Chapter – V

EXPERIMENTATION

5.1 Experimental Programme

To achieve the objectives of the proposed work, following experimental programme was formularized. Batch experiments were conducted for treatment of simulated wastewater containing pesticide (methyl parathion, chlorpyrifos and carbofuran) using various type of treatment technologies as summarized in Figure 5.1.

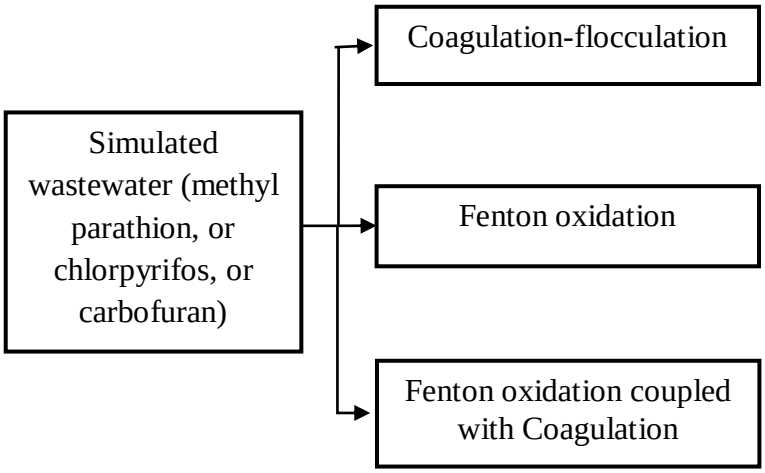


Figure 5.1: Flow chart of experimental program for pesticide removal using various treatment technologies.

5.2 Preparation of Simulated Wastewater

Simulated pesticide (methyl parathion, chlorpyrifos and carbofuran) bearing wastewater of concentration 30 mg/L was prepared in double distilled water for further experiments. Fresh solution in each case was prepared weekly and stored at 4 ºC. The characteristics of simulated wastewater are listed in Table 5.1.

Table 5.1: Characterisation of simulated wastewater at initial stage (Concentration = 30 mg/L).

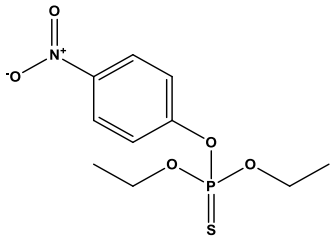
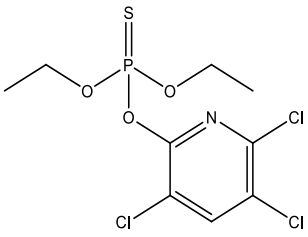
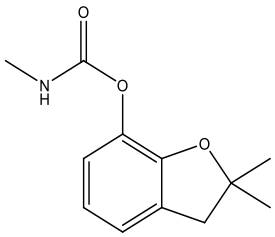
Pesticides	Symbol	Initial pH	Initial COD (mg/L)
Methyl parathion	MP	6.5	440
Chlorpyrifos	CPF	6.7	385
Carbofuran	CF	6.2	510

5.3 Chemicals Used

The analytical grade pesticides − methyl parathion (assay = 99.7%), chlorpyrifos (assay = 99.8%) and carbofuran (assay ≥ 98%) used were acquired from Sigma-Aldrich, Mumbai, India. All other chemicals used in the experiments were also of analytical reagent (AR) grade and obtained from Sigma-Aldrich, Mumbai, India. Double distilled water was used to prepare the stock solution (prepared in lab). The coagulants used in the experiments were alum ($Al_2(SO_4)_3 \cdot 18H_2O$) and ferric chloride ($FeCl_3$). Alum (M = 666. 39 g/mol; assay ≥ 97%) and ferric chloride (M = 162.2 g/mol; assay ≥ 96%) of AR grade were procured from Fisher Scientific, Mumbai, India. The pH of the test solutions was adjusted by using 1.0 M H_2SO_4 and 1.0 M NaOH. The stock solutions of alum and ferric chloride were prepared as per the required concentration.

Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) and hydrogen peroxide (H_2O_2, 30% w/v) were purchased from Merck (Mumbai, India). Manganese(II) sulfate dihydrate ($MnSO_4.2H_2O$), potassium iodide (KI), sodium azide (NaN_3) and sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) were procured from Fisher Scientific, Mumbai, India. High purity HPLC water and acetonitrile used as mobile phases for HPLC analysis were obtained from Sigma–Aldrich, Mumbai, India. The physio-chemical properties of the pesticide used in this work are presented in Table 5.2.

Table 5.2: Physico-chemical properties of pesticides.

Properties	Methyl parathion	Chlorpyrifos	Carbofuran
Molecular formula	$C_8H_{10}NO_5PS$	$C_9H_{11}Cl_3NO_3PS$	$C_{12}H_{15}NO_3$
Molecular structure			
Molecular weight	263.2 g/mol	350.6 g/mol	221.26 g/mol
Chemical name	O,O-Diethyl O-4-nitrophenyl phosphorothioate	O, O-Diethyl O-3, 5,6-trichloro-2-pyridyl phosphorothioate	2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate
Half-life	In aqueous solution at 20 ºC was about 175 day	In aqueous solution > 2 months	In soil was about 78 days, In aqueous solution 450-1200 days
Solubility in water	Solubility in water 55 - 60 mg/L at 20°C; soluble in most organic solvents, slightly soluble in petroleum and mineral oils.	0.39 mg/L at 19°C, 0.941-1.05 mg/L at 20°C, 2 mg/L (25 °C)	320 mg/L at 25 °C
Chemical Type	Organophosphate	Organophosphate	Carbamate
Density	1.4 g/cm^3	1.398 g/cm^3	1.18 g/cm^3
Melting point	35-36 °C	42 °C	151 °C
Boiling point	143 °C	160 °C	313.3 °C
Flash point	46.1 °C	189 °C	143.3 °C
Appearance	White crystals (pure form)	Colourless crystals	White, crystalline solid

5.4 Coagulation−Flocculation Study

5.4.1 Experimental programme

In the present study, batch experiments have been conducted for the reduction of pesticides. Figure 5.2 demonstrates the complete sequence of the coagulation−flocculation experiments performed for reduction of pesticides through batch studies.

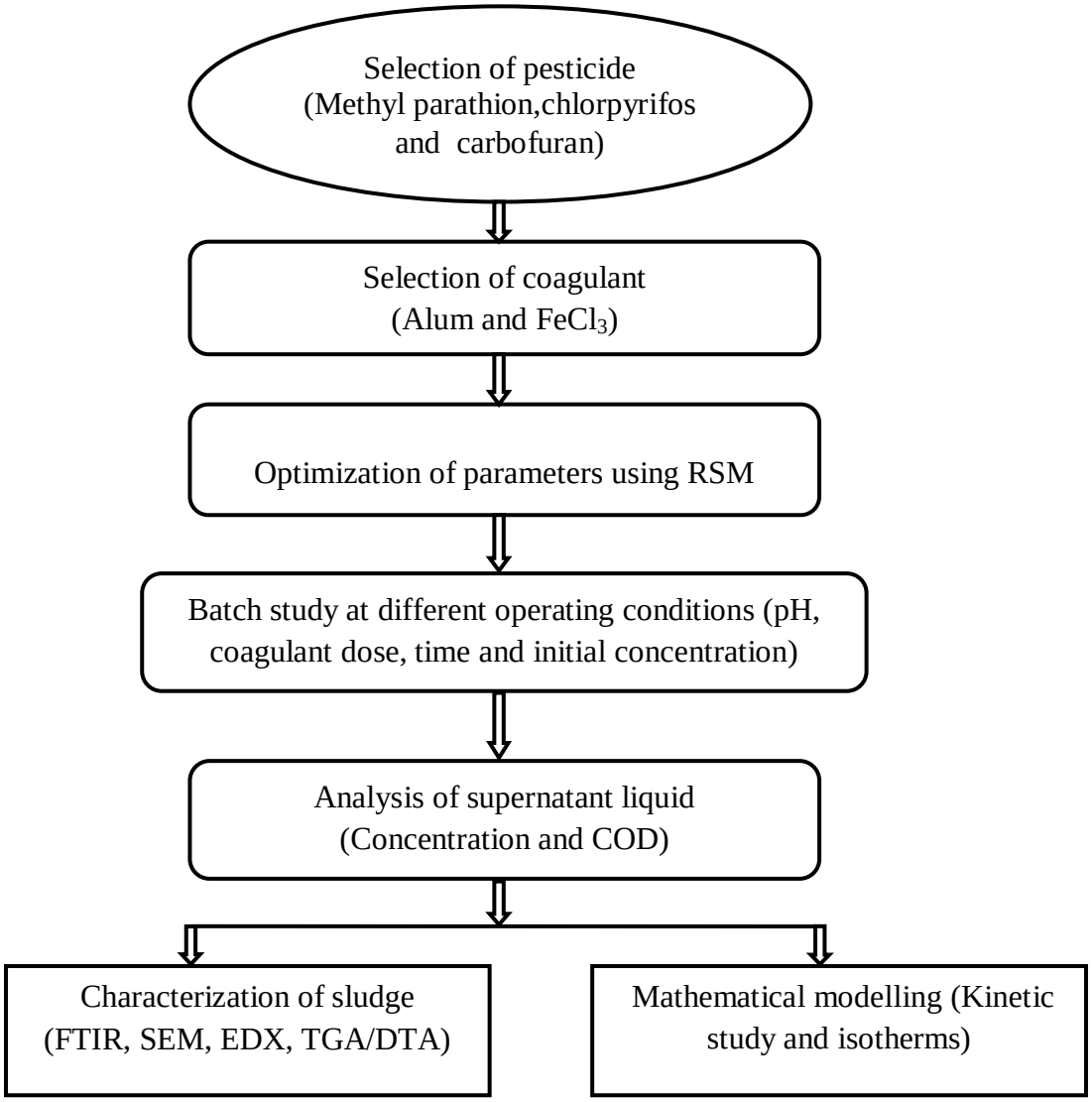


Figure 5.2: Schematic block diagram for treatment of simulated wastewater using coagulation-flocculation process.

5.4.2 Experimental set-up

Batch studies of coagulation/flocculation process on methyl parathion, chlorpyrifos and carbofuran reduction from simulated wastewater were conducted. The experiments were carried out using a standard jar-test apparatus (Model: IK-155, IKON, Delhi, India) equipped with six paddle gang stirrers and jars as shown in Figure 5.3. All runs were conducted at 25 ºC. In jar-test apparatus, six beakers of 1000 mL capacity were placed and filled with 200 mL of simulated wastewaters for further studies. Synthetic wastewater of methyl parathion 11.4×10^{-5} M (30 mg/L), chlorpyrifos 8.65×10^{-5} M (30 mg/L) and carbofuran 13.5×10^{-5} M (30 mg/L) concentrations were prepared in double distilled water. Coagulant doses were varied in the range 12.3×10^{-5} to 7.40×10^{-5} M for $FeCl_3$ and 3×10^{-5} to 18×10^{-5} M for alum, respectively and the pH varied between 4 and 9. The initial pH of the wastewater was adjusted by adding either 1M H_2SO_4 (for acidic region) or 1 M NaOH (for basic region). After pH adjustment, coagulants, alum or ferric chloride were added in various doses into each beaker. The test solutions of pesticides were treated with various coagulant dosages and analyzed. The coagulation/flocculation process was organized in three stages: in the first stage flash mixing of the coagulant was done at 120 rpm for 2 min. This was followed by the second stage of slow mixing at 40 rpm for 30 min. In the final stage the flocs were allowed to settle down for half an hour and then filtered using Whatman No. 42 filter paper prior to analysis. reduction efficiency of pesticides was calculated by the given Equation (5.1):

$$\text{Reduction } (\%) = \frac{C_i - C_t}{C_i} \times 100 \qquad (5.1)$$

where, C_i are the initial COD and concentration in influent and C_t are the COD and concentration value at time t in treated samples. Pesticide concentration was measured

using HPLC technique as detailed in Section 5.8.2 and COD was measured using photometer as detailed in Section 5.8.3.

Figure 5.3: Jar-test apparatus for coagulation-flocculation process.

5.4.2.1 Effect of pH

The effect of pH on COD and concentration reduction of pesticides was observed in the pH range of 4 to 9. The required values of pH were maintained by adding known volume of 1 M H_2SO_4 or 1 M NaOH.

5.4.2.2 Effect of coagulant dose

Coagulant doses were varied from 12.3×10^{-5} to 74×10^{-5} M (20−120 mg/L) for $FeCl_3$ and 3×10^{-5} to 18×10^{-5} M (20−120 mg/L) for alum, to investigate the influence of coagulant doses on COD and concentration reduction of pesticide.

5.4.2.3 Effect of operating time and initial pesticide concentration

The initial concentrations of pesticides were varied in the range of $3.8 \times 10^{-5} - 19 \times 10^{-5}$ M for methyl parathion, $2.85 \times 10^{-5} – 14.3 \times 10^{-5}$ M for

chlorpyrifos and $4.52 \times 10^{-5} - 22.6 \times 10^{-5}$ M for carbofuran to observe the effect on reduction efficiency of pesticides. Time was varied from 0 − 120 min in each case.

5.5 Fenton Oxidation Study

5.5.1 Experimental programme

Figure 5.4 depicts the complete sequence of the Fenton oxidation steps for the reduction of pesticides in batch process.

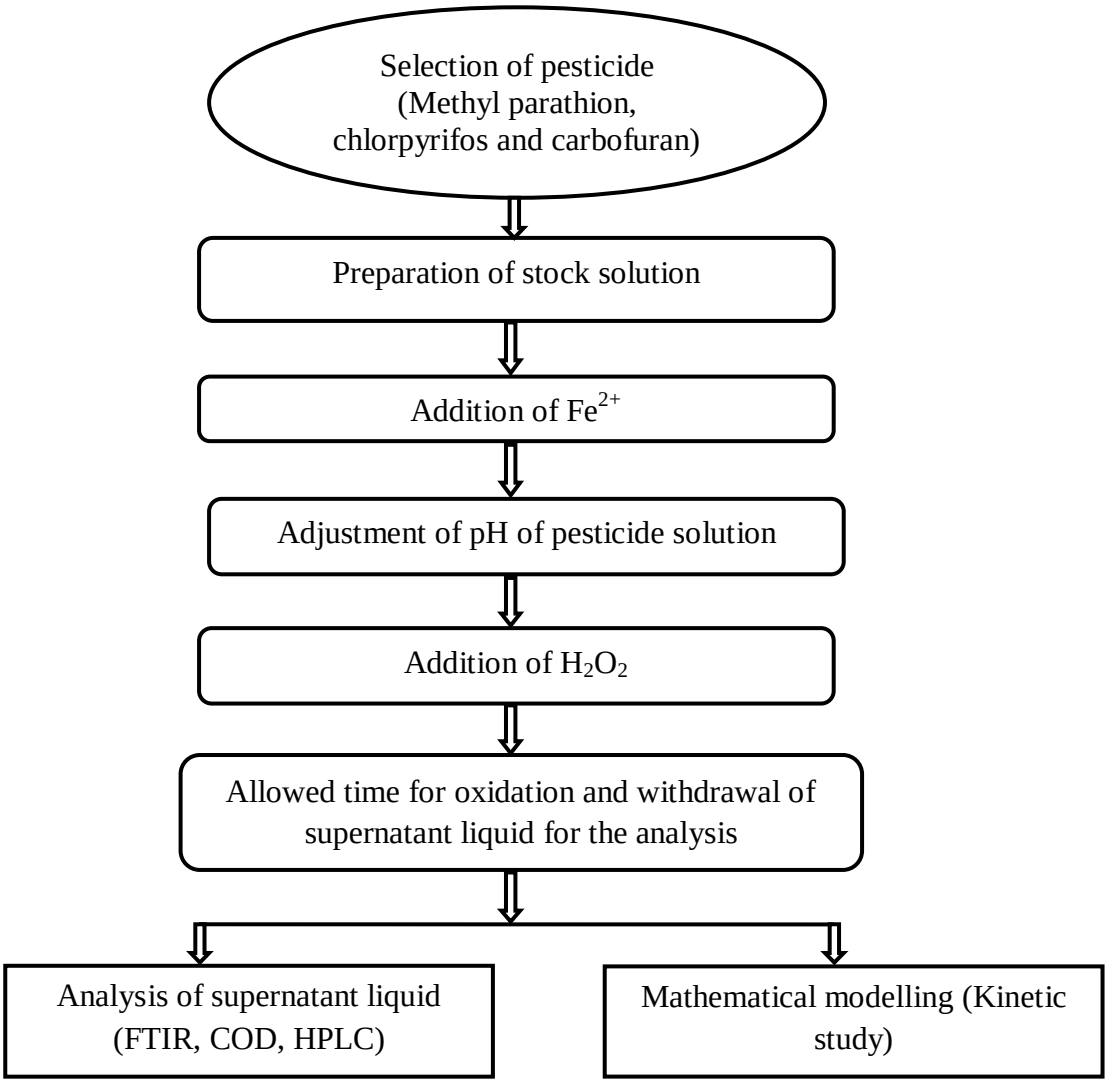


Figure 5.4: Schematic block diagram for treatment of simulated wastewater using Fenton oxidation.

5.5.2 Experimental procedure

In each case hydrolysis experiments were performed at an initial pesticide concentration of 30 mg/L. The equivalent molar concentration were 11.4×10^{-5} M for methyl parathion, 8.65×10^{-5} M for chlorpyrifos and 13.5×10^{-5} M for carbofuran. The Fenton experiments were conducted in a 250 mL reactor with 100 mL solution at a 25 ºC. Schematic diagram of the system is shown in Figure 5.5. For each run fresh stock solution was prepared. In the first step, $FeSO_4.7H_2O$ was added for the generation of catalyst (Fe^{2+}) in simulated wastewater. The solution pH was adjusted by adding sulfuric acid (1 M H_2SO_4) or sodium hydroxide (1 M NaOH) as per the requirement. Then H_2O_2 was added to the sample after the complete dissolution of $FeSO_4.7H_2O$ for facilitating the oxidation reaction. Magnetic stirrer was used to conduct the Fenton oxidation at 300 rpm. Effects of pH, Fe^{2+} and H_2O_2 dosages, initial pesticide concentration, temperature and reaction time were studied. Reaction time was kept at 60 min in each experiment.

The supernatant liquid was withdrawn periodically at intervals of 0, 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 min. The pH of supernatant liquid was immediately increased above 9 before filtering and analysis to prevent further oxidation of organic compounds during the measurement process (concentration, COD and H_2O_2). Above pH 9, hydrogen peroxide decomposes to oxygen and water (Shen et al., 2013; Sanchis et al., 2014). Also, before COD analysis, the supernatant liquid was heated on the water bath at 80 ºC for 40 min to eliminate the residual H_2O_2. The residual concentration of pesticide (methyl parathion, chlorpyrifos and carbofuran) was quantified by HPLC method (Shen et al., 2013).

The following equations were used to calculate the percent reduction of pesticide concentration (E_1) and COD (E_2):

$$E_1\ (\%) = \left(1 - \frac{C_t}{C_i}\right) \times 100 \tag{5.2}$$

$$E_2(\%) = \left(1 - \frac{COD_t}{COD_i}\right) \times 100 \tag{5.3}$$

where, C_i and COD_i are the initial pesticide concentration and COD in simulated wastewater, respectively, whereas the C_t and COD_t are the pesticide concentration and COD at time t in treated solution.

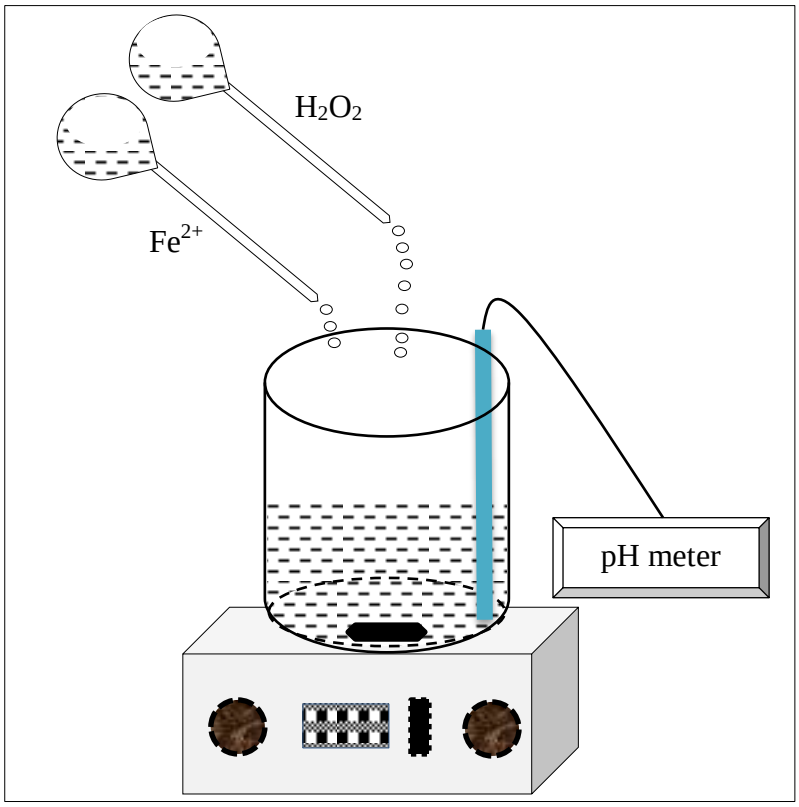


Figure 5.5: Schematic diagram for treatment of simulated wastewater by Fenton process.

5.5.2.1 Effect of pH

The effect of pH of the solution on reduction efficiency of pesticides was investigated in the pH range of 2 to 7 in a batch reactor. The pH was adjusted using required amount of 1 M H_2SO_4 or 1 M NaOH.

5.5.2.2 Effect of H_2O_2 concentration

The influence of H_2O_2 concentration on reduction efficiency of pesticides was investigated by varying its concentration in the range of 3.36×10^{-1} to 8.16×10^{-1} M in a batch reactor.

5.5.2.3 Effect of ferrous iron dosage

Batch experiment was performed to study the effect of Fe^{2+} doses which varied in the range of 3.60×10^{-3} to 14.4×10^{-3} M.

5.5.2.4 Effect of pesticide concentration

Initial pesticides concentrations were varied in the range of $3.8 \times 10^{-5} - 19 \times 10^{-5}$ M for methyl parathion, $2.85 \times 10^{-5} - 14.3 \times 10^{-5}$ M for chlorpyrifos and $4.52 \times 10^{-5} - 22.6 \times 10^{-5}$ M for carbofuran to observe the effect of concentration.

5.5.2.5 Effect of temperature

The effect of temperature on reduction of COD and concentration of pesticides by Fenton oxidation was investigated in the range of 15 to 45°C.

5.5.4 Kinetic models

Fenton oxidation includes multiple-step reactions with different rate constants; therefore the study of the kinetics of Fenton oxidation is intricate. It has been reported (Lu et al., 2011b; Gozzi et al., 2012), that the degradation of pesticides follows pseudo-first order kinetics. The reaction between degraded substance and hydroxyl radicals ($HO^{\bullet}$) determines the overall rate of reaction. Hydroxyl radicals ($HO^{\bullet}$) in the reaction are consumed and supposed to reach a constant concentration in the solution (Mitsika et al., 2013). In the present study first order, second order and Behnajady-Modirshahla-Ghanbery (B-M-G) kinetic relations, were used to study the degradation kinetics of methyl parathion, chlorpyrifos and carbofuran by the Fenton process

(Behnajady et al., 2007; Ertugay and Acar, 2013; Arat and Bicer, 2015). The relevant expression are given below.

First-order kinetic model:

$$-\frac{dC}{dt} = k_1 C \tag{5.4}$$

$$\ln\left(\frac{C_0}{C}\right) = k_1 t \tag{5.5}$$

Second-order kinetic model:

$$-\frac{dC}{dt} = k_2 (C)^2 \tag{5.6}$$

$$C = C_0 e^{-k_1 t} \tag{5.7}$$

$$\frac{1}{C} = \frac{1}{C_0} + k_2 t \tag{5.8}$$

Behnajady-Modirshahla-Ghanbery kinetic model:

$$\frac{C}{C_0} = 1 - \frac{t}{m+b} \tag{5.9}$$

$$\frac{t}{1-(C/C_0)} = m + bt \tag{5.10}$$

where, C_0 is the initial concentration of pesticide and C is the final concentration of pesticide in solution at reaction time t. k_1 and k_2 are reaction rate constants of first-order and second order kinetic models, respectively; m and b are the two characteristic constants of the B-M-G kinetic model relating the oxidation capacities and reaction kinetics.

5.6 Coupled Fenton and Coagulation Study

Experiments using Fenton oxidation followed by coagulation-flocculation were performed (both at their respective optimum conditions) to observe the effect on the reduction efficiency of pesticides concentration and COD. Figure 5.6 shows the complete sequence of the coagulation process followed by Fenton oxidation.

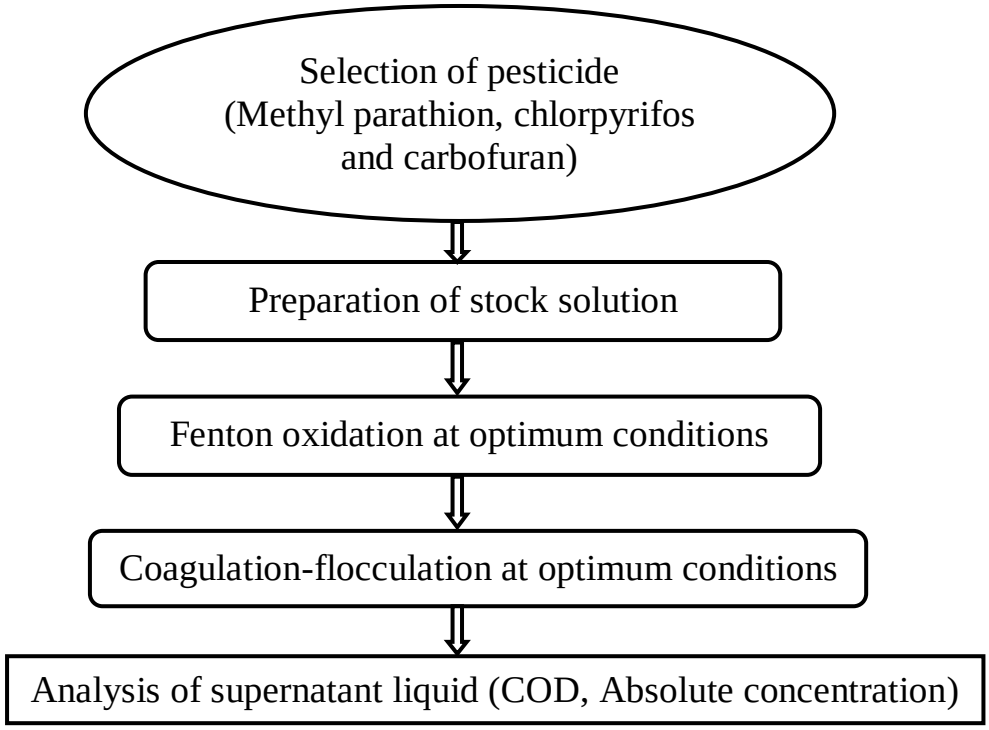


Figure 5.6: Schematic block diagram for treatment of simulated wastewater using coupled Fenton and coagulation process.

5.7 Toxicity Study

The toxic effects of methyl parathion, chlorpyrifos and carbofuran on human cells and red blood count (RBC) were investigated. Details of the procedure adopted for toxicity analysis is described below.

5.7.1 Cell lines and cell culture

Human glioblastoma cells U-87 were originally purchased from American Type Culture Collection (ATCC), Manassas, Virginia, USA. The tumor cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA) with supplements including 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA). This culture medium was hence forth considered as complete medium. The tumor cells were mycoplasma free. Human peripheral blood mononuclear cells

(PBMC) and dendritic cells (DC) were isolated from peripheral blood as described elsewhere (Manna et al., 2002).

5.7.2 Cell proliferation assay

Proliferation of U-87 cells in presence of methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives formed after coagulation and Fenton oxidation was studied by MTT assay. 5×10^3 U-87 cells/well were added in a 96-well tissue culture plate and were exposed to serial concentrations of 1, 2, 4, 8, and 16 μM of methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives (Manna et al., 2002). Plates were incubated at 37 °C with 5% CO_2, for 48 h. CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega, USA) was used to assess the cell proliferation. The tissue culture plates were incubated for 4 h in presence of MTT reagent and the absorbance was measured at 570 nm using Synergy HT Multi-Mode Micro plate Reader BioTek®, USA. Growth inhibition of tumor cells was determined from the formula (Hira et al., 2014):

$$\%\ \text{Growth Inhibition} = [\,1 - \frac{\text{Experimental OD570}}{\text{Target OD570}}\,] \times 100 \qquad (5.11)$$

The 'experimental OD' value is the reading of the tumor cells exposed to various concentrations of carbofuran and its derivatives and the 'target OD' represents the corresponding value of tumor cells cultured in medium only.

5.7.3 In-vitro cytotoxicity assay

The lytic activity of methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives against tumor cells was measured by non-radioactive cytotoxicity assay using the CytoTox 96 Non-Radioactive Cytotoxicity assay kit obtained from Promega, USA (Manna et al., 2013). Tumor (U-87) target cells (5×10^3) was added to 96-well tissue culture plate and exposed to serial concentrations of methyl

parathion, chlorpyrifos and carbofuran and their corresponding derivatives and incubated for 18 h at 37 ºC, 5% CO_2. Percent-specific lysis was determined using the following formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous})}{(\text{Target Maximum} - \text{Target Spontaneous})} \times 100 \qquad (5.12)$$

5.7.4 Cell viability assay

The effect of methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives on the viability of normal human DC and PBMC was evaluated by a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) assay (Roche Molecular Biochemicals, Indianapolis, IN). Cells were added (5×10^3 cells/well) in a 96-well culture plate and exposed to different concentrations of methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives and incubated at 37 ºC, in 5% CO_2, for 18 h. Optical density (OD) was measured at 450 nm using Synergy HT Multi-Mode Micro-plate Reader BioTek, USA (Hira et al., 2014). The data was presented as the percentage of viable cell calculated from the following formula:

$$\% \text{ Cell Viability} = \frac{\text{Experimental OD450}}{\text{Target OD450}} \times 100 \qquad (5.13)$$

5.7.5 Hemolysis assay

For time dependent kinetics, 5 μM methyl parathion, chlorpyrifos and carbofuran and their corresponding derivatives were incubated with the blood sample. For concentration dependent kinetics, the blood sample was incubated with varying concentrations (2.5, 5 and 10 μM) of the compounds for 4 h. Hemolysis assay was performed according to the standard protocol (Kuznetsova et al., 2012; Kumar et al., 2015). In brief, an aliquot of each blood sample was centrifuged for 5 min. 25 μL plasma aliquot was diluted with 225 μL Drabkin's reagent (Sigma) in a 96-well

culture plate and mixed for 2 min under lateral agitation (300 rpm). After 10 min equilibration at room temperature, optical density was recorded at 540 nm in Synergy HT Multi-Mode Microplate Reader BioTek, USA. Blood hemoglobin was determined by measuring the absorbance of 100 fold dilution of the whole blood in Drabkin's reagent at 540 nm. Saponin (2 mg/mL final blood concentration) and PBS were used as positive and negative controls, respectively. A sample of plasma without additives was considered as the basal condition. The standard calibration curve was obtained with the solution containing 0.07–3.8 mg/mL bovine hemoglobin treated with a Drabkin's reagent. The results are presented as percent hemolysis indicating the free plasma hemoglobin (mg/ml) and measured as released hemoglobin divided by the total blood hemoglobin (mg/mL) multiplied by 100. All measurements were performed in triplicate.

5.7.6 Statistical analysis

Experimental data on cytotoxicity, cell viability and hemolysis were stastically analyzed. In this study, n reflects the number of times the experiments were performed independently in triplicate. The mean ± SD were calculated for each experimental group (n = 3). Differences between the groups were analyzed by unpaired Student's t-test and one- or two-way ANOVA analysis of variance depending on the requirement. One or two-way ANOVA followed by Holm-Sidak post-hoc multiple comparison tests were used to conduct pair wise comparisons using PRISM statistical analysis software (Graph Pad Software, Inc., San Diego, CA, USA). Significant differences among the groups were calculated at $P<0.05$ or less (*=<0.05, **=<0.001).

5.8 Analysis and Instrumentation

In the present work high performance liquid chromatography was used for the analysis of pesticide concentration (methyl parathion, chlorpyrifos and carbofuran) in simulated wastewater. COD of the samples were determined by the microwave digestion method using photometer. pH meter was used for measurement of the pH of the solution.

The flocs generated after coagulation-flocculation process were characterized by using Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Thermo-Gravimetric Analysis (TGA) and Differential Thermal Analysis (DTA). The details of procedure and equipment used are discussed below.

5.8.1 pH meter

Digital pH meter (LI 614, ELICO) was used to measure the pH of the solution, using buffer solutions (pH 4.0, 7.0 and 9.1) for its calibration.

5.8.2 Determination of pesticide concentration

High performance liquid chromatography (HPLC: Thermo Ultima 3000) was used for the measurement of concentration of pesticides in fresh sample and treated water. UV–Visible detector and a C-18 column of Acclaim^{TM} 120 (4.6 × 250 mm, 5 µm, 120 Å) was used as the stationary phase in HPLC. Acetonitrile and HPLC grade water (70:30, v/v) were used as a mobile phase at a flow rate of 1 mL/min. The peaks observed at wavelength of 225 nm represented methyl parathion, at 220 nm for chlorpyrifos and at 276 nm for carbofurn. Data were collected at a pressure of 83 bar and frequency of 50 Hz.

5.8.3 COD analysis

COD values of methyl parathion, chlorpyrifos and carbofuran bearing samples, before and after the reactions were determined by the microwave digestion method

(RD 125, Loviband, Germany) using Lovibond 2420721 COD vario vials as per the procedure described in the operating manual using a double wavelength (430 nm, 610 nm) photometer system (Figure 5.7).

COD vario vials

Appropriate vial for the desired range were selected:

LR-Range: 0 - 150 mg/L

MR- Range: 0 - 1500 mg/L

HR- Range: 0 - 15000 mg/L

Preparation of samples and measurement

Blank was prepared using deionized water instead of the sample (LR/MR: 2 mL water sample, HR: 0.2 mL water sample). Then the contents were digested for 120 minutes in the reactor at a temperature of 150 ºC. The vials were removed from the reactor and cooled to 60 ºC or less. The contents were mixed by inverting each vial several times, while still warm. The vials were cooled to ambient temperature before measuring. The required test was selected using the [MODE] key in the adapter. Blank was placed in the adapter then press the [ZERO/TEST] key. After calibration the blank vial from the adapter was removed and sample vial was placed for COD analysis. The photometer measured the COD in mg/L for LR/MR-range samples and in g/L for HR-range samples.

Figure 5.7: Digester and photometer for COD measurement.

5.8.4 FTIR analysis

FTIR (Thermo: NICOLET 5700) spectrophotometer was employed to determine the presence of functional groups/organic bonds at room temperature from FTIR spectra. The pellets for analysis were prepared by mixing the adsorbent in KBr pellets.

5.8.5 SEM/EDX analysis of flocs

The settled flocs obtained from jar-tests were composed and dried for SEM analysis. In order to determine the surface morphology of flocs after coagulation-flocculation, the samples were scrutinized using SEM. The SEM and EDX analysis was performed using model EVO 18 research ZEISS, with an X-rays detector of energy dispersion.

5.8.6 TGA/DTA analysis of flocs

Thermal analysis was conducted using the thermal analysis instrument (NETZSCH: STA 449 *F*3 Jupiter). The TGA and DTA data and plots obtained from the instrument were used to obtain relevant information. The high quality heating element of the furnace of a rhodium alloy allows maximum furnace temperature of 1650 ºC. DTA measures the difference in temperature between a sample and a thermally inert

reference as the temperature is raised. The plot of this differential provides information on exothermic and endothermic reactions taking place in the sample. Temperature for phase transitions melting point, crystallization can be determined using the computer controlled graphics package. Typical heating rate is 10 ºC per minute, optimum sample size was 40 to 80 mg, ideally, the sample should be ground to 100 mesh. TGA measures the change in weight of sample with increasing temperature. Moisture content and presence of volatile matter can be determined by this technique. Maximum temperature is 1200 ºC. Typical heating rate is 10 ºC per minute. Optimum sample size was 40−80 mg. Gas input pressure was maintained at 0.5 bar.

5.8.7 H_2O_2 determination

Residual H_2O_2 concentration was determined through the vanadate method using double beam UV−Visible spectrophotometer (LMSP−UV1900) at 450 nm (Brandhubber et al., 2009).

Chemicals: The colouring reagent, vanadium pentoxide solution, was prepared by dissolving 0.2 g of vanadium pentaoxide (V_2O_5) in 100 mL 0.5 mol/L H_2SO_4.

Analytical procedure: A 5 mL of each effluent sample was mixed with 1 mL of 0.5 mol/L H_2SO_4 in a vial and then, the mixture was centrifuged at 8000 rpm for 5 min. A micro-syringe was used to take a measured volume of supernatant and add it to a 5mL aliquot of colour agent. Mixing of the solutions was achieved by manual shaking (Zhang et al., 2013).

Determination of H_2O_2: H_2O_2 reacts with V_2O_5 in H_2SO_4 solution and generates a peroxovanadate complex of red-brown colour. It gives maximum absorbance in the UV−Vis at 450 nm (Brandhubber and Korshin, 2009). A simple standard calibration curve was developed for H_2O_2 determination. In the present work, the calibration curve

was obtained based on adding different contents (0 to 0.9 mg/L) of the standard H_2O_2 solution in colour reagent. The results are shown in Figure 5.8. From the data of standard calibration curve, the value of residual H_2O_2 in treated sample after Fenton oxidation was found out.

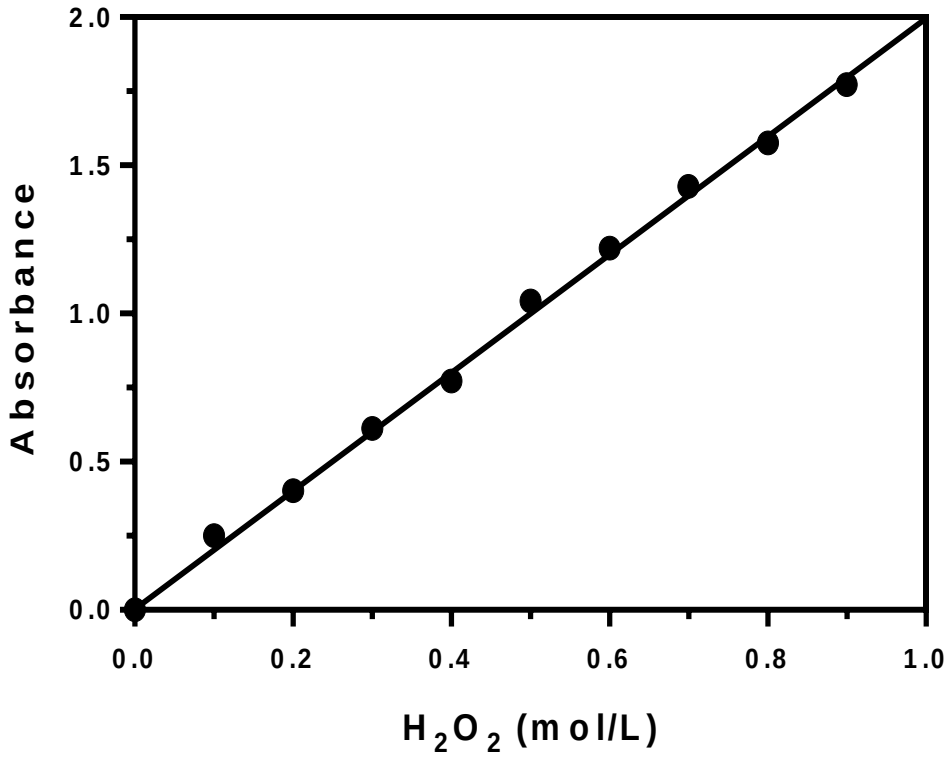


Figure 5.8: Standard calibration curve for H_2O_2.