM of the water samples
- Subtract the FEEM of the water samples with the FEEM of the Milli-Q water.
- Convert the fluorescent intensity of the subtracted FEEM of the water samples into QSU unit.
- Eliminate the influence of the primary and secondary scatter fluorescence and highlight the target peak by discarding the FEEM data when the excitation wavelength (Ex) $\geq$ emission wavelength (Em) or Ex X 2 $\leq$ Em (Komatsu *et al.* 2005)
- Remove the Rayleight and Raman scattering peaks at Em ± 10-15nm of each Ex (Zepp *et al.* 2004)

FEEM interpretation

FEEM can provide information on the putative origin of fluorescent organic matter of DOM in water.

Moreover, fluorescent excitation-emission wavelengths that exhibited fluorescent emission intensities were classified as fluorescent peaks as illustrated in Figure 3.10. In this study, the use of FEEM fluorescent emission intensities at peak position was utilized to evaluate the reduction of fluorescent organic matter of DOM after treatment process.

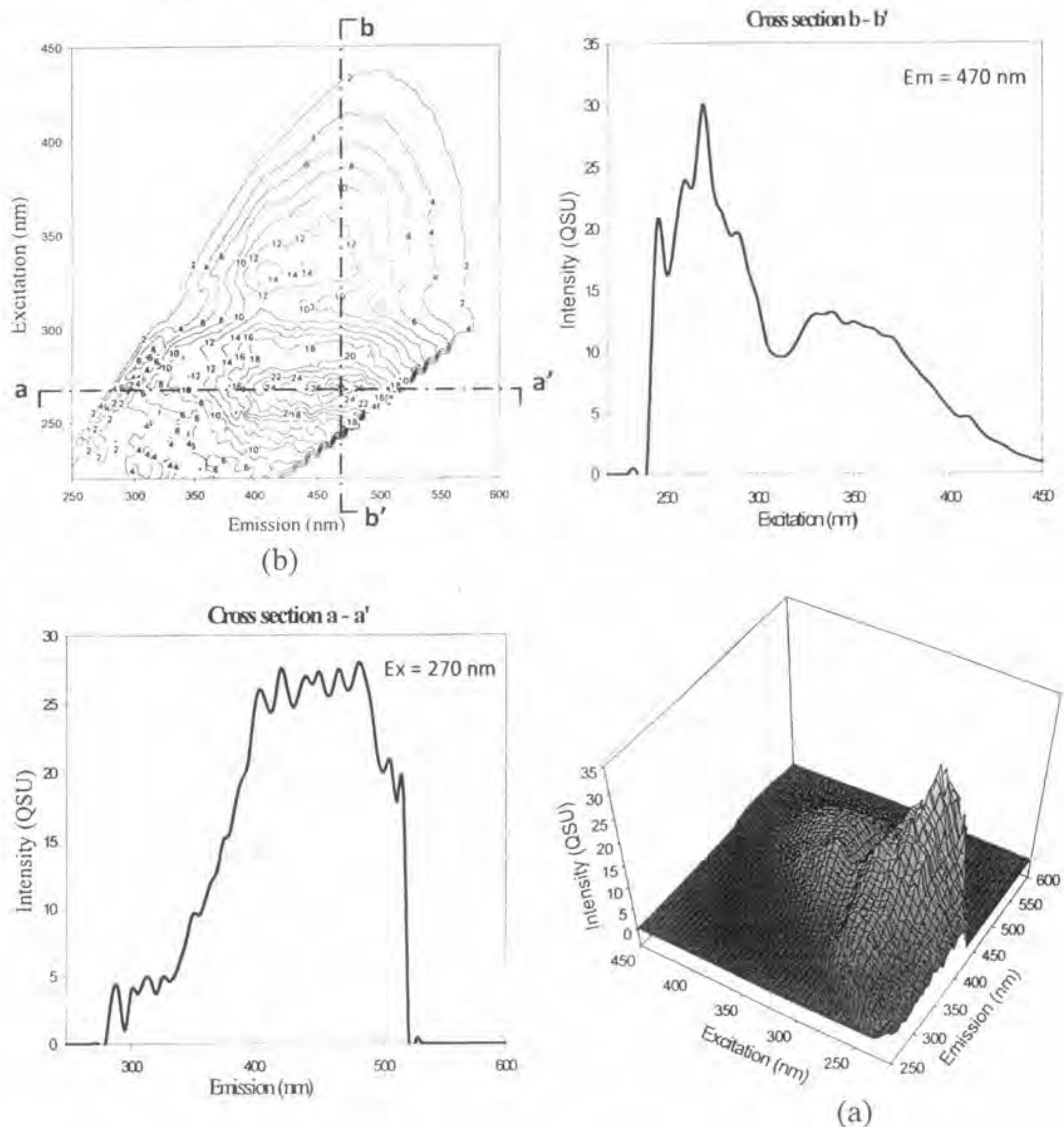

Figure 3.10 Sample of the three-dimensional view (a) and contour view (contour interval of 2 QSU) with fluorescent peaks and its sections (b) of a FEEM

Table 3.3 The summary of analytical methods and instruments

Parameters	Analytical methods	Standards	Analytical Instruments
Temperature	Direct measurement	-	Horiba Thermometer, Model D-13E
pH	Direct measurement	-	Horiba pH meter, Model F-21
EC	Direct measurement	-	WTW Cond. meter, Model cond.330i
Turbidity	Direct measurement	-	HACH, 2100 Turbidity Meter
Alkalinity	Titration Method	Standard method 2320B*	-
UV-254	Ultraviolet Absorption Method	Standard method 5910 B*	Jasco, Model UV-530, UV-spectrometer
DOC	Wet Oxidation Method	Standard method 5310C*	O.I. analytical 1010 TOC Analyzer
FEEM analysis	-	-	JASCO FP-6200 spectrofluorometer
Free chlorine residual	Colorimetric Method	Standard method 4500-Cl G*	Jasco, Model UV-530, UV-spectrometer
THMFP	Formation of Trihalomethane and Other Disinfection By-Products and Liquid-Liquid Extraction Gas Chromatography Method	Standard method 5710 and 6232B*	Agilent 6890 Series Gas Chromatography with ECD detector

3.6 Analysis of Microorganisms

The methods for detection and enumeration of bacteria and bacteriophages were mentioned below. Culture media, reagents and diluents of each analysis were refered in topic 3.6.3.

3.6.1 Analysis of Bacteria

Analysis of total coliform and *Escherichia coli* (*E. coli*)

Total coliform and *E. coli* were analyzed by single agar layer method using Chromocult Coliform agar (Merck, USA). Add 1 ml of the water sample or dilued sample by LB Broth in the petri dish. Pour approximately 15 ml of the agar solution into petri dish. The microbes were assayed after incubated at 37 °C for 18-24 hrs. This agar performed three different colored colonies. Salmon to red colonies and dark-blue

to violet colonies were counted as total coliforms. Dark-blue to violet colonies were counted as *E.coli*. The concentration of microbes was reported as CFU/ml (Colonies Forming Unit/ ml).

3.6.2 Analysis of Bacteriophages

Somatic coliphage

E.coli WG5 grown on Modified Scholten's Broth (MSB) was used as host strain for enumeration of somatic coliphages. The procedures are described in the corresponding standardized protocol (ISO, 1998). Somatic coliphages were quantified by using double layer method. Briefly, 1 milliliter of sample or diluted sample was added to prepared bottom agar layer in Petri dish. The host strain 1 milliliter of WG5 host strain was added to 2.5 milliliter of molten Semi-solid Modified Scholten's Agar (ssMSA). This mixture was mixed and poured onto bottom agar (Modified Scholten's Agar; MSA) in 70 mm diameter petri dishs. When higher bacterial backgroun flora may interfere with growth of the host and replication of phages, the addition of nalidixic acid is recommended to suppress contaminant growth. The overlays were incubated overnight at 37 °C. The concentration of microbe was reported as PFU/mL.

In addition, *E. coli* K12 A/λ (F+) was used as host strain at a concentration of amount 10^8 CFU/milliliter for enumeration of somatic coliphages. Briefly, *E. coli* K12 A/λ (F+) host strain was prepared by incubated in LB broth for 4-5 hrs at 37 °C with shaking at 150 rpm. Autoclaved bottom agar was poured in petri dish and waiting until becoming solid. One milliliter of samples was added on the bottom agar layer. *E. coli* K12 A/λ (F+) host culture was mixed with autoclaved upper agar. Mixing solution was poured into the pretri dish. The overlays were incubated overnight at 37 °C. The concentration of microbe was reported as PFU/mL.

F-specific bacteriophage

Indigenous F-specific RNA bacteriophages were analyzed in double agar layer method by using *Salmonella typhimurium* WG 49 as host strains. The procedures followed the standardized protocol (ISO, 1997).

Briefly, WG49 host strain was incubated in Tryptone-yeast extract-glucose-broth for 18±2 h at 37 °C with shaking at 150 rpm. 1 milliliter of dilution sample and 1 milliliter of exponentially growing WG 49 host culture were added to 2.5 milliliter of molten Semi-solid tryptone-yeast extract-glucose agar. This was mixed and poured onto a previous Tryptone-yeast extract-glucose agar base in a 70-mm-diameter petri dish. When higher bacterial background flora may interfere with growth of the host and replication of phages, the addition of nalidixic acid and kanamycin is recommended to suppress contaminant growth. The overlays were incubated overnight at 37 °C. The concentration of microbe was reported as PFU/mL. In addition, *E. coli* K12 A/λ (F+) was used as host strain for enumeration of F-specific RNA bacteriophages. The procedures to enumerate *E. coli* K12 A/λ (F+) were described the same as above.

3.6.2.1 Host Preparation

WG 5: *E.coli* strain CN

2 ml of working culture of WG5 from freezer that previously thawed at room temperature were added to 25 ml of MSB. The culture was incubated at 37 °C with shaking at 150 rpm for 3.5 - 4 hours.

WG 49: *Salmonella typhimurium*

Working culture

Stock from freezer was diluted around 10^{-7} – 10^{-8}. Working culture was prepared by using pour plate technique that using MacConkey agar as a medium. The culture plate was incubated overnight at 37 °C. Working culture plate was kept in the refrigerator at 4 °C within 3 weeks.

The concentration of red colonies in MacConkey agar must be more than 10^8 CFU/mL and the concentration of white colonies in the agar should be less than 10% of the colonies in agar.

Inoculum culture

3-4 red colonies with agar were picked up from working culture plate and added into the 10 ml TYGB. The culture was incubated with shaking for 24 hours at 37 °C. 10 ml of inoculum culture could use with 500 ml of TYGA agar or use for preparation of working plate for next step.

K12: *E. coli* K12 A/λ (F+)

Working culture

Stock of *E. coli* K12 A/λ (F+) from the freezer that previously thawed at room temperature was added in 10 ml of LB broth. The culture was incubated at 37 °C with shaking at 150 rpm for 2-3 hours. 100 µl of culture was spread on the solidified bottom agar (LB agar 2) that should be prepared at the same day of preparation of working culture. The culture plate was incubated overnight at 37 °C. Working culture plate was kept in the refrigerator at 4 °C within 3 weeks.

Inoculum culture

Colonies from the working plate were smeared and added into 10 ml of LB broth. The culture was incubated at 37 °C while shaking for 2-3 hours. 10 ml of inoculum culture could use with 500 ml of top agar (LB agar 1) or use for preparation of working plate for next step.

3.6.3 Culture Media, Reagents and Diluents

LB Broth base for dilution

LB Broth (Invitrogen)	20	g
Milli-Q water	1000	ml

The LB Broth was dissolved in the Milli-Q water while heating gently. The media was transferred to the vials and autoclaved at 121 °C for 15 min. The solution was stored for further use.

Chromocult Coliform agar

Chromocult Coliform agar (Merck, USA)	23.7 g
Milli-Q water	1000 ml

The Chromocult Coliform agar was dissolved in the Milli-Q water while heating gently without autoclaved. Cool medium to 50-55 °C and keep for further processing.

Modified Scholten's broth (MSB), Modified Scholten's Agar (MSA) and Semi-solid modified Scholten's agar (ssMSA)

MSB : Broth for inoculum culture

MSA : Bottom Agar Media

ssMSA : Upper Agar Media

The ingredients were shown in Table 3.4. The ingredients were dissolved in hot water. The mediums were distributed into bottles or vials and sterilized in the autoclave at 121 °C for 15 min.

Table 3.4 The ingredients of MSB, MSA, and ssMSA

Components	Unit	MSB (Broth)	ssMSA (Upper layer agar)	MSA (Bottom layer agar)
Peptone	g	10	10	10
Yeast extract	g	3	3	3
Meat extract	g	12	12	12
NaCl	g	3	3	3
Na_2CO_3 solution (150 g/l)	ml	5	5	5
$MgCl_2$ solution (100 g/ 50 ml)	ml	0.3	0.3	0.3
Bacto agar	g	-	7	15
$CaCl_2$* 14.7 g/ 100 ml	ml	-	6	6

Note: * $CaCl_2$ solution was pre-warmed and added to top agar prior adding agar to petri dish.

Na_2CO_3 solution

The 15 g of Na_2CO_3 was dissolved in 100 ml of Milli-Q water. The solution was decontaminated by 0.22 μm membrane filtration.

$MgCl_2$ solution

The 100 g of $MgCl_2.6H_2O$ was dissolved in 50 ml of Milli-Q water. The final concentration of Mg^{2+} in this solution will be 4.14 mol/l. The solution was sterilized by autoclaving and stored at room temperature in the dark.

$CaCl_2$ solution

The 1 M of $CaCl_2$ was prepared as stock solution by dissolving 14.7 g of $CaCl_2.2H_2O$ in 100 ml of Milli-Q water by gentle heating. The solution was decontaminated by 0.22 μm membrane filtration and stored at 4±2 °C: maximum 6 months.

Nalidixic acid stock solution

Nalixidic acid	100 mg
NaOH (1M)	2 mg
Milli-Q water	98 ml

The nalixidic acid was dissolved in the solution of NaOH in Milli-Q water. The solution was sterilized in the autoclave at 121 °C for not longer than six months.

Trytone -Yeast extract-Glucose-Broth (TYGB), Trytone-Yeast extract-Glucose-Agar (TYGA), and Semi-solid Trytone -Yeast extract-Glucose-Agar (ssTYGA)

TYGB : Broth for inoculum culture

TYGA : Bottom agar media

ssTYGA : Upper agar media

The ingredients were shown in Table 3.5. The ingredients were dissolved in hot water. The mediums were distributed into bottles or vials and sterilized in the autoclave at 121 °C for 15 min.

Table 3.5 The ingredients of TYGB, TYGA, and ssTYGA

Components	Unit	TYGB	ssTYGA (Upper layer agar)	TYGA (Bottom layer agar)
Tryptone	g	10	10	10
Yeast extract	g	1	1	1
Glucose	g	1	1	1
NaCl	g	8	8	8
$CaCl_2$ 0.3 mg/L	ml	1	1	1
$MgSO_4$ 0.15 mg/L	ml	1	1	1
Bacto agar	g	-	9	15

LB agar 1 and LB agar 2 for detection and enumeration of coliphage using *E. coli* K12 A/λ (F+) as host strain

LB agar 1: Upper agar media

LB agar 2: Bottom agar media

The ingredients of the agar for enumeration and detection coliphage by using *E. coli* K12 A/λ (F+) as host strain were mentioned in Table 3.6.

Table 3.6 The ingredients of top and bottom agar for *E. coli* K12 as host strain

Components	Unit	LB agar 1 (Upper layer agar)	LB agar 2 (Bottom layer agar)
LB broth	g	20	20
Bacto agar	g	8	11
$CaCl_2$	g	1	-
E. coli K12 A/λ (F+)	ml	20	-
Milli-Q water	ml	1000	1000