CHAPTER 2
LITERATURE REVIEW

2.1 Assimilable Organic Carbon (AOC)
2.1.1 AOC Methods

AOC is the fraction of biodegradable organic carbon that can be used by microorganisms to increase cell mass and is expressed as a carbon concentration using a calibration yield factor (APHA, 1998). This biomass-based method first proposed by van der Kooij et al. (1982) was developed to investigate the bacterial regrowth potential in finished water. In this original method, *Pseudomonas fluorescence* strain P17 is solely applied as a surrogate microorganism since this strain is commonly found in drinking water, surface water and groundwater. In addition, this strain is capable of using a variety of substrates at very low concentrations.

The AOC procedure began with collecting water samples in organic-free glasswares. Then, the water samples were pasteurized (heated at 60°C for 30 minutes) in order to kill indigenous microorganisms. After cooling, samples were inoculated with 50 to 500 CFU/mL of P17 inoculum before incubated at 15 ± 5°C under darkness. Determination of cell densities were periodically performed by plate counts until cell densities had reached a maximum value (stationary phase, N_{max}). Incubation time was dependent upon AOC concentration. Low AOC concentrations would require long incubation time and the procedure may take up to 25 days for the cell density to reach N_{max}. To obtain an AOC value (µg acetate C/L), the N_{max} value was converted using a growth yield which is obtained from the slope of a plot between N_{max} values and acetate carbon concentrations in the standard samples.

van der Kooij et al. (1982) reported that using AOC-P17 bioassay could assess concentration of a wide range of biodegradable compounds including amino acids, carboxylic acids, alcohols, and carbohydrates (polysaccharides excluded). With the exception that strain P17 could not grow on some ozonation byproducts such as formic, glyoxylic, and oxalic acids, van der Kooij and Hijnen (1984) modified the original method by using *Spirillum* strain NOX as an additional surrogate microorganism. In this AOC method, NOX strain was inoculated after cell mass of P17 strain had reached N_{max}.

Incubation time of NOX strain was reported ranging from 5 to 10 days. Therefore, the overall incubation time might take up to more than 30 days. The growth yield of NOX strain can be obtained from the standard calibration curve of oxalate or acetate added versus N_{max}. Hence, AOC_{NOX} is expressed in terms of μg oxalate or acetate C/L. van der Kooij and Hijnen (1984) pointed out that the addition of the AOC-NOX bioassay to the original-test version should increase the accuracy in quantifying readily usable carbon for bacterial growth in water.

Jago-Stanfield (1987) introduced the use of an ATP technique to quantify the increase of biomass. This AOC method used a mixed culture of indigenous microorganisms, which was removed from water samples by filtration, as an inoculum. After the sample was inoculated, it was placed in a luminometer for ATP measurement until a maximum ATP value was reached, approximately 3 to 5 days. ATP concentration was thus converted to an AOC value by means of a standard conversion factor, which is determined from the slope of a calibration curve derived from plotting ATP values versus acetate carbon concentrations in the standard sample.

Kaplan and Bott (1988) attempted to simplify the method of van der Kooij and Hijnen (1984) by using an inoculum that is in a log-growth phase. Enumeration of this method was conducted by microscopic counts. The results showed that a log-phase culture did not make the test more rapid; on the other hand, it complicated the handling procedure. The use of microscopic counts made the test slower and more complex. Water sterilization (heated at 121 °C for 15 minute) was used since water pasteurization did not destroy indigenous bacteria spores. It was found that sterilization could change original characteristic of water, thereby contributing to the change in AOC. They also recommended that the step of pasteurization be maintained since the interference from pasteurized bacteria spores was observed to be negligible.

Kemmy et al. (1989) proposed an AOC method using a mixture inoculum, which was comprised of four-defined bacteria; namely, *P. fluorescens*, *Curtobacterium sp.*, *Corynebacterium sp.*, and *Coryneform*. This mixed culture shorten the incubation time to around 6 days. The incubation temperature was at 20°C. Enumeration of the bacteria was carried out by plate counts. This method did not yield consistent AOC values. Occasionally, AOC values were higher than DOC values.

In 1992, van der Kooij modified the two-strain bioassay of van der Kooij and Hijnen, (1984) by inoculating P17 and NOX simultaneously. After the water samples

had been pasteurized, it is inoculated with 100 to 300 CFU/mL of each inoculum. The distinctive difference of their colony forming units allows enumeration by plate counts to be performed at the same time. In this method, AOC_{NOX} and AOC_{P17} are expressed in the unit of μg acetate C/L. AOC_{total} is a sum of AOC_{NOX} with AOC_{P17}. LeChevallier et al. (1993) followed the method of van der Kooij (1992) but using ATP technique for enumeration. The incubation temperature of 20 to 23°C reduced incubation time to around 1 to 3 days. It was observed that AOC concentrations assessed by the ATP technique were consistent with those quantified by spread plate technique although sometimes the ATP technique tended to underestimate AOC concentration. Moreover, the results obtained by using combined inoculation were not significantly different from those given by separate inoculation.

APHA (1998) approved an AOC standard method for determining the regrowth potential in water. In general, this approved method is similar to the method of van der Kooij (1992), which applied P17 and NOX strains as surrogate organisms. This method requires chlorinated water samples be dechlorinated and filtered through a 0.2-μm filter prior to performing the assay since disinfectant residual and turbidity could have interference with AOC results. Inoculations of P17 and NOX were simultaneous at 500 CFU/mL of each. It takes around 7 to 9 days for cell densities to achieve N_{max}. Enumeration of cell densities is carried out by plate counts. To determine AOC_{NOX} values, this method allows the use of oxalate or acetate yield factors. However, when the oxalate yield is used to convert N_{max} for AOC_{NOX} values, this use should be noted along with the value of AOC_{total} since AOC_{P17} and AOC_{NOX} would have a different carbon-based yield.

2.1.2 AOC Applications

AOC method has been widely used in Western Europe since the first method (van der Kooij et al., 1982) was developed. AOC measurement started to gain popularity in the United State in the 1996 (Nitisoravut et al., 1997). The applications of these biomass methods can broadly be classified into two areas: 1) prediction of bacterial growth in the distribution systems, and 2) evaluation of the performance of treatment processes. Nevertheless, it should be noted that AOC tests are suitable for water containing low-level organic contents.

1) Prediction of bacterial growth in the distribution systems: In 1982, van der Kooij et al. applied the P17 bioassay to determine biological stabilities of several raw water sources. The results revealed that groundwater samples had higher AOC than surface water samples. Moreover, van der Kooij et al. (1982) pointed out that pipelines made of poly vinyl chloride promoted biofilm formation during prolonged contact time. LeChevallier et al. (1987) applied the method of van der Kooij et al. (1982) to determine AOC of water in a distribution system that has long been plagued with taste and odor problems. Results showed that AOC value in water decreased along the distance from the treatment plant. *E. coli* inoculated in water samples obtained from the distribution system showed limited growth when AOC concentration was about 85 to 130 μg C/L and no growths when AOC concentration was 54 μg C/L.

van der Kooij (1992) indicated that AOC increased with the travel time in the distribution system. This result is in contrast with that reported by LeChevallier et al. (1987) and can be explained by two reasons. First, van der Kooij (1992) used the two-strain bioassay (P17 and NOX), whereas LeChevallier et al. (1987) applied the one-strain bioassay (P17). Therefore, AOC data from LeChevallier et al. (1987) did not cover AOC_{NOX}, which was the major portion as observed from data of van der Kooij (1992). Second, for the investigation of LeChevallier et al. (1987), water in mains was in a poor biological condition. Therefore, the decrease of AOC was attributed to the use of available substrates by indigenous microorganisms along the distribution system. To prevent bacterial regrowth, van der Kooij (1992) suggested that finished water is required to have AOC of 10 μg C/L or less.

White and LeChevallier (1993) examined the effect of using oil-lubricated well pumps on biological quality of finished water. The results indicated that normal operation oil-lubricated well pumps provided opportunities to leach significant amount of AOC in distribution systems. Yeh et al. (1998) related the effects of chlorine residuals to microbial regrowth problems. When AOC concentrations were between 30 and 70 µg C/L and chlorine residual was maintained at 1 mg/L, the regrowth problems were not significant in pipelines. On the contrary, the regrowth occurred when AOC concentrations were higher than 110 µg C/L although water was provided with 1 mg/L of chlorine residual. These results indicated that solely applying chlorination was not sufficient to repress microbial growth in distribution systems.

2) <u>Evaluation of the performance of treatment processes</u>: Kemmy et al. (1989) assessed AOC removal performance of conventional processes: coagulation, sedimentation, and rapid sand filtration. The result showed that a major AOC decrease was observed after sedimentation while rapid sand filtration offered only a small further decrease of AOC. LeChevallier et al. (1990) reported that the addition of powder activated carbon in a sludge blanket reactor contributed to high AOC reduction. Noble et al. (1996) compared biological stabilities in effluent water from three different types of filtration; dual media, granular activated carbon, and membrane, which were used for treating groundwater. AOC could not be removed by membrane filtration especially AOC_{P17}. Between the two conventional processes, dual media filtration showed more satisfactory results in removing AOC.

Uhl et al. (1996) applied an AOC method to evaluate a two-step process for removing organic in surface water. The process consisted of biofiltration and GAC filtration, respectively. AOC was significantly reduced after biofiltration, while longer service time of GAC was obtained. Trace organics (detected in terms of AOC) were almost all removed during biofiltration and the remaining was subsequently eliminated by GAC filtration. Major AOC removal by biofiltration was also observed in the study of Nitisoravut et al. (1997). It was noted in their study that supporting media made of inorganic material could contribute to more AOC removal than those made of organic material. In addition, an AOC threshold of 30 µg C/L would be more appropriate than 10 µg C/L as proposed by van der Kooij (1992) because of the sensitivity of AOC determination.

AOC was employed as an indicator to assess nanofiltering performance in restricting bacterial regrowth potential (Escobar et al., 1999). DOC and BDOC could significantly be removed by nanofiltration. However, the AOC fraction could still pass through nanofiltration. Escobar et al. (1999) concluded that AOC consists of organics, which have very small molecular sizes and could not be retained by nanofiltration. Volk et al. (2000) used AOC as an indicator to assess BOM removal by enhanced coagulation. It was found that using poly aluminum chloride as a coagulant could result in high removal of refractory DOC (> 40%) and BDOC (> 30%) in all samples. However, most of the results showed that the AOC fraction could not efficiently be removed by this enhanced coagulation (AOC removal < 10%).

Hem et al. (2001) examined the effectiveness of using membrane filtration to treat water containing a wide array of humic substances. This study employed the two-strain AOC bioassay to identify biological characteristic of the effluent. The result showed that nanofiltration greatly removed around 80 to 90 % of color, while more than 50% of AOC still remained after the treatment.

2.2 Advance Oxidation Processes (AOPs) that Have Been Reported to Affect AOC

2.2.1 Ozonation

Ozonation has been reported to increase biodegradability (BOD_5/COD and BDOC/DOC) and/or BOD_5 and BDOC in water (Gilbert, 1988; Servais et al. 1989; and Chamapro et al. 2001). The effect of ozonation on AOC was first observed by van der Kooij et al. (1982). Ozonation substantially increases AOC, which is removed by biofiltration, a process normally employed after ozonation. Similar result was also reported by Jansens et al. (1984); AOC_{P17} increased as a function of ozone dosage. van der Kooij and Hijnen (1984) reported that ozonation provided higher increase of AOC_{NOX} than that of AOC_{P17}. This suggests that ozonation by-products are more usable substrates for strain NOX than for strain P17.

Huck et al. (1991) reported that ozone doses of 0.5 and 1.0 mg/L mainly promoted the increase of AOC_{NOX}. For AOC_{P17}, the increase was not always a result. Occasional decreases of AOC_{P17} may be attributed to the occurrence of unfavorable substrates for strain P_{17}. Preozone dosage of 2.5 mg/L provided an average AOC

increase of 2.3 fold (LeChevallier et al., 1993). The increase of AOC_{NOX} was higher than AOC_{P17}. More than 80% of the increased AOC was reduced by GAC filtration particularly with prolonged contact time.

Bradford et al. (1993) used ozonation to treat highly colored groundwater and surface water. After ozonation, AOC of groundwater was much higher than that of surface water. High color in groundwater was much reduced along with an AOC increase. For both water types, increasing ozone dosage resulted in higher production of AOC. When ozone dosage of 1 mg/L was applied, the increase of AOC_{NOX} was higher than the AOC_{P17} increase. However, when the applied dosage was changed to 7 mg/L, AOC_{P17} concentration was higher than AOC_{NOX} concentration.

Price et al. (1993) investigated AOC production induced by various ozone doses. Higher doses of ozone produced higher AOC concentrations. However, ozone doses greater than 2 mg/L provided small increases of AOC production. The most effective ozone dosage for significant AOC_{NOX} enhancement was 1 mg/L. When higher ozone dosage was applied, AOC_{P17} appeared to increase more than AOC_{NOX}. Lykins et al. (1994) compared AOC concentrations resulting from ozonation and chlorination. Ozone dosage of 2.5 mg/L with the contact time of 30 minutes produced an average AOC of 166 µg C/L. This level was reduced to 39 µg C/L by sand filtration. However, when the chlorine dosage of 1.8 mg/L was provided in place of ozonation, AOC production reduced to 5 µg C/L after the contact time of 30 minutes. Lykins et al. (1994) suggested that if ozonation is applied in water treatment, subsequent filtrations are required to provide biostability to the finished water.

Vahala et al. (1998) found that the maximum AOC increase was at an ozone dosage of 0.4 to 0.5 mg/mg TOC. With this ozone dosage, AOC_{P17} increased up to 7 folds, whereas more than 10 fold increases in AOC_{NOX} were achieved. This AOC increase was significantly removed through the subsequent GAC biofilter. In comparing with another treatment train without ozonation, it was found that the effluent from the biofiler following ozonation had lower AOC than that from the biofilter without preozonation.

Escobar et al. (2001) investigated biological quality of water in distribution system before and after applying ozonation. This study was conducted at the Pine Hills Water Treatment Plant located in Orlando, Florida, USA. Raw water was withdrawn from high-quality aquifer (DOC of 1.10 mg/L). The original treatment process comprised only of chlorination before distribution. After ozonation was

introduced to this treatment process, around 100% AOC increase was observed in the distribution system. This AOC increase correlated with a significant increase of bacteria counts. However, chlorine dosage of 2 mg/L showed high efficiency in repressing bacterial regrowth before and after switching to ozonation. Based on these results, they concluded that bacteria proliferation could possibly occur if the chlorine residual was not maintained at that level.

2.2.2 Other AOPs

Besides ozonation, only peroxone (ozonation + hydrogen peroxide) was reported for their effects on AOC production. Hacker et al. (1994) compared the effects of peroxone and ozone on AOC production in surface water. They found that the maximum increase of AOC was obtained from the H_2O_2/O_3 ratio of 0.5. At this ratio, AOC generation was approximately 100% of that obtained from ozonation alone. Out of the total AOC increase, 60% was from AOC_{NOX}, whereas 40% was contributed by AOC_{P17}. To reduce this high AOC product, biofilters were used and able to reduce AOC up to the level equal or lower than that of the influent.
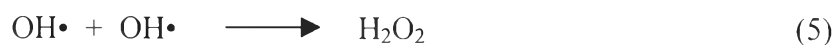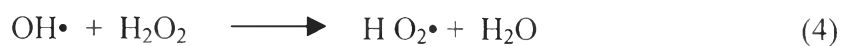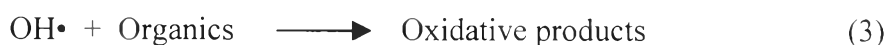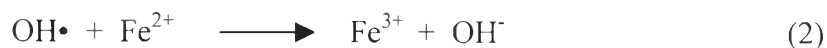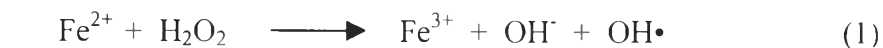
2.3 Fenton's Reagent
2.3.1 Principle

The use of hydrogen peroxide combined with an iron salt, commonly known as Fenton's reagent, was first observed by Fenton in 1894. During the early time, Fenton's reagent was recognized as an odor control agent (Lu et al., 1999). Principally, the reaction between hydrogen peroxide and an iron species, more powerful if ferrous ion, will generate hydroxyl radicals (OH•), which are capable of oxidizing non-selective organic matter. The intensity as well as the rate of oxidation is generally affected by concentrations of hydrogen peroxide and iron catalyst, pH, and organic content (Beltran et al., 1998 and Chamapro et al., 2001).

When Fenton's reagent is used to degrade organics, a series of reactions occurs.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH\bullet \qquad (1)$$

$$OH\bullet + Fe^{2+} \longrightarrow Fe^{3+} + OH^- \qquad (2)$$

$$OH\bullet + Organics \longrightarrow Oxidative\ products \qquad (3)$$

$$OH\bullet + H_2O_2 \longrightarrow HO_2\bullet + H_2O \qquad (4)$$

$$OH\bullet + OH\bullet \longrightarrow H_2O_2 \qquad (5)$$

$$Fe^{3+} + H_2O_2 \longrightarrow FeOOH^{2+} + H^+ \qquad (6)$$

$$FeOOH^{2+} \longrightarrow HO_2\bullet + Fe^{2+} \qquad (7)$$

(Chamapro et al., 2001; Lu et al., 1999; Beltran et al., 1998; and Flaherty and Huang, 1992)

$OH\bullet$ is generated through the reaction between Fe^{2+} and H_2O_2 as shown in eq. (1). In addition to oxidizing organics to produce oxidative products (eq.3), $OH\bullet$ oxidizes Fe^{2+} to give ferric ions (Fe^{3+}) as shown in eq. (2). Fe^{3+} can be reduced to reform Fe^{2+} via eq. (6) and (7). However, the Fe^{2+} regeneration rate is much slower than its consumption rate. Therefore, when the amount of Fe^{2+} is used up, Fenton's oxidation will depend on the Fe^{2+} regeneration rate. In eq. (4) and (5), if $OH\bullet$ is produced in excess, it can react with H_2O_2 to generate peroxyl radical ($HO_2\bullet$) or may combine another $OH\bullet$ to form H_2O_2.

Several researchers stated that acidic condition; pH between 2 and 5, is preferable for Fenton's reaction (Lunar et al., 2000; Lin et al., 2000; and Lu et al., 1999). Besides pH, peroxide and ferrous doses are also important since they affect the magnitude of $OH\bullet$ production and rate of reaction (Chamapro et al, 2001).

2.3.2 Applications

Since Fenton's reagent has powerful oxidizing capabilities, its utilities have usually been found in the area of wastewater treatment. Hölf et al. (1997) compared the efficiencies of H_2O_2/Fe^{2+}, O_3/UV, H_2O_2/UV, and O_3 to treat pharmaceutical wastewater, which contained high content of adsorbable organic halide (AOX = 3 mg/L). Fe^{2+}/H_2O_2 showed the highest efficiency, requiring less than 10 minutes to completely remove AOX, whereas the other AOPs required longer than 60 minutes of reaction time to obtain the same result. Beltran et al. (1998) used Fenton's reagent to treat fluorene, phenanthrene, and acenaphthene in water. They found that the removal of those aromatic hydrocarbons (PAHs) correlated with the increase of H_2O_2 concentration. Nevertheless, the removal was limited to a certain level of H_2O_2 concentration. It was because excessive H_2O_2 added reacted with OH• thereby forming water. The increase of Fe^{2+} dose resulted in faster rate of the PAH removal as well as higher removal. The highest removal occurred under neutral pH. This disagrees with most Fenton's degradation studies, which reported optimal pH of 3 to 5.

Lu et al. (1999) used Fenton's reagent to degrade an organic insecticide. pH 3 to 4 was the most preferable range. pH higher than this range could contribute to precipitation of Fe^{2+} in the form of $Fe(OH)_3$. The degree of pesticide removal varied proportionately with H_2O_2 concentration. The role of Fe^{2+} was to accelerate the degradation rate particularly in the first 5 minutes. Benitez et al. (2000) applied Fenton's reagent to oxidize 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) in aqueous solutions. The degree of degradation in descending order was 4-CP > 2,4-DCP > 2,4,6-TCP. This was because OH• would first attack the molecular sites without chlorine before dissociating chlorine atoms. Therefore, chlorinated organics with higher number of chlorine atoms tended to be more resistant to Fenton's reaction. The degradation kinetics of the chlorophenols significantly complied with the first-order rates. However, this kinetic order did not agree with the report of Hagg and Yao (1992), which stated that the kinetics of Fenton's oxidation of halogenated aromatics follows the second-order model.

Lunar et al. (2000) showed that Fenton's reagent efficiently reduced COD in synthetic wastewater containing developing agents (N-methyl-p-aminophenol). With a reaction time of 2 hours, Fenton's reagent was capable of reducing COD from 2,000 mg/L to below 1,000 mg/L. Further reduction did not seem feasible since the oxidation was mainly responsible for the ring cleavage of aromatics. Kang and Hwang (2000) purported that the use of Fenton's reaction not only offers high COD removal in landfill leachate (COD > 4,000 mg/L) but also enhances the efficiency of coagulation used as a next treatment step. pH 4 was optimal for COD removal. H_2O_2 was the main component that significantly influenced the COD removal. Fe^{2+} dosage higher than 500 mg/L did not contribute to the removal efficiency. Instead, it contributed to the precipitation of iron complexes.

Spent caustic from Olefin plants containing high concentrations of H_2S, NaOH, emulsified oil and phenols was successfully treated by Fenton's reaction (Sheu and Weng, 2001). It was necessary to adjust pH to a strong acidic range (pH 2 to 3), the condition that significantly improved Fenton's efficiency. Under the H_2O_2/COD ratio of 1.1, more than 95% COD removal along with other pollutant reductions was achieved. Sheu and Weng, (2001) pointed out that Fe^{2+} under a strong acidic condition could spontaneously be regenerated; consequently, continuous refilling of Fe^{2+} was not required.

Pérez et al. (2002) applied Fenton' reagent to treat bleaching effluents from paper pulp factories. Different H_2O_2 doses provided different TOC removal after 15 minutes of reaction time; increasing H_2O_2 concentration resulted in higher TOC reduction. However, it took longer than 3 hours to achieve more than 50% TOC removal. This was because refractory organics in waste stream were fractionated into low-molecular weight before undergoing mineralization. To achieve mineralization, the authors suggested that much higher H_2O_2 concentration would be required. It was also learned that too high H_2O_2 and Fe^{2+} did not improve TOC removal since they would react with OH• instead of organic compounds.

Chamapro et al. (2001) used Fenton's reagent to enhance biodegradability of synthetic wastewater. COD was not significantly removed for wastewater containing 4-CP and 2,4-DCP. On the contrary, biodegradability (BOD_5/COD) in this synthetic wastewater was greatly improved. H_2O_2 dose controlled the amount of biodegradability increase while Fe^{2+} was responsible for the improvement of the

reaction kinetics. The H_2O_2:organic (mol/mol) of 2 was the optimal ratio for the degradation of the chlorophenols tested.

Teel et al. (2001) found that Fenton's reaction were a promising process for remediating groundwater and soil contaminated with trichloroethene (TCE). In their study, although iron mineral (Fe^{3+} complexes) offered lower TCE removal than soluble Fe^{2+}, it provided high dechlorination efficiencies under pH 3. H_2O_2 fed with 2 minutes interval could remove more TCE than H_2O_2 fed with pulsing system. This result suggested that oversupplying H_2O_2 to Fenton's system would lead to the reduction of its oxidizing capabilities.

2.3.3 Light-Enhanced Fenton' s Reagent

The efficiency of Fenton's reagent can be improved by supplementing UV-visible irradiation. The interaction between Fe^{3+} and radiation will lead to the regeneration of Fe^{2+} (eq. 8 and 9), whereby it will react with H_2O_2 to yield OH• (Pignatello et al., 1999; and Oliveros et al., 1997).

$$Fe^{3+}(L^-) \xrightarrow{h\nu} Fe^{2+} + L• \quad (L = \text{organic ligand}) \qquad (8)$$

$$FeOOH^{2+} \xrightarrow{h\nu} HO_2• + Fe^{2+} \qquad (9)$$

Oliver et al., (1997) stated that the degradation rate of organic contaminants such as 4-CP, polychlorinated biphenyls (PCBs), and herbicides could be accelerated by enhancing Fenton's reagent with UV light. Pignatello et al. (1999) reported that the degradation rates of chlorinated organics obtained from UV-enhanced Fenton's reagent increased 1.2 to 1.4 times from normal Fenton' s reaction. Dichloroacetic acid (DCA) and 2,4-DCP were successfully degraded under ferrioxalate $((Fe(C_2O_4)_3)^{3-})$/H_2O_2 system assisted with UV irradiation (Nogueira and Guimaraes, 2000). Increasing light intensity and using pulsing feed of H_2O_2 resulted in high dechlorination efficiency. However, the system did not achieve complete dechlorination. pH 2.5 to 2.8 was the optimum range for this photodegradation. During the reaction, Fe^{2+} could not be detected because it was rapidly used up. The

authors recommended using this system because it required low cost and small exposition area.