CHAPTER 3

METHODOLOGY

3.1 Sample collection

3.1.1 Shrimp farm effluents

All samples were collected from inland shrimp farms located in the Bangpakong watershed, Chachoengsao province; Ban Pho and Bangkla district, from May to November, 2003 (see Figure 3-1). Different 16 shrimp farms (Table 3-1) were chosen at harvest day when the entire volume of the ponds was discharged. The culture days per one crops ranged from 90 to 140 days. At that day, farmers usually use diesel motors for pumping water out or opening gates at the pond banks and allowing the water to flow out into a canal or reservoir. Samples were collected approximately 10-15 litter from the effluent points (grab sampling) when a half of water in the pond were drained out and then directly contained in amber glass with TFE-lined screw caps. Samples were stored in the refrigerator at 4 °C throughout the 14-day holding time. Milli-Q water was used for all dilutions, solution preparation, and final glassware washing.

3.1.2 Bangpakong River

The river samples from Bangpakong River were collected as grab sampling from upstream to downstream locations from the shrimp farm areas as shown in Figure 3-1 and Table 3-2.

3.2 Physico-chemical parameters measurement

The basic parameters of physico-chemical characters were measured for all shrimp effluents and river at the field as shown in Table 3-3: pH, turbidity, temperature, conductivity, salinity, and alkalinity. Physical-chemical parameters of shrimp farm effluents and Bangpakong River were reported in Tables 3-4 and 3-5.

3.3 Fractionation

3.3.1 Hydrophobic and hydrophilic fractionation

3.3.1.1. Filtrated samples through three different kinds of filter paper: Whatman 40, GFC, and 0.45-μm-cellulose nitrate, respectively.

3.3.1.2. Acidified filtrated sample to pH 2 with H_2SO_4 and pumped through the column at 30 bed volumes/h or less (XAD-8 passed the hydrophilic materials).

3.3.1.3. Back eluted resin with 0.1 and 0.01 N NaOH (XAD-8 passed the hydrophobic materials).

3.3.1.4. All fractions were preserved and refrigerated at 4°C.

3.3.2 Prepare XAD-8 resin

3.3.2.1. Slurring the resin with 0.1 N NaOH for 24 h., decanting off the floating resin.

3.3.2.2. Rinsed the resin with Mill Q water to remove all NaoH.

3.3.2.3. Purified the resin by putting in soxhlet with acetone and hexane for 24 h. After acetone extraction and before hexane extraction, the whole thimble was put on a funnel and the resin was with hexane. Similarly, after hexane extraction, the whole timble was transfered on a funnel and the resin was rinsed with methanol.

3.3.2.4. Packed the resin into the column and passed NaOH 0.1 N , HCl 0.1 N , and Mill Q correspondingly through column until DOC and conductivity of the passed water less than 0.2 mg/L and 10 μs/cm.

3.4 Organic carbon measurement

Analyzing Trihalomethane precursors in terms of organic carbon by using the TOC analyzer (O.I. analytical college station Texas model 1051) for all four water fractions: the original water itself, the filtrated water, the hydrophilic and the hydrophobic fraction. The analytical method followed standard methods 5310-D, sodium persulfate oxidation. Potassium hydrogen phthalate (KHP) in concentrations 2.5, 5, 10, 15 mg/L were used for setting the calibration curve. The two reagents, phosphoric acid and sodium persulfate, were regularly changed every two weeks. Each sample was suitably diluted and prepared prior to being analyzed. The programs,

the amount of acid, oxidant, and reaction time, were set appropriately as recommended by the manufacturer. Duplicates were run for every sample.

3.5 Ultraviolet adsorbance

All four water fractions were measured at a single wavelength of 254 nm which serves as a rough indication of overall NOM concentration. All samples were analyzed by using the Helios Alpha Thermo Electron Corporation. The instrument always adjusts to read zero absorbance with the organic-free water blank. The UV 254 values (1/cm) for all samples were read in comparison with the organic-free water blank.

3.6 Specific ultraviolet absorption

$$\text{SUVA (L/mg. m)} = UV_{254}\text{ (1/m)} / \text{DOC (mg/L)} \qquad (3\text{-}1)$$

3.7 THMs formation potential

To have some quantitative measure of the proclivity of NOM to from DBPs, a test of the THM formation potential or THMFP was devised in accordance with the Standard Method 5710-B. The formation potential was determined by exposing all fractions: raw water, filtrated water, hydrophobic, hydrophilic, and coagulated water samples, to an excess of oxidizing disinfectant, calcium hypochlorite solution, for 7 days at 25 °C. Before their incubation for 7 days, samples were adjusted to pH between 6-7 and added phosphate buffer. After the 7-day holding time, sulfite was added to the samples in order to de-chlorinated free chlorine. The change in their THM concentration relative to time zero was the THMFP. The total concentration of THMs at any time is expressible as

$$[\text{THMs}] = [CHCl_3] + [CHBrCl_2] + [CHBr_2Cl] + [CHBr_3] \qquad (3\text{-}2)$$

Thus, the THMFP is given by

$$\text{THMFP} = [\text{THMs}]_7 - [\text{THMs}]_0 \qquad (3\text{-}3)$$

3.8 Gas chromatography for THM measurement

THMs were analyzed in according with the EPA method 551.1 by using gas chromatography which is equipped with HP-1 columns, micro electron capture Detector. THMs mixed standard containing four species: chloroform, bromodichloromethane, Dibromochloromethane, bromoform concentrations of 200 and 2,000 mg/ml were used during all experiments. The standard THMs was prepared in concentrations as follows: 50, 100, 200, 500, 1000, and 1500 μg/L, in order to draw the calibration curve. The calibration curve was reset before the analysis of each sample. Pentane was used as the only extraction solvent in this method. Bromofluorobenzene and decafluorobiphenyl were an internal and a surrogate standard, respectively. GC signals were interpreted by using the ChemStation program, Agilent.

3.9 Statistical analysis

3.9.1 Pearson correlation

Pearson correlation was used to illustrate the relation of two parameters without controlling other parameters (zero-order correlation). The Pearson correlation coefficient is a measure of linear association between two variables. The values of the correlation coefficient range from -1 to 1. The sign of the correlation coefficient indicates the direction of the relationship (positive or negative). The absolute value of the correlation coefficient indicates the strength, with larger absolute values indicating stronger relationships. The correlation coefficients on the main diagonal are always 1.0, because each variable has a perfect positive linear relationship with itself. The significance level (sig. or p-value) is the probability of obtaining results as extreme as the one observed. If the significance level is very small (less than 0.05) then the correlation is significant and the two variables are linearly related. If the significance level is relatively large (for example, 0.50) the correlation is not significant and the two variables are not linearly related.

3.9.2 Linear regression model

Multiple regression analyses (SPSS) were used to construct a mathematical model for THMFP prediction. Method "stepwise regression" selects the independent variables in a step-by-step manner and determines which group of parameters can suitably predict THMFP. All parameters from the experiments needed for this model development were set as follows: probability F Entry = 0.05, Removal= 0.10, F value Entry = 3.84, and Removal = 2.71.

R, a multiple correlation coefficient, is the correlation between the observed and predicted values of the dependent variable. The values of R for models produced by the regression procedure range from 0 to 1, the larger values of R indicate the stronger relationships. R represents the correlation among independent variable or surrogate parameters.

R squared is the proportion of variation in the dependent variable explained by the regression model. The values of R squared range from 0 to 1. Small values indicate that the model does not fit the data well.

The sample R squared tends to optimistically estimate how well the model fits the population. Adjusted R squared attempts to correct R squared to more closely reflect the goodness of fit of the model in the population. It is recommended to choose a model with a high value of R squared that does not contain too many variables. Models with too many variables are often over fit and hard to interpret.

3.10 Quality control

QA/QC plans were set for all steps of the experiments to obtain accurate and precise results.

3.9.1 Chemicals in this work were all analytical grade.

3.9.2 The entire stock of glassware used was of high quality grade. Every piece was neatly cleaned with the particular washing liquid for laboratory use, rinsed with M.Q. water, and than heated at 105 C for 3 hours before being used.

3.9.3 TFE-screw cap with amber glass bottles were used to store samples for THMFP. However, when samples were collected out in the field, sometimes TFE-screw caps were not used.

3.9.4 Reagent blanks were tested at the beginning of experiment to ensure their purity.

3.9.5 Duplicate measurement was programmed into all instruments for detecting an error that might happen in the experiments.

3.9.6 Regularly calibrated the instruments step by step as noted in instrument guideline: pH, conductivity, turbidity, UV etc.

3.9.7 Fractionation process, resins and glass wool were cleaned and purified by soxhlet extraction as described in the approved research paper of Leenheer 1981.

3.9.8 For the7- day THMFP, the reagent blank was held in the cool incubator together with the samples to make sure that no cross contamination occurred between samples.

3.9.9 Bromofluorobenzene (internal standard) and decafluorobiphenyl (surrogate standard) were used in accordance with the QA/QC plan mentioned in the EPA method.

Table 3-1 Details of each shrimp farm

Source water	Farm Name	Collecting date	Shrimp type	Pond type	Tel.
No 1	Mr. Somkorn	28-May-03	black tiger	Clay	
No 2	Ms. Pook	20 -Jun-03	black tiger	Clay	
No 3	Mr. Aumnat	5-Jun-03	black tiger	Clay	
No 4	Mr.Somboon	10-Jun-03	white tiger	Clay	
No 5		19-Jun-03	black tiger	Clay	
No 6	Mr.Akeachai Srijarenwongwan	28-Jun-03	black tiger	Clay	
No 7	Mr.Boonlee Srisamai	3-July-03	black tiger	Clay	06-5001053
No 8	Mr.Vichai Tongsutti	12 Jul-03	white tiger	Clay	038-478145
No 9	Mr.Montree	22-Jul-03	black tiger	Clay	
No 10		29-Jul-03	black tiger	Clay	
No 11	Mr. Peak	6-Aug-03	black tiger	Clay	09-4505101
No 12		20-Aug-03	black tiger	Clay	
No 13		10-Sep-03	black tiger	Clay	
No 14		26 -Sep-03	black tiger	Clay	
No 15		17-Oct-03	black tiger	Clay	
No 16		28 -Oct-03	black tiger	Clay	

Table 3-2 Details of river sampling location

Source water	Collecting date	Location
Upstream 1 (No 1)	1-Nov-03	Near city hall of Nakornayok province
Upstream 2 (No 2)	1-Nov-03	Bang num preaw district where 2 rivers are joined
Upstream 3 (No 3)	1-Nov-03	Bang num preaw district where 2 rivers are joined
Downstream 1 (No 4)	1-Nov-03	Near Sotorn Temple
Downstream 2 (No 5)	1-Nov-03	Near Ms. Mai 's house and police station
Downstream 3 (No 6)	1-Nov-03	Near Bangpakong River delta under crossing Bangpakong River bridge

Table 3-3 Analytical method and analytical instrument for each parameter

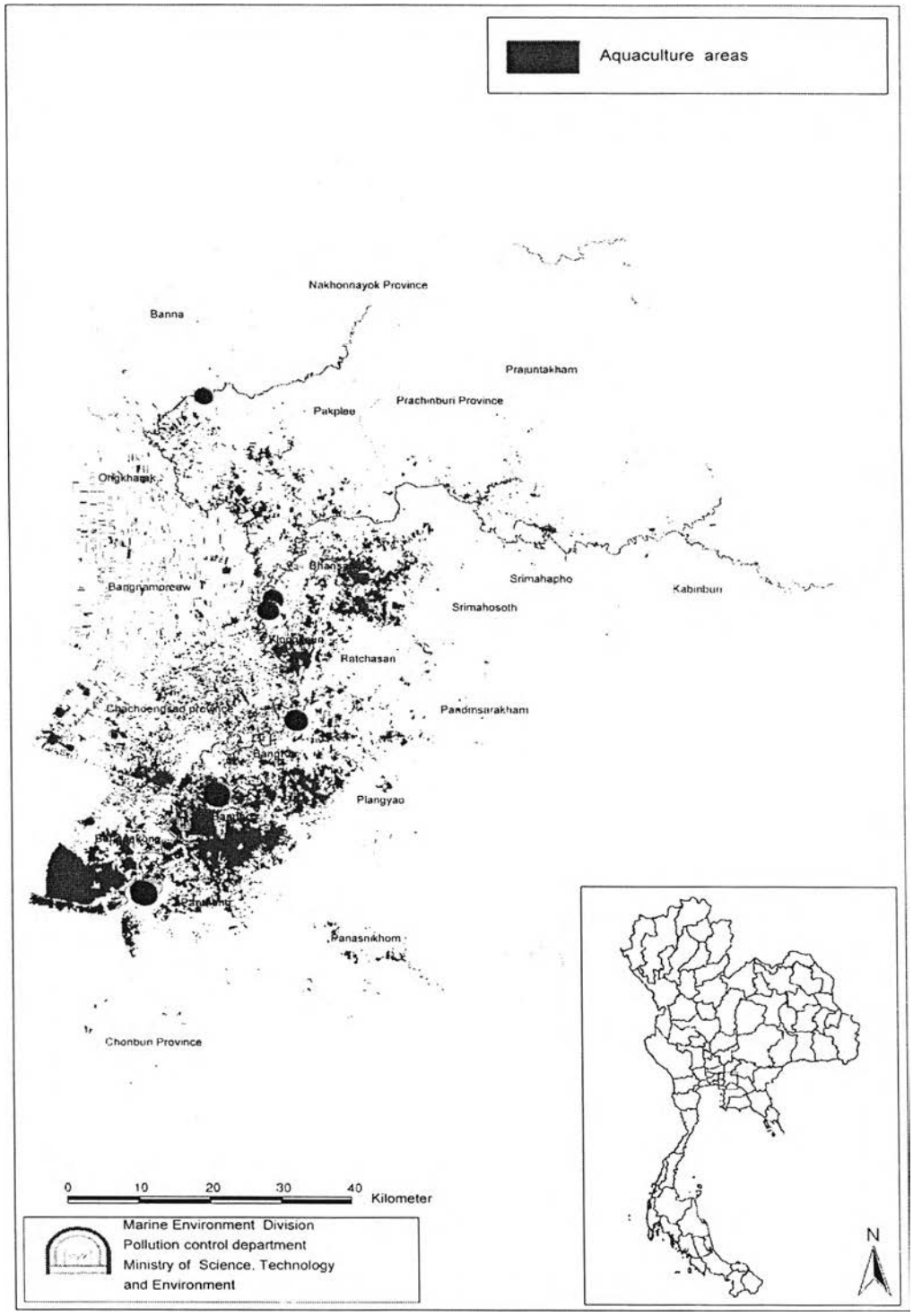
Parameters	Analytical method	Analytical instruments
pH	-	pH meter
Turbidity	-	Turbidity meter
Temperature	-	pH meter
Conductivity	-	Conductivity meter
Salinity	-	Conductivity meter
Alkalinity	Titration method, Standard method 2320B	-

Table 3-4 Characteristic of shrimp farm effluents

Source water	Temperature (°C)	Culture days (days)	pH	Salinity (ppt)	Conductivity (µs/cm)	Turbidity (NTU)	Alkalinity (mg/L as $CaCO_3$)
No 1	30.2	105	7.91	0.4	860	47.1	82.5
No 2	27.6	100	8.13	0.5	947	86.4	110.0
No 3	27.5	95	7.88	1.4	2660	27.1	135.0
No 4	29.0	96	8.16	6.4	11250	41.9	105.0
No 5	23.2	75	7.94	14.0	31700	49.2	55.0
No 6	32.3	75	6.82	14.5	24100	20.0	40.0
No 7	29.8	100	8.66	0.3	680	64.4	75.0
No 8	26.1	90	7.24	1.0	1954	21.7	92.5
No 9	32.3	85	8.78	0.7	1385	15.7	40.0
No 10	33.7	120	7.58	0.6	1278	163.3	92.5
No 11	30.7	125	7.48	0.7	1433	150.3	65.0
No 12	30.2	135	8.52	1.8	3400	102.0	130.0
No 13	30.6	98	8.17	6.9	12080	58.2	180.0
No 14	32.2	135	7.36	11.8	19870	111.8	120.0
No 15	30.7	93	8.73	4.7	8450	33.5	65.0
No 16	32.3	109	9.91	0.5	1086	49.0	90.0

Table 3-5 Characteristic of Bangpakong River

Source water	Temperature °C	pH	Salinity (ppt)	Conductivity (µs/cm)	Turbidity (NTU)	Alkalinity (mg/L as CaCO3)
Upstream 1 (No 1)	27.3	6.53	0	50.2	12.3	27.5
Upstream 2 (No 2)	30.1	6.14	0.1	150.3	228.2	40
Upstream 3 (No 3)	29.2	6.65	0.1	183.7	286.4	55
Downstream 1 (No 4)	27.1	7.00	0.1	233.0	106.4	40
Downstream 2 (No 5)	31.4	7.02	0.1	245.0	66.21	50
Downstream 3 (No 6)	30.0	6.89	5.8	10380.0	52.58	77.5



● Sampling points in River
Source: Pollution Control Department, 2002

Figure 3-1 Studied areas

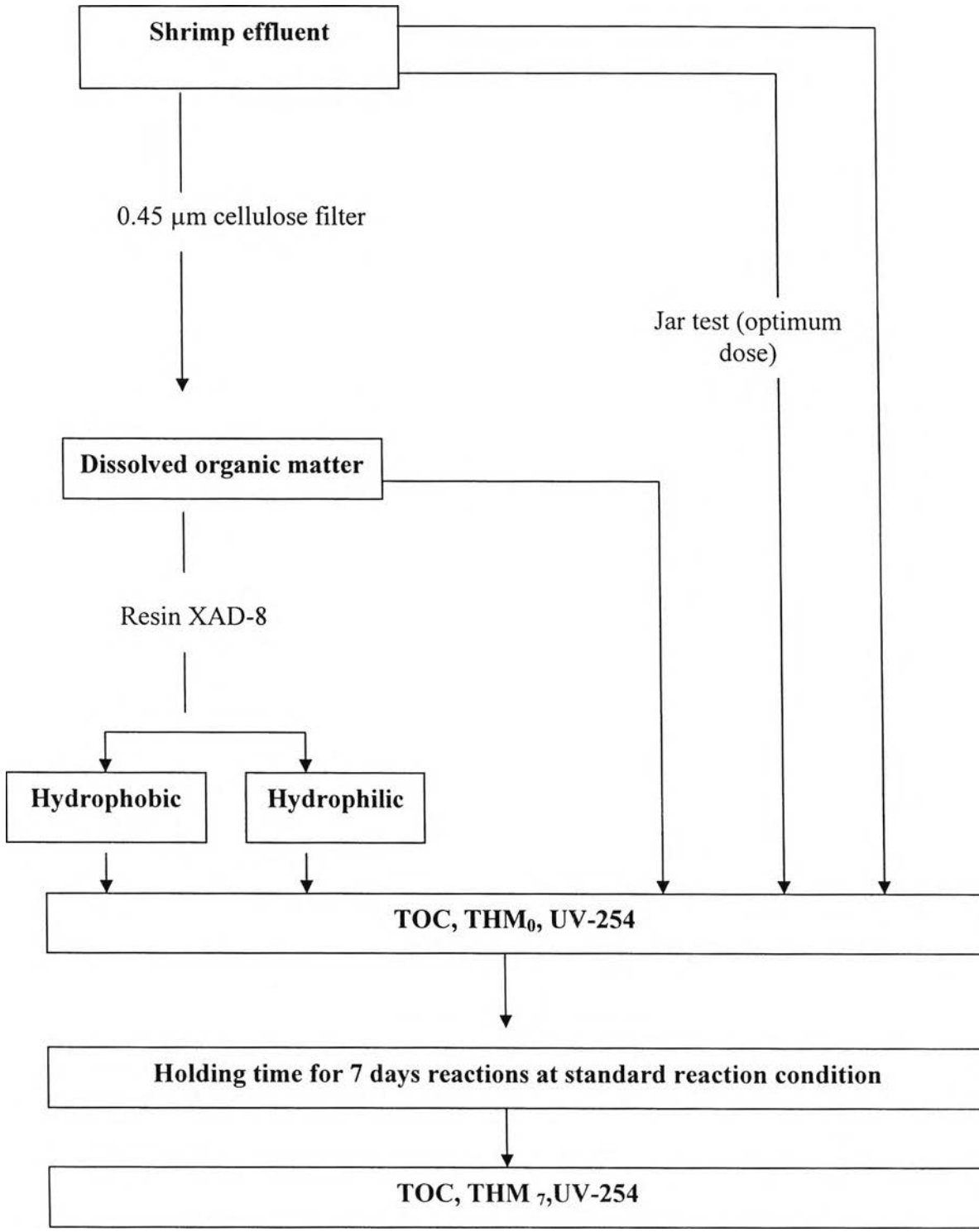


Figure 3-2 Diagram of experimental procedure