CHAPTER III

METHODOLOGY

3.1 Study Site

The central wastewater treatment plant of the Northern-Region Industrial Estate is located in Tambon Banklang, Amphur Muang, Lamphun province, Thailand. This industrial estate is the largest industrial estate located in the northern part of Thailand. The total area of this industrial estate is approximately 286 hectares and consists of about 76 factories in electronics, machinery parts and equipment, agricultural products, leatherwear, food products, wooden products, jewelry and accessories, construction, and more.

3.2 The Central Wastewater Treatment Plant

Figure 3.1 shows a diagram of the stabilization pond system of the central wastewater treatment plant of the studied industrial estate.

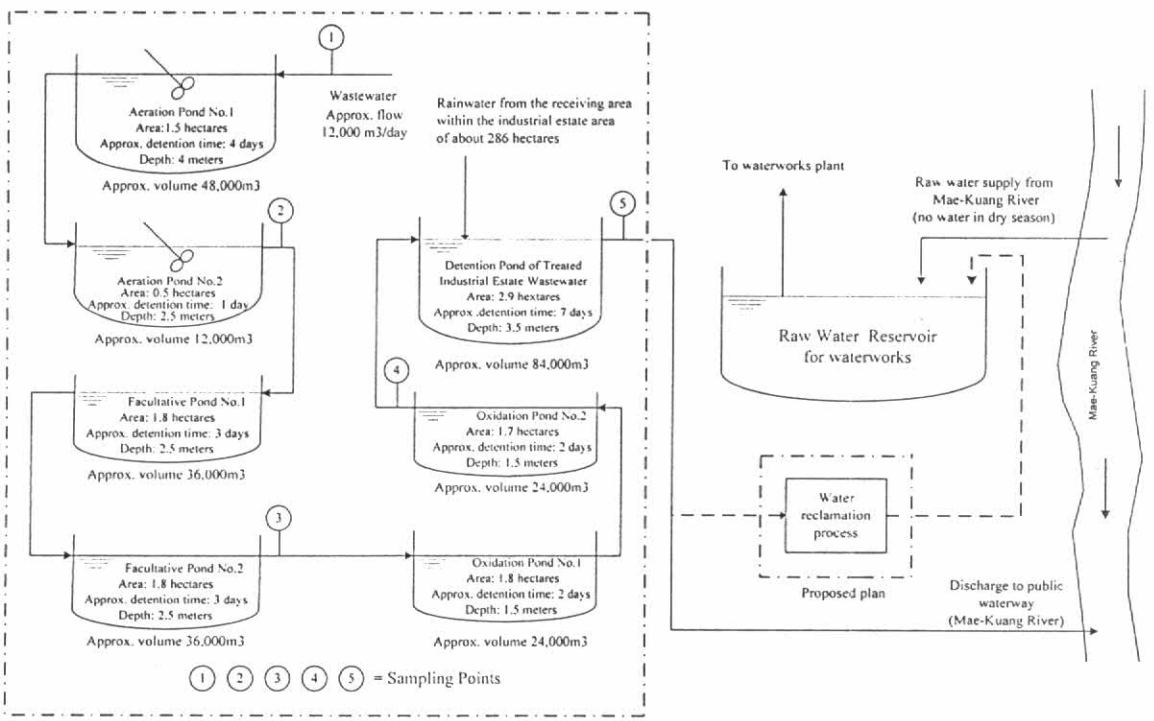

Figure 3.1: Diagram of stabilization pond system of the central wastewater treatment plant of the studied industrial estate.

The stabilization pond system of the central wastewater treatment plant has about a 12,000 m^3 per day capacity. It consists of two aeration ponds (with depths of 4 and 2.5 m, respectively, and a total detention time of 5 days), two facultative ponds (with depths of 2.5 m each, and a total detention time of 6 days) and two oxidation ponds (with depths of 1.5 m each, and a total detention time of 4 days). Effluent water from the oxidation ponds and rainwater from the receiving area within the industrial estate directly flows into a detention pond (with a depth of 4 m, for a total detention time of 7 days; the calculation was based on 12,000 m^3/day of influent wastewater) and is discharged downstream into the Mae-Kuang River. Due to the water shortage problem during the dry season, a reclamation process will be operated to treat the treated wastewater from the detention pond prior to its storage in the raw water supply reservoirs and use as raw water for the water supply plant to produce water supply of about 14,000 m^3/day.

3.3 Sample Collections

Water samples were collected five times from June 24, 2004 to July 27, 2005 from the sampling points as shown in Figure 3.1 and Table 3.1.

Table 3.1 Details of the sampling dates and sampling points

Sampling dates	Sampling points				
	Influent wastewater ①	Eff.[1] water from aeration ponds ②	Eff. water from facultative ponds ③	Eff. water from oxidation Ponds ④	Eff. water after detention Pond ⑤
Jun. 24, 2004	X	X	X	X	√
Sep. 16, 2004	√	√	√	√	X
Oct. 22, 2004	√	√	√	√	X
Feb. 18, 2005	√	√	√	√	√
Jul. 27, 2005	√	√	√	√	√

Remark: [1]Eff. = Effluent, √ = Water samples were collected and X = Water samples were not collected.
① ② ③ ④ ⑤ = Sampling points as indicated in Figure 3.1.

The water sample collected on June 24, 2004 was filtered thought a well-washed cellulose acetate membrane (0.45 μm pore size) and the water samples collected on September 16

and October 22, 2004, and February 18 and July 27, 2005 were filtered thought a pre-combusted (550 °C for 2 h) Whatman GF/F (nominal pore size 0.7 μm). The filtered waters were kept at 4 °C until analysis. It must be noted that all water samples were measured for pH, alkalinity, turbidity, and temperature prior to being filtered.

3.4 Experimental Procedure

Effluent water from the detention pond collected in June 24, 2004 was measured for ultraviolet absorbance at 254 nm (UV-254), dissolved organic carbon (DOC), specific ultraviolet absorption (SUVA), trihalomethane formation potential (THMFP), and fluorescent excitation-emission matrix (FEEM). Subsequently, resin adsorption procedures were used to fractionate five liters of filtered water into six dissolved organic matter (DOM) fractions; namely the hydrophobic neutral (HPON), hydrophobic base (HPOB), hydrophobic acid (HPOA), hydrophilic base (HPIB), hydrophilic acid (HPIA) and hydrophilic neutral (HPIN) fractions. The six DOM fractions were analyzed for their UV-254, SUVA, DOC, THMFP and FEEM.

All water samples collected on September 16, 2004, October 22, 2004 and July 27, 2005 were measured for UV-254, DOC, SUVA, THMFP and FEEM.

The influent wastewater and effluent water collected on February 18, 2005 from the aeration, facultative, oxidation, and detention ponds were put through UV-254, DOC, SUVA, THMFP, FEEM, and pyrolysis gas chromatography mass spectrometry (GC/MS) analysis. Subsequently, resin adsorption procedures were used to fractionate fifteen liters of filtered influent wastewater and effluent water from the aeration, facultative, oxidation, and detention ponds into six DOM fractions, HPON, HPOB, HPOA, HPIB, HPIA and HPIN. The six DOM fractions of each water sample were analyzed for their UV-254, SUVA, DOC, THMFP, FEEM and pyrolysis GC/MS results. A schematic diagram of the experimental procedure of the water samples collected on February 18, 2004 is depicted in Figure 3.2.

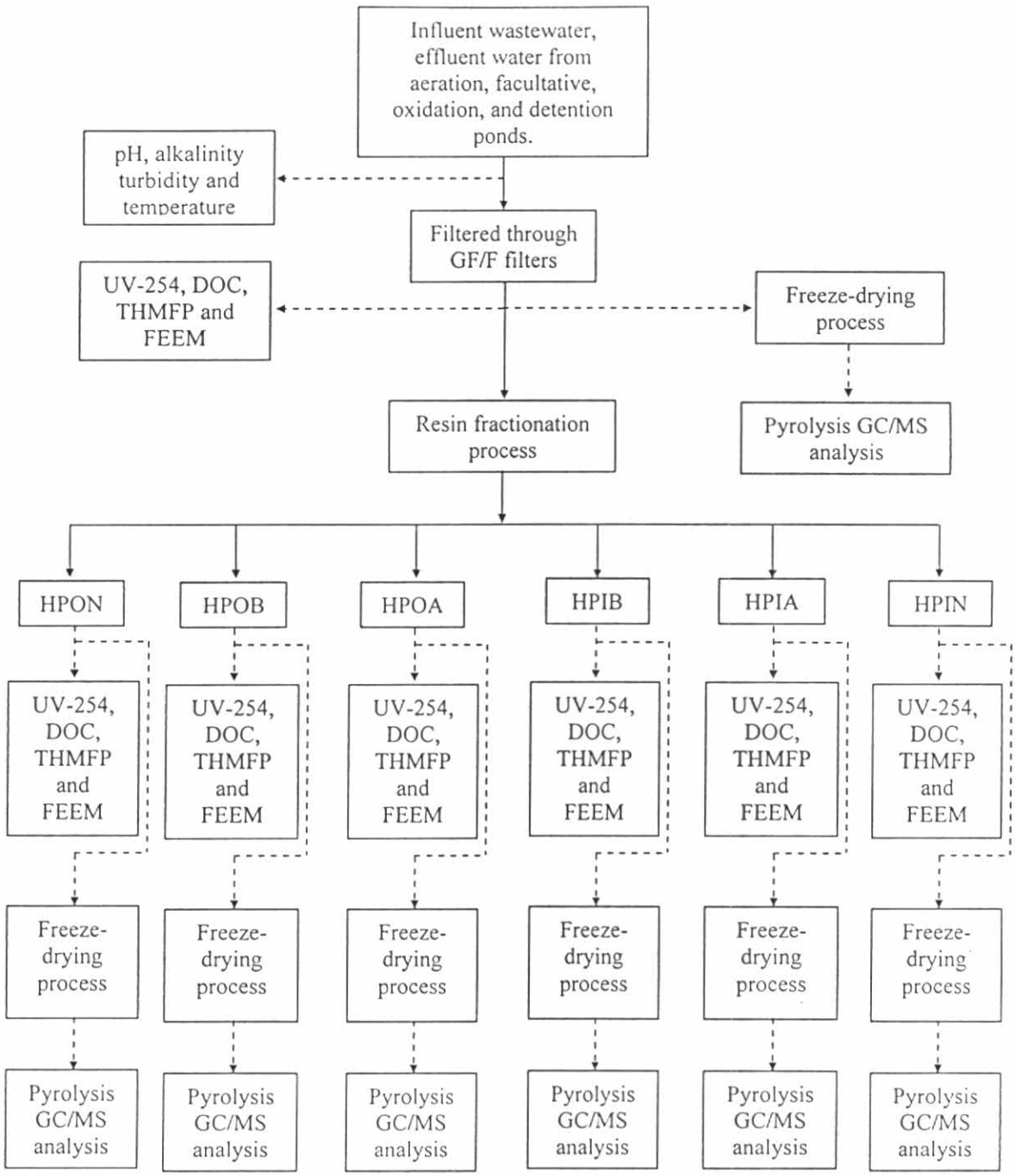

Figure 3.2: Schematic diagram of experimental procedure of water samples collected on February 18, 2005

3.5 Resin Fractionation Procedure

The resin fractionation procedure developed by Leenheer (1981) and Marhaba and Bengraine (2003) was utilized to fractionate DOM in the water samples into six DOM fractions, namely the HPON, HPOB, HPOA, HPIB, HPIA and HPIN fractions, by using a

series of three resins (DAX-8, AG-MP-50 and WA-10). The specifications of the three resins are described as follows:

DAX-8:

- Nonionic resin (SUPELCO)
- 60% porosity
- 40-60 mesh
- 160 square meters per dry gram

AG-MP-50:

- Strong acid cation exchange resins (BIO-RAD)
- Sulfonic acid functional groups attached to a styrene divinylbenzene copolymer lattice
- Effective surface area approximates 35 square meters per dry gram
- 30-50% porosity

WA-10:

- Weak anionic resin (SUPELCO)
- Strong physical and chemical chemistry

3.5.1 Resin Preparation

3.5.1.1 DAX-8

- Refine the resin with 0.1 N NaOH for 24 h.
- Rinse the resin with Milli-Q water to remove NaOH.
- Purify the resin with acetone for 24 h in a set of Soxhlet extraction apparatus
- Purify the resin with hexane for 24 h in a set of Soxhlet extraction apparatus
- Rinse the resin with methanol until the effluent is free of hexane
- Transfer the purified resin into the column with glass wool packed at the bottom as supported in a slurry of methanol. It must be noted that glass wool used in the experiment was purified by a set of Soxhlet extraction apparatus for 24 h prior to its being packed into the column.

- Rinse the packed column with more than 2.5 bed volume (BV) of 0.1 N NaOH, followed with 0.1 N HCl and Milli-Q water, respectively, until the DOC and conductivity of the effluent water is less than 0.1 mg/L and 10 μs/cm, respectively.

3.5.1.2 AG-MP-50

- Purify the resin with methanol for 24 h in a set of Soxhlet extraction apparatus
- Transfer the purified resin into the column with glass wool packed at the bottom as supported in a slurry of methanol
- Rinse the packed column with more than 2 BV of 1 N NaOH, followed by more than 2 BV of 2 N HCl and Milli-Q water, respectively, until the DOC and conductivity of the effluent water is less than 0.1 mg/L and 10 μs/cm, respectively

3.5.1.3 Diaion WA 10

- Refine the resin with methanol for 24 h
- Transfer the purified resin into the column with glass wool packed at the bottom as supported in a slurry of methanol
- Rinse the packed column with more than 1 BV of 1 N HCl, followed by more than 2.5 BV of 1 N NaOH and Milli-Q water, respectively, until DOC and conductivity of the effluent water is less than 0.1 mg/L and 10 μs/cm, respectively

3.5.2 Fractionation Procedure

The fractionation procedure as shown in Figure 3.3 can be described step by step as follows:

1. The filtered water samples, which were adjusted to pH 7±0.2, were pumped through the first DAX-8 resin column with a flow rate of less than 12 BV/h. They were displaced quickly with 1 BV of Milli-Q water. The HPON fraction was retained in the DAX-8 resin. HPON was then extracted from the DAX-8 resin by methanol and the rotary evaporator was utilized to extract methanol from the extracted sample.

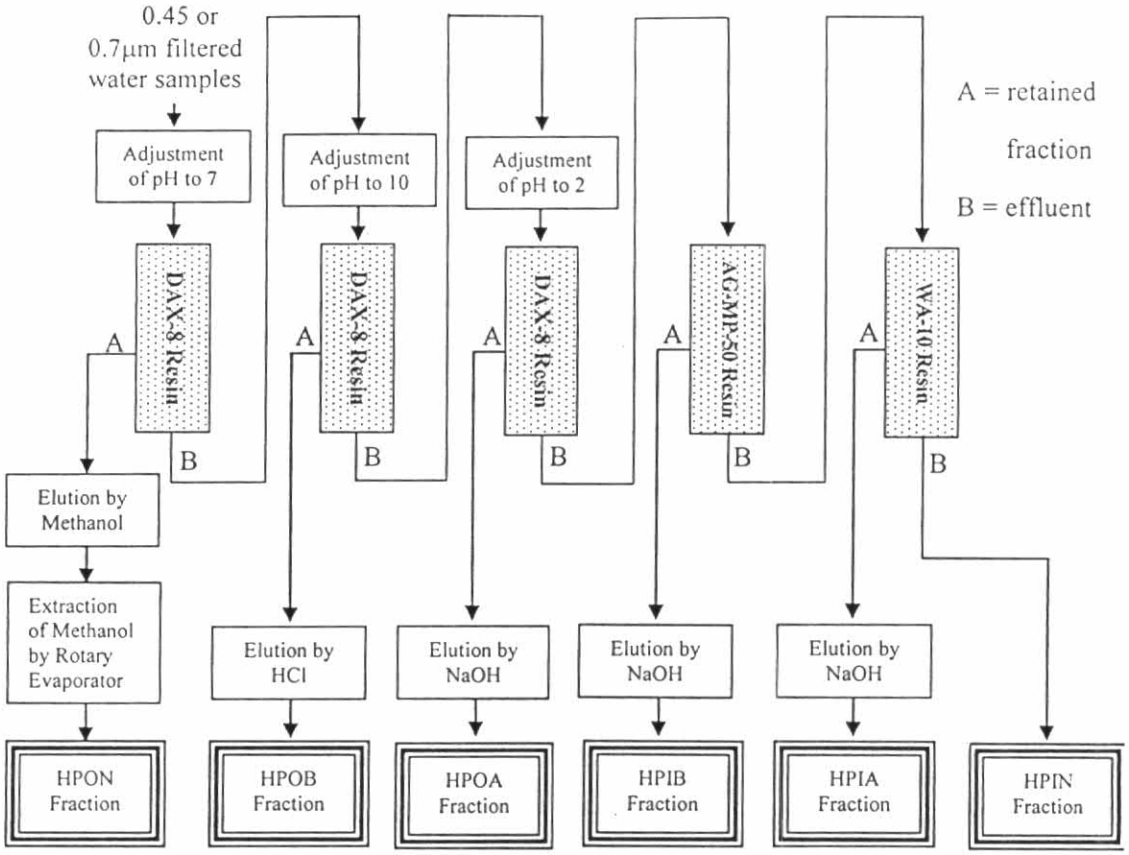

Figure 3.3: Diagram of the fractionation procedure for the DOM fractions

2. The sample effluent from Step 1 was then adjusted to pH 10±0.2 by NaOH and pumped through the second DAX-8 resin column with the flow rate at less than 12 BV/h. It was displaced quickly with 1 BV of Milli-Q water. The HPOB fraction was retained and eluted from the DAX-8 resin with 0.25 BV of 0.1 N HCl followed by 1.5 BV of 0.01 N HCl at a flow rate of less than 2 BV/h.
3. The effluent was then acidified to pH 2±0.2 with H_2SO_4 and recycled to the third DAX-8 resin column with the flow rate of less than 12 BV/h. It was then displaced quickly with 1 BV of Milli-Q water. HPOA was subsequently eluted from the DAX-8 resin with 0.25 BV of 0.1 N NaOH followed by 1.25 BV of 0.01 N NaOH at a flow rate of less than 2 BV/h.
4. The effluent was pumped through the AG-MP-50 resin column to separate the HPIB fraction with a flow rate of less than 5 BV/h. It was then displaced quickly with 1 BV of Milli-Q water. This organic fraction was then eluted

from the AG-MP-50 resin with 1 BV of 1 N NaOH at a flow rate of less than 2 BV/h.

5. Finally, the effluent was pumped through the WA-10 resin column with a flow rate of less than 8 BV/h. It was displaced quickly with 1 BV of Milli-Q water. The final effluent contained the HPIN fraction; whereas the adsorbate eluted from the WA-10 resin, with 1.5 BV of 0.1 N NaOH followed by 1 BV of 0.01 N NaOH at a flow rate of less than 4 BV/h, was the HPIA fraction. The ratios between the resin volume and the water sample of 15mL:1L, 4mL:1L and 85mL:1L for DAX-8, AG-MP-50 and WA-10, respectively, were used in this study.

3.6 Pyrolysis GC/MS Analysis

The filtered influent wastewater and filtered effluent water from the aeration, facultative, oxidation and detention ponds were poured in special glass bottles that were resistant to the Freeze-Drying unit. Due to the very low DOC concentration of HPOB, a rotary evaporator was employed to evaporate the HPOB after the elution process at 45 °C for approximately 4 h in order to gain concentrated HPOB samples. Concentrated HPON and HPOB after evaporation, and HPOA, HPIB, HPIA and HPIN after the elution process of all water samples were put in the glass bottles. Then, all samples were placed in a pre-freeze unit at -20 °C for at least 12 h. After that, they were placed in a freeze-drying unit at -57 °C and 0.004 bar for at least 24 h in order to produce a uniform fine powder for determining the organic compounds in the water samples using a pyrolysis GC/MS.

A few milligrams of uniform powder of all the water samples were placed in platinum buckets and attached to sample holders in the quartz tube of the pyrolyzer. The pyrolyzer was attached to the injection port of a Shimadzu GC/MS QP-5050 equipped with a Rtx-VMS column (Restek, thickness: 1.4μm, length: 30m, diameter: 0.25mm, maximum usable temperature: 240 °C) for pyrolysis GC/MS analysis. The operating pyrolyzer, GC and MS conditions are shown as follows:

3.6.1 Pyrolysis Conditions

Presure:150 psi

Spilt flow: 8 cm/s

Intermediate temp: 220 °C

Initial temperature. 220 °C

Final temperature: 700 °C, final time 10 second

Gas type: Helium.

3.6.2 GC Conditions

Initial temp: 40 °C

Ramp#1: Rate 2.0 °C/min, final temperature 80 °C

Ramp#2: Rate 3.0 °C/min, final temperature 140 °C

Ramp#3: Rate 5.0 °C/min, final temperature 220 °C, final time 30 min.

Run time: 86.0 min

3.6.3 MS Conditions

Acquisition mode: Scan

Interface Temp: 220 °C

Solvent cut time: 0.1 min

Detector voltage: Relative to tuning results

Start time: 0.1 min

End time: 86.0 min

Start (m/z): 40

End (m/z): 650

Scan speed: 2000

3.7 Spectrofluorometry Analysis

3.7.1 Sample Preparation

The filtered influent wastewater and filtered effluent water from the aeration, facultative, oxidation, and detention ponds were adjusted to a pH of about 7±0.2. In the case of the DOM fractions, concentrated HPON, HPOB, HPOA, HPIB and HPIA fractions from the resin fractionation process were diluted with Milli-Q water to their

original DOCs, which was calculated by using the mass balance from the resin fractionation results. The six DOM fractions were then adjusted to a pH of about 7±0.2.

3.7.2 Quinine Sulfate Standard

The quinine sulfate $[(C_{20}H_{24}N_2O_2)_2H_2SO_4 2H_2O]$ 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).

3.7.3 Spectrofluorometor Operating Conditions

A JASCO FP-6200 spectrofluorometer was used to measure the FEEM of all water samples in this study using the following operating conditions:

Measurement Mode: Emission
Band with excitation: 5 nm
Band with emission: 5 nm
Response: Fast
Sensitivity: High
Scanning speed: 2000 nm/min
Excitation wavelength: Start at 220 nm, end at 720 nm
Emission wavelength: Start at 220 nm, end at 720 nm
Excitation wavelength interval: 5 nm
Emission wavelength interval: 1 nm

3.7.4 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) ≥ emission wavelength (Em) or Ex X 2 ≤ Em (Komatsu *et al.* 2005)
- Remove the Rayleight and Raman scattering peaks at Em ± 10-15nm of each Ex (Zepp *et al.* 2004)

3.8 Analytical Methods

3.8.1 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.

3.8.2 Turbidity

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

3.8.3 Alkalinity

Alkalinity was measured in accordance with Standard Method 2320 B.

3.8.4 UV-254

UV-254 was analyzed in accordance with Standard Method 5910B (Standard Methods, 1995) using a UV/VIS spectrometer: a Jasco V-350 spectrophotometer (Jasco Corporation, Tokyo, Japan) at 253.7 nm with matched quartz cells that provided a path length of 10 mm. At least two replications of each measurement of the UV-254 analysis were performed.

3.8.5 DOC

DOCs were analyzed in accordance with Standard Method 5310D (Standard Method, 1995) using a TOC analyzer (O.I. analytical, College Station, Texas, USA). Milli-Q water (ELGA, Lane End, High Wycomebe, UK) was used on every sample to clean the system. At least two replications of each measurement of the DOC analysis were performed.

3.8.6 THMFP

THMFP measurements were carried out according to Standard Method 5710B (Standard Method, 1995). The neutralized solution was buffered by a phosphate solution before incubation at 25 ± 2 °C in amber bottles with PTFE liners. At the end of the 7-day reaction period, samples should have a remaining free chlorine residual of between 3 and 5 mg/L. The residual chlorine was measured according to the procedures mentioned in Standard Method 4500-Cl G, the *N,N*-dechthyl-p-phenylenediamine colorimetric method. The level of chlorine was then represented by the light absorbance at 515 nm using a UV/VIS spectrometer: a Jasco V-350 spectrophotometer with matched quartz cells that provided a path length of 10 mm. THMs were extracted with pentane in accordance with Standard Method 6232B (Standard Method, 1995). Agilent Gas Chromatography-6890 with an electron capture detector (ECD) (Agilent technologies Inc., Wilmington, Delaware, USA) and chromatographic column (J&W Science DB-624, DE, USA) with 0.2-mm X 25 m 1.12 μm film was used to analyze THMs under the following operating conditions:

Inlet conditions

Mode: Split

Initial temp: 225°C.

Pressure: 31.33 psi,

Split ratio: 10:1

Split flow 15.9 mL/min

Gas Type: Helium

Total flow: 20.5 mL/min

Oven conditions

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

Table 3.2 Temperature programs for analyzing THMs

Ramp	Rate (°C/min)	Final temp. (°C)	Holding time of final temp (minute)	Remark
1	15	100	1.00	Initial temp.: 75°C, Initial temp. Holding Time 1.00 min
2	15	130	1.00	-
3	15	180	1.00	-

Detector Conditions

Temperature: 300 °C

Mode: Constant make up flow

Makeup flow: 60 mL/min,

Makeup Gas Type: Nitrogen

At least two replications of each measurement for the THMFP analysis were performed and Milli-Q water was used for all dilutions, sample and chemical preparations, and final glassware cleaning.

A summary of the analytical methods and instruments used in this study is illustrated in Table 3.3.

Table 3.3 Analytical methods and instruments

Parameter	Analytical method	Standard	Analytical Instrument
pH	Direct measurement	-	Horiba pH-meter, Model F-21
Turbidity	Direct measurement	-	HACH, 2100 Turbidity Meter
Alkalinity	Titration Method	Standard method 2320B*	-
Temperature	Direct measurement	-	Horiba Thermometer, Model D-13E
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
Free chlorine residual	Colorimetric Method	Standard method 4500-Cl G*	Jasco, Model UV-530, UV-spectrometer
$TTHM_0$, $TTHM_7$ (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
FEEM analysis	-	-	JASCO FP-6200 spectrofluorometer
Pyrolysis GC/MS analysis	-	-	Shimadzu GC/MS QP-5050

(*Standard Methods, 1995)