CHAPTER II

BACKGROUND AND LITERTURE REVIEW

2.1 Dissolved Organic Matter

Dissolved organic matter (DOM) is defined as the complex matrix of organic material present in natural waters. The term "organic" is used to define general compounds that contain carbon (C) and one or more of the following elements: hydrogen (H), nitrogen (N), and oxygen (O). DOM is a dominant reactant in and product of biogeochemical processes in which the material serves as a carbon and energy source for biota and controls levels of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity (Leenheer and Croue, 2003).

2.1.1 Defining Dissolved Organic Matter

DOM is typically dominated by humic substances generated by biological activity both in a watershed surrounding a water source (allochthonous DOM) and within the water source itself (autochthonous DOM) (Croue *et al.*, 2000). Humic substances include humic and fulvic acids, while non-humic substances include hydrophilic acids, proteins, carbohydrates, carboxylic acids, amino acids, and hydrocarbons. Humic and non-humic substances are the major component of DOM in water (Thurman, 1985; and Amy, 1994). DOM consists of humic substances, amino acids, sugars aliphatic acids, and a large number of organic molecules (Malcolm Pirnie Inc., 1993). Leenheer and Croues (2003) defined DOM as a complex mixture of aromatic and aliphatic hydrocarbon structures with attached amide, carboxyl, hydroxyl, ketone, and various minor functional groups.

The humic fraction has a more hydrophobic character than the non-humic fraction. The humic fraction consists of humic and fulvic acids. The non-humic consists of hydrophilic acids, proteins, amino acids and carbohydrate. However, in terms of their chemical properties and implications for water treatment, humic substances are more important (Owen, 1995). DOM consisting of humic and fulvic acids (aquatic humic) that cause natural color is the most important (Edzwald, 1993). DOM in surface water is mainly composed of humic substances (50-65%) (Collin *et al.* 1986; Leenheer and Croue, 2003) and non-humic substances. Thurman and Malcolm (1991) defined the humic or hydrophobic substances that precipitated at pH 1 as polar, straw-colored, organic acids

derived from soil humas and terrestrial/aquatic plants. It contributed from of about 50% to more than 90% of the organic matter in natural waters. Humic substances can be further classified in to humic acids, fulvic acids and humin.

Wastewater and treated wastewater are the major allochthonous DOM in natural water sources. Sirivedkhin and Gray (2005) presented that effluent-derived organic matter (EfOM) was found to be dominated by more aliphatic compounds and had higher organic nitrogen and halogen content when compared with organic material derived from natural sources. Conclusive results from past research show that EfOM contained substantially higher nitrogen (Peschel and Wildt, 1988; and Debroux, 1998), halogens (Gray *et al.*, 1996a), and sulfurs (Poerschmann *et al.,* 1998) when compared with natural organic matter (NOM). Nevertheless, the determination of aromaticity of EfOM was inconclusive since some researchers found EfOM to be aromatic-dominated (Dignac *et al.*, 2000), whereas others found it to be aliphatic-dominated (Peschel and Wildt, 1988; Gray 1966a; and Debroux, 1998).

2.1.2 Disinfection by-Products

Disinfection by-products (DBPs) such as trihalomethans (THMs), which has been classified as potentially carcinogenic substances, in produced water are generated from the reaction between DOM and chlorine (Rook, 1974; and Bellar *et al.* 1974). Rook (1974) was the pioneer researcher who discovered the DBPs in chlorinated drinking water. Since then there have been several studies that have led to a better understanding of DBPs, their precursors, the kinetic yield of DBP forming reactions, and the active chemical classes for forming DBPs. (Kavanaugh *et al.*, 1980; Christman *et al.*, 1989; Miller and Uden, 1983; Steven, 1982; and White *et al.*, 2003). The reaction of DOM with chlorine produces the major DBPs; they include THMs, haloacetic acids (HAAs), haloacetronitriles (HANs), haloketones (HKs), chloral hydrate (CH) and chloripicrin (CP). The general reaction of DOM with chlorine is as follows (Marhaba and Washington, 1998):

$$\text{DOC} + \text{free chlorine} \rightarrow \text{THMs} + \text{HAAs} + \text{HANs} + \text{other DBPs}$$

Factors influencing DBPs formation are the contact time of chlorine with DOM, chlorine or disinfectant dosage, turbidity, water temperature, pH, presence of other ions such as bromide, DOM concentration, and complex compositions of DOM. Among these factors, the complex composition of DOM is one of the important parameters. Harrington *et al.* (1996) and White *et al.* (2003) proposed that pyrolysis fragments of phenol classes were the best indicator of chlorine reactivity. Phenol correlated well with chloroform formations (Harrington *et al.* 1996). The THMs and total organic halides (TOX) formation were observed to be related to the organic nitrogen content that expressed the presence of proteins and/or elevated algal content (Reckhow *et al.*, 1990; Scully *et al.*, 1988; Gehr *et al.*, 1993; and Young and Uden, 1994). Sirivedhin and Gray (2005) found that the combination of aromatic and aliphatic structures including some substituted with nitrogen and chlorine had a linear relationship with disinfection by product formation potential (DBPFP). With regard to the previous study, it can be stated that the complex composition of DOM was highly influential in the formation of DBPFP. The identification of chemical classes of DOM and DOM fractions, therefore, were the major objectives in this study.

2.2 Quantification and Characterization of DOM

With regard to the heterogeneous character of DOM, there are two approaches for identifying the composition of DOM. DOM has been commonly quantified by using surrogate, nonspecific parameters such as total organic carbon (TOC), dissolved organic carbon (DOC), ultraviolet absorbance at wavelength of 254 nm (UV- 254) and trihalomathane formation potential (THMFP) (USEPA, 1999). For a more complicated approach, resin fractionation can be used to isolate bulks of DOM into DOM fractions that are chemically similar (AWWA, 1993). By conducting THMFP tests on DOM fractions, the ability of each DOM fraction for reacting with chlorine to form carcinogenic substances such as THMs and HAAs can be determined.

In addition, DOM can be characterized on the basis of its apparent molecular weight (AMW) by using ultrafiltration. Amy *et al.* (1987) describes the procedure using a series of hydrophilic ultrafiltration membranes. That approach yielded a series of corresponding permeated for analysis with the following AWW ranges: < 500 Daltons, < 1,000 Daltons, < 3,000 Daltons, < 5,000 Daltons, < 10,000 Daltons, < 30,000 Daltons.

Another method that is commonly used to characterize NOM according to the AMW is gel permeation chromatography (GPC). GPC can be used to chromatographically separate large and small amounts of DOM. The size separation of DOM is dependent upon the types of gel. For example, a Sephadex G-75 gel is capable of size separation in the range of approximately 1,000 to 50,000 Daltons (AWWA, 1993).

Three-dimensional fluorescent spectroscopy (fluorescent excitation-emission matrix, FEEM) provides information on the putative origin of fluorescent organic matter in water. It may identify the matter as a tyrosine-like substances, tryotophan-like substances, humic acid and fulvic acid-like substances, and so on (Coble 1996; Nakajima et al. 2002; Chen *et al.* 2003; and Sierra *et al.* 2005).

In order to identify the nature and abundance of structural units in DOM molecules, element composition analysis, ^{13}C- and 1H-neuclear magnetic resonance (NMR) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy and pyrolysis gas chromatography mass spectrometry (pyrolysis GC/MS) were deliberately employed (AWWA, 1993). Among theses methods, pyrolysis GC/MS, seems to be one of the most advanced techniques commonly used. The pyrolysis GC/MS provides information on the pyrolysis fragments of chemical classes of DOM in water.

As seen at the outset, nonspecific parameters such as DOC, UV- 254, SUVA and THMFP were utilized to quantify the level of DOM in the industrial wastewater treated by stabilization ponds in this study. Due to the lack of information on the DOM fractions in the wastewater and treated wastewater, resin fractionation was used to separate the DOM in the raw and treated wastewater. In addition, FEEM and pyrolysis GC/MS were utilized to identify the nature and abundance of structural units in the DOM molecule. The background and literature reviews in the next section, therefore, will focus on the above-mentioned parameters and methods.

2.3 Characterization of DOM by Nonspecific Parameters

TOC is the measure of all organic substances contained in water samples including suspended fractions that could be removed by coagulation and sedimentation. DOC is defined as the fraction of TOC that passes through a 0.45 μm filter paper. Since some

types of 0.45 μm filter paper are produced using cellulose nitrite or cellulose acetate membrane, organic substances could leach from these filter papers after the filtration process. Thus, GF/F filter paper with a pore size of 0.7 μm is used in DOC analysis.

UV-254 is used to provide an indication of the aggregate concentration of UV-absorbing organic constituents, such as humic substances and various aromatic compounds (APHA, AWWA, WEF, 1995). As noted by Edzwald *et al.* (1985), humic aromatic compounds and molecules with conjugated double bonds absorb UV light, whereas simple aliphatic acids, alcohol, and sugars do not. Most research has utilized the measurement at the UV-visible at the wavelength of 254nm as the representative for the relative quantity of aromatic-humic organic substances (Leenheer and Croue 2003). UV absorbance is a well-known technique for measuring the presence of naturally occurring organic matter such as humic substances. UV analysis is also affected by pH and turbidity (Edzwald, Becker, and Wattier, 1985). UV absorption is a useful surrogate measure for DOM or precursor of THMs because humic substrates strongly absorb ultraviolet radiation (Eaton, 1995).

The ratio between UV absorbance to DOC, referred to as specific ultraviolet absorbance (SUVA) (L/mg-m) demonstrates a relative index of humic content (Edzwald, 1993; and Owen *et al.,* 1993). SUVA could suggest the nature of DOM and its consequent THM formation (Krasner *et al.,* 1996). Higher SUVA values tend to indicate higher humic content. The SUVA of a humic sample depends upon the molecular weight of the substance (Petterson *et al.,* 1995). SUVA can be used as an indicator of its coagulation (or softening) ability to remove THM precursors. Water having a high SUVA value (SUVA > 3 L/mg-m) has been found to contain organic matter that is more humic-like in character, higher in AMW, and more readily removed by coagulation (Edzwald, 1993) whereas lower SUVA values (< 3 L/mg-m) indicate the presence of organic matter of lower AMW that is more fulvic-like in character and more difficult to remove. With regard to wastewater treatment plants, Fukushima *et al.* (1996) reported that the SUVA of total DOM increased as the lake water, influenced by pedogenic DOC, was allowed to further stabilize through biodegradation over a long period of time. Imai *et al.* (2002) reported that since a biological treatment was employed in sewage treatment plants, it should produce effluent water with higher SUVA values than that of influent wastewater.

Total trihalomethanes ($TTHM_T$) is the sum of all four compound concentrations, chloroform, dichlorobromomethane, dibromochloromethane and bromoform, produced at any time T (usually measured in days). $TTHM_0$ is the total THMs concentration at the time of sampling. It can range between non-detectable to several hundred micrograms per liter if the samples have been chlorinated. $TTHM_7$ is the total concentration of all four THMs compounds produced during the reactions of the sample precursors with excess free chlorine over a 7-day reaction time at the standard reaction conditions, which were as follows: free chlorine residual at least 3 mg/L and not more than 5 mg/L at the end of a 7-day reaction (incubation) period with sample incubation temperature of 25± 2°C, and pH controlled at 7± 0.2°C with phosphate buffer. THMFP or ΔTHMFP is the difference between the final $TTHM_T$ concentration and the initial $TTHM_0$ concentration. For samples that do not contain chlorine at the time of sampling, $TTHM_0$ will be close to zero. Therefore the term THMFP may be used. For samples that contain chlorine at the time of sampling, a $TTHM_0$ value will be detected. Therefore the term ΔTHMFP may be used when reporting the difference between the TTHM concentrations (Standard method, 1995).

THMFP has also only been commonly employed to measure the DOM in water at drinking water supply facilities. The THMFP of treated wastewater has not been widely evaluated due to treated wastewater being perceived as less aromatic (Aieta, 1998). Nevertheless, treated wastewater seems to have much higher DOC concentrations than most surface water used as drinking water supplies. A survey of five wastewater treatment plants in southern California, USA demonstrated that DBPs in treated wastewater was found to be higher than that of influent wastewater (National Research Council, 1998). Musikavong *et al.* (2005) reported that the level of THMFP in the treated wastewater of the industrial estate in northern Thailand was moderately high when compared with other surface waters in Thailand (Wattanachira *et al.* 2003, Homklin 2004, Janhom 2004, Panyapinyopol *et al.* 2005; and Phumpaisanchai 2005).

2.4 Resin Fractionations

DOM in water could be separated into DOM fractions that are chemically similar by using the resin fractionation process (AWWA, 1993). The fractionation of DOM using the resin adsorption technique was developed for characterizing DOM and differentiating the problematic organic fractions from other organic fractions (Leenheer, 1981; and Marhaba *et al.* 2003). The resin fractionation process could help researchers gain a better understanding of DOM in the formation of DBPs. By conducting THMFP tests on DOM fractions, the ability of each DOM fraction to react with chlorine to form carcinogenic substances such as THMs and HAAs was evaluated.

Leenheer (1981), one of the pioneers in the area of the fractionation method, developed the resin fractionation procedure for separated DOM by using a series of three resins (DAX-8, AG-MP-50 and Duolite A7) on six fractions (i.e., hydrophobic neutral (HPON), hydrophobic base (HPOB), hydrophobic acid (HPOA), hydrophilic base (HPIB), hydrophilic acid (HPIA) and hydrophilic neutral (HPIN)). Imai *et al.* 2001 modified the resin fractionation procedure developed by Leenheer (1981) by replacing one of the resins, Duolite A7, with AG-MP-1. Later on, the resin fractionation procedure proposed by Leenheer (1981) was modified by Marhaba *et al.* (2003) by replacing one of the resins, Duolite A7,with WA-10 as can be seen in Figure 2.1.

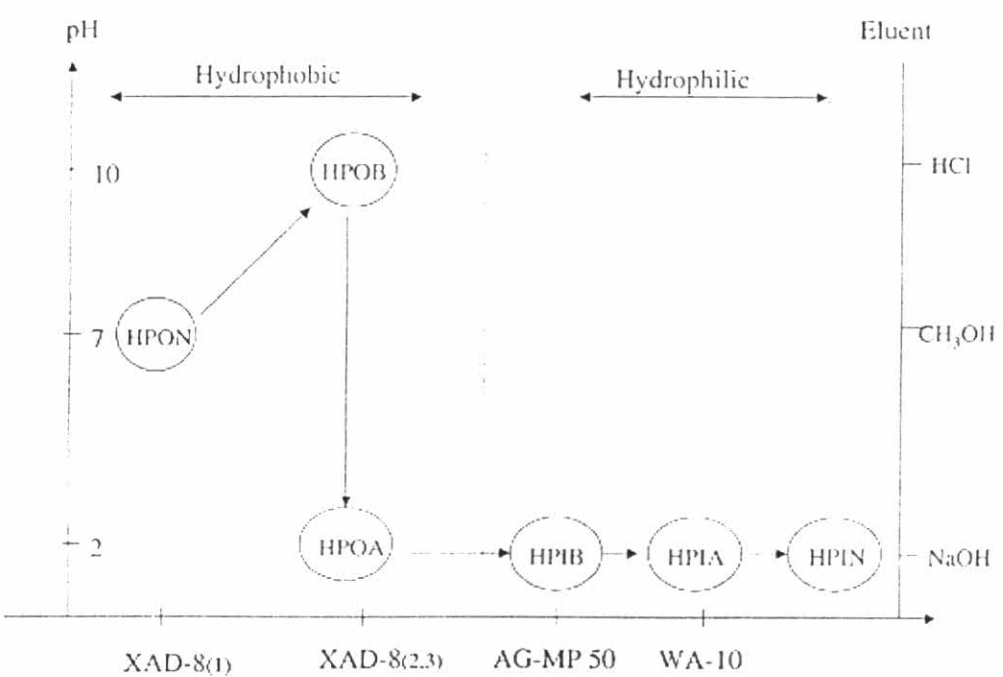


Figure 2.1 Resin fractionation process (Marhaba *et al.*, 2003)

The resin fractionation technique has been utilized by many researchers for characterizing DOM in water sources, such as underground water (Swietlik *et al.* 2004), reservoirs, lakes, (Imai *et al.* 2001; Imai *et al.*, 2003; and Janhom 2004) and rivers (Day 1991; Marhaba and Van, 1999; Croue *et al.* 2000; Marhaba and Van, 2000; Imai *et al.* 2001; Kimura *et al.* 2004; and Panyapinyopol *et al.* 2005), for drinking water facilities. As mentioned previously, wastewater and treated wastewater are major allochthonous sources of DOM in drinking water sources. The resin fractionation technique has been applied in wastewater and treated wastewater research. Barber *et al.* (2001) fractionated the effluent water from three wetlands in the USA. HPOA and HPIA were found to be the major DOM fractions. Imai *et al.* (2001) and Imai *et al.* (2002) employed the resin fractionation technique to separate the DOM in effluent water from wastewater treatment plants in Japan in which HPOA and HPIA were the dominant fractions. Hu *et al.* (2003) fractionated treated secondary effluent in Singapore. HPOA and HPIN were found to be the major DOM fractions.

The DOC, UV-254, SUVA and percent distribution of DOC of DOM fractions of groundwater and reservoir water obtained form literature data is shown in Table 2.1, while that of river water is depicted in Table 2.2. In Table 2.1, in the case of groundwater, only three data were available; hence, they could not be summarized. In the case of reservoir and lake water, DOC values ranged from 2.0 and 147.0 mg/L. The lowest DOC reading was obtained from a water sample from the Mae-Kuang Reservoir, Chiangmai, Thailand (Homklin, 2004); whereas the highest DOC reading of 147.0 mg/L came from a water sample from Inkpot Lake, Victoria, Australia (Day *et al.*1991). The UV-254 and SUVA in reservoir and lake water ranged from 0.049 to 0.602 cm^{-1} and from 1.4 to 5.9 L/mg-m, respectively. DOC values in river water ranged from 1.1 and 26.5 mg/L (Table 2.2). The lowest DOC value was found in a water sample from the South Fork Tolt River, Seattle, WA, USA (Liang and Singer, 2003); whilst the highest DOC value of 26.5 mg/L, came from Red Water Creek, Victoria, Australia (Day *et al.* 1991). The UV-254 and SUVA in river water ranged from 0.023 to 0.295 cm^{-1} and from 1.3 to 4.7 L/mg- m, respectively.

Table 2.1: DOC, UV-254, SUVA and percent distribution of DOC in groundwater and reservoir water obtained from literature data.

No	Water Sources	DOC mg/L	UV-254 cm^{-1}	SUVA L mg^{-1} m^{-1}	Percent distribution of DOC							
					Percent		Percent					
					HPO	HPI	HPON	HPOB	HPOA	HPIB	HPIA	HPIN
1	Underground water, Poland (Swietlik *et al.* 2004)	3.8-6.5	NA	NA	85	15	12	Less	73	5	7	3
2	Shallow well nearby closed damping sites, Chiangmai, Thailand, (Jiarsirikul, 2003)	1.7	0.27	16.3	56	44	NA	NA	NA	NA	NA	NA
3	Shallow well nearby closed damping sites, Chiangmai, Thailand(Jiarsirikul, 2003)	3.1	0.28	9.0	58	42	NA	NA	NA	NA	NA	NA
1	Mae-Kuang Reservoir, Chinagmai, Thailand(Homklin, 2004)	2.0	0.049	2.4	60	40	NA	NA	NA	NA	NA	NA
2	Aung-Keaw Reservoir, Chiangmai, Thailand (Homklin, 2004)	2.4	0.109	4.6	53	47	NA	NA	NA	NA	NA	NA
3	Bhumibol Dam, Tak, Thailand (Phumpaisanchai, 2005)	2.5	0.077	3.1	49	51	NA	NA	NA	NA	NA	NA
4	Tomhannock Reservior, Newyork, USA (Kitis *et al.* 2002)	3.3	0.069	2.1	36	64	NA	NA	NA	NA	NA	NA
5	Poquonnock Reservoir, Groto, CT, USA (Liang and Singer, 2003)	3.3	0.119	3.6	44	56	NA	NA	NA	NA	NA	NA
6	Kasumigaura Lake, Japan (a shallow eutrophic lake) (Imai *et al.*2003)**	3.5	NA	NA	40	60	8	NA	32	9	46	6
7	The Ijssel Lake, Andijk, North Holland (Kenedy *el at.* 2005)	4.0	0.084	2.1	54	46*	NA	NA	NA	NA	NA	NA
8	Kasumigaura Lake, Japan (a shallow eutrophic lake) (Imai *et. al.* 2001)	4.1	0.065	1.6	41	57	9	NA	32	10	43	4
9	Cazua Lake, Bordeaux, French (Lee *et al.* 2004)	5.0	0.069	1.4	39	61*	NA	NA	NA	NA	NA	NA
10	Minaga Reservoir, Japan (Galapate *et al.* 2001)	5.0	NA	NA	38	62	NA	NA	NA	NA	NA	NA
11	Raw water supply supply reservoir, Thailand (Janhom *et al.* 2005)	5.4	0.139	2.6	57	43	12	3	42	5	21	18
12	Rivington WTW (February 2001), UK(Goslan *et al.* 2004)***	6.1	0.246	4.0	66	34	NA	NA	NA	NA	NA	NA
13	Mae-Hia Reservoir, Chinagmai, Thailand (Phumpaisanchai, 2005)	6.4	0.191	3.0	49	51	NA	NA	NA	NA	NA	NA
14	Bultiere Reservoir, French (Lee *et al.* 2004)	6.9	0.177	2.6	44	56*	NA	NA	NA	NA	NA	NA
15	Albert WTW (November,2002), Yorkshire, UK (Goslan *et al.* 2004)***	7.5	0.381	5.1	72	28	NA	NA	NA	NA	NA	NA
16	Lake Manatee Reservoir, Bradenton, FL, USA (Liang and Singer, 2003)	8.2	0.359	4.4	52	48	NA	NA	NA	NA	NA	NA
17	Albert WTW (November,2001), Yorkshire, UK (Goslan *et al.* 2004)***	9.9	0.480	4.8	66	34	NA	NA	NA	NA	NA	NA
18	Albert WTW (November,2000), Yorkshire, UK (Goslan *et al.* 2004)***	10.2	0.602	5.9	79	21	NA	NA	NA	NA	NA	NA
19	The Inkpot Lake, Victoria, Australia, (Day *et al.* 1991)	147.0	NA	NA	77	23	NA	NA	NA	NA	NA	NA

Remark; In case of the water sample was fractionated into six fractions, HPO= HPON+HPOB+HPOA and HPI = HPIB+HPIA+HPIN

NA = Not avialable,

* Hydrophilic organic fraction was calculated from the summation of tranphilic and hydrophilic orgnaic fractions

** Average value from the original paper and HPOB was negligible

*** WTW = Water treatment plant, hydrophobic fraction was calculated from humic acid fraction and fulvic acid fraction

Table 2.2:DOC, UV-254, SUVA and percent distribution of DOC in river water obtained from literature data.

No.	Water Sources	DOC mg/L	UV-254 cm^{-1}	SUVA L mg^{-1} m^{-1}	Percent distribution of DOC							
					Percent		Percent					
					HPO	HPI	HPON	HPOB	HPOA	HPIB	HPIA	HPIN
1	South Fork Tolt River, Seattle, WA, USA (Liang and Singer, 2003)	1.1	0.052	4.7	58	42	NA	NA	NA	NA	NA	NA
2	The Slip Creek, Victoria, Australia (Day *et al.* 1991)	1.4	NA	NA	20	80	NA	NA	NA	NA	NA	NA
3	The Mae-Sa River, Chiangmai, Thailand(Homklin, 2004)	1.8	0.023	1.3	69	31	NA	NA	NA	NA	NA	NA
4	The Da Cha Creek, Tai Chung, Taiwan (Chang *et al.* 2001)	2.0	NA	NA	43	57*	NA	NA	NA	NA	NA	NA
5	The Myrtle Creek, Victoria, Australia (Day *et al.* 1991)	2.1	NA	NA	52	48	NA	NA	NA	NA	NA	NA
6	The Han River, Korea Kim and Yu, 2005)	2.1	0.036	1.7	47	53	NA	NA	NA	NA	NA	NA
7	The Chitose River, Sapporo, Japan (Kimura *et al.* 2004)	2.4	0.090	3.8	51	49	20	5	27	4	23	22
8	The Marne River, Paris, French (Lee *et al.* 2004)	2.7	0.057	2.1	50	50*	NA	NA	NA	NA	NA	NA
9	The Koise, Sakura, Hanamuro and Ono Rivers, Japan (Imai *et. al.* 2001)***	2.8	0.058	2.1	48	53	9	0	39	11	37	6
10	White River, Indianapolis, USA (Liang and Singer, 2003)	2.8	0.087	3.1	33	67	NA	NA	NA	NA	NA	NA
11	Confluence of the Raritan and Millstone River, USA (Marhaba and Van 1999)	3.8	NA	NA	33	67	17	6	11	3	44	19
12	The Passaic River, New Jersey USA (Marhaba and van 2000)	4.6	NA	NA	29	71	10	7	12	5	53	13
13	The Chao Phraya River, Bangkok, Thailand (Panyapinyopol *et al.*, 2003)	4.7	0.122	2.6	40	60	5	3	32	3	16	41
14	Mississippi River, East St.Louis, IL, USA (Liang and Singer, 2003)	5.0	0.163	3.3	43	57	NA	NA	NA	NA	NA	NA
15	Yffinaiac River, Brittany, French (N.Lee *et al.* 2004)	8.4	0.295	3.5	40	60*	NA	NA	NA	NA	NA	NA
16	The Red Water Creek, Victoria, Auatralia (Day *et al.* 1991)	26.5	NA	NA	84	16**	8	1	75	2	9	6

Remark; In case of the water sample was fractionated into six fractions, HPO= HPON+HPOB+HPOA and HPI = HPIB+HPIA+HPIN

NA = Not avialable,

* Hydrophilic organic fraction was calculated from the summation of tranphilic and hydrophilic orgnaic fractions

**The percent distribution was adjusted to 100 percent recovery

***The Koise, Sakura, Hanamuro and Ono Rivers dischrage to Kasumigaura Lake, Japan and HPOB was negligible.

2.5 Three-Dimensional Fluorescence Spectroscopy

An FEEM is obtained by fluorescent spectrometry, recording the matrix of fluorescent intensity in coordinates of excitation and emission wavelengths (Coble *et al.* 1990; and Coble 1996). Fluorescent excitation-emission wavelengths that exhibited outstanding fluorescent emission intensities were classified as fluorescent peaks as depicted in Figure 2.2. Three-dimensional fluorescent spectroscopy analysis--in this case, the use of a FEEM--has the advantage of its simplicity due to its minimal sample amount, pretreatment and analysis time requirements.

2.5.1 FEEM of DOM in Water Sources

FEEM can provide information on the putative origin of fluorescent organic matter of DOM in water. Coble *et al.* (1993) demonstrated that FEEM offers several major advantages over single-scan methodologies. Once the FEEM has been fully corrected for instrumental configuration, data can be analyzed as excitation spectra, emission spectra or synchronous scan spectra, even though originally collected as emission scans. This method was then utilized by many researchers to scrutinize the fluorescent organic matter in water from several water sources. Two dominant types of fluorescent organic matters have been determined; the first one has fluorescence properties similar to those of proteins (Tranganza, 1969; Ewald *et al.*, 1986; Coble *et al.*, 1990; and Mopper and Park, 1993) and the other has properties similar to those of humic substances from terrestrial sources (Coble *et al.*, 1990; and Mopper and Park, 1993). Wolfbeis (1985) presented that the location of fluorescent peaks of tyrosine-like and protein-like substances was detected at excitation wavelength/emission wavelengths of (Ex/Em) 220-275nm_{Ex}/300-305nm_{Em} whereas that of tryptophan-like and protein-like substances was found at 220-275nm_{Ex}/340-350nm_{Em}.

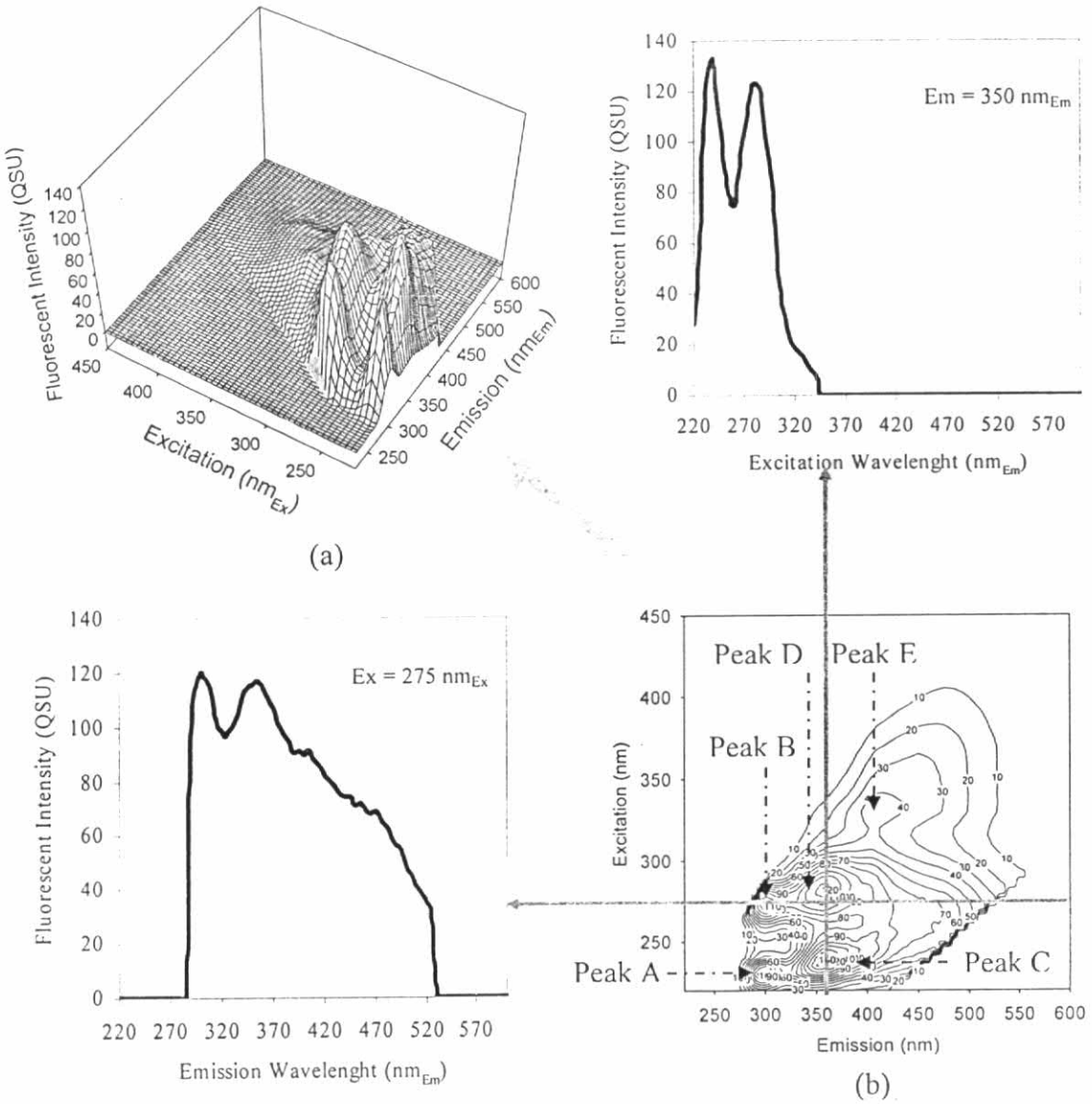


Figure 2.2: Sample of the three-dimensional view (a) and contour view (contour interval of 10 QSU) with fluorescent peaks and its sections (b) of a FEEM

Coble *et al.* (1993) proposed that the fluorescent peak of humic-like and protein-like substances were at 230nm$_{Ex}$/420-450nm$_{Em}$ and 340nm$_{Ex}$/420-450nm$_{Em}$, and 220nm$_{Ex}$/300-340nm$_{Em}$ and 275nm$_{Ex}$/300-340nm$_{Em}$, respectively. Coble (1996) utilized high-resolution fluorescent spectroscopy to characterize the DOM in water samples from a wide variety of freshwater, coastal and marine environments. The fluorescent signals of humic-like, tyrosine-like, and tryptophan-like were observed. The fluorescent peak positions of the major fluorescent component in bulk seawater were as follows: tyrosine-like and protein-like (275nm$_{Ex}$/310nm$_{Em}$), tryptophan-like and protein-like

($275nm_{Ex}/340nm_{Em}$), humic-like ($260nm_{Ex}/380\text{-}460nm_{Em}$), marine humic-like ($312nm_{Ex}/380\text{-}420nm_{Em}$) and humic-like ($250nm_{Ex}/420\text{-}480nm_{Em}$).

Nakajima *et al.* (2002) applied three-dimensional fluorescent spectroscopy to investigate the water quality in the Tama River, Japan. It was found that FEEM of the water downstream of a lake exhibited a small but strong fluorescent peak at $225nm_{Ex}/295nm_{Em}$ and a weak fluorescent peak at $270nm_{Ex}/295nm_{Em}$. These peaks were largely derived from algae. The FEEM of the water receiving treated sewage exhibited two distinctive peaks at around $345nm_{Ex}/430nm_{Em}$ and $240nm_{Ex}/330\text{-}430nm_{Em}$. When compared with the fluorescent peaks of human urine, humic acid and a laundry detergent with fluorescent whitening agents, the fluorescent peak of the water receiving treated sewage at $345nm_{Ex}/430nm_{Em}$ was also detected in the FEEM of a laundry detergent.

Yamashita and Tanoue (2003) reported that the fluorescent peak of tyrosine-like and protein-like substances was found at $270\text{-}275nm_{Ex}/300\text{-}302nm_{Em}$ whereas that of tryptophan-like and protein-like substances was found at $280nm_{Ex}/342\text{-}346nm_{Em}$. In addition, fulvic acid-like substances and humic acid-like substances exhibited fluorescent peaks at $215nm_{Ex}/437\text{-}441nm_{Em}$ and $350\text{-}365nm_{Ex}/446\text{-}465nm_{Em}$, respectively. Chen *et al.* (2003) divided excitation and emission boundaries into five regions based largely upon supporting literature. Fluorescent peaks have been associated with humic-like, tyrosine-like, tryptophan-like, and phenol-like organic compounds. In general, fluorescent peaks at shorter excitation wavelengths (<250 nm) and shorter emission wavelengths (<350 nm) are related to simple aromatic proteins such as tyrosine (Regions I and II). Fluorescent peaks at intermediate excitation wavelengths (250-280 nm) and shorter emission wavelengths (<380 nm) are related to soluble microbial byproduct-like material (Region IV). Fluorescent peaks at longer excitation wavelengths (>280 nm) and longer emission wavelengths (>380 nm) are related to humic acid-like organics (Region V). For fulvic acids, fluorescent peaks with minimum excitation wavelengths of 250 nm indicated shoulders of fluorescent peaks located at shorter excitation wavelengths. Therefore, fluorescent peaks at shorter excitation wavelengths (<250 nm) and longer emission wavelengths (>350 nm) are related to fulvic acid-like materials (Region III).

Leenheer and Croue (2003) presented the major fluorescent components in a FEEM. Humic-like substances exhibited fluorescent peaks at 330-350nm$_{Ex}$/420-480nm$_{Em}$ and 250-260nm$_{Ex}$/380-480nm$_{Em}$, whereas the fluorescent peak of marine humic-like substances was found at 310-320nm$_{Ex}$/380-420nm$_{Em}$. Protein-like and tyrosine-like substances exhibited a fluorescent peak at 270-280nm$_{Ex}$/300-320nm$_{Em}$, while tyrptophan-like substances exhibited a fluorescent peak at 270-280nm$_{Ex}$/320-350nm$_{Em}$.

Sierra *et al.* (2005) used single-scan and FEEM techniques to identify the fluorescent fingerprints of fulvic and humic acids from various origins. They noted that the in the case of fulvic acids, the Ex/Em pairs were approximately 260nm$_{Ex}$/460nm$_{Em}$ and 310nm$_{Ex}$/440nm$_{Em}$, whereas in the case of humic acids, their excitation and emission maxima were red-shifted. The corresponding Ex/Em pairs were located at approximately 265nm$_{Ex}$/525nm$_{Em}$ and 360nm$_{Ex}$/520nm$_{Em}$.

In Thailand, FEEM was utilized to characterize fluorescent organic matter in some water sources. Janhom (2004) characterized the fluorescent organic matter in reservoir water of the Northern-Region Industrial Estate, Lumphun province, Thailand. A fluorescent peak at 275nm$_{EX}$/410nm$_{Em}$ representing humic-like substances was detected. Homklin (2004) utilized FEEM for characterizing fluorescent organic matter in water samples from Aung-Keaw Reservoir, Mea-Kuang Reservoir and Mae-Sa River, Chiang Mai Province, Thailand. Fluorescent peaks of these respective waters were found at 260nm$_{EX}$/460nm$_{Em}$ and 330nm$_{EX}$/440nm$_{Em}$, 260nm$_{EX}$/460nm$_{Em}$ and 310nm$_{EX}$/400nm$_{Em}$, and 270nm$_{EX}$/450nm$_{Em}$ and 340nm$_{EX}$/440nm$_{Em}$. These obtained peaks showed the presence of humic acid-like and fulvic acid-like substances. Phumpaisanchai (2005) used the FEEM to characterize the fluorescent organic matter in water from the Mae-Hia reservoir, Chiang Mai province and Bhumibol Dam reservoir in Tak province, Thailand. Fluorescent peaks at 260nm$_{EX}$/420nm$_{Em}$ and 330nm$_{EX}$/400nm$_{Em}$ were detected in the water from the Mae-Hia reservoir, whereas fluorescent peaks at 260nm$_{EX}$/420nm$_{Em}$ and 330nm$_{EX}$/410nm$_{Em}$ were found in the water from the Bhumibol Dam reservoir. These observation lead to the conclusion that humic acid-like and fulvic acid-like substances were the major fluorescent organic matter in both the Mae-Hia reservoir and Bhumibol Dam reservoir.

FEEMs have been used to analyze wastewater and treated wastewater. Baker (2001) studied the FEEM characterization of sewage-impacted rivers in England. Sewage treatment work (STW) discharge samples from two STW showed that the high fluorescent intensity of tryptophan-like and fulvic-like fluorescent were detected. Baker (2002) used FEEM for characterizing river water impacted by effluent from a tissue mill. FEEMs of the river water samples from both the tissue mill effluent and the impacted river were dominated by tryptophan-like fluorescent and a FEEM center may due to the presence of fluorescent whitening agents. Lee and Ahn (2004) utilized FEEM to monitor the chemical oxygen demand (COD) in wastewater and treated effluent. Fluorescent peaks of protein-like substances at $220nm_{Ex}/350nm_{Em}$ and $270nm_{Ex}/350nm_{Em}$ and that of humic-like substances at $240nm_{Ex}/450nm_{Em}$ and $340nm_{Ex}/450nm_{Em}$ were found in the FEEMs of domestic wastewater, treated effluent and receiving river water.

There are some studies that developed the relationship between the fluorescent intensity of outstanding peaks and DOM surrogates parameters such as DOC and THMFP. Marhaba and Kootchars (2000) used the fluorescent intensity to predict the disinfection by-product formation potential. The results show that the total THMs, total HAN and HAA6 (total of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, dibromoacetic acid and tribromoacetic acid) for the river humic acid and fulvic acid correlated with the fluorescent intensity at $250nm_{Ex}/450nm_{Em}$. Nakajima *et al.* (2002) found that the fluorescent intensity correlated well with total THMFP for a wide range of excitation/emission wavelengths. The fluorescent intensity at $255\text{-}295nm_{Ex}/245\text{-}385nm_{Em}$ correlated with total THMFP better than UV-260. The maximum value of the determination correlation coefficient (R^2) of 0.90 at $260nm_{Ex}/355nm_{Em}$ was determined. Lee and Ahn (2004) proposed that the fluorescent peak of protein-like substances at $270nm_{Ex}/350nm_{Em}$ showed the best correlation with the Chemical COD values obtained by wet oxidation methods.

2.5.2 FEEM of DOM Fractions

Recently, FEEMs have been commonly employed to characterize the fluorescent organic matter in DOM fractions in water for drinking water facilities. Marhaba (2000) utilized the fluorescence technique for the rapid identification of DOM fractions. The

water samples were collected along the entire treatment train, from influent to effluent, of the Canal Road (CR) water treatment plant in Somerset, N.J. USA. Janhom (2004) characterized the fluorescent organic matter of DOM fractions in reservoir water of the Northern-Region Industrial Estate, Thailand. Kimura *et al.*, (2004) utilized FEEM to characterize the fluorescent organic matter of DOM fractions in water from the Chitose River, Japan. The fluorescent peak positions of DOM fractions from literature data are shown in Table 2.3.

Table 2.3 Summary of fluorescent peak position of DOM fractions from literature data

DOM Fractions	Fluorescent peaks positions
HPON	225-237nm$_{Ex}$/309-321nm$_{Em}$ (major peak),(Marhaba, 2000) 230-300nm$_{Ex}$/340-520nm$_{Em}$ (Janhom., 2004) 280nm$_{Ex}$/310nm$_{Em}$ (Kimura *et al.*, 2004) 225nm$_{Ex}$/350nm$_{Em}$ (Chen *et al.*, 2003)
HPOB	225-237nm$_{Ex}$/369-381nm$_{Em}$ (major peak), 273-285nm$_{Ex}$/369-381nm$_{Em}$ (minor peak) (Marhaba, 2000) 315nm$_{Ex}$/355nm$_{Em}$ (Kimura *et al.*, 2004)
HPOA	237-249nm$_{Ex}$/417-429nm$_{Em}$ (major peak), 297-309nm$_{Ex}$/417-429nm$_{Em}$ (minor peak) (Marhaba, 2000) 255nm$_{Ex}$/420nm$_{Em}$ (Janhom., 2004) 290nm$_{Ex}$/445nm$_{Em}$ (Kimura *et al.*, 2004) 215nm$_{Ex}$/415nm$_{Em}$ and 220nm$_{Ex}$/355nm$_{Em}$ (Chen *et al.*, 2003)
HPIB	225-237nm$_{Ex}$/357-369nm$_{Em}$ (major peak), 273-285nm$_{Ex}$/357-381nm$_{Em}$ (minor peak) (Marhaba, 2000) 290nm$_{Ex}$/380nm$_{Em}$ (Kimura *et al.*, 2004)
HPIA	225-237nm$_{Ex}$/345-357nm$_{Em}$ (major peak), 237nm$_{Ex}$/357-369nm$_{Em}$ (minor peak) (Marhaba, 2000) 270nm$_{Ex}$/410nm$_{Em}$ and 330nm$_{Ex}$/410m$_{Em}$(Janhom, 2004) 310nm$_{Ex}$/430nm$_{Em}$ (Kimura *et al.*, 2004) 205nm$_{Ex}$/405nm$_{Em}$, 220nm$_{Ex}$/355nm$_{Em}$ 275nm$_{Ex}$/325nm$_{Em}$, and 300nm$_{Ex}$/400nm$_{Em}$ (Chen *et al.*, 2003)
HPIN	225nm$_{Ex}$/609-621nm$_{Em}$ (major peak),(Marhaba, 2000) 230-300nm$_{Ex}$/340-520nm$_{Em}$ (Janhom., 2004) 295nm$_{Ex}$/315nm$_{Em}$ (Kimura *et al.*, 2004)

Conclusive results from literature data established that the utilization of FEEM for the evaluation of DOM fractions in wastewater and treated wastewater has not been widely evaluated. Chen *et al.* (2003) presented the FEEM of wastewater effluent DOM fractions in USA as can be seen in Table 2.3

2.6 Pyrolysis GC/MS Analysis

Pyrolysis GC/MS is the analytical technique that has been generally utilized in geochemistry and soil-science to identify the structure of complex non-volatile organic macromolecules. Pyrolysis is a method that thermally cleaves an organic molecule into volatile fragments which are then separated by gas chromatography and identified by mass spectroscopy (MS). Pyrolysis GC/MS yields a reproducible fragmentation pattern or fingerprint, which is highly characteristic of the parent organic matter. Saiz-Jimenez, (1994) proposed the advantages of pyrolysis GC/MS. First, it required a small sample amount; only a few hundred micrograms of organic carbon was enough for analysis. Furthermore, an elaborate sample preparation such as derivatization and extraction was not required. Finally, it was capable to providing detailed molecular weight information.

Recently, pyrolysis GC/MS has been applied to identify the pyrolysis fragment and chemical classes of DOM occurring in systems of interest to environmental engineering (Gray *et al.*, 1996). This is attributed to the fact that numerous compounds can appear in the refractory, nonchomatographable (Thurman, 1985). The use of a pyrolyzer coupled with GC/MS is able to provide a chemical fingerprint (pyrolysis fragments) of both chromatographable and nonchomatographable fractions, which could be used to identify the original mixture of DOM in water. The qualitative identification of polysaccharides, proteins, lipids, lignins and other biomarkers can be accomplished using pyrolysis GC/MS (Almedros *et al.*, 1997; Fabbri *et al.*, 1998; Hatcher *et al.*, 2001; Kogel-Knabner, 2000; Pouwels *et al.*, 1989; and, Ralph and Hatfield, 1991).

Pyrolysis GC/MS has been utilized by many researchers in studying different aspects of DOM. The major differences between humic substances from aquatic plants such as algae, aquatic phanerograms and lagoon, and marine and lacustrine deposits were identified by using pyrolysis GC/MS. Proteins and numerous nitrogen by-products (alkylpyrroles, nitriles and alkylpyrridines) along with aromatic compounds that are tough to reflect the decomposition of individual amino acids (styrene, toluene, phenol and *p*-cresol) were the dominate chemical classes in algae. These compounds were less abundant in the pyrolysis products of humic substances (Gadfl and Bruchet, 1986). Abbt-Braun *et al.* (1989) utilized pyrolysis-field ionization mass spectrometry and pyrolysis

GC/MS to investigate the structures of aquatic humic substances. Bruchet *et al.* (1990) developed the pyrolysis GC/MS technique for investigating high molecular weight THM precursors and other refractory organics. The four main types of biopolymers, carbohydrates, protienaceous materials, *N*-acetylamino sugars and polyhydroxy aromatics, were proposed. The pyrochromatogram of a mixture of biopolymers (bovine serum albumin, cellulose acetate, fluka humic acid, and chitin) and the details of their pyrolysis fragments are shown in Figure 2.3 and tabulated in Table 2.4, respectively.

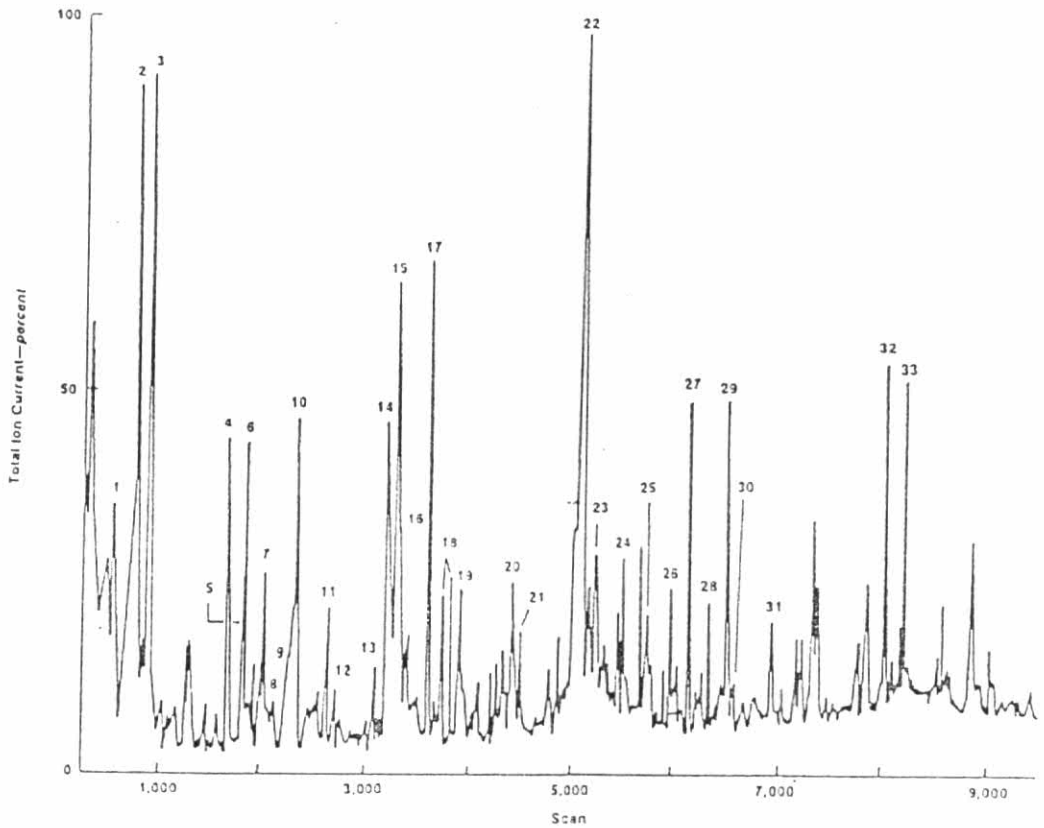


Figure 2.3 Pyrochromatogram of a mixture of biopolymers: (bovine serum albumin, cellulose acetate, fluka humic acid, and chitin) (Bruchet *et al,* 1990)

Table 2.4 Pyrolysis fragment from a mixture of biopolymers (bovine serum albumin, cellulose acetate, fluka humic acid, and chitin) for the pyrochromatograms shown in Figure 2.2 (Bruchet *et al,* 1990)

Peak	Pyrolysis fragment	Biopolymer type*	Peak	Pyrolysis fragment	Biopolymer type*
1	Benzene	PH	18	Methypyrrole	Pr
2	Acetronitrile	Pr, As	19	Methylfurfural	Ps
3	Toluene	Pr	20	Acetophenone	
4	Pyridine	Pr	21	Furfuryl alcohol	Ps
5	2-Methylfuran	Ps	22	Acetamide	As
6	Methylpyridine	Pr	23	Methylnaphthalene	
7	Styrene	Pr	24	N-methylacetamide	As
8	Unknown polysaccharide fragment	Ps	25	Phenylacetronitrile	Pr
9	Methylpiridine	Pr	26	Levoglucosenone	Ps
10	Hydroxypropanone	Ps	27	Phenol	PH, Pr
11	2-Cyclopenten-1-one	Ps	28	Unknown chitin fragment	As
12	2-Cyclopenten-1-one-3-methyl	Ps	29	p-cresol	Pr, PH
13	Furfural	Ps	30	m-cresol	PH
14	Acetic acid	Ps, As	31	C2-Phenol	PH
15	2-Furaldehyde	Ps	32	Indole	Pr
16	2-Acetylfuran	Ps	33	Methylindole	Pr
17	Pyrrole	Pr			

Remark: *Pr = protein, Ps = polysaccharide, PH = polyhydroxy aromatic, As = amino sugar

Widrig *et al.* (1996) utilized pyrolysis GC/MS for monitoring the removal of algal-derived organic material by preozonation and coagulations. Although the use of ozone may not have improved the removal of DOC, in pyrolysis results, the chemical character of the organic matrix produced by algae was dramatically changed using preozonation. Biber *et al.* (1996) studied seasonal variation in principal groups of organic matter in a eutrophic lake using pyrolysis GC/MS. The main groups of biopolymers were classified into five groups including polysaccharides, proteins, lignins, amino sugars, and polyhydroxy aromatics. AWWA (1998) utilized identified chemical classes in water samples from the Ohio River, Mississippi River, Passaic River, Lake Gailland and groundwater in the USA. The pyrolysis fragments were classified into five categories: aromatic, aliphatic, nitrogen containing, halogen substituted and unknown compounds. A summary of this data is presented in Chapter V. Christy *et al.* (1999) characterized NOM from 9 different water sources in Norway. Reverse osmosis and evaporation techniques were utilized to concentrate the organic matter from each water source. The pyrolysis fragments were identified with one of fives biopolymer including carbohydrates, proteins, amino sugars, polyhydroxy aromatics, and others. The NOM separated by evaporation

preserved most of the organic material when compared with the reverse osmosis technique.

Cunha *et al.* (2000) used pyrolysis GC/MS to characterize riverine particulate organic matter. Particulate matter samples were collected in the mountainous section and river mouth at the Tech River basin in the south of France during floods (December 1996) and the summer (September 1997). Twenty-three identified pyrolysis fragments were classified into five main biopolymers: amino sugars, aromatic hydrocarbons, polysaccharides, phenols, and nitrogeneuous compounds. Neither significant spatial nor temporal changes in the particular organic matter composition were determined. Croue *et al.*, (2000) has summarized the methods used to characterize DOMs; for example, the use of chemical components such as amino acids and carbohydrates, molecular weight/size distribution, pyro-chromatogram and fluorescence spectrum. Thapa *et al.* (2002) characterized the natural organic matter in Lake Kasumigaura (a shallow eutrophic lake) in Japan. Distinctive characteristics of NOM near the mouth of the Sakura River could be better explained by the difference in pyrochromatograms rather than the general water quality. White *et al.* (2003) determined the nature of natural organic matter (NOM) contributing to DBPs in Alaskan water supplies using pyrolysis GC/MS. It was found that NOM contributing to DBPs were primarily phenolic compounds. Leenheer and Croue (2003) proposed the predominant pyrolysis by-products from aquatic NOM, as can be seen in Table 2.5. Komatsu *et al.* (2005) utilized the pyrolysis-GC/MS to characterize the complex position of DOM in a eutrophic lake in Japan. Their study revealed that 16 fragment compounds were detected. Some of them possibly originated from polyhydroxy aromatics (PHA), polysaccharides (Ps), proteins (Pr) and amino sugars (As)

Table 2.5 Specific pyrolysis fragments of biopolymers (Leenheer and Croue, 2003)

Type of Biopolymer	Common Pyrolysis Fragments
Polysaccharides	Methylfuran, furfural, acetylfuran, methylfurfural, levglucosenone, hydroxypropanone, cyclopentanone, methylcyclopentanone, acitic acid
Amino sugars	Acetamide, N-methylacetamide, propionamide, acetic acid
Proteins	Acetronitrile, benzonitrile, pyridine, methylpiradine, pyrrole, methylpyrrole, indole, methylindole (from tryptophan) toluene, styrene (from phenylalanine), phenol and *p*-cresol (from tyrosine)
Polyphenolic compounds	Phenol; *o-m-p*-cresol, methylphenol, dimethylphenol
Lignin	Methoxyphenols
Tannins	Catechol
DNA	Furfural alcohol
Polyhydroxybutyrates	Butenoic acid
(Source; Bruchet, 1985)	

To consider the application of pyrolysis GC/MS for coagulation-flocculation, Ritter *et al.* (1999) studied the removal of NOM in water from the Seine River, France, by coagulation-flocculation. The pyrochomatogram of water from the Seine River is depicted in Figure 2.4. Pyrolysis fragments were defined into four biopolymer normally found in natural water: polysaccharides (PS), proteins (PR), aminosugars (AS), and polyhydroxyaromatics (PHA).

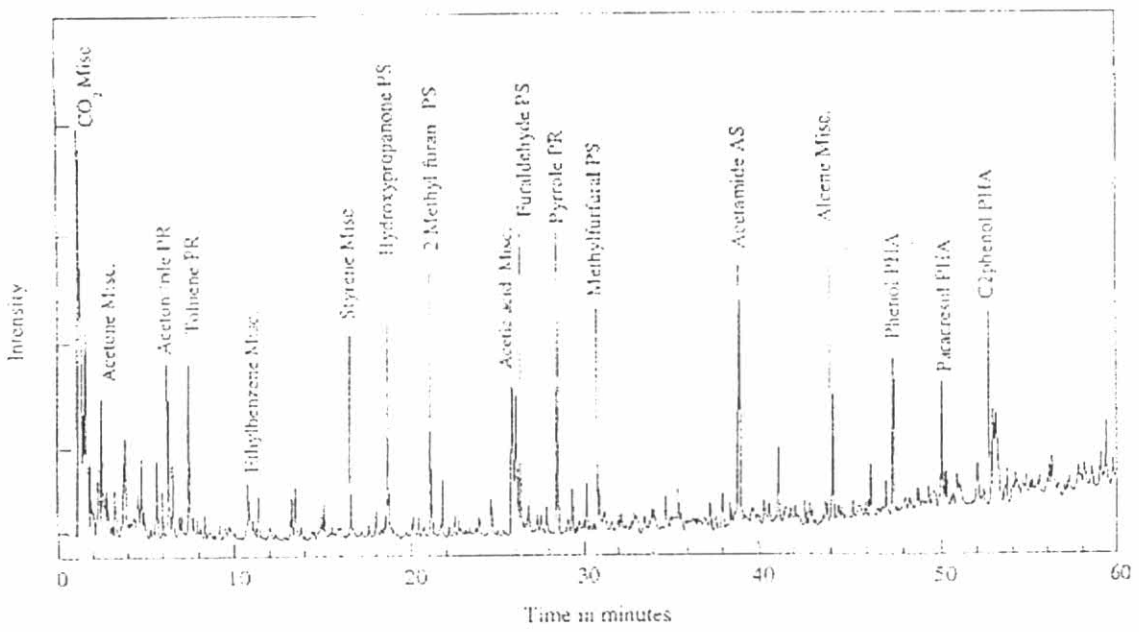


Figure 2.4 Pyrochromatograms of water from the Seine River, France (Ritter et al., 1999)

Page *et al.* (2003) studied the application of pyrolysis GC/MS for the characterization of DOM before and after alum treatment. The pyrolysis fragments were

defined into four basic groups: the polysaccharide-derived marker compounds, *N*-containing compounds, lignin-derived marker compound, and other compounds. Due to the vast quantity and range of pyrolysis products obtained, it was very difficult to quantify the pyrolysis products. In addition, the absolute integration of the peak area was unreliable since the weights of the samples and relative amounts of organic material in samples were different. The relative ratio of area between the fragments and one normalizing fragment, therefore, was utilized to accomplish a fingerprint of the pyrolysis data. Large amounts of alkylbenzenes, alkylphenols and polycyclic hydrocarbons were produced in all samples. The aromatic, producing methoxyphenols, which were possibly derived from lignin and tannin-like materials, were found as the predominant organic matter that flocculated with alum.

When pyrolysis GC/MS was used to measure the six DOM fractions present in the Suwannee and South Platte Rivers, in a study by the AWWA (2000), Phenol and *p*-cresol, which can be used to represent the presence of PHA type structure, were the dominant pyrolysis fragments of HPOA. This observation corresponded well with the studies of Croue *et al.* (1993b), Martin (1995), Harigton *et al.* (1996). The fatty acids (C_{12}, C_{16}) were found as the dominant species in HPON. In case of HPIA, phenol was the major fragment followed by acetic acids. For HPIB, the abundance and intensity of pyrolysis fragments such as acetronitrile, pyridine and alkylpyridine, pyrrole and alkylpyrrole were the representative of protienaceous organic structures. The abundance of acetamide also indicated the presence of amino sugars. In the case of HPIN, it included a large amount of amino sugar as indicated by the exceedingly large acetamide peak. Since a large acetamide peak was not found in the pyrochromatograms of the other fractions, this indicated that amino sugars were the major type of organic structure in HPIN (AWWA, 2000).

Upon review of past research, it can be concluded that the application of pyrolysis GC/MS on wastewater and treated wastewater has not been widely evaluated; particularly the use of pyrolysis GC/MS for characterizing the six DOM fractions of wastewater and treated wastewater. Dignac *et.al.* (2000) studied the fate of wastewater organic pollution during activated sludge treatment and in particular, the nature of residual organic matter.

The water and sludge samples were collected from the Compiegne treatment plant in France. Pyrolysis fragments were defined into five basic groups: the polysaccharides, proteins, lipids, polyphenolic compounds, and volatile fatty acids. The major pyrolysis fragments of the wastewater and treated wastewater were significantly similar. Some of the difficulties in identifying the organic matter of the treated water using common methods resulted from the presence of complex structures that were refractory to hydrolysis. These structures may have been recalcitrant to microbial degradation because they had concentrated during the biological treatment of the wastewater. Sirivedhin and Dray (2005) identified an anthropogenic marker in surface waters influenced by treated effluents as a tool in potable water reuse. The water samples were collected from four sampling sites: the South Platte River (SPR) above the Strinia Spring Reservoir, the SPR above Marcy Gulch, effluent from Bi-Cities wastewater treatment plants and the SPR at the Burlington Canal headgate (SPBC). Pyrolysis fragments were classified into one of the five chemical classes: halogen-substituted, nitrogen-containing, aromatic, aliphatic, and unknown. The percent of the total peak height was calculated for each category. This developing approach corresponded well with the approach developed by Bruchet *et al.* (1990), i.e. polysaccharide-aliphatic, nitrogen-containing-protein, and aromatic-polyhydroxyaromatic. Effluent-derived organic matter (EfOM) was found to be dominated by more aliphatic structure and had higher organic nitrogen and halogen content compared to organic material derived from natural sources. However, as mentioned earlier, no data is currently available with regard to the use of pyrolysis GC/MS for characterizing the six DOM fractions in wastewater and treated wastewater.

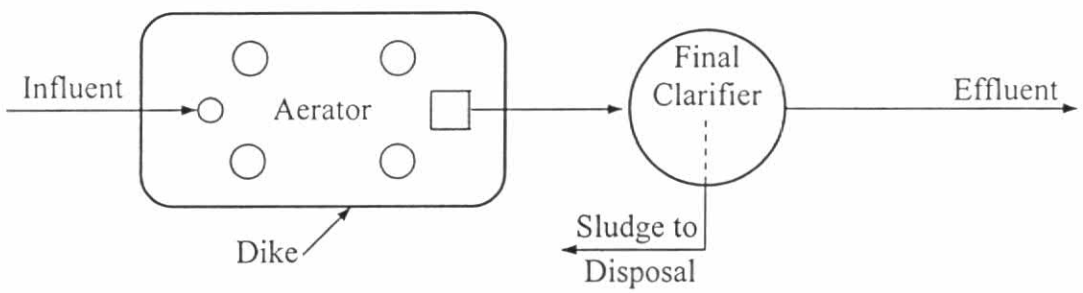
2.7 Aerated Lagoons and Stabilization Ponds

Aerated lagoons and waste stabilization ponds are commonly employed as efficient means of wastewater treatment that involves only slightly complicated technology and minimal regular maintenance. Their low capital and operation costs and ability to handle fluctuating organic and hydraulic loads result in their common use in rural regions and in many tropical countries where suitable land is available at reasonable cost (Nameche and Vasel, 1998). Since Thailand is located in a tropical zone with an abundance of sunlight, waste stabilization ponds is an appropriate method for the treatment of wastewater, especially in the provincial areas where land is available (Muttamara and Puetpaiboon,

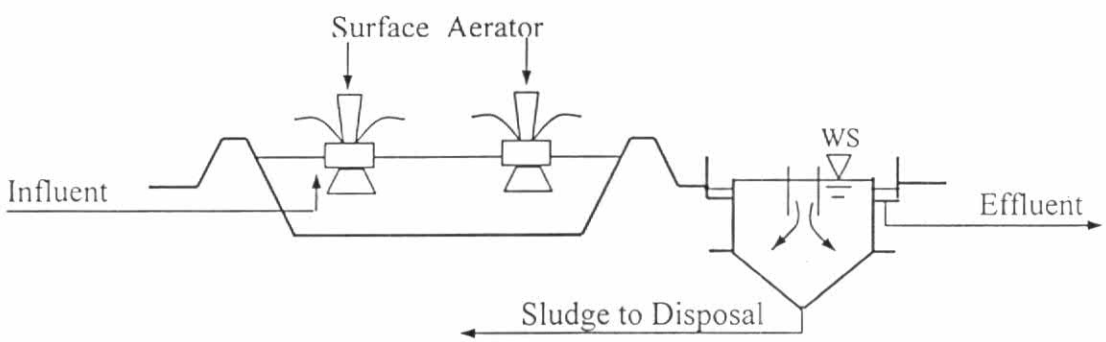
1996, 1997). Detailed information on the design and operation of aerated lagoons and stabilization ponds are presented in the following sections.

2.7.1 Aerated Lagoons

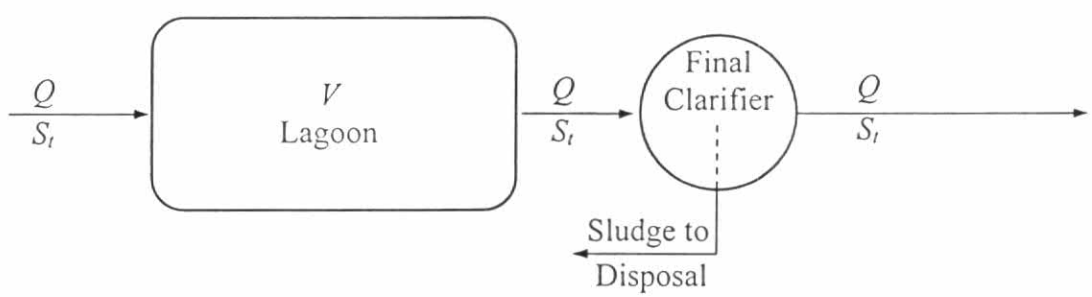
An aerated lagoon, as shown in Figure 2.5, consists of a diked pond with artificial aeration, usually by floating pump-type aerators, and a downline settling tank or facultative stabilization pond that serves as the final clarifier (Reynolds and Richards, 1996).



(a) Layout for an aerated lagoon system



(b) Profile through an aerated lagoon system



(c) Flowsheet of an aerated lagoon system

Figure 2.5 Aerated lagoon systems (Reynolds and Richards, 1996)

The biological solids developed in an aerated lagoon are removed from the effluent by the settling tank or stabilization pond. If a settling tank is used, it is usually a poured-in-place concrete clarifier with mechanical sludge rakes for continuous sludge removal. If a stabilization pond is used, the biological solid settles to the pond bottom and undergoes anaerobic decomposition. The pond is drained periodically, usually every two or three years, and the digested solids are removed and disposed of by sanitary landfill. The aerated lagoon processes are essentially non-recycle activated that usually has biological solids at a concentration of about 200 to 500 mg/L. Aerated lagoons are widely used in industrial wastewater treatment because they are less expensive than the activated sludge process. However, they have much greater land requirements, which must be considered. Frequently an industrial wastewater treatment facility is initially a stabilization pond system, and as the waste load increase, artificial aeration is added to convert some of the ponds into the aerated lagoons. Aerated lagoons are also used in these regions because the lagoon temperature is relatively high as a result of the climate and sunlight intensity (Reynolds and Richards, 1996).

Aerated lagoons may be classified as aerobic or facultative lagoons according to their dissolved oxygen profile. In aerated lagoons, the oxygen furnished is sufficient to maintain dissolved oxygen throughout the pond depth, and the mixing is sufficient to keep the biological solids in suspension. In an aerobic lagoon, final clarification is provided by a settling tank or a facultative stabilization pond. The facultative lagoon has dissolved oxygen in the upper depths of the lagoon; however, no dissolved organic matter is present in the lower depths. In the facultative lagoon, the mixing is insufficient to maintain all of the biological solids in suspension, and solid decomposition occurs on the lagoon bottom. These solids undergo anaerobic decomposition and are removed at infrequent intervals, such as every few years. The facultative lagoon usually has no final clarification except that which occurs in the aerated lagoon itself; consequently, the solid content in the effluent often precludes their use (Reynolds and Richards, 1996). In the following discussion, the fundamentals and application of aerobic lagoon are presented..

Aerated lagoons are widely used in the treatment of biodegradable organic industrial wastewater because they occupy less land area than stabilization ponds and have less construction and operational costs than activated sludge plants. However, they

have an appreciable land requirement when compared with activated sludge plants. The cost of an aerated lagoon is between that of a stabilization pond system and that of an activated sludge plant. The oxygen requirements for an aerobic lagoon are furnished by artificial aeration and, in particular, by mechanical surface aerators such as the floating pump-type. Occasionally, perforated pipes are laid on the lagoon bottom to disperse compressed air. Earthen dikes are used to form a lagoon and to exclude surface runoff. The aerated lagoon is essentially an activated sludge system without recycle; thus biochemical kinetics may be used for design formulations. Because the biological solids in lagoons do not vary appreciably, the pseudo first-order reaction representing the rate of removal is

$$-\frac{dS_t}{dt} = KS_t \tag{2.1}$$

Where

dS_t/dt = rate of substrate utilization, mass/(volume)(time)

K = reaction rate constant

S_t = substrate concentration at any time, mass/volume

(Reynolds and Richards, 1996)

A material balance on the substrate is given by

$$[\text{Accumulation}] = [\text{Input}] - \begin{bmatrix}\text{Decrease} \\ \text{due to reaction}\end{bmatrix} - [\text{Output}] \tag{2.2}$$

Assuming the system is completely mixed and using the designations as shown in Figure 2.5 give

$$V(dS_t) = QS_i d_t - V[dS_t]_{Growth} - QdS_t d_t \tag{2.3}$$

From Eq. (2.1) $[dS_t]_{Growth} = KS_t d_t$; thus substituting this expression in Eq. (2.3) gives

$$V(dS_t) = QS_i d_t - V\,KS_t d_t - QdS_t d_t \tag{2.4}$$

Dividing Eq. (2.4) by Vd_t yields

$$\frac{dS_t}{d_t} = \left(\frac{Q}{V}\right)S_i - KS_t - \left(\frac{Q}{V}\right)S_t \tag{2.5}$$

Since the accumulation term $(dS_t/d_t) = 0$ for steady state and $(V/Q) = \theta t$, Eq.(2.5) may be rearranged to give the following formulation (Eckenfelder, 1970; Eckenfelder and Ford, 1970):

$$\frac{S_t}{S_i} = \frac{1}{1 + K\theta_i} \tag{2.6}$$

which is the design equation for an aerated lagoon. Experimental studies can be performed using a completely mixed activated sludge unit without recycle to obtain the reaction rate constant, K. The unit should be operated at a detention time of several days until acclimation is attached and the substrate and biological solid concentrations in the effluent are measured. The flowrate is then increased, and once a steady state occurs, the substrate and biological solids in the effluent are again measured. Four or five increases in the flowrate should be made to obtain sufficient data for plotting. To evaluate the data, Eq. (2.6) may be rearranged to give

$$K = \frac{S_i - S_t}{S_t\theta_i} \tag{2.7}$$

Thus, plotting S_i-S_t on the y-axis, as in Figure 2.6 will give a straight line with a slope of K. To evaluate the data to determine the yield coefficient, Y, and the endogenous decay constant, K_e, as shown in Figure 2.7, the following equation developed by Reynolds and Yang (1966) as well as others researchers (Metcalf & Eddy, Inc., 1979; Wu and Kao, 1976), may be used:

$$\frac{S_i - S_t}{\overline{X}\theta_i} = \frac{k_e}{Y} + \left(\frac{1}{Y}\right)\frac{1}{\theta_c} \tag{2.8}$$

Where

$\overline{X}$ = average cell mass concentration during the reaction

θ_i = detention time based on the influent flow

Y = yield coefficient, mass of cells produced per mass of substrate used

K_e = endogenous decay constant, time

θ_c = mean cell residence time, time

The mean cell residence time, θ_c, is equal to the detention time for a nonrecycle reactor. A plot of $(S_i-S_t)/(X\theta_t)$ on the y-axis versus $1/\theta_c$ on the x-axis will be a straight line with a slope of $1/Y$ and a y-axis intercept of k_e/Y, as shown in Figure 2.7 (Reynolds and Richards, 1996).

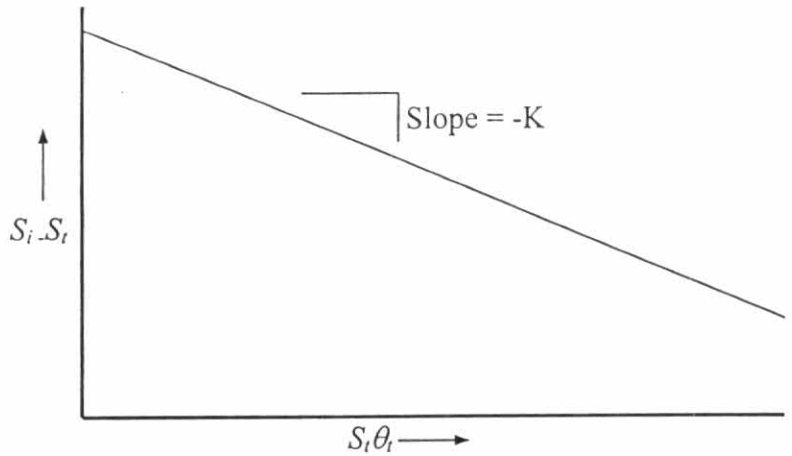


Figure 2.6 Graph for determining K (Reynolds and Richards, 1996)

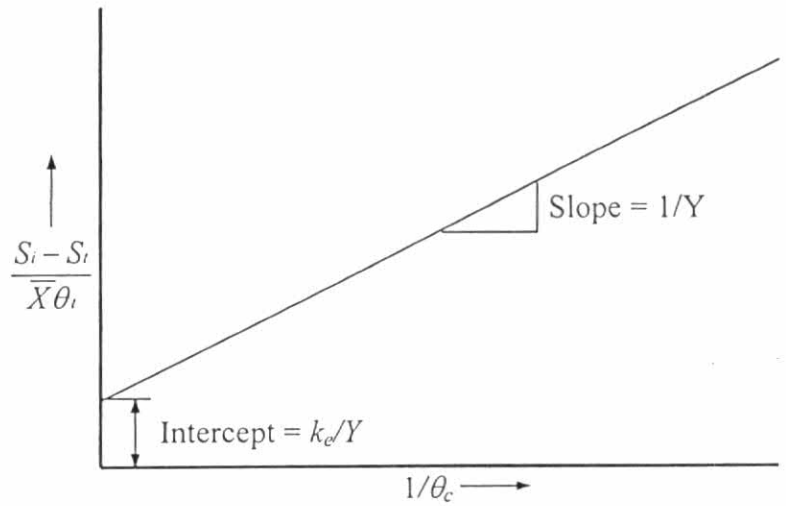


Figure 2.7 Graph for determining Y and k_e (Reynolds and Richards, 1996)

Since the biological solid concentration in an aerated lagoon is relatively low, the water temperature has a significant effect upon the lagoon's performance. The variation of the rate constant over the temperature range of 10 °C to 30 °C has been found to be represented by

$$K_{T_2} = K_{T_1}\theta^{(T_2-T_1)} \tag{2.9}$$

Where

K_{T2} = rate constant at temperature T_2, °C

K_{T1} = rate constant at temperature T_1, °C

θ = temperature correction coefficient, θ = 1.06 to 1.10 (Eckenfelder and Ford, 1970)

The operating temperature of a pond depends on the temperature of the influent wastewater; the heat loss by convection, radiation, and evaporation; and the heat gain by solar radiation. Mancini and Barnhart (1968) have found heat loss by evaporation and the heat gain by solar radiation to be relatively small. Their findings show that the temperature of a lagoon is given by

$$Ti - Tw = \frac{fA(T_w - T_a)}{Q} \tag{2.10}$$

Where

T_i = influent wastewater temperature, °F (°C)

T_w = temperature of water in the lagoon, °F (°C)

T_a = temperature of air, °F (°C)

A = lagoon area, ft^2 (m^2)

Q = influent wastewater flow, MGD (m^3/day)

f = experimental factor

For USCS units, they found the value of the factor f to be 12 x 10-6 for the central and eastern part of the United States and 20 x 10-6 for the Gulf States. For SI units, f is 0.489 for the central and eastern parts of the United States and 0.815 for the Gulf States. Equation (18.12) may also be used to obtain the temperature of stabilization ponds. The total oxygen requirements of the lagoon are given by the equation

$$O_r = Y' S_r + k'_e \overline{X} + O_n \tag{2.11}$$

where

O_r = total oxygen required, lb/day (kg/day)

Y' = oxygen coefficient, pound of oxygen required per pounds of BOD_5 or COD removed (kg/day)

S_r = substrate removed per day, pound of BOD_5 or COD removed (kg/kg)

k'_e = endogenous oxygen coefficient, pounds of O_2 required per pound of cells decayed-day (kg/kg-day)

$\overline{X}$ = mass of biological solids in lagoon, lb (kg)

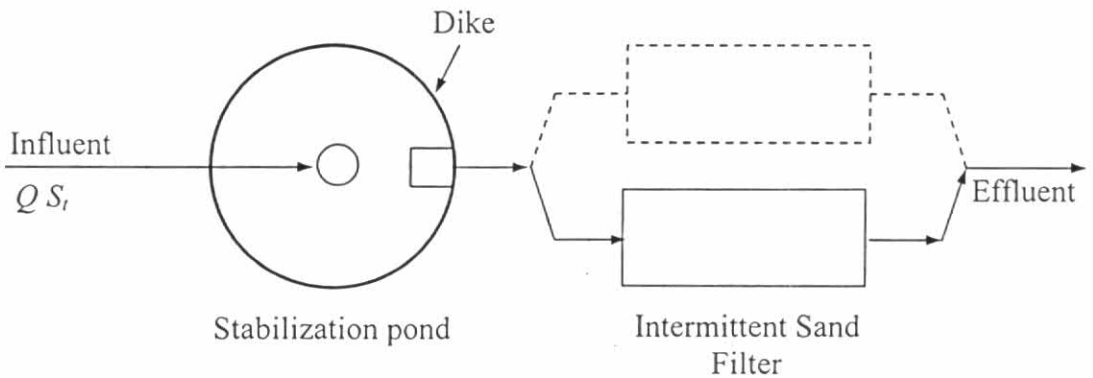
O_n = oxygen required for nitrification, lb (kg)

The term k'_e may be assumed to equal to 1.42 k_e, where k_e is the endogenous decay coefficient as $time^{-1}$. Frequently, the term $k'_e \overline{X}$ is very small compared to $Y' S_r$, and in such cases it may be neglected. The oxygen requirement should be as least 1.6ib oxygen per lb BOD_5 (kg/kg) applied to the lagoon. The power level supplied by artificial aeration should be at least 0.15hp/1000ft^3 (3.95 kW/1000m^3) to ensure the maintenance of the biological solids in suspension. Since aerated lagoon are completely mixed activated sludge system without recycle, The equation used to design the completely mixed activated sludge system also could be used to design the aerated lagoon (Reynolds and Richards, 1996).

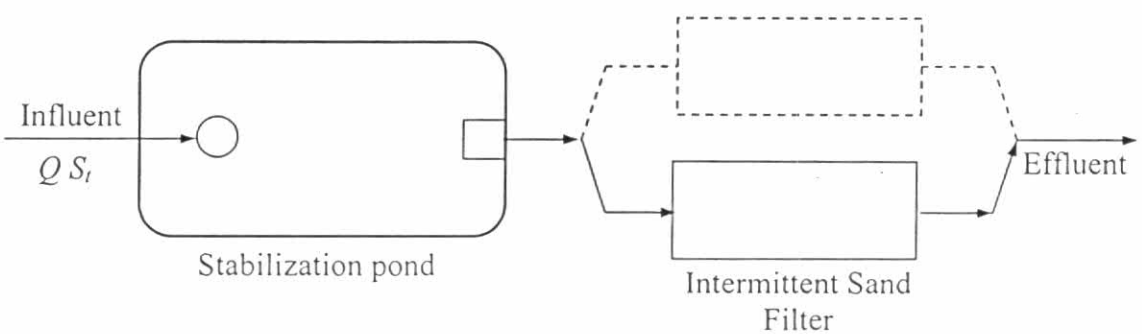
Aerated lagoons usually have a rectangular layout except where the terrain precludes the use of this geometry. When they are rectangular in layout, the length-to-width ratio is usually 2:1. At least two lagoons should be provided for flexibility, and piping should be arranged so that parallel or series operation is possible. Surface drainage must be excludes by dikes and ditching. The earthen dikes forming the lagoon usually have side slopes of 1:3 or less, and riprap or other suitable protection to avoid wave erosion. The outside slopes should be sodded to avoid erosion due to rainfall runoff. The lagoon and outlets should have a clay liner as a sealer if the soil is pervious. Inlets must distribute the influent outward into the lagoon, and outlets should be baffled to avoid floating matter from leaving the effluent. A freeboard of at least 3ft (0.91m) should be provided. Concrete pads must be furnished under each aerator to protect the bottom from erosion. Final clarifiers or stabilization ponds should be provided to remove biological solids from the final effluent. Lagoon depths are from 8 to 16ft (2.43 to 4.88m) (Reynolds and Richards, 1996).

2.7.2 Stabilization Ponds

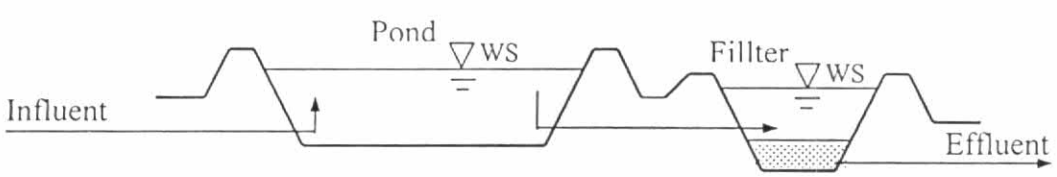
A stabilization or oxidation pond, shown in Figure 2.8, consists of a quiescent diked pond in which the wastewater enters and, as a results of microbial action, the organic material is bio-oxidized, giving CO_2; NH_3, in organic radicals such as SO_4^{-2} and PO_4^{-3}; and new microbial cells as end products. The algae population uses the CO_2, inorganic radicals, and sunlight to produce dissolved oxygen and new algal as end products. Thus the microbial and algal populations have a synergistic relationship in which both groups benefit from each other (Reynolds and Richards, 1996).



(a) Layout for a circular pond and filter



(b) Layout for a rectangular pond and filter



(c) Profile of stabilization pond and intermittent sand filter

Figure 2.8 Stabilization pond systems (Reynolds and Richards, 1996)

Although most stabilization ponds have effluents discharging directly into the receiving body of water, the future trend in design is to use polishing treatments such as intermittent sand beds to remove algal growths from the effluent, thus giving a better degree of treatment. Stabilization ponds are used for both municipal and industrial wastewater treatment, particularly for small municipalities and seasonal industrial wastewater. Stabilization ponds are widely used in the southern and southwestern United States because the climate is warm and there is high sunlight intensity. Stabilization ponds may be classified as aerobic, facultative, or anaerobic according to their oxygen profile. An aerobic pond has no dissolved oxygen in the upper zone of water and no dissolved oxygen in the lower zone. Most ponds are facultative since these are loads higher than aerobic ponds, yet few odors are produced because the upper pond depth is aerobic (Reynolds and Richards, 1996).

The amount of oxygen present in a facultative pond depends on the organic loading and sunlight intensity. There will be a diurnal variation in the dissolved oxygen concentration in the upper zone, and the variation will be greater during the summer. During the night, the dissolved oxygen will be low because the microbial population and some of algal population use dissolved oxygen. After the sun rises, photosynthesis starts to occur and the dissolved oxygen, which is an algal end product, begins to increase, with the maximum concentration occurring during the middle of the day. Shortly after sunset, photosynthesis ceases, dissolved oxygen production stops, and the dissolved oxygen concentration decreases because of the oxygen demand of the microbial and algal population. In the summer in the southern and southwestern United States, the dissolved oxygen may vary from 2 to 3 mg/L at night to above 14 to 16 mg/L during the day. Even for the facultative ponds, anaerobic conditions may occur throughout the pond depth during overcast days. Odors, however, are usually not a problem unless there are windy conditions that cause waves on the surface. (Reynolds and Richards, 1996)

As the algal and microbial cells die, they lyse and the cell fragments settle to the bottom of the pond, where they undergo anaerobic decomposition. For the usual facultative pond loadings, the sludge accumulation is very slow and may be only a fraction of an inch (several millimeters) per year. Approximately one-third to one-half the influent organic carbon in the raw wastewater is synthesized into microbial and algal

cells that live within the pond effluent unless an effluent polishing treatment is used. Many times in the past, the BOD_5 reported for an effluent was for a filtered effluent sample, which was misleading because the algal and microbial growths exerted an oxygen demand in the receiving body of water. The BOD_5 removal based on filtered effluent samples will be from 80% to 90%, whereas the BOD_5 removal for unfiltered samples will be from about 45% to 65%. Coliform removal on an unfiltered sample is usually from 85% to 95% (Reynolds and Richards, 1996).

Facultative ponds may range from 3 to 8 feet (0.91 to 2.44 m) deep, with the usual water depth being from 3 to 6 feet (0.91 to 1.83 m). When future conversion to an aerobic lagoon system is anticipated, the pond should be deep to accommodate surface aerators. Ponds are usually circular or rectangular in plain view, as shown in Figure 18.1 (a) and (b), unless the terrain necessitates some other geometry. Usually, two or more ponds are provided and the piping is arranged so that series or parallel operation is possible. For single ponds or ponds operated in parallel when treating municipal wastewater, an organic loading of 25 to 35 lb BOD_5/ac-day (28.0 to 39.2 kg BOD_5/ha-day) is frequently used. For series operation when treating municipal wastewaters, the first pond is usually loaded as high as 75 to 80 lbBOD_5/ac-day (84 to 89.7 kg BOD_5/ha-day), and a downstream pond receives a loading of 25 to 35 lbBOD_5/ac-day (28.0 to 39.2 kg BOD_5/ha-day) (Reynolds and Richards, 1996). Gloyna (1976) recommends the following equation for determining the volume of a facultative stabilization pond treating municipal wastewater:

$$V = CQS_i[\theta^{(35-T)}]ff' \qquad (2.12)$$

Where

V = pond volume, ft^3 (m^3)

$C = 4.7 \times 10^{-3}$ for USCS units and 3.5×10^{-5} for SI units

Q = flow, gal/day (L/day)

S_i = ultimate influent BOD or COD, mg/L.

f = algal toxicity factor; $f = 1$ for municipal wastewater and many industrial wastewaters

f = sulfide or other immediate chemical oxygen demand; f' = 1 for SO4-2 equivalent ion concentration of less than 500 mg/L

θ = temperature coefficient

T = average water temperature for the pond during winter months, °C

The value of θ ranges from 1.036 to 1.085. The value of 1.805 is recommended because it is conservative and field data support this analysis (Gloyna, 1976). For the case where θ = 1.085 and the effective depth = 5 ft (1.52 m), the area of the pond may be computed using Eq. (2.12). The area can be found as follows:

$$A = CQS_i[1.085^{(35-T)}]ff' \tag{2.13}$$

Where

A = area of pond, acres (ha), for depth of 5 ft + 1 ft of sludge storage (1.5 m + 0.3 m)

$C = 2.148 \times 10^{-2}$ for USCS units and 2.299×10^{-3} for SI units

Q = flow, MGD (MLD)

The BOD_5 removal efficiency can be expected to be 80% to 90% based on unfiltered influent samples and filtered effluent samples. The efficiency based on unfiltered effluent samples can be expressed to vary unless a maturation pond is used as a followup unit. The recommended minimum depth of a facultative pond is 3.28 ft (1 m). Additional depth to compensate for sludge storage is desirable. The minimum depth of about 3.28 ft (1 m) is required to control the potential growth of emergent vegetation. If the depth is too great, there will be inadequate surface area to support photosynthetic action; also deep ponds tend to stratify during hot periods. The suggested design guidelines for depth are shown in Table 2.6 (Reynolds and Richards, 1996).

For wastewaters containing considerable amounts of biodegradable settleable solids, Gloyna (1996) recommends that an anaerobic pond precede the facultative pond. The high temperature coefficient, 1.085, indicates that pond performance is very sensitive to temperature changes. To determine the design pond loading for industrial wastewater, for which no field data exist, Gloya (1968) and Eckenfelder and Ford (1970) developed laboratory procedures using bench-scale stabilization ponds.

Table 2.6 Design guidelines for the depth of stabilization ponds (Reynolds and Richards, 1996).

Case	Depth	Related conditions
1	3.28ft (1m)	Generally ideal condition, very uniform temperature, tropical to subtropical, minimum settleable solids
2	4.10ft (1.25m)	Same as Case 1 but with modest amounts of settleable solid. Surface design based on 3.28-ft depth and 0.82ft used for reserve volume (1m and 0.25m)
3	4.29ft (1.5m)	Same as Case 2 except for significant seasonal variation in temperature and major fluctuations in daily flow. Surface design based on 3.28-ft (1-m) depth
4	6.56ft and greater (2m and greater)	For soluble wastewaters that are slowly biodegradable and retention is controlling

Ponds should be located so that the prevailing winds passing over the ponds are not directed toward populated areas where possible odors would create a problem. The pond bottom and sides should be relatively impervious, and if the soil is porous, a clay liner should be used. The inlet to the pond should be designed so that the influent is distributed outward into the pond, and the outlet structures should be baffled to prevent floating materials from leaving with effluent. The earthen dikes usually have side slopes of 1:3 or less, and the inside slope is protected against wave erosion by riprap. The outside slopes should be sodded to prevent erosion due to surface runoff. Surface drainage must be excluded by dikes or ditching. At least 3 ft (0.91 m) of freeboard should be provided above the maximum water surface, and the ponds should be designed so that the water level can be varied by 6 in. (150mm) to assist in mosquito control (Reynolds and Richards, 1996).

There have been some researchers who have succeeded in improving the performance capability of stabilization ponds for removing contaminants. Muttamara and Puetpaiboon (1996 and 1997) modified the waste stabilization ponds to improve its performance or to reduce the land area requirement by the addition of baffles to waste stabilization ponds. These baffles provide additional submerged surface area to which microorganisms could attach themselves, thus increasing the concentration of microorganism in the ponds and, theoretically, the rate of organic stabilization as well.

The baffles should also affect the hydraulic flow pattern of the system and could reduce short circuiting. Total nitrogen (TN) and NH_3-Nitrogen (NH_3-N) removal increased with the number of baffles in the pond units. In a 6-baffled pond unit, more than 65% TN and 90% NH_3-N removal were achieved at a hydraulic detention time of 5 days. In terms of COD removal, the baffled wastes stabilization pond units with 6 baffles seem to reach their maximum removal efficiency. Green *et.al* (1996) proposed the Advanced Integrated Wastewater Pond Systems for permanently removing significant amounts of nitrogen from wastewater streams.

The reuse of treated wastewater from waste stabilization ponds is increasingly becoming more attractive. In South Africa for example, wastewater reuse is a limited practice, but when allowed, it should generally be on the condition of some form of treatment that achieved a certain standard (Department of Water Affairs, 1986). Mara (1996) stated that the reuse of treated wastewater is becoming increasing more common in arid and semi-arid regions. However, waste stabilization ponds and other treatment processes have the disadvantage of only contributing effluent for irrigation during the irrigation season. During other times of the year treated wastewater usually is discharged to surface waters. Storage is more sensible as its permits a much greater area of land to be irrigated during the irrigation season. Aquifer recharge is one way to store effluent; however, the treatment process must produce an effluent of very low suspended solids.