CHAPTER 3

MATERIAL AND METHODS

3.1 Chemicals

All solutions were prepared from 18 MΩ/cm deionization water. Analytical grade formaldehyde 37% (with approximately 10% 0f methanol), hydrogen peroxide 35%, and heptahydrated ferrous sulfates were purchased from the Merck Company. All other reagents used in this study were at least reagent grade.

3.2 Experimental Apparatus

All experiments were performed in a 1.2 liter double-jacked quartz reactor; the volume of the solution mixture was 1.1 liter. The outer compartment contained the solution mixture and the chemical reagents. The inner one was used to insert a 10 W low pressure mercury lamp with a major emission at 254 nm. Cooling water was provided for the inner part to keep a constant temperature (30 °C). Thermometer and pH meter were fixed onto the sampling part of the photo-reactor. The sampling solution was well mixed with a magnetic stirrer bar (Figure 3.1).

3.3 Experimental Procedures

All solutions, including formaldehyde, methanol, and ferrous sulfate heptahydrate, were adjusted to the designed pH with sulfuric acid and sodium hydroxide. For all experiments, the initial concentration of formaldehyde was 0.333 M, which 7.96×10^{-2}M of methanol was already existed. The reaction was initiated by adding the calculated amount of hydrogen peroxide. For UV/H_2O_2 and photo-Fenton

experiments, the UV-light was turned on for 5 min before initiating the oxidation reaction in order to reach its stability.

One-millilitre aliquots were withdrawn at selected time intervals and quenched instantly by adding 2-ml of 0.28 M Na_2SO_3 and 1-ml of NaOH 0.5 M. After adding Na_2SO_3 and NaOH, the solution was filtered through 0.45 μm syringe microfilters before analysis. For formaldehyde and methanol analysis, the samples were taken at 0, 5, 10, 20, 40, 60 and 80 min while pH and temperature were recorded. Besides, 1- ml aliquot samples were withdrawn at 0, 5, 10, 20, 40, 80, 120, 140, 180, 240 min for total organic carbon and toxicity test. And 0.5-ml samples were taken for H_2O_2 measurement.

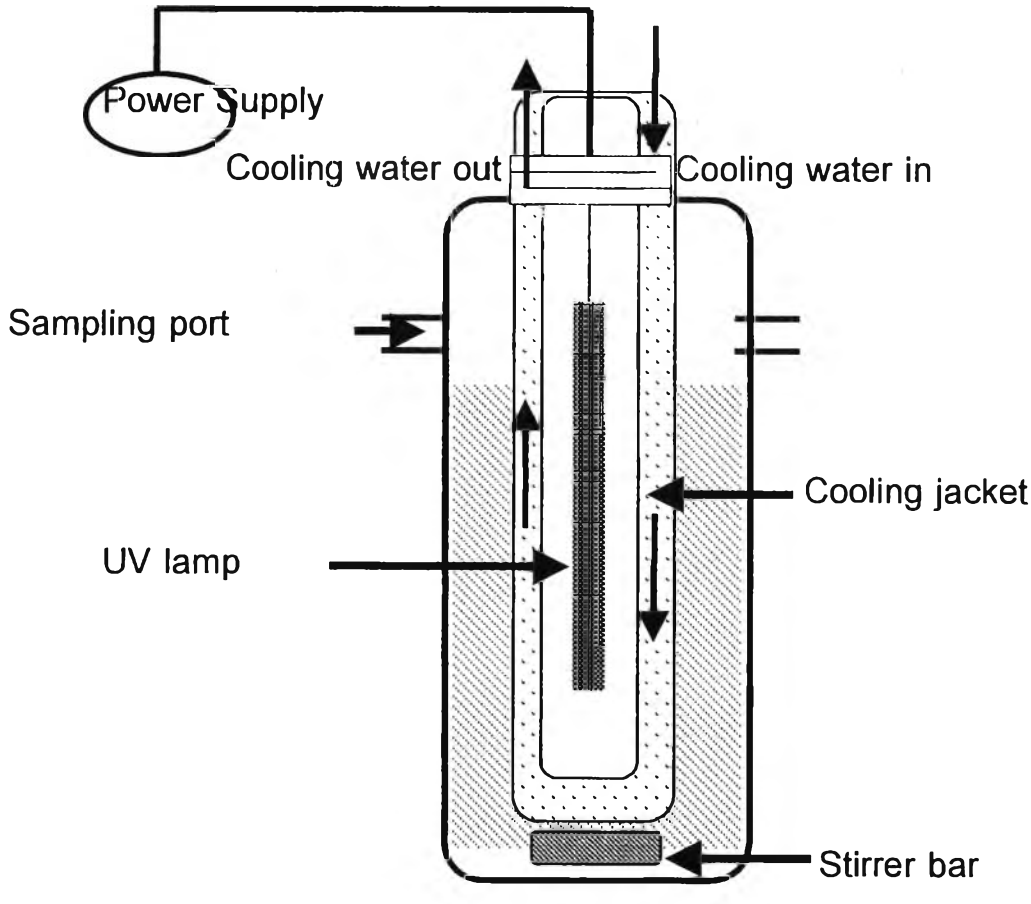

Figure 3.1 Photo-reactor used for all experiments.

3.4 Analytical Methods

3.4.1 Measurement of Formaldehyde and Methanol

Before measurement, liquid samples were first filtered through 0.45 μm syringe microfilters in order to separate precipitated iron from the solutions. Then formaldehyde was analyzed by a gas chromatograph equipped with a frame ionization detector (HP 4890), a pack column (5% diphenyl and dimethylpolysiloxane) with the following dimension, 60 m, and 0.53 μm was used. The temperature program during the GC analysis was used as follows: 35 °C (1min), 0.8 °C•min^{-1} until 40 °C (3min), 65 °C•min^{-1} until 200 °C (1min).

3.4.2 Analysis of Total Organic Carbon

The mineralization of the solution effluence was determined by mean of total organic carbon. Before analysis, samples were filtered through 0.45 μm microfilters in order to separate iron from the solutions. All supernatants were diluted 160 times by 18 MΩ/cm deionization water before measurement.

3.4.3 Toxicity Analysis

For the toxicity test, all samples were diluted 16 times before analysis. Then the toxicity of solutions were evaluated by The CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay, a colorimetric method for determining the number of viable cells in proliferation or chemosensitivity assays. The CellTiter 96® AQ_{ueous} Assay is composed of solutions of a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(phenazine methosulfate; PMS). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm can be measured directly from 96 well assay plates without additional processing (Riss and Moravec, 1992). The conversion of MTS into aqueous, soluble formazan is accomplished by

dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product as measured by the account of 490 nm absorbance is directly proportional to the number of living cells in culture.

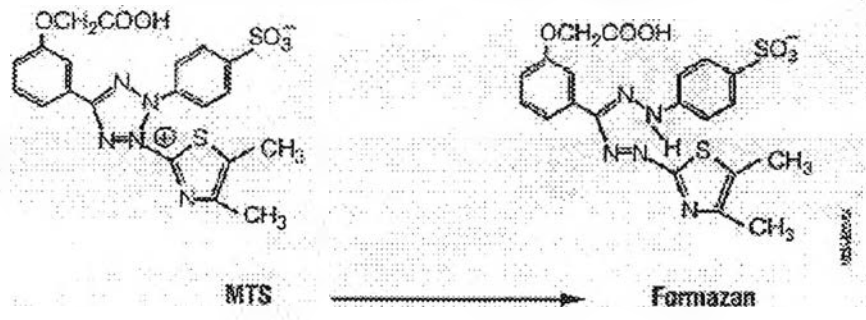

Figure 3.2 Structures of MTS tetrazolium salt and its formazan product.

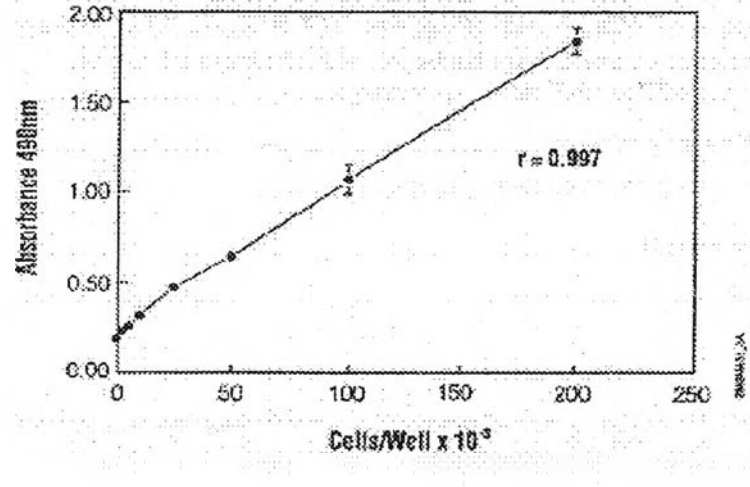

Figure 3.3 Effect of cell number on absorbance at 490 nm measured using the CellTiter 96® Aqueous Assay.

Various numbers of K562 (human chronic myelogenous leukemia) cells in RPMI supplemented with 5% fetal bovine serum (FBS) were added to the wells of a 96 well plate. The medium was allowed to equilibrate for 1 hour, then 20 μl/well of combined MTS/PMS solution were added. After1 hour at 37°C in a humidified, 5% CO_2 atmosphere, the absorbance at 490 nm was recorded using an ELISA plate reader. Each point represents the mean ±SD of 4 replicates. The correlation coefficient of the line was 0.997, indicating that there was a linear response between cell number and absorbance at 490 nm. The background absorbance shown at zero cells/well was not subtracted from these data.

3.4.4 Other Measurements

The concentration of hydrogen peroxide was determined by standard iodometric method, which potassium iodide was used as a reactant as describe in Appendix A. The pH meter was used to determine the pH variation during the reaction time period.