CHAPTER III

EXPERIMENTAL AND METHODOLOGY

3.1 Chemical substances

All the reagents used in the experiments were in analytical quality. All the preparations and experiments were at the room conditions.

- o-Toluidine: C_7H_9N
- ferrous sulfate heptahydrated: $FeSO_4.7H_2O$
- hydrogen peroxide (35%): H_2O_2
- perchloric acid (70-72%): $HClO_4$
- sodium hydroxide: $NaOH$
- calcium chloride anhydrous: $CaCl_2$
- magnesium sulfate: $MgSO_4.7H_2O$
- manganese sulfate: $MnSO_4.4H_2O$
- ammonium Chloride: NH_4Cl
- potassium hydroxide: KOH
- potassium iodide: KI
- potassium diphosphate: KH_2PO_4
- potassium phosphate dibasic: K_2HPO_4
- sodium phosphate dibasic: Na_2HPO_4
- sodium azide: NaN_3
- ferric chloride: $FeCl_3.6H_2O$
- acetonitrile (99.9%): CH_3CN
- silver sulfate: $AgSO_4$
- mercury Sulfate: $HgSO4$
- sulfuric acid: H_2SO_4
- ammonium iron(III) sulfate hexahydrate: $FeH_8N_2O_8S_2.6H_2O$
- potassium dichromate: $Cr_2K_2O_7$
- ferroin indicator
- hydrochloric acid: 0.64% HCl
- ammonium acetate: $NH_4C_2H_3O_2$
- 1,10 – phenanthroline monohydrate: $C_{12}H_8N_2.H_2O$
- potassium titanium oxalate dihydrate: $K_2TiC_4O_9$
- alkaline-azide-Iodide: AIA
- sodium thiosulfate: 0.025N $Na_2S_2O_3$
- starch

3.2 Experimental Instruments

1. pH meter: a SUNTEX pH/mV/TEMP (SP-701) meter.
2. UV-VIS Spectrophotometer: SHIMADZU UV-1201
3. Thermometer: 0-100 °C; ± 1°C
4. Auto Pipette (adjustable): 1,000 µl and 5 ml
5. Weighing Machine (4-Digits Balance): METTLER TOLEDO (AB 204-5)
6. Magnetic Stirrer: Fargo (MS-90)
7. Magnetic Bar: 30 x 8 mm

8. Glassware
9. Clock Timer
10. Membrane Filter 0.45 μm and 0.2 μm
11. Incubator (temperature controllable)
12. Total organic carbon analyzer: liquiTOC (elementar) with high temperature combustion method
13. High performance liquid chromatography (HPLC): with SpectraSYSTEM model SN4000 pump and Asahipak ODP-506D column (150mm × 6mm × 5μm).
14. Ion Chromatograph (IC): (Dionex DX-120) equipped with RFC-30 EGCII (KOH), IonPac®AG11 guard column(4x50mm), IonPac®AS11 analytical column (4x250mm), ASRS®-ULTRA II (4mm) suppressor and conductivity detector.
15. UV light: The UV bult bought from SUNBEAM with tube diameter 3 mm, length 300 mm. The input voltage of inverter is 12 V, output is 1.80 V, current 5 mA. The brightness is between 28000 to 30000 cd/m^2 with the lifetime 30,000 hours.
16. Electrode:
- Anode: Mesh-type titanium metal coated with IrO_2/RuO_2: diameter = 7 cm and height = 35 cm
(DSA - Dimensionally Stable Anodes)
- Cathode: Stainless steel mesh: diameter = 13 cm and height = 35 cm
17. Power supply: The electrodes were connected to a Topward 33010D power supply operated at the desired electric current.
18. Power supply for UV light: The UV light were connected to the power supply E-safe 2003, Switching Power Supply (Max. 300W), Model: LC-B300AT

3.3 Analytical methods
3.3.1 Measurement of *o*-toluidine concentration

o-toluidine was analyzed by high performance liquid chromatography (HPLC) with Spectra SYSTEM model SN4000 pump and Asahipak ODP-506D column (150mm × 6mm × 5μm). The mobile phase was 60% Acetonitrile with 40% DI water. Before the analysis, all supernatants were diluted 10 times by 0.1 N NaOH, and then the solutions were filtered by 0.2 μm microfilters to separate iron sludge from the solutions before injected to HPLC.

3.3.2 TOC analysis

Mineralization of the solution effluent will be determined by means of total organic carbon using SHIMADZU TOC-V$_{CPH}$ Model ASI-V SA. Before the analysis, all supernatants were diluted 10 times by 0.1 N NaOH, and then the solutions were filtered by 0.2 μm microfilters to separate iron sludge from the solutions before analyses.

3.3.3 Ferrous analysis

The sample was determined with phenanthroline reagent based on the Standard Methods (APHP, 1992). Then, the DI water was added to make up the volume to 50 ml. The DI water mixed with sample without phenanthroline was used as the blank.

3.3.4 Hydrogen peroxide analysis

The samples were analyzed using titanium dioxide method. The DI water was added to make up the volume to 25 ml. For blank, the DI water was mixed with titanium dioxide.

3.3.5 COD analysis

COD was determined by using a closed-reflux titrimetric method based on the Standard Methods (APHA, 1998). The sample was refluxed in strongly acidic solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion the remaining unreduced $K_2Cr_2O_7$ was titrated with ferrous ammonium sulphate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter was calculated in terms of oxygen equivalent.

The samples were filtrated by 0.45 μm filters, and kept for 24 hours in the refrigerator before the COD analysis was conducted. This work was carried out to correct quantitative the effect of the concentration of hydrogen peroxide on the COD value.

3.3.6 BOD analysis

The samples were diluted 10 times and quench the reaction by increasing pH up to 11 with NaOH. Then, the samples were kept in the water bath at 65°C for 24 hours in order to decompose H_2O_2. After that, the samples were diluted to 100 times with dilution water and incubated at 20°C for 5 days. All samples were determined DO_0 and DO_5 with Azide Modification Method.

3.3.7 Organic acid analysis

Organic acids were analyzed using Dionex DX-120 ion chromatograph with an IonPac® AS 11 anion column at 30°C. Before the analysis, all supernatants were diluted 10 times by 0.1 N NaOH, and then the solutions were filtered by 0.2 μm microfilters to separate iron sludge from the solutions, then all supernatants were diluted 10 times by DI water and injected into IC.

3.4 Reactor
3.4.1 Fenton reactor

The reactor was made of stainless steel (radius = 6.5 and height = 35 cm). The pH meter was used to control the pH of the solution as shown in Figure 3.1.

pH meter

Thermometer

recirculation
pump
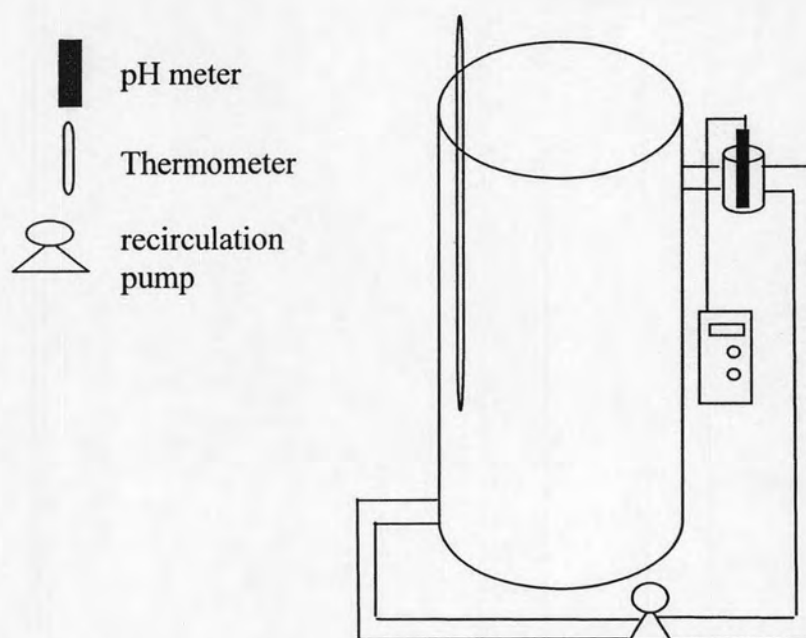
Figure 3.1: Reactor configuration of Fenton process

3.4.2 Electro-Fenton Reactor

The cylindrical reactor was operated at a constant current mode as shown in Figure 3.2 below. The material of electrodes were use titanium net coated with RuO_2/IrO_2 (DSA) as the anode, and the stainless steel as the cathode. All of electrodes were connected with DC power.

pH meter

Thermometer

Recirculation
pump

DC
POWERSUPPLY

+   -
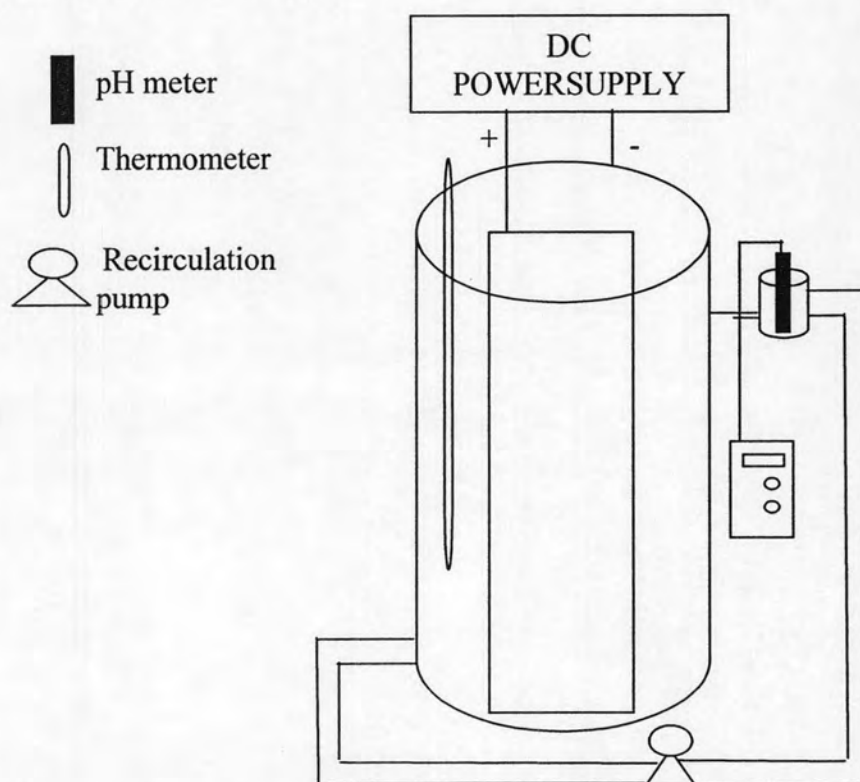
Figure 3.2: Reactor configuration of electro-Fenton process

3.4.3 Photoelectro-Fenton reactor

The reactor for photoelectron-Fenton process was the same as electro-Fenton process except for the addition of six UV bulbs inside the reactor as shown in Figure 3.3.

3.5 Experimental procedures

The experiment procedures can be divided into 2 parts. The first part is for determination of the optimum condition by using Design-Expert software as shown in Figure 3.4. The second part is for the degradation and detoxification of *o*-toluidine (OT) as shown in Figure 3.5.

Figure 3.3: Reactor configuration of photoelectro-Fenton process

```
          ┌──────────────────────────────────┐
          │      Design-Expert Software       │
          └──────────────────────────────────┘
                          │
                          ▼
          ┌──────────────────────────────────┐      ┌──────────────────┐
          │    Key parameters determination  │──────│  2-Level Factorial│
          └──────────────────────────────────┘      │      Design       │
                          │                          └──────────────────┘
                          ▼
          ┌──────────────────────────────────┐      ┌──────────────────┐
          │   Optimum condition predication  │──────│   Box-Behnken    │
          └──────────────────────────────────┘      │ Response Surface │
                          │                          └──────────────────┘
                          ▼
          ┌──────────────────────────────────┐
          │   -  Kinetic information          │
          │   -  H₂O₂ availability            │
          │   -  Control experiments          │
          └──────────────────────────────────┘
```
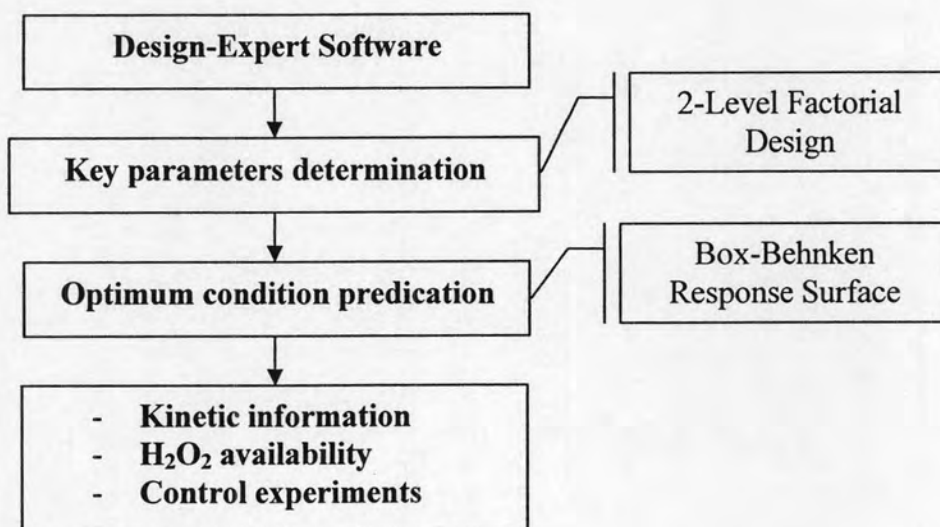
Figure 3.4: Flow chart for determination of the optimum condition

3.5.1 Determination the optimum condition by Design-Expert software

The electro-Fenton process was selected as the main process to find the optimum condition by this software:

3.5.1.1 Identification of key parameters by using 2-levels factorial function

The variables were pH, the amount of Fe^{2+}, H_2O_2 concentration and current of which were varied as shown in Table 3.1.

```
                          ┌──────────┐
                          │    OT    │
                          └──────────┘
                                │
          ┌─────────────────────┼─────────────────────┐
          ▼                     ▼                     ▼
  ┌───────────────┐   ┌──────────────────────┐   ┌────────────────────┐
  │ Fenton process│   │ electro-Fenton process│   │ photoelectro-Fenton│
  └───────────────┘   └──────────────────────┘   └────────────────────┘
          │                     │      ┌───────┐          │
          │                     ├─────▶│  TOC  │◀─────────┤
          │                     ▼      └───────┘
          │         ┌────────────────────────────────┐
          └────────▶│  OT concentration, BOD and COD │
                    └────────────────────────────────┘
                                  │
                                  ▼
                    ┌────────────────────────────────┐
                    │   Biodegradability efficiency   │
                    └────────────────────────────────┘
```
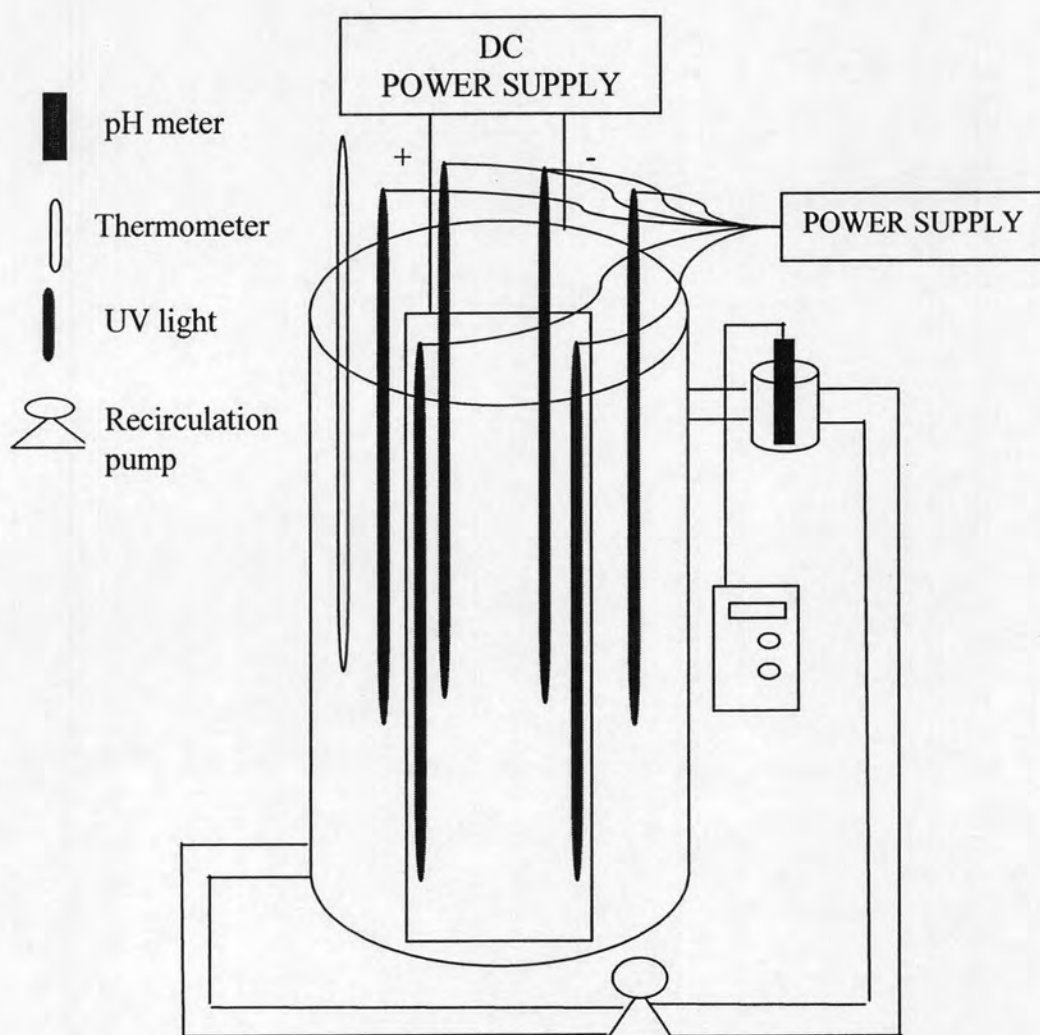
Figure 3.5: Experimental design for degradation and detoxification of OT

Table 3.1: Experimental setting for 2-levels factorial function

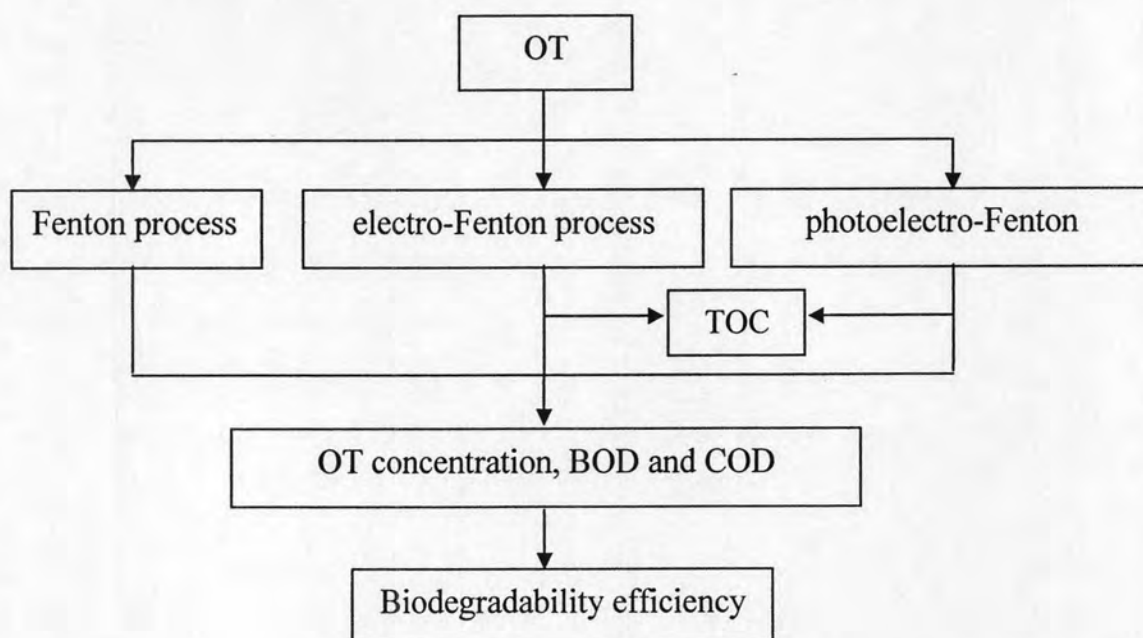| Input/factor	Range		Output/Response (%)
	Minimum	Maximum	
pH	2	4	1. OT removal 2. COD removal
$[Fe^{2+}]$ (mM)	0.2	1	
$[H_2O_2]$ (mM)	1	5	
Current (A)	1	4	

3.5.1.2 Optimization condition for OT and COD removal by using Box-Behnken response surface function

The significant parameters obtained from 3.5.1.1 including the center point of each factor were used to find the optimum condition and responses. Table 3.2 shows an example of independent variables when they were chosen as key parameter.

3.5.2 Degradation and detoxification of OT

The degradation and detoxification of OT were studied at the optimum condition obtained from 3.5.1.2 by several Fenton processes including conventional Fenton, electro-Fenton and photoelectron-Fenton processes.

3.5.2.1 Degradation of OT procedure

All solutions with OT solution were prepared in the reactor. Ferrous sulfate heptahydrate was then completely mixed with the prepared solution in the reactor. The initial pH was adjusted to the desired pH with $HClO_4$ and NaOH. The reaction was initiated by adding H_2O_2 (for Fenton process; for electro-Fenton, H_2O_2 and current were supplied concurrently; for photoelectro-Fenton, H_2O_2, current and UV light were also supplied concurrently). The sample was taken at time interval 0, 1, 2, 5, 10, 15, 30, 60, 90 and 120 minutes and was increased pH around 11 by NaOH in order to quench the reaction. The sample was then filtrated with 0.2 μm membrane filter before determine OT concentration by HPLC.

Table 3.2: Experimental setting for Box-Behnken response surface function

Input/factor	Level			Output/response
	Minimum	Centre	Maximum	
pH	2	3	4	1.OT removal 2.COD removal
$[Fe^{2+}]$ (mM)	0.2	0.6	1	
$[H_2O_2]$ (mM)	1	3	5	
Current (A)	1	2.5	4	

3.5.2.2 Biodegradability of OT procedure

BOD_5/COD ratio was used as an index for the biodegradability of OT. After the reaction was stopped and kept 24 hours for decomposition of the oxidant. Then, the sample was removed precipitation by filtrate with 0.45 μm membrane filter before COD analysis was conducted by closed-reflux titrimetric method. For BOD_5; after the reaction was stopped and kept in the water bath at 65°C for 24 hours. The samples were then diluted with dilution water and incubated at 20°C for 5 days. All samples were determined DO_0 and DO_5 with Azide Modification Method.

3.5.2.3 Conventional Fenton experiment

As shown in Figure 3.6, the OT solution was adjusted to the desired pH by an addition of $HClO_4$. After that, a predetermined amount of catalytic ferrous sulfate was added into the solution. The solution pH was rechecked again prior to the addition of H_2O_2 solution and the reaction was simultaneously started. Samples of 1 ml were taken at every predetermined time interval of 0, 1, 2, 5, 10, 15, 30, 60, 90 and 120 minutes and were immediately injected into a tube containing 9 ml of NaOH to stop the reaction. Samples were analyzed for COD, TOC and OT concentration. For BOD analysis, the samples were taken at 10 ml at predetermined time interval of 0, 5, 10, 15, 30, 45, 60, 90 and 120 minutes and quenched the reaction by increasing pH greater than 10 with NaOH.


```
┌─────────────────────────────────────────────────────────────┐
│  OT solution was adjusted pH to the desired value by HClO₄   │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│  Add a predetermined amount of FeSO₄.7H₂O into the solution  │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│  Add a predetermined amount of H₂O₂ into the reactor and     │
│  switched on simultaneously to start the experiment          │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│  Sample was taken at 0, 1, 2, 5, 10, 15, 30, 60, 90 and 120  │
│  min and immediately mixed with NaOH solution                │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│  Filtrate through 0.2 or 0.45 μm membrane filter and analyze │
│  for H₂O₂ residual, Fe²⁺ concentration, COD, TOC and OT      │
│  concentration                                               │
└─────────────────────────────────────────────────────────────┘
```
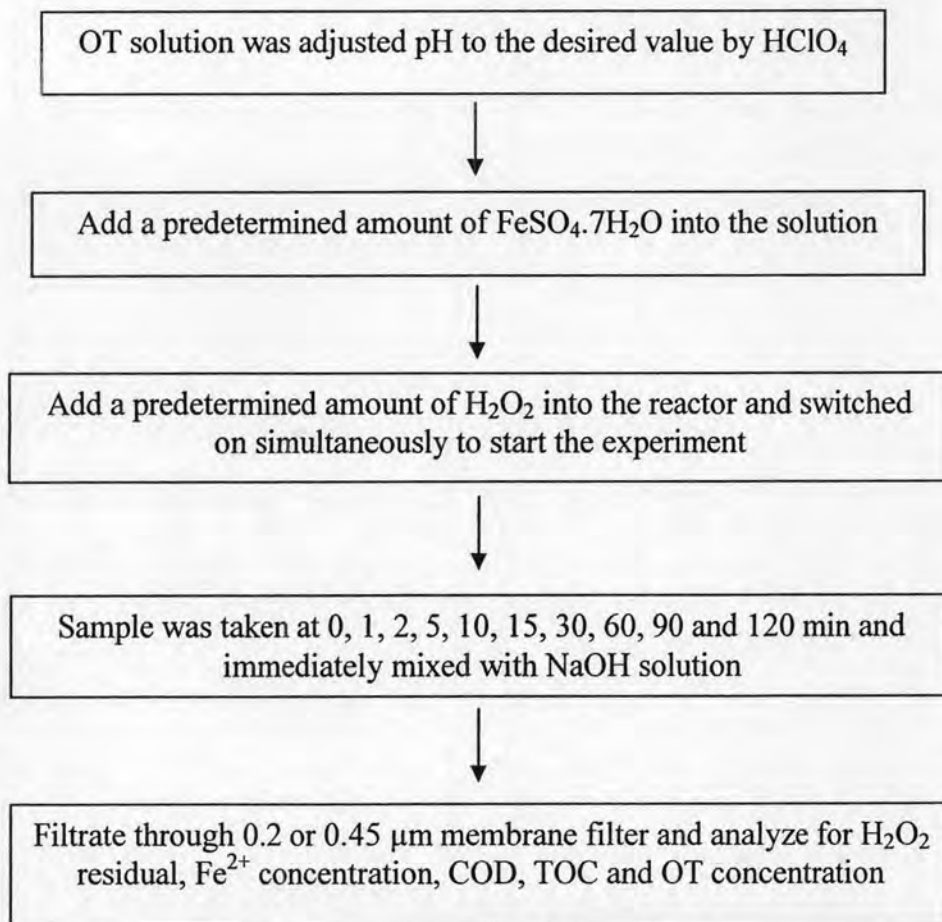
Figure 3.6: Flow chart for Fenton process experiment

3.5.2.4 Electro-Fenton experiment

In this experiment, an electrical supply unit which consisted of a stainless steel cathode, a IrO_2/RuO_2 (DSA) anode, and a regulator DC power supply were installed additionally in the reactor. Most procedures were similar to those of typical Fenton reaction, however, in this scenario, the electrical current was delivered through out the experiment period. The reaction was initiated by turning on the current supply and adding of H_2O_2 as shown in Figure 3.7.

3.5.2.5 Photoelectro-Fenton experiment

In this experiment, the process was quite similar to the electro-Fenton process except for the irradiation of UV light. The reaction was initiated by turning on both the current supply and UV light and adding of H_2O_2 as shown in Figure 3.8.

OT solution was adjusted pH to the desired value by $HClO_4$

↓

Add a predetermined amount of $FeSO_4.7H_2O$ into the solution

↓

Both predetermined amount H_2O_2 and current were supplied into the reactor simultaneously to start the experiment

↓

Sample was taken at 0, 1, 2, 5, 10, 15, 30, 60, 90 and 120 min and immediately mixed with NaOH solution

↓

Filtrate through 0.2 or 0.45 μm membrane filter and analyze for H_2O_2 residual, Fe^{2+} concentration, COD, TOC and OT concentration

Figure 3.7: Flow chart for electro-Fenton process experiment

```
┌─────────────────────────────────────────────────────────────┐
│   OT solution was adjusted pH to the desired value by HClO₄   │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│   Add a predetermined amount of FeSO₄.7H₂O into the solution  │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│   A predetermined amount H₂O₂, current and UV light were      │
│   supplied into the reactor simultaneously to start the       │
│   experiment                                                  │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│   Sample was taken at 0, 1, 2, 5, 10, 15, 30, 60, 90 and      │
│   120 min and immediately mixed with NaOH solution            │
└─────────────────────────────────────────────────────────────┘
                              │
                              ▼
┌─────────────────────────────────────────────────────────────┐
│   Filtrate through 0.2 or 0.45 µm membrane filter and         │
│   analyze for H₂O₂ residual, Fe²⁺ concentration, COD, TOC     │
│   and OT concentration                                        │
└─────────────────────────────────────────────────────────────┘
```
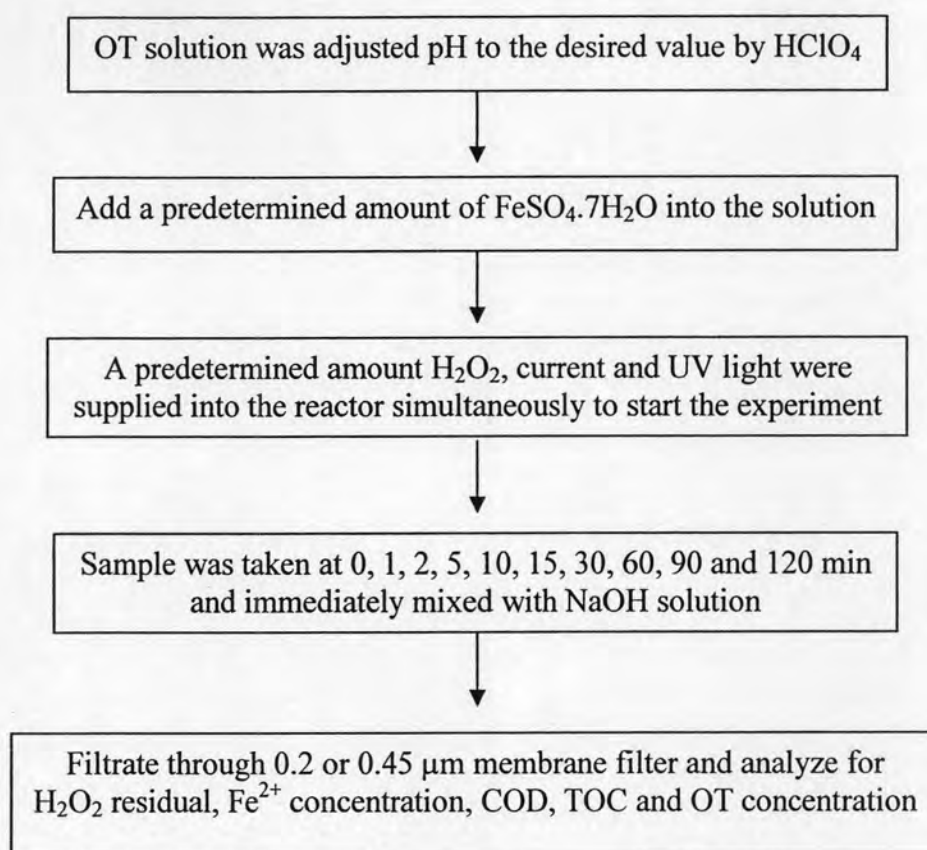
Figure 3.8: Flow chart for photoelectro-Fenton process experiment

3.6 Experimental scenarios

Scenario A: Determination of key parameter for OT and COD removal with electro-Fenton process by using 2-levels factorial function from Design-Expert software. The details are showed in Table 3.3.

Scenario B: Determination of the optimum condition for OT and COD removal with electro-Fenton process by using Box-Behnken responses surface from Design-Expert software.

Scenario C: Control experiments for optimum condition. The details are showed in Table 3.4.

Scenario D: Determination of the degradation and detoxification efficiency by Fenton process at the optimum condition. The details are showed in Table 3.5.

Scenario E: Determination of the degradation and detoxification efficiency by electro-Fenton process at the optimum condition. The details are showed in Table 3.6.

Scenario F: Determination of the degradation and detoxification efficiency by photoelectro-Fenton process at the optimum condition. The details are showed in Table 3.7.

Table 3.3: Details for scenario A

	Factor			
Run	pH	Fe^{2+} (mM)	H_2O_2 (mM)	Current (A)
1	4.00	0.20	5.00	4.00
2	2.00	1.00	5.00	1.00
3	4.00	0.20	5.00	1.00
4	4.00	1.00	5.00	4.00
5	2.00	0.20	5.00	1.00
6	2.00	1.00	5.00	4.00
7	2.00	1.00	1.00	1.00
8	2.00	1.00	1.00	4.00
9	4.00	1.00	1.00	4.00
10	4.00	0.20	1.00	1.00
11	2.00	0.20	5.00	4.00
12	2.00	0.20	1.00	1.00
13	4.00	0.20	1.00	4.00
14	4.00	1.00	5.00	1.00
15	2.00	0.20	1.00	4.00
16	4.00	1.00	1.00	1.00

Table 3.4: Details for scenario C

Experimental	Parameter			
	Fe^{2+}	H_2O_2	UV-light	Current
Electrolysis	X	X	X	From scenario A or B
Photolysis	X	X	✓	X
Photo-electrolysis	X	X	✓	From scenario A or B
H_2O_2	X	From scenario A or B	X	X
H_2O_2 + current	X	From scenario A or B	X	From scenario A or B
H_2O_2 + UV	X	From scenario A or B	✓	X
Fe^{2+} + current	From scenario A or B	X	X	From scenario A or B
Fe^{2+} + UV	From scenario A or B	X	✓	X

Table 3.5: Details for scenario D

[OT]	pH	[Fe^{2+}]	[H$_2$O$_2$]
1 mM	From scenario A or B	From scenario A or B	From scenario A or B

Table 3.6: Details for scenario E

[OT]	pH	[Fe^{2+}]	[H$_2$O$_2$]	current
1 mM	From scenario A or B	From scenario A or B	From scenario A or B	From scenario A or B

Table 3.7: Details for scenario F

[OT]	pH	[Fe^{2+}]	[H$_2$O$_2$]	current	UV light
1 mM	From scenario A or B	From scenario A or B	From scenario A or B	From scenario A or B	✓