CERTIFICATION OF APPROVAL

Modified Photo-Fenton (Uv-Vis/Ferrioxalate/H_2O_2) Treatment of Recalcitrant Wastewater

By
Muhammad Zulhasri Bin Abd Wahap

A project dissertation submitted to the
Civil Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
(CIVIL ENGINEERING)

Approved by,

(Prof. Dr. Malay Chaudhuri)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
September 2011

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

Muhammad Zulhasri Bin Abd Wahap

ABSTRACT

Antibiotics can reach the aquatic environment through various sources such as antibiotic industry, hospital effluent and excretion from humans and livestock. The removal of antibiotics from wastewater has not been effective due to their relatively low concentrations and because the conventional wastewater treatment plant (WTP) is not designed to remove them. The problem associated with the presence of antibiotics in low concentrations in the environment is the development of antibiotic resistant bacteria which could lead to severe health problem. The study examined the effectiveness of modified photo-Fenton treatment of an antibiotic wastewater containing amoxicillin and cloxacillin and determined the optimum operating conditions (H_2O_2/COD molar ratio 2.75, H_2O_2/Fe^{3+} molar ratio 75, $H_2O_2/C_2H_2O_4$ molar ratio 37.5, irradiation time 90 min and pH 3) of the treatment. A series of experiments modeled by the RSM software were conducted to obtain the optimum operating conditions. Then, under the optimum operating conditions, efficiency of modified photo-Fenton treatment of the antibiotic wastewater was tested. The efficiency and optimum operating conditions of the modified photo-Fenton treatment were then compared to the efficiency and optimum operating conditions of Fenton and photo-Fenton treatments. The study has found that the biodegradability improved from ~0 to 0.35 in 90 min, and the COD, NH_3-N and TOC removal were 78.85%, 46.85% and 52.30%, respectively.

ACKNOWLEDGEMENT

First and foremost, the highest gratitude goes to God the Almighty for His mercy and kindness for allowing me to successfully complete the project within the time frame. I believe that the experience gained from the project will be very useful for me in the future career and endeavors. Hopefully the information provided in this dissertation will be a good reference for future research especially in the research related to the treatment of recalcitrant wastewater and the application of advance oxidation processes.

Apart from the efforts of me, the successful completion of this project has been made possible through the help and support from many individuals and organizations. Here, I would like to take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this project.

The utmost appreciation goes to the most influential person behind the successful completion of this project, my FYP supervisor, Prof. Dr. Malay Chaudhuri. Prof. Dr. Malay Chaudhuri has been very supportive and helpful throughout the two semesters of the FYP and I have learned a lot of things both related and nonrelated to the FYP.

In addition to that, I would also like to acknowledge the assistance and guidance from Mr. Affam Agustine, the Graduated Assistance who I am attached to. His relentless help and support will always in my memory and will not be forgotten.

At this opportunity, I would also like to thank the cooperation given by the lab technicians, friends and other individuals. Besides, thanks to my family members and also friends who always believed in and constantly supported the work throughout the two semesters. All of the support and help were vital for the success of the project.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

1.1.1 Recalcitrant Wastewater

The term recalcitrant means difficult or obstinate, not easy to control (Mara & Horan, 2003). Recalcitrant wastewater can be defined as the wastewater that is difficult to be degraded by microorganisms in ordinary biological treatment. This happens because the recalcitrant wastewater is toxic to the microorganisms. The examples of recalcitrant substances are pharmaceuticals, synthetic fragrances, pesticides and drugs.

Pharmaceuticals can reach the aquatic environment through various sources, such as pharmaceutical wastewater, hospital effluent and excretion from humans and livestock (Ikehata et al., 2006; Nikolaou et al., 2007; Yang et al., 2008). Pharmaceuticals including antibiotics and other drugs have been observed in surface water (Kolpin et al., 2002; Anderson et al., 2004; Rabiet et al., 2006), groundwater (Rabiet et al., 2006), sewage effluents (Carballa et al., 2004; Nikolaou et al., 2007) and even in drinking water (Stackelberg et al., 2004).

Among all the pharmaceutical drugs that cause contamination of the environment, antibiotics occupy an important place due to their high consumption rates in both veterinary and human medicine (Elmolla & Chaudhuri, 2009). Antibiotics are a group of chemical produced by microorganisms and commercially produced synthetically or semi-synthetically (Ashnaga & Naseri, 2007). From the various types of antibiotics, Penicillin has since become the most widely used antibiotic to date and is still used for many Gram-positive bacterial infections (Ashnaga & Naseri, 2007). Amoxicillin and cloxacillin are semi-synthetic penicillin obtaining their antimicrobial properties from the presence of a beta-lactam ring (Elmolla & Chaudhuri, 2009).

1.1.2 Photocatalytic Oxidation

Biological treatment is limited to wastewaters which contain biodegradable substances and which are not toxic to the biological culture. Antibiotic wastewater has high chemical oxygen demand (COD) and low biochemical oxygen demand (BOD), and hence biological treatment is unsuitable for the wastewater (Elmolla & Chaudhuri, 2009). Photocatalytic oxidation or advanced oxidation processes (AOPs) such as Fenton, photo-Fenton and Fenton-like processes constitute a promising technology for the treatment of wastewaters containing non-easily removable (non-biodegradable) organic compounds (Pera-Titus et al., 2004).

Photocatalytic oxidation enables the advanced destruction of the majority of organic pollutants into carbon dioxide (CO_2), water and some inorganic compounds at room temperature and atmospheric pressure (Pasieczna-Patkowska et al., 2009). It also has been demonstrated that semiconductor photocatalytic oxidation of organic substances can be an alternative to conventional methods of removal of organic pollutants from water (Ollis et al., 1991). Additional advantages of the photocatalytic process are its mild operating conditions and the fact that the semiconductor can be activated by sunlight (near UV), thus reducing significantly the electric power requirement and, hence the operating cost (Goswami, 1995).

1.2 Problem Statement

1.2.1 Problem Identification

There are growing concerns about the fate of antibiotics in the environment and their possible effects on the aquatic ecosystem. This is because, in recent years, antibiotics are extensively used in human and veterinary medicine (>50,000,000 lbs/yr produced in US (Karthikeyan & Meyer, 2006), >700,000 kg/yr used in Australia (JETACAR, 1999)). In addition, antibiotics including their precursor compounds and transformation products are discharged into the environment intentionally and unintentionally during manufacturing processes and through consumption or disposal of used and unwanted drugs (Daughton & Ternes, 1999). Besides, most of the antibiotics are poorly absorbed by humans and animals after intake, with about 25% to 75% of added compounds leaving the organisms unaltered via feces or urine (Chee-Sanford et al., 2001). Pharmaceuticals including antibiotics and other drugs have been observed in surface water (Kolpin et al., 2002; Anderson et al., 2004; Rabiet et al., 2006), groundwater (Rabiet et al., 2006), sewage effluents (Carballa et al., 2004; Nikolaou et al., 2007) and even in drinking water (Stackelberg et al., 2004).

Problem that may be created by the presence of antibiotics at low concentration in the environment is the development of antibiotic resistant bacteria (Walter & Vennes, 1985). In recent years, the incidence of antibiotic resistant bacteria has increased and many people believe the increase is due to the use of antibiotics (Alexy et al., 2004). Antibiotic-resistant bacteria are emerging as important waterborne contaminants (Pruden et al., 2006; Sapkota et al., 2007; Li et al., 2009). Acquisition and further spread of antibiotic resistance determinants among pathogens is becoming one of the most relevant problems for treatment of infectious diseases (WHO, 2007; Kumarasamy et al., 2010). In addition, antibiotic resistance in organisms which are not considered primary pathogens is also important because of their ability to potentially transmit resistance to other organisms by means of transmissible resistance factors (Se´veno et al., 2002; Bennett, 2008; Martı´nez, 2008).

1.2.2 Significance of the Project

Various treatment techniques can be applied to purify the wastewater containing pharmaceuticals compound. The advanced oxidation processes (AOPs) appear more practical in comparison with other techniques (activated carbon adsorption, air stripping and reverse osmosis) because these techniques only transfer the pollutants from one phase to another without destroying them. Biological treatment is limited to wastewater which contains biodegradable substances and which are not toxic to the biological culture (Elmolla & Chaudhuri, 2009).

For the time being, Fenton and photo-Fenton processes are regarded as promising technology for the treatment of wastewater containing recalcitrant (non-biodegradable) organic compounds (Chamarro et al., 2001). However, the modified-photo Fenton (UV-vis/ferrioxalate/H_2O_2) process was found to be more effective than the Fenton and photo-Fenton processes in the treatment of process water containing chlorobenzene, a tank-bottom water containing a mixture of benzene, toluene and xylenes (BTX), a wastewater containing 1,4-dioxane and another wastewater containing methanol, formaldehyde and formic acid (Safarzadeh-Amiri et al., 1997) and degradation of aniline wastewater (Zhang et al., 2002). The process was also applied in decolourisation of complex azo dyes and treatment of a dyehouse waste (Tripathi & Chauduri, 2004) and decolourisation of reactive dyes (Chaudhuri & Wei, 2009).

The ferrioxalate semiconductor absorbs light strongly at longer wavelength (up to $\lambda \approx 550$ nm) and generates hydroxyl radicals with high quantum yield (Safarzadeh-Amiri et al., 1997). The ferrioxalate complex, $Fe^{III}(C_2O_4)_3^{3-}$, is highly photosensitive and reduction of Fe(III) to Fe(II) through a photoinduced ligand to metal charge transfer can occur over the ultraviolet ray and into the visible light (out to $\lambda \approx 550$ nm) (Tripathi & Chauduri, 2004), thus reducing significantly the electric power requirement and hence, the operating cost (Goswami, 1995).

1.3 Objective of the Study

To obtain the optimum operating conditions and efficiency of modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process in treating and improving biodegradability of recalcitrant wastewater (antibiotic aqueous solution).

1.4 Scopes of the Study

- Experimental procedures and optimum operating conditions in treating antibiotic aqueous solution by modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process.

- Comparison between the effectiveness and optimum operating conditions of modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process and other AOPs in treating antibiotic aqueous solution by referring to the literature.

1.5 Relevancy of the Project

The presence of antibiotics at low concentration in the environment will cause the development of antibiotic resistant bacteria (Walter & Vennes, 1985). Antibiotic resistant genes and antibiotic resistant bacteria have been detected in wastewater samples (Zhang et al., 2009a,b; Auerbach et al., 2007; Brooks et al., 2007; Pruden et al., 2006; Reinthaler et al., 2003). Also, the release of antibiotic resistant organisms through wastewater effluents into streams has been previously reported (Gallert et al., 2005; Iwane et al., 2001). This is a very serious problem as the antibiotic resistant microorganism might be absorbed into the food chain and possibly reached the humans and animals. Therefore, treatment of the antibiotics wastewater is essential in providing clean water to the environment. This study is important because the modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process is expectantly having higher efficiency for antibiotic wastewater treatment.

1.6 Feasibility of the Project within the Scope and Time Frame

The following are the goals to be achieved for the project during the first four months (FYP 1) period:

- Review of literature related to the topic.
- Perform preliminary experiment to confirm that \modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process is able to oxidize the antibiotic aqueous solution.

In the remaining four months of the project (FYP 2), detailed oxidation experiments and data analysis will be conducted. The detailed oxidation experiments will focus on obtaining the following:

- Optimum pH
- Optimum irradiation time
- Optimum concentration of oxidant and catalyst

Basically, the project is feasible within the scope and time frame as proper planning has been done. In addition, all of the equipments and materials needed for the project are available in the Environmental Engineering Laboratory.

CHAPTER 2

LITERATURE REVIEW

2.1 Antibiotics

Antibiotic was first discovered by Alexander Fleming in 1928. Antibiotics are chemicals produced by microorganisms that kill other microorganisms by breaking down their cell walls. They are used to fight infections in humans and animals, since both serve as hosts to disease causing organisms such as bacteria, viruses, fungi and protozoa. Since the discovery of antibiotic by Alexander Fleming, a lot of researches had been done for development of antibiotics. In fact, those research and development activities are still rapidly continuing until today. Consequently, various types of antibiotics have been produced. The following Table 1 shows the classification of antibiotics according to their mode of action and structure.

Table 1: Classification of antibiotics (Morse, 2003).

Class	Mode of Action & Structure
β-lactams	Includes penicillin (amoxicillin, ampicillin and cloxacillin). They have low toxicity and quite effective. It contains a carboxyl group which makes them weak acids and has high water solubility.
Sulfa drugs	Includes sulfanilamide and sulfamethoxazole class of synthetic antibiotics. They could block folic acid synthesis & subsequently kill bacteria.
Quinolones	Such as ciproflaxacin, cipro, norfloxacin etc. They are DNA inhibitors which block action of bacterial enzymes that relaxes the coils of DNA for replication and repair
Aminoglycosides & Tetracyclines	These are products of actinomycetes, which are soil bacteria and synthetic products. It includes streptomycin, neomycin, chlorotetracycline, terramycine, e.t.c. They interfare with the sub-unit bateria ribosome. They also prevent activated amino acids to the ribosome thus halting protein synthesis

2.2 Antibiotics in the Environment

Nowadays, antibiotics are very important and essential as they are extensively used in human therapy and veterinary medicine. This situation is proven as more than 50,000,000 lbs/yr of antibiotics are produced in United State of America (Karthikeyan & Meyer, 2006) and more than 700,000 kg/yr of antibiotics are used in Australia (JETACAR, 1999)). Due to the high production and consumption of antibiotics, it presence in the environment also had become a world concern.

Antibiotics can reach the aquatic environment through various sources, such as pharmaceutical wastewater, hospital effluent and excretion from humans and livestock (Ikehata et al., 2006; Nikolaou et al., 2007; Yang et al., 2008). The presence of antibiotics and other pharmaceuticals had been observed in surface water (Kolpin et al., 2002; Anderson et al., 2004; Rabiet et al., 2006), groundwater (Rabiet et al., 2006), sewage effluents (Carballa et al., 2004; Nikolaou et al., 2007) and even in drinking water (Stackelberg et al., 2004). The following Figure 1 shows the exposure routes of human-use antibiotics into wastewater and the environment.

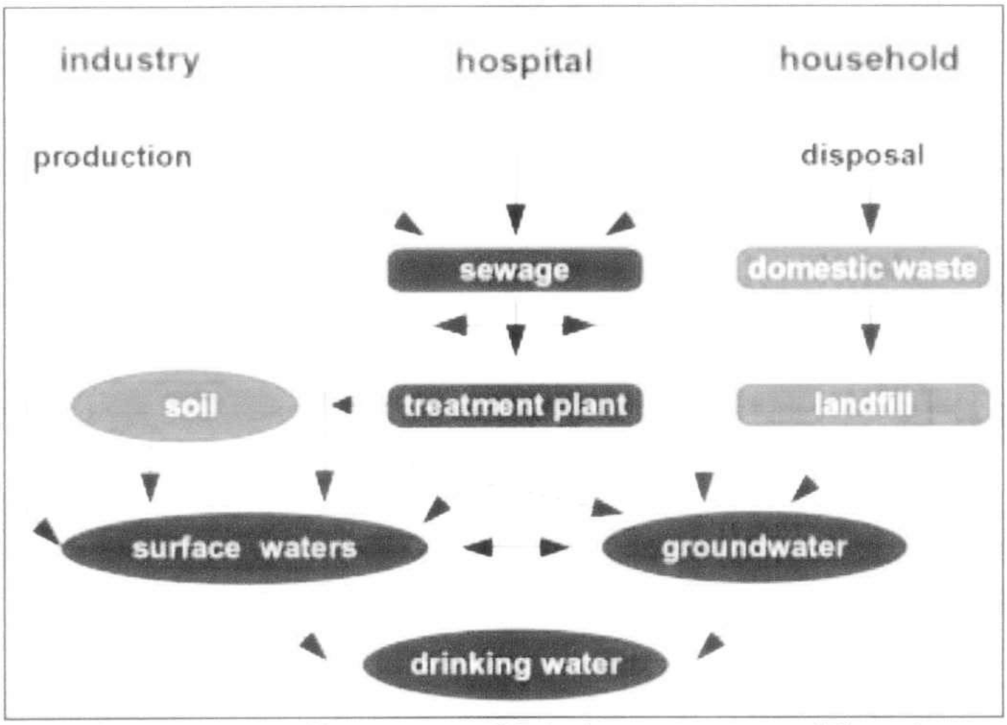


Figure 1: Exposure routes of human-use antibiotics into wastewaters and the environment (Giger et al., 2003).

2.3 Antibiotic Resistant Microorganisms

Due to the widespread use of antibiotics by humans, dramatic and global increase is observed in aquatic environments in reference to the number of antibiotics resistant bacteria, multiple antibiotic resistance, pathogenic bacteria resistance and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens (Meirelles-Pereira et al., 2002; Park et al., 2003; Schwartz et al., 2003; Dang et al., 2006). Antibiotic resistance has been observed in various aquatic environments including river and coastal areas, domestic sewage, surface water and sediments, lakes, sewage polluted ocean water, and drinking water (Merzioui & Baleux, 1994). In Malaysia, presence of antibiotic resistant bacteria in three (3) sewage treatment plants near Kuala Lumpur has been reported (Yaziz, 1981).

Resistance determinants present in gene-transfer units are auto-replicative elements that can be maintained in microbial populations unless they confer a fitness cost to the recipient bacteria. It has been shown that reducing the antibiotic load in natural ecosystems may reduce as well the amount of pollutant antibiotic resistant genes (Martinez, 2009). However, the declination is slow and part of the resistant population remains (Andersson, 2003) in the environment. This situation should be taken into attention as the incidence of outbreaks involving waterborne antibiotic-resistant bacteria has led to a serious problem of death of patients who do not respond to antibiotics (Morse, 2003).

2.4 Treatment of Antibiotic Wastewater

Previously, antibiotics received comparatively little attention as pollutants in the aquatic environment (Costanzo, 2005). However, their occurrence in aquatic systems has become a concern as biological impacts and potential risks to the environment, as well as to human health, have been reported (Hirsch et al., 1999; Boxall et al., 2003; Banik & Hossain, 2006). Because of that, it is very important to treat the antibiotic wastewater before it is discharged.

Conventional wastewater treatment processes are not designed to remove trace quantities of chemicals such as antibiotics and given the increasing number of reports of their presence in the environment. Because of that, it is essential that alternative technologies be developed which effectively degrade or remove these compounds (Stackelberg et al., 2004; Westerhoff et al., 2005; Choi et al., 2008). One such alternative is advanced oxidation processes (AOPs). AOPs will produce hydroxyl ($^{\cdot}OH$) radicals, which are capable of oxidizing almost any organic molecule yielding short-chain organic acids, inorganic ions and CO_2 as final products. Thus, AOPs can be employed as pretreatment before conventional biological treatment for discharging them to the receiving water bodies.

Table 2: Summary of selected antibiotic wastewater pretreatment by AOPs.

Wastewater	Treatment Method	Initial Conc.	pH	Removals Efficiency	Reference
Penicillin	Fenton/Photo-Fenton	1390 mg/L	3	COD 66%; TOC 52%	Alaton & Dogruel, (2004)
Amoxillin, Ampicillin & Cloxacillin	Fenton	520 mg/L	3	COD 81.4%; DOC 54.3% BOD_5/COD 0.37	Elmolla & Chaudhuri (2009a)
Amoxillin, Ampicillin & Cloxacillin	Photo-Fenton	520 mg/L	3	COD 80.8%; DOC 58.4% BOD_5/COD 0.40	Elmolla & Chaudhuri (2009b)
Amoxillin, Ampicillin & Cloxacillin	UV/ZnO	520 mg/L	11	COD 23.9 %; DOC 9.7% BOD_5/COD 0.036	Elmolla & Chaudhuri (2010a)
Amoxillin, Ampicillin & Cloxacillin	UV/TiO2 & UV/H2O2/ TiO2	520 mg/L	5	COD 26.3%; DOC 13.5% BOD_5/COD 0.10	Elmolla & Chaudhuri (2010b)

2.5 Modified Photo-Fenton Process

One of the latest developments of AOPs is the introduction of modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process. A few studies have been conducted on this AOP for treatment of recalcitrant wastewater such as water containing chlorobenzene, a tank-bottom water containing a mixture of benzene, toluene and xylenes (BTX), a wastewater containing 1,4-dioxane and another wastewater containing methanol, formaldehyde and formic acid (Safarzadeh-Amiri et al., 1997), aniline wastewater (Zhang et al., 2002), complex azo dyes and a dyehouse waste (Tripathi & Chauduri, 2004) and reactive dyes (Chaudhuri & Wei, 2009). No study has been conducted yet on this AOP for antibiotic wastewater treatment.

The modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process is expectantly having higher degradation efficiency because the ferrioxalate, is able to absorb light strongly at longer wavelength (up to $\lambda \approx 550$ nm) and generates hydroxyl radicals with high quantum yield (Safarzadeh-Amiri et al. 1997).

The ferrioxalate complex, $Fe^{III}(C_2O_4)_3^{3-}$ is highly photosensitive, and reduction of Fe(III) to Fe(II) through a photoinduced ligand to metal charge transfer can occur over the ultraviolet and into the visible (out to $\lambda \approx 550$ nm):

$$Fe^{III}(C_2O_4)_3^{3-} + h\nu \longrightarrow Fe^{2+} + 2C_2O_4^{2-} + C_2O_4^{\cdot -} \tag{1}$$

$$C_2O_4^{\cdot -} \longrightarrow CO_2^{\cdot -} + CO_2 \tag{2}$$

$$CO_2^{\cdot -} + Fe^{III}(C_2O_4)_3^{3-} \longrightarrow Fe^{2+} + CO_2 + 3C_2O_4^{2-} \tag{3}$$

The reactions can be collapsed into one reaction, since the short lifetime of the oxyl radical, $C_2O_4^{\cdot -}$, should preclude it from participation in other reactions, and its decarboxylation product, $CO_2^{\cdot -}$, is not involved in any other significant reactions:

$$Fe^{III}(C_2O_4)_3^{3-} + h\nu \longrightarrow Fe^{2+} + CO_2 + 2.5C_2O_4^{2-} \tag{4}$$

There are no other significant photochemical reactions (e.g., H_2O_2 photolysis) because the molar extinction coefficients of the reactants are such that ferrioxalate is the predominant absorber. The Fe^{2+} produced then generates hydroxyl radical, $^{\cdot}OH$, via the Fenton reaction:

$$Fe^{2+} + H_2O_2 + 3C_2O_4^{2-} \longrightarrow Fe^{III}(C_2O_4)_3^{3-} + OH^{-} + {}^{\cdot}OH \tag{5}$$

In presence of a sufficient excess of oxalate, Fe(III) will coordinate with either two or three oxalate ligands. As with the photo-Fenton reaction, iron cycles between oxidation states and so the production of hydroxyl radicals is limited only by the availability of light, H_2O_2 and oxalate, the latter two of which are depleted during the reaction.

CHAPTER 3

METHODOLOGY

3.1 Research Methodology

The response surface methodology (RSM) (Khuri & Cornell, 1996) was adopted for optimizing the treatment. This method is essentially a particular set of mathematical and statistical methods for designing experiments, building models, evaluating the effects of variables, and searching optimum conditions of variables to predict targeted responses. Specifically, central composite design (CCD), which is the most widely used form of RSM was employed to evaluate the effect of important process variables (irradiation time, catalyst and oxidant concentration) on chemical oxygen demand (COD), ammonia nitrogen (NH_3-N) and total organic carbon (TOC) removals efficiency. The following are the steps for project of the modified photo-Fenton treatment of antibiotic aqueous solution.

3.1.1 Determination of Range of the Variables

The appropriate ranges for the variables were obtained from preliminary experiment with reference to prior studies which also involved the modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process. Previously, the process had been used for treating process water containing chlorobenzene, a tank-bottom water containing a mixture ofbenzene, toluene and xylenes (BTX), a wastewater containing 1,4-dioxane and another wastewater containing methanol, formaldehyde and formic acid (Safarzadeh-Amiri et al., 1997), degradation of aniline wastewater (Zhang et al., 2002), decolourisation of complex azo dyes and treatment of a dyehouse waste (Tripathi & Chauduri, 2004) and decolourisation of reactive dyes (Chaudhuri & Wei, 2009).

Initially, the pH of the aqueous solution was fixed at pH 3 because the optimum pH for the previous studies was in the range of pH 2.8 to pH 4. Table 3 shows the appropriate range for the other variables.

Table 3: Appropriate range for process variables.

Process Variable	Unit	Low Level (-1)	High Level (+1)
H_2O_2/COD	Molar Ratio	1.5	4.0
H_2O_2/Fe^{3+}	Molar Ratio	50	100
$H_2O_2/C_2H_2O_4$	Molar Ratio	25	50
Irradiation time	Min	60	120

3.1.2 Design of the Experiment by Using RSM

The CCD was used for the RSM in the experimental design. The CCD with three factors at three levels was applied using Design-Expert® software 6.0 (trial version) with the bounds of the independent variables. Each independent variable was coded at three levels between −1 and +1 at the selected ranges: H_2O_2/COD, 1.5-4.0 molar ratio; H_2O_2/Fe^{3+}, 50-100 molar ratio; $H_2O_2/H_2C_2O_4$, 25-50 molar ratio; irradiation time, 60-120 min. The total number of experiments with three factors was obtained as 30 ($=2^k+2k + 6$), where k is the number of factors (=4). Twenty four experiments were augmented with six replications at the design center to evaluate the pure error and carried in randomized order as required in many design procedure.

3.1.3 Experimental Procedure

Batch experiments were conducted in a SolSim solar simulator photoreactor (Luzchem Research Inc. Gloucester, ON, Canada) with a 250 mL beaker containing 200 mL of the antibiotic aqueous solution. The required amount of $H_2C_2O_4$ and Fe^{3+} were added to the aqueous solution and mixed by a magnetic stirrer to ensure complete homogeneity during reaction. Thereafter, necessary amount of H_2O_2 was added to the mixture simultaneously with pH adjustment to the required value by using H_2SO_4 or NaOH. The time at which H_2O_2 was added to the solution is considered as the beginning of the experiment. Sample was taken out from the SolSim photoreactor when the pre-selected time is reached and filtered through 0.45 μm membrane filters and tested for chemical oxygen demand (COD), ammonia nitrogen (NH_3-N) and total organic carbon (TOC).

3.1.4 Optimization of the Operating Conditions

The results of each experiment obtained from step 3.1.3 were extracted to get the efficiency. The efficiency was then entered into the RMS software and the software through the CCD obtained the optimum operating conditions of the treatment. Besides, the results also were evaluated by the application of analysis of variance (ANOVA). The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent to the measurements of the generated responses. It is important to be performed because ANOVA will determine whether the results obtained are reproducible model or not.

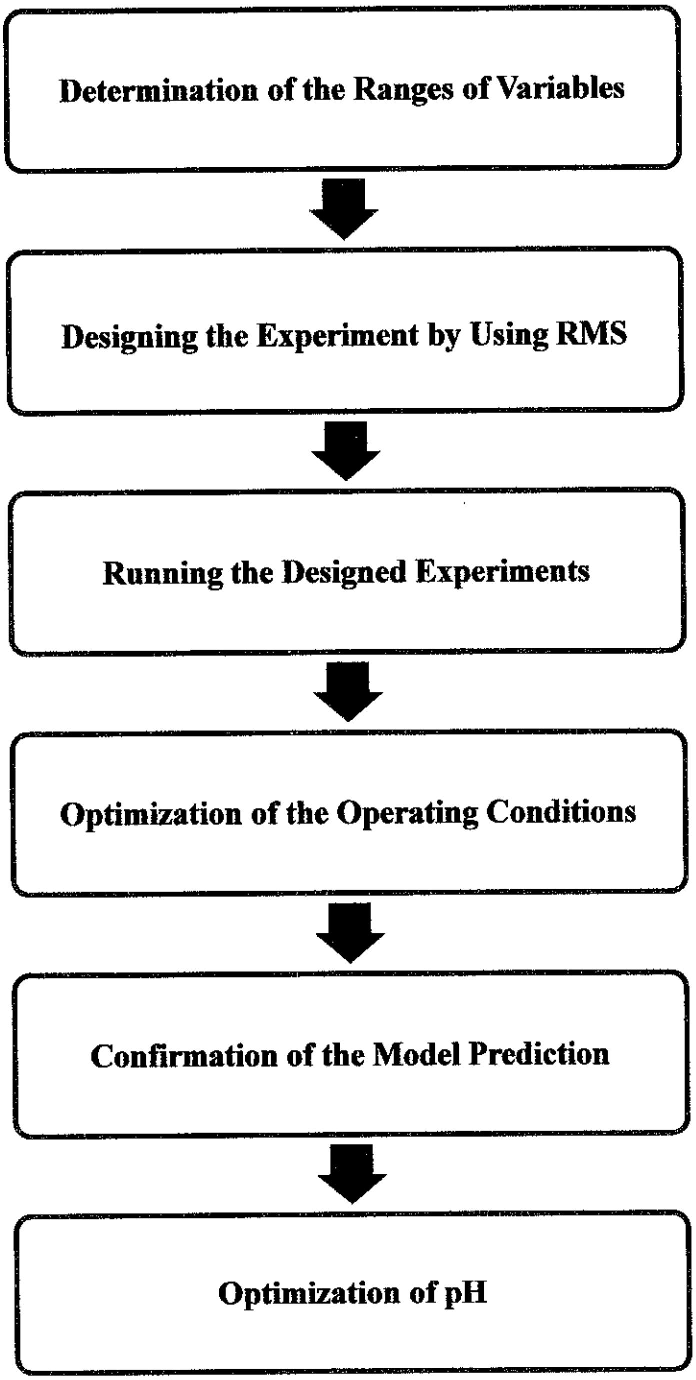
3.1.5 Confirmation of the Model Prediction

Experiments were conducted under the optimum operating conditions in order to know the experimental removals efficiency of the modified photo-Fenton process on antibiotic aqueous solution. Then, all data will be recorded and the model prediction comparison under optimum operating conditions was done.

3.1.6 Optimization of pH

After the optimum concentration of $H_2C_2O_4$, concentration of Fe^{3+} concentration of H_2O_2 and the irradiation time were obtained; the optimum operating conditions were used in order to get the optimum operating pH of the modified photo-Fenton treatment of the antibiotic aqueous solution. A series of experiments were conducted at different pH (2, 3, 4 and 5). The efficiency of the treatment for each experiment was compared and the pH for the highest treatment efficiency was employed as the optimum operating pH.

3.2 Process Flow of Methodology



3.3 Apparatus and Chemicals Required

3.3.1 Apparatus

- Pipette
- 500 mL beaker
- 0.45 μm membrane filter
- 0.20 μm membrane filter
- SolSim solar simulator photoreactor (Luzchem Research Inc. Gloucester, ON, Canada)
- pH meter
- Magnetic stirrer
- Syringe
- COD reactor
- COD vial adapter
- Volumetric flask
- Spectrophotometer
- Test vial
- Reagent bottle
- DO meter

3.3.2 Chemicals

- Sulphuric acid, H_2SO_4
- Hydrogen peroxide, H_2O_2
- Oxalic acid, $H_2C_2O_4$
- Iron(III) Sulphate, $Fe_2(SO_4)_3$
- Amoxicillin
- Cloxacillin
- Distilled water
- Nessler reagent

3.4 Project Activities and Key Milestones

Several targets have been set for the FYP I and FYP II. Table 4 and Table 5 show the project activities and key milestones for FYP I and FYP II respectively.

Table 4: Project activities and key milestones for FYP I.

No.	Detail/ Week	1	2	3	4	5	6	7		8	9	10	11	12	13	14
1	Selection of Project Topic	▨							MID SEM BREAK							
2	Literatures Review		▨	▨	▨	▨										
3	Submission of Extended Proposal						■									
4	Modeling the Experiment by Using RSM Software							▨		▨						
5	Proposal Defense										■					
6	Modeled Experiments Execution											▨	▨	▨	▨	
7	Submission of Interim Draft Report														■	
8	Submission of Interim Report															■

Legends:

▨ Project Activity

■ Key Milestone

Table 5: Project activities and key milestones for FYP II.

No.	Detail/ Week	1	2	3	4	5	6	7		8	9	10	11	12	13	14	15
1	Modeled Experiments Execution and Optimization	▨	▨	▨	▨	▨	▨	▨	MID SEM BREAK	▨	▨	▨	▨				
2	Submission of Progress Report									■							
4	Compare the Efficiency with Other Treatment Methods												▨	▨			
5	Pre-EDX												■				
6	Submission of Draft Report													■			
7	Submission of Dissertation (soft bound)														■		
8	Submission of Technical Paper														■		
9	Oral Presentation															■	
10	Submission of Project Dissertation (Hard Bound)																■

Legends:

▨ Project Activity

■ Key Milestone

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Experimental Design and Observed Removals

The following Table 6 shows the experimental design and response parameters (observed removals of COD, NH_3-N and TOC).

Table 6: Experimental design and observed removals.

Process Variables				Removals (%)		
H_2O_2/COD (molar ratio)	H_2O_2/Fe^{3+} (molar ratio)	$H_2O_2/C_2H_2O_4$ (molar ratio)	Time (min)	COD	NH_3-N	TOC
4.00	50.00	25.00	120.00	60.23	40.00	57.29
1.50	50.00	50.00	120.00	74.69	50.74	53.25
1.50	100.00	50.00	60.00	60.81	52.40	23.82
0.25	75.00	37.50	90.00	64.71	49.75	29.80
1.50	100.00	25.00	120.00	65.27	45.21	56.32
2.75	75.00	12.50	90.00	75.09	57.26	43.12
5.25	75.00	37.50	90.00	54.25	33.15	44.85
1.50	50.00	25.00	60.00	70.32	55.07	25.17
2.75	75.00	37.50	90.00	78.23	48.49	49.76
2.75	75.00	37.50	90.00	77.21	48.77	51.79
2.75	75.00	37.50	90.00	70.07	38.36	50.07
4.00	50.00	50.00	120.00	72.02	36.44	48.01
4.00	100.00	25.00	60.00	76.28	52.60	42.98
1.50	50.00	25.00	120.00	69.64	45.02	34.17
1.50	100.00	50.00	120.00	71.34	52.70	61.93
1.50	50.00	50.00	60.00	61.82	36.99	25.28
2.75	125.00	37.50	90.00	69.88	48.86	38.75
4.00	50.00	50.00	60.00	65.12	36.16	70.91
4.00	100.00	50.00	60.00	59.18	40.82	41.90
4.00	50.00	25.00	60.00	58.25	35.89	55.07
1.50	100.00	25.00	60.00	66.92	64.99	39.13
2.75	75.00	37.50	90.00	77.30	43.84	60.66
4.00	100.00	25.00	120.00	69.98	50.41	47.94
4.00	100.00	50.00	120.00	75.85	54.47	40.26
2.75	75.00	37.50	150.00	83.33	38.63	57.39
2.75	25.00	37.50	90.00	72.62	45.21	52.47
2.75	75.00	62.50	90.00	70.83	46.03	52.39
2.75	75.00	37.50	30.00	65.48	47.67	48.79
2.75	75.00	37.50	90.00	73.05	40.55	53.51
2.75	75.00	37.50	90.00	72.24	43.29	41.32

All of the experiments in Table 6 were done with the following fixed parameters:

- pH = 3
- Light Intensity = 900 watts/m^2
- Initial COD concentration = 392 mg/L
- Initial NH_3-N concentration = 7.3 mg/L
- Initial TOC concentration = 144.4 ppm

4.2 Statistic Analysis

Analysis of variance (ANOVA) in the CCD was used to evaluate the "goodness of fit" of the COD, NH_3-N and TOC removals toward the experimental design. The summary of the ANOVA is shown in Table 7.

Table 7: ANOVA of the response parameters.

Parameter	Prob > F value	R^2	Adequate Precision
COD	0.0037	0.8031	7.856
NH_3-N	0.0132	0.7579	8.803
TOC	0.0022	0.8196	8.992

Prob > F value represents the proportion of time or probability you would expect to get the stated F value (Noordin et al., 2004). Basically, F value is a measure of variation of the data about the mean. The general rule mentioned that the model is significant if the prob > F values equal to or less than 0.05. From Table 7, ANOVA of all response parameters are less than 0.05 and thus imply the experimental design is significant. Meanwhile, R^2 is the coefficient of determination which estimates the fraction (a number between zero and one) of the overall variation in the data accounted for by the model. Since R^2 values for all parameters are numbers between zero and one, the model is good enough for the quadratic fits to navigate the design space defined by the CCD (Ghafari et al., 2009). Adequate precision compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination and can be used to navigate the design space defined by the CCD (Ghafari et al., 2009). From Table 7, the adequate precision for all responses are more than 4 and prove that the model is significant and good.

Other that ANOVA, the CCD also performed some diagnosis on the model. The following Figure 2, 3, 4, 5, 6, 7, show the results of the diagnostics.

4.3 Model Validation

In the CCD, the model is also diagnosed in order to validate it. The following Figure 2, 3, 4, 5, 6, 7, show the results of the diagnostics.

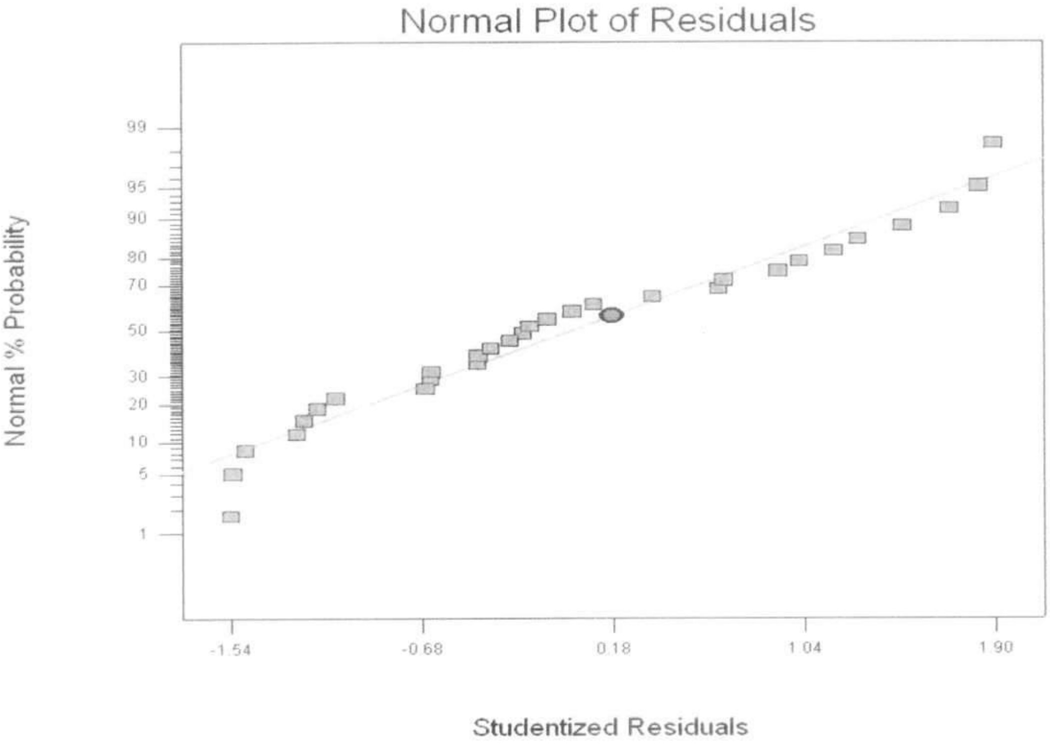


Figure 2: Normal Probability plot of the studentized residuals of COD removal.

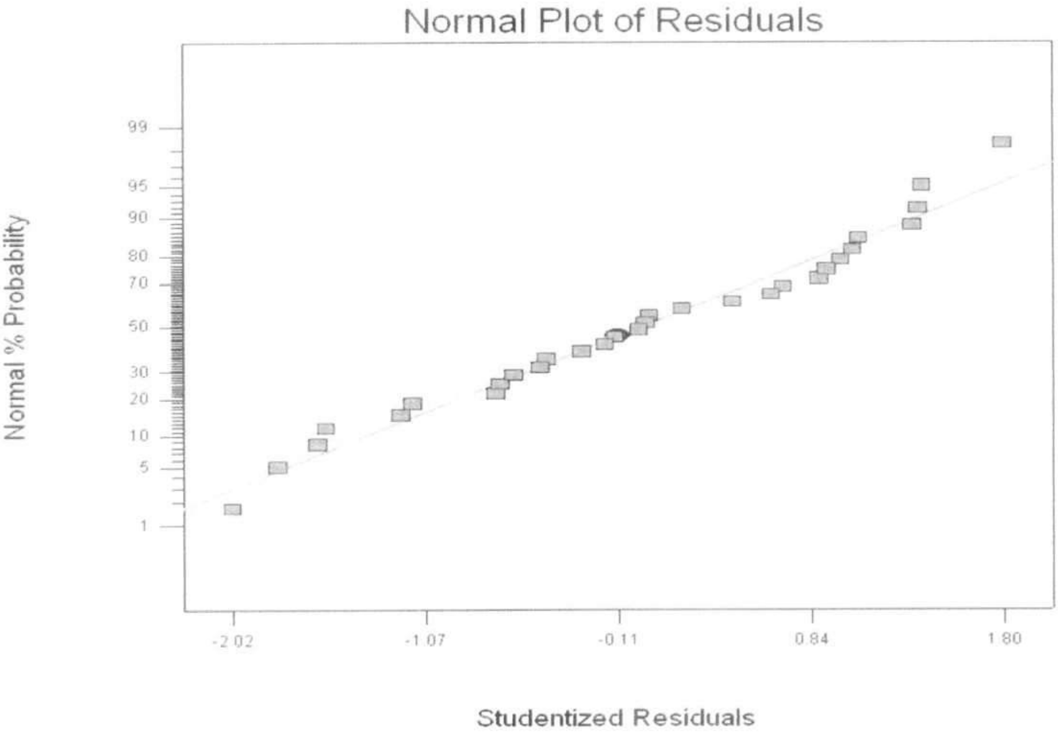


Figure 3: Normal Probability plot of the studentized residuals of NH_3-N removal.

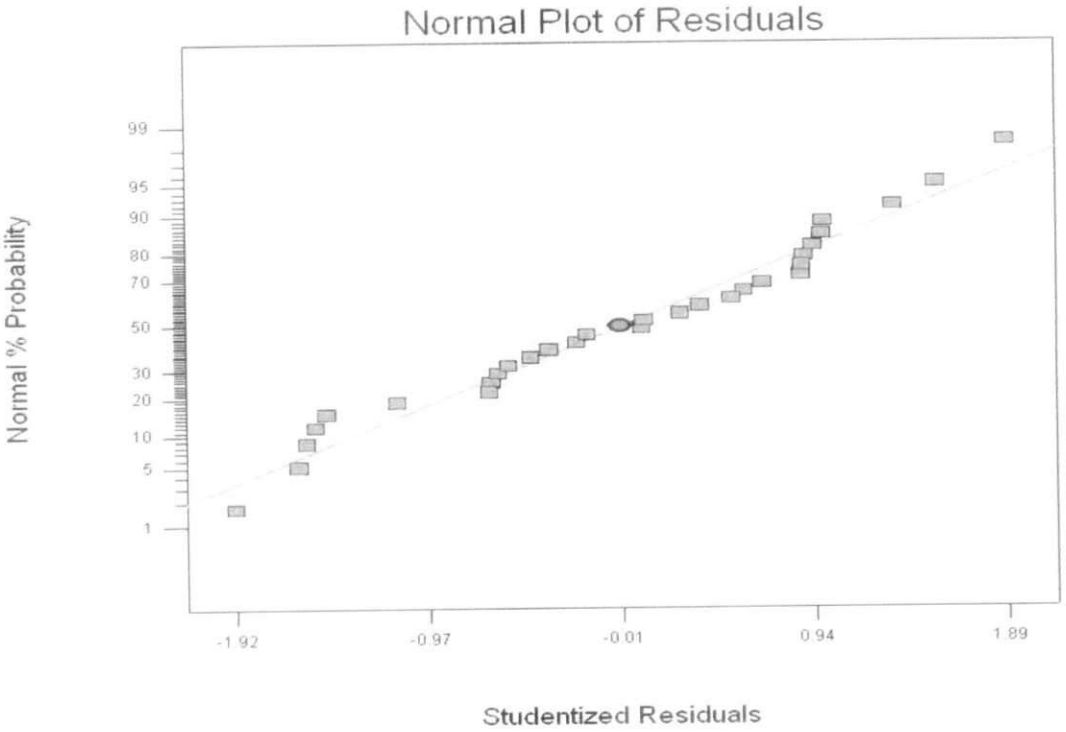


Figure 4: Normal Probability plot of the studentized residuals of TOC removal.

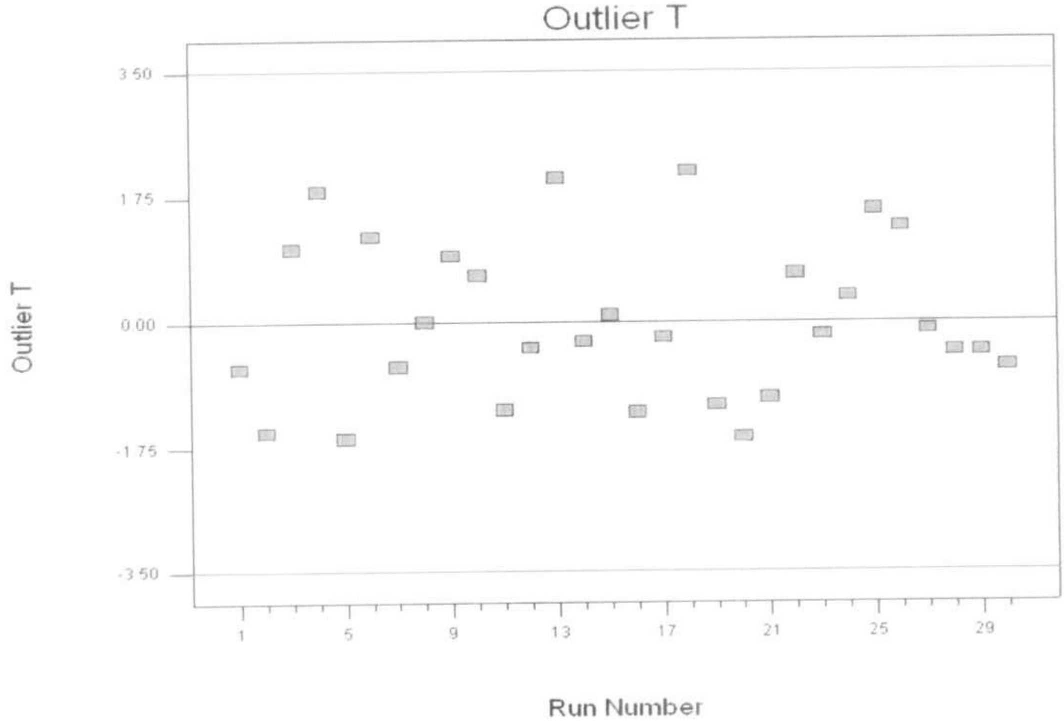


Figure 5: Outlier T plots of COD removal.

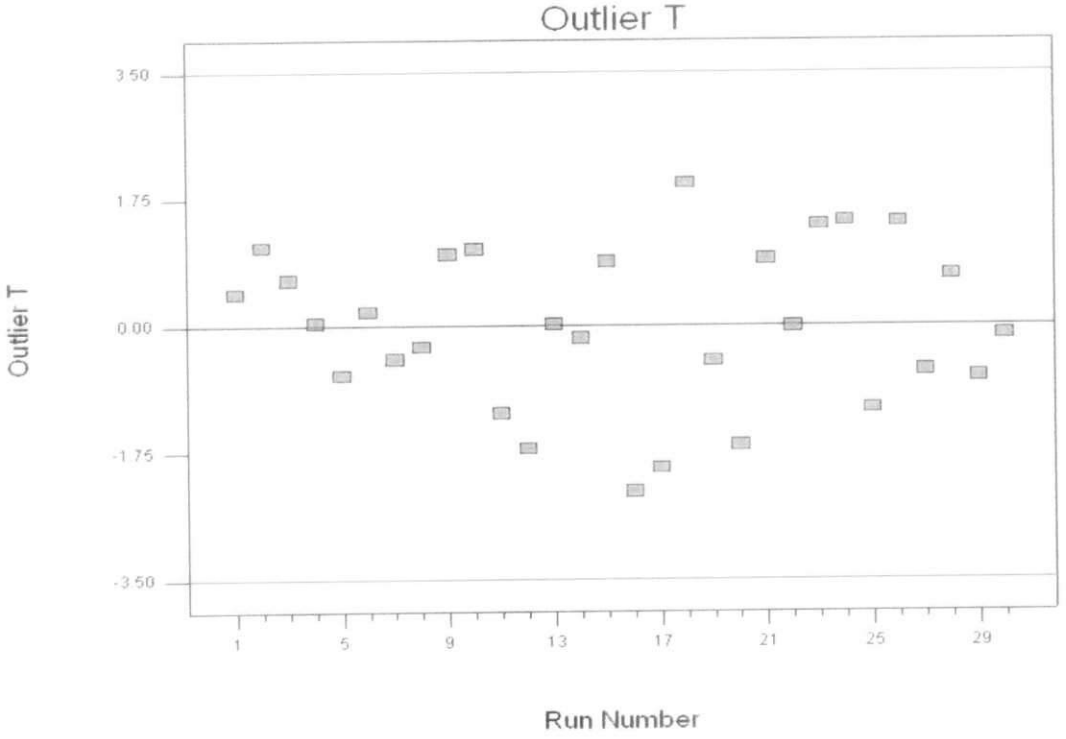


Figure 6: Outlier T plots of NH_3-N removal.

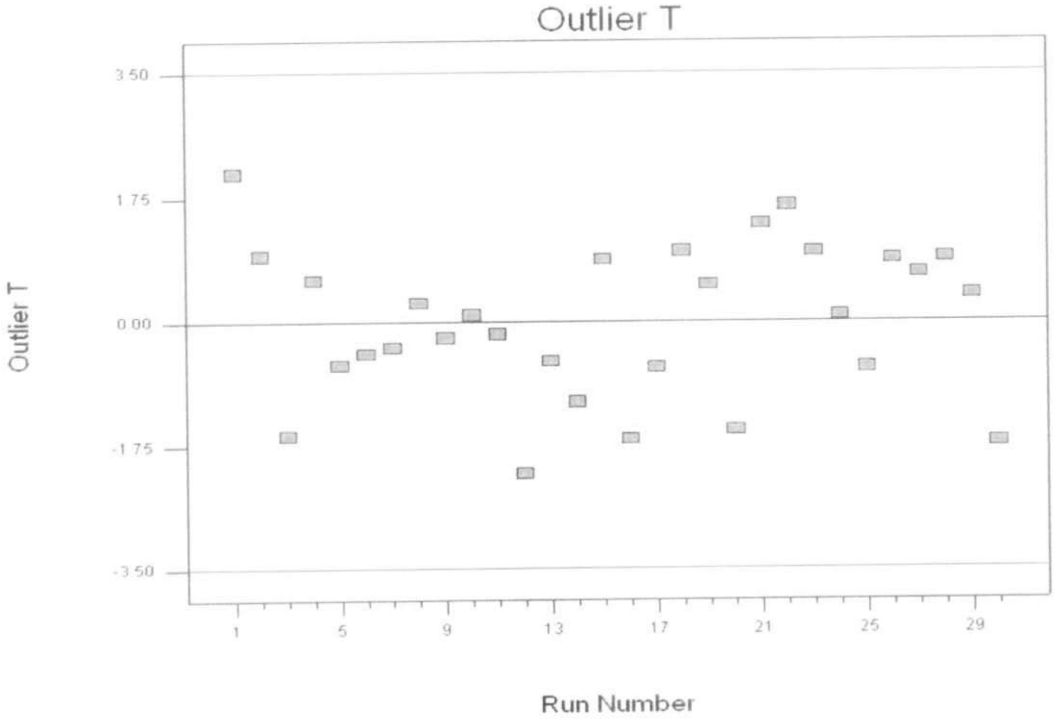


Figure 7: Outlier T plots of TOC removal.

The straight line in Figure 2, 3 and 4 indicate that there is no abnormality in the model and the model is valid. From Figure 5, 6 and 7, we can see that there is no point beyond the red lines which were set at plus-or-minus 3.5. The red lines are the boundary for a valid model, thus model is applicable and acceptable.

4.4 Model Optimization and Prediction

Based on the experimental design and the observed responses data, an optimized model and prediction are generated by the CCD.

4.4.1 Optimization and Prediction of COD Removal

The optimization and prediction are presented in various views. The following Figure 8, 9 and 10 show the different views of the COD removal's optimization and prediction.

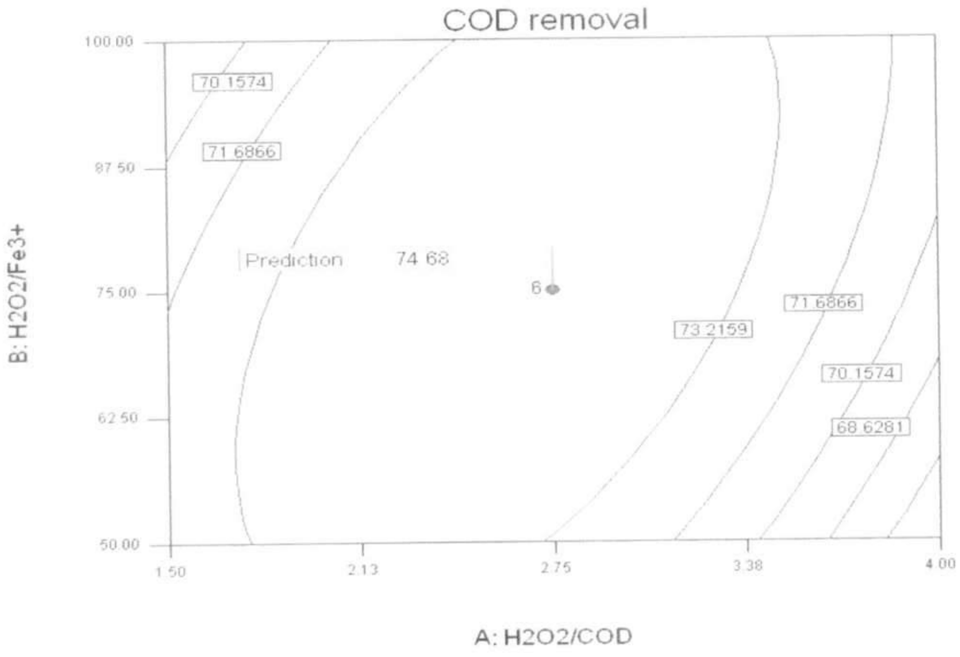


Figure 8: Response surface plot for COD removal.

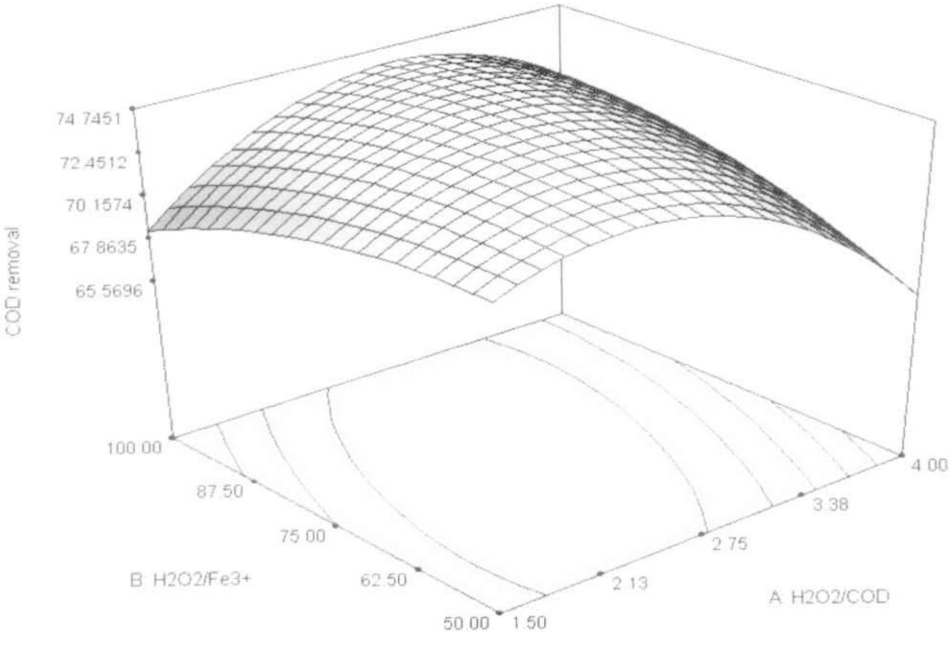


Figure 9: 3D view of the COD removal prediction.

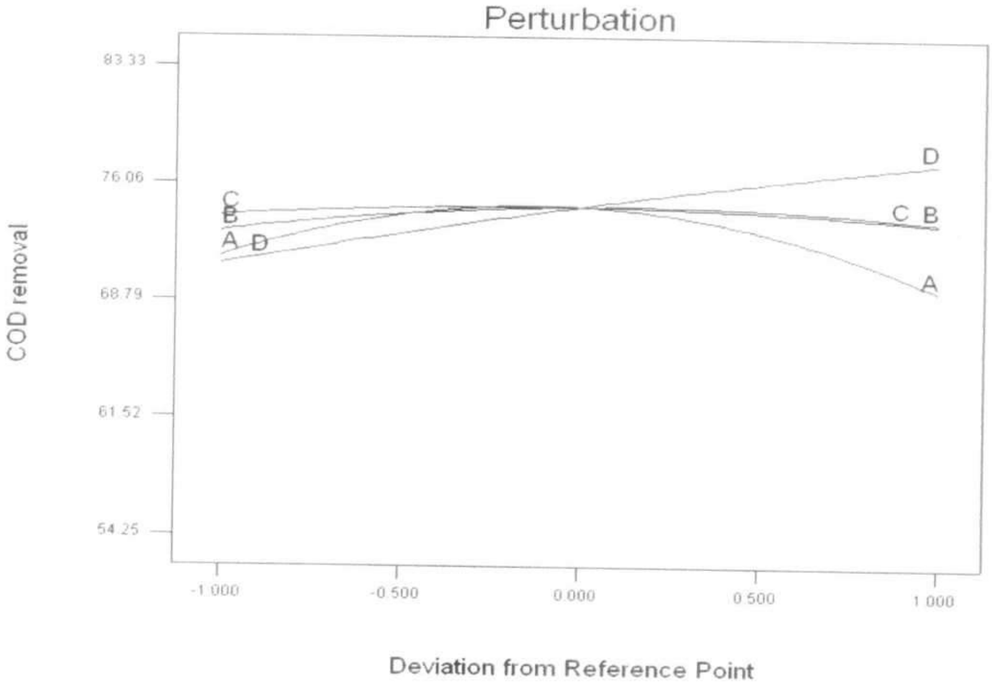


Figure 10: Perturbation view of COD removal prediction.

From Figure 8, 9 and 10, we can see that the CCD predicted that the optimum COD removal is at 74.68 %.

4.4.2 Optimization and Prediction of NH_3-N Removal

The following Figure 11, 12 and 13 show the different views of the NH_3-N removal's optimization and prediction.

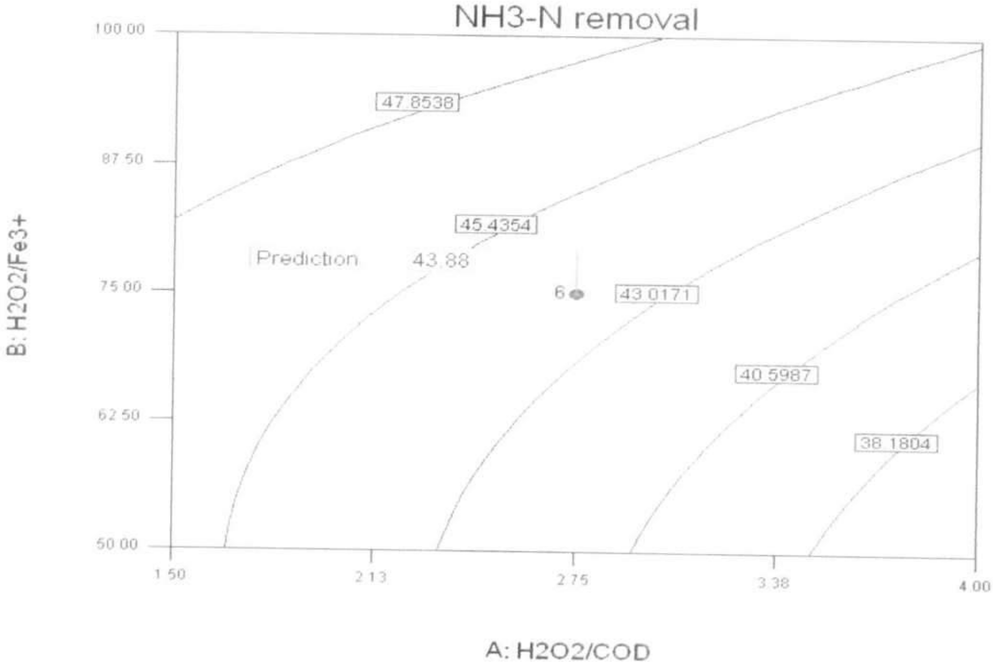


Figure 11: Response surface plot for NH_3-N removal.

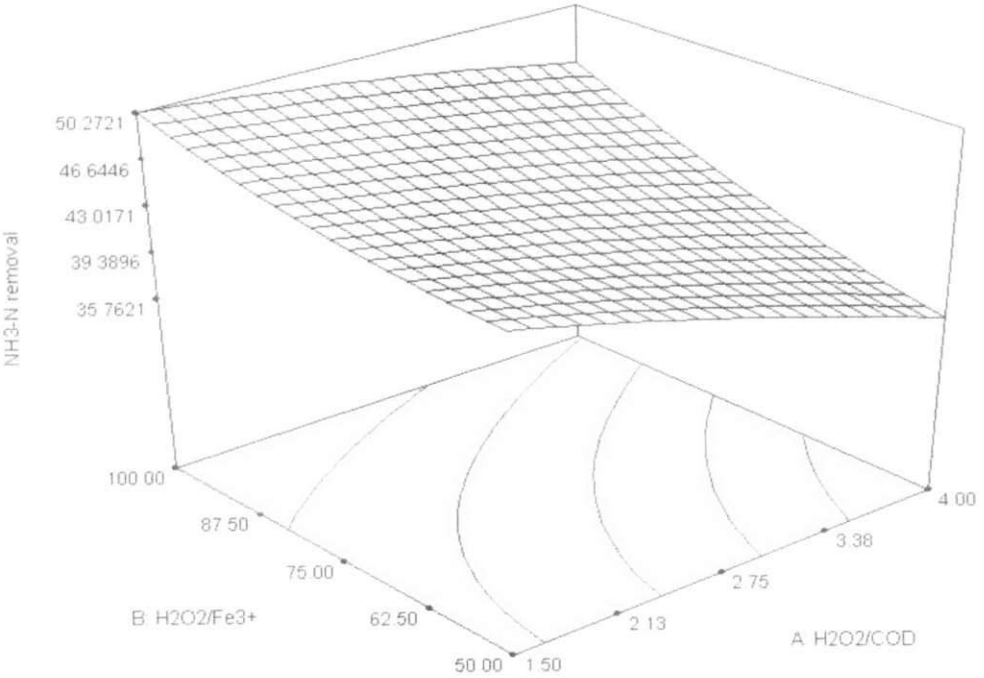


Figure 12: 3D view of the NH_3-N removal prediction.

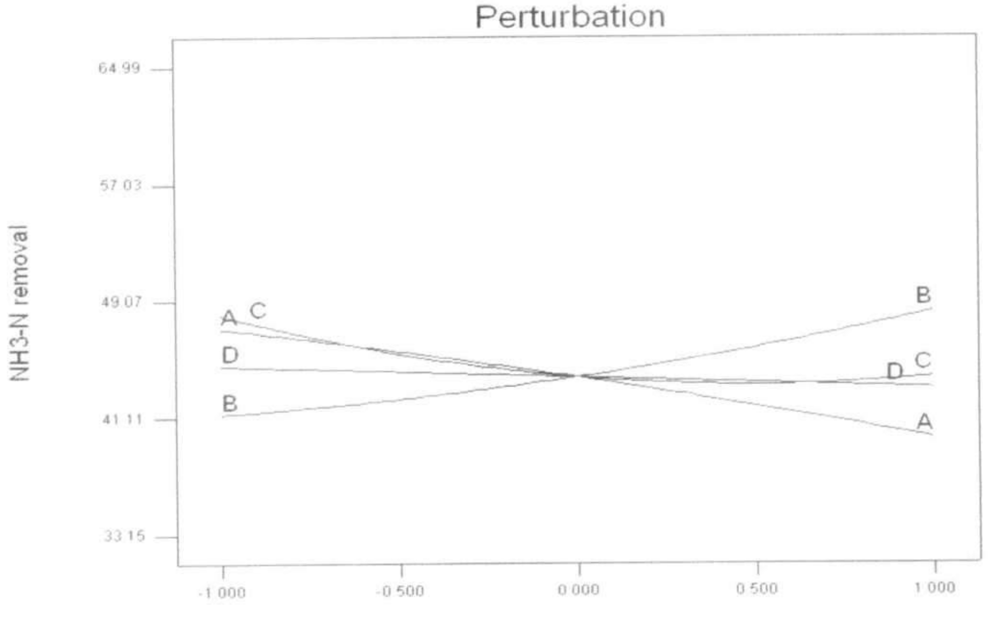


Figure 13: Perturbation view of NH_3-N removal prediction.

From Figure 11, 12 and 13, we can see that the CCD predicted that the optimum NH_3-N removal is at 43.88 %.

4.4.3 Optimization and Prediction of TOC Removal

The following Figure 14, 15 and 16 show the different views of the TOC removal's optimization and prediction.

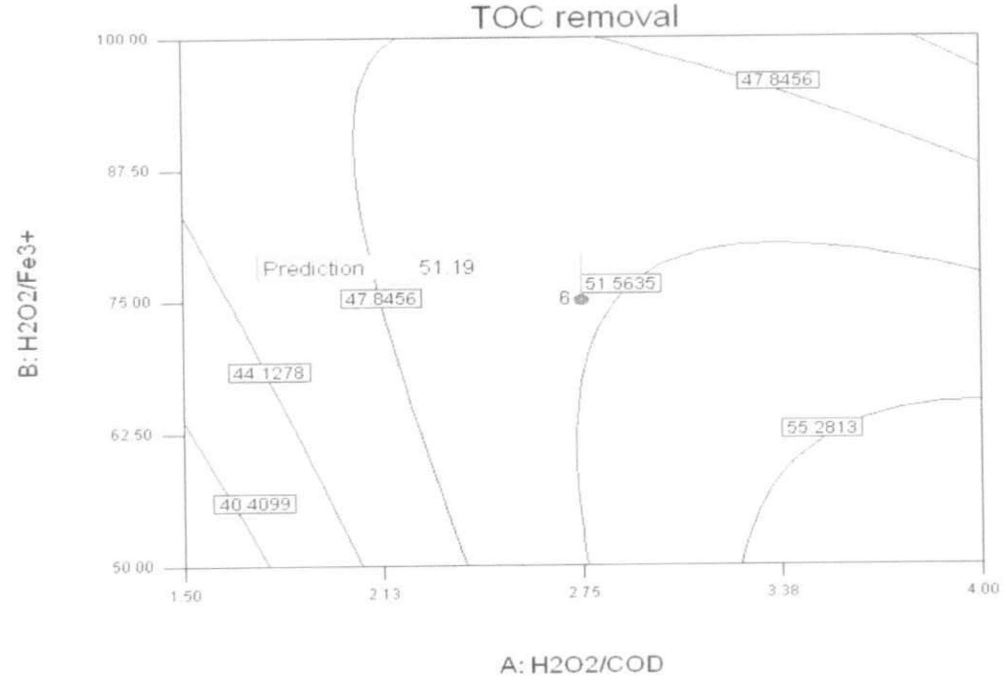


Figure 14: Response surface plot for TOC removal.

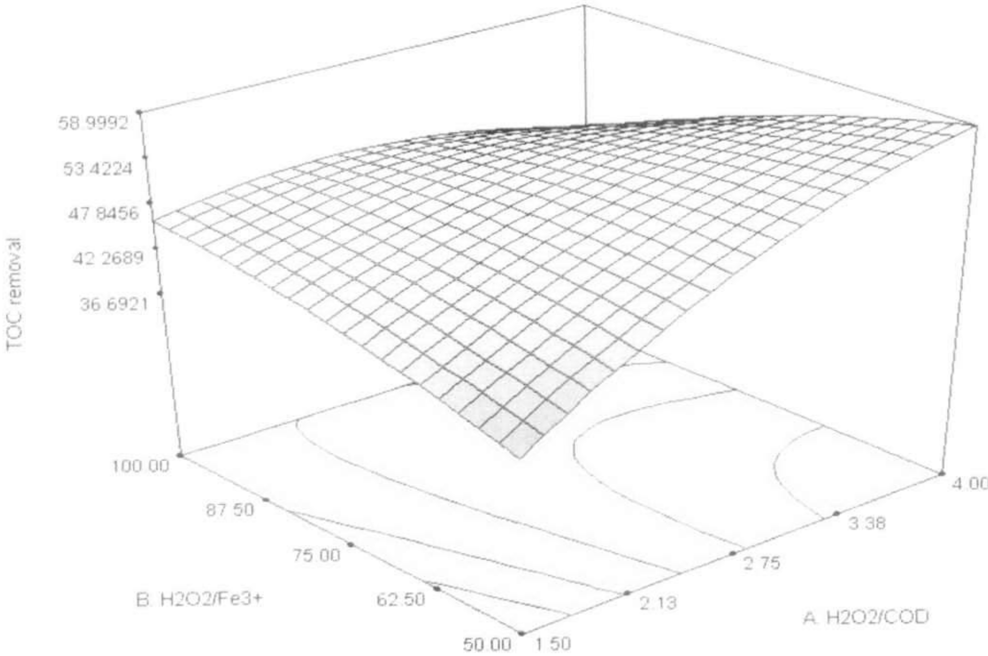


Figure 15: 3D view of the TOC removal prediction.

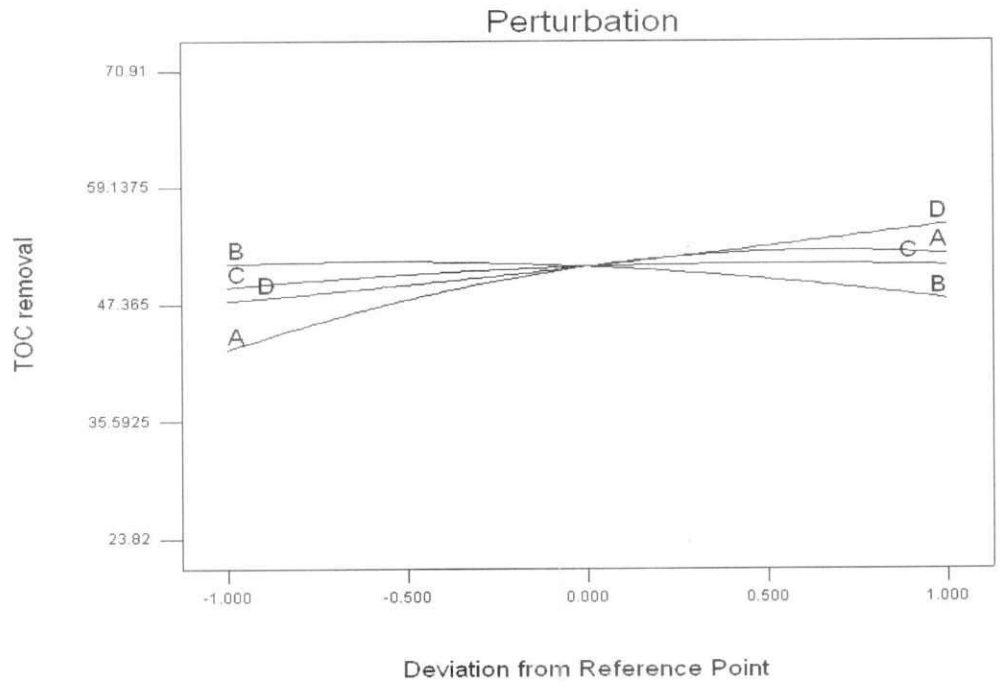


Figure 16: Perturbation view of TOC removal prediction.

From Figure 14, 15 and 16, we can see that the CCD predicted that the optimum TOC removal is at 51.19 %.

4.4.4 Optimization and Prediction Summary

In addition to the removal prediction, the CCD also comes out with the predicted optimum operating conditions for the experiment. The experiment which will be conducted under the predicted optimum operating conditions is expectedly to produce the COD, NH_3-N and TOC removals as close as possible to the removal prediction. The subsequent Table 8 shows the summary of the model prediction.

Table 8: Summary of model optimization and prediction.

Predicted Optimum Operating Condition				Removal Prediction (%)		
H_2O_2/COD (molar ratio)	H_2O_2/Fe^{3+} (molar ratio)	$H_2O_2/C_2H_2O_4$ (molar ratio)	Time (min)	COD	NH_3-N	TOC
2.75	75.00	37.5	90.00	74.68	43.88	51.19

4.5 Optimum Model Verification

Verification experiments had been conducted in order to validate the removal prediction made by the CCD application. The experiments were conducted under the optimum operating conditions and at pH 3. The Table 9 on the next page compares the experimental removals with the predicted removals.

Table 9: Comparison of predicted removals and experimental removals.

	COD	NH_3-N	TOC
Predicted Removals (%)	74.68	43.88	51.19
Experimental Removals (%)	78.37	45.94	52.30
Percentage Difference (%)	4.95	4.69	2.17

The tolerance for the percentage difference according to the CCD application is ±5.00 %. From the Table 9 above, all responses are acceptable because the percentage differences between the actual and the predicted removals are less than 5.00 %.

4.6 pH Optimization

A series of experiments had been performed under the optimum operating conditions but at different pH (2, 3, 4 and 5). The purpose of these experiments was to determine the optimum pH for the modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process. The Figure 17 below shows the effect of pH on the COD, NH_3-N and TOC removals.

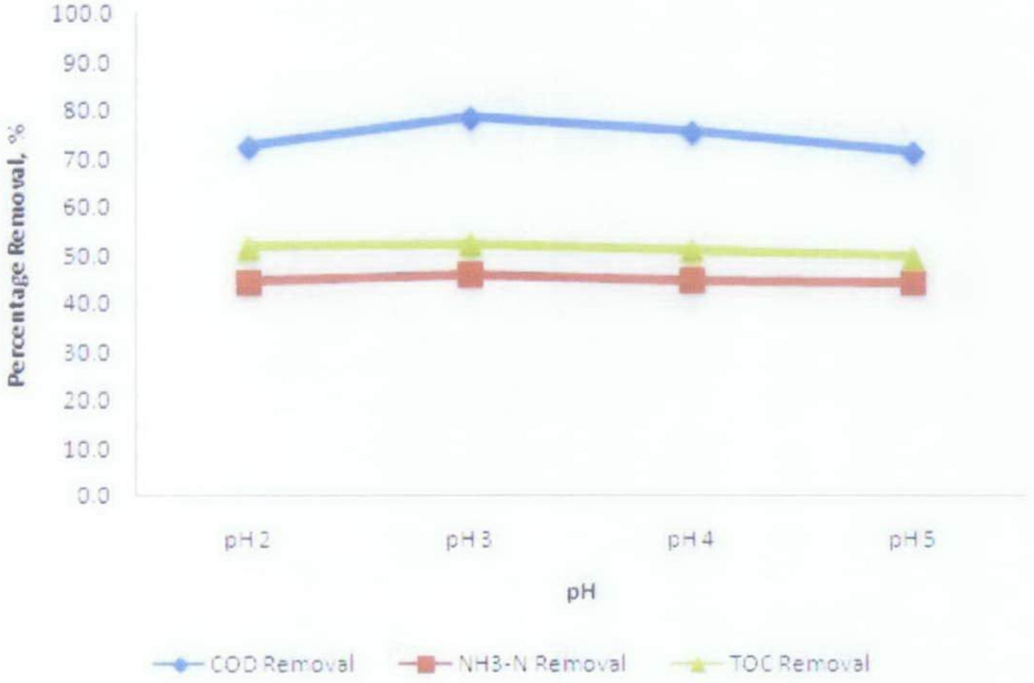


Figure 17: Effect of pH on the COD, NH_3-N and TOC removals.

From the Figure 17 above, we can see that not much difference in the COD, NH_3-N and TOC removals between the pH 2 – pH 5. However, it is apparent that the highest removals were happening when the experiment was conducted at pH 3. Therefore, we can say that the optimum pH for the modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process is pH 3.

4.7 Comparison with Fenton and photo-Fenton Processes

The comparison was performed with respect to optimum operating conditions and removals efficiency. The following Table 10 shows the comparison for the optimum operating conditions.

Table 10: Comparison with respect to optimum operating conditions.

Process	OPTIMUM OPERATING CONDITIONS			
	H_2O_2/COD	$H_2O_2/(Fe^{2+}/Fe^{3+})$	$H_2O_2/C_2H_2O_4$	Irradiation time
Fenton	3.0	10	NA	60
Photo-Fenton	2.5	20	NA	50
Modified Photo-Fenton	2.75	75	37.5	90

With respect to H_2O_2/COD (Molar Ratio), modified photo-Fenton requires an amount that is less than the Fenton but more than the photo-Fenton process. Meanwhile, with respect to $H_2O_2/(Fe^{2+}/Fe^{3+})$ (Molar Ratio), the modified photo-Fenton requires significantly less than those Fenton and photo-Fenton processes. However, for Fenton and photo-Fenton, $H_2O_2/C_2H_2O_4$ (Molar Ratio) is not applicable and the modified photo-Fenton requires 37.5 molar ratio. In term of irradiation time, modified photo-Fenton needs the longest irradiation time as compare with the other processes. Table 11 shows the comparison for the removals efficiency.

Table 11: Comparison with respect to removals efficiency.

Process	Removals (%)			BOD_5/COD
	COD	NH_3-N	TOC/COD	
Fenton	81.4	NA	54.3	0.37
Photo-Fenton	80.8	NA	51	0.40
Modified Photo-Fenton	78.37	45.94	52.3	0.35

From Table 11, the overall performance of the modified photo-Fenton in term of the removals efficiency and biodegradability improvement is slightly lower than the previous two methods in the treatment of antibiotics aqueous solution. However, the difference is small to the extent of negligible.

From comparison above, the operation of modified photo-Fenton process is more economical than the Fenton and photo-Fenton processes and yet producing a comparable removals efficiency and biodegradability improvement. This is because, the modified photo-Fenton process requires less amount of oxidant and catalyst than the Fenton process. Meanwhile, comparing the modified photo-Fenton to the photo-Fenton process, the required amount of oxidant and catalyst is more but the modified photo-Fenton uses sunlight which is free and safe, whereas the photo-Fenton process needs the irradiation of ultraviolet (UV) rays which is expensive and unsafe.

4.8 Other Observations

During conducting the experiments, some other interesting observations were made.

1. The pH of the solution must be increased up to pH 10 immediately after the specified irradiation time for the model is reached. The increase in pH up to pH 10 will stop the reaction in the solution and thus stop the treatment or otherwise the reaction will still continue and the results obtained will not be accurate.

2. Brownish colored iron sludge was produced after the treatment process (Figure 18). Thus, a settling or sedimentation tank should be provided after the modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) treatment.

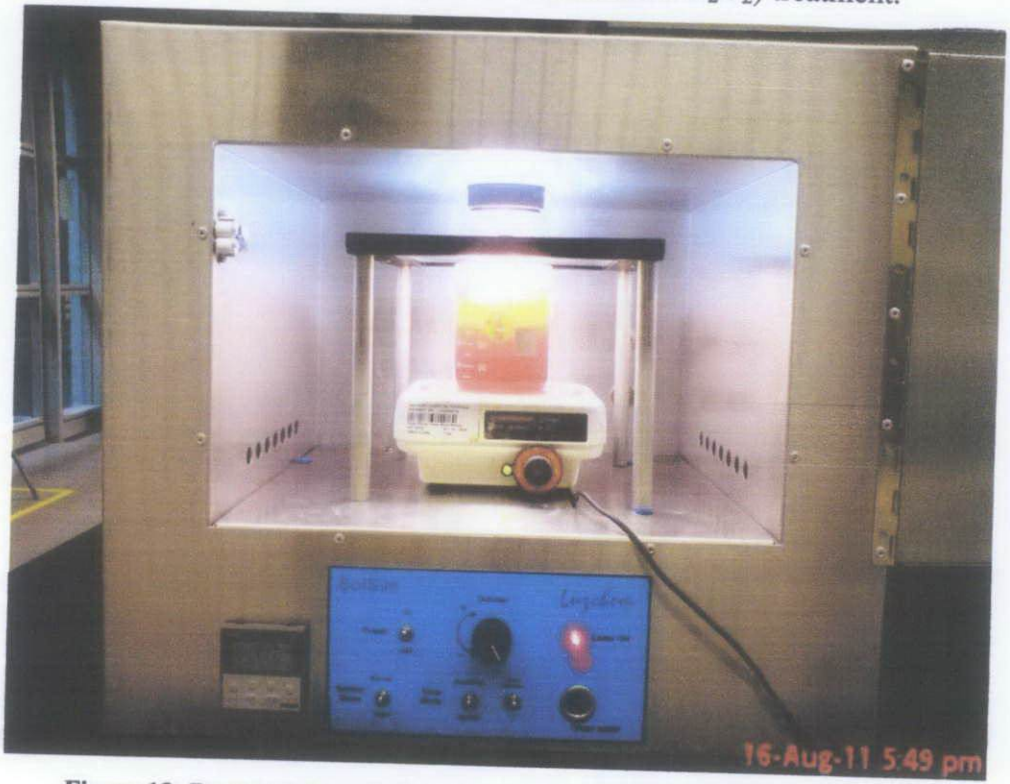


Figure 18: Brownish iron sludge observed in one of the model experiments.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- Under optimum operating conditions (H_2O_2/COD 2.75 molar ratio, H_2O_2/Fe^{3+} 75 molar ratio and $H_2O_2/C_2H_2O_4$ 37.5 molar ratio) at pH 3, the biodegradability improved from ~0 to 0.35 in 90 min, and the COD, NH_3-N and TOC removals were 78.37%, 45.94% and 52.30%, respectively.
- The modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process is comparable with Fenton and photo-Fenton processes in terms of removals efficiency and biodegradability improvement. In addition, operation of the modified photo-Fenton process is more economical than the other two processes.
- Modified Photo-Fenton process is effective and can be used as pretreatment of antibiotic wastewater for biological treatment.

5.2 Recommendations

For further research of this topic, the following is recommended:

- Expanding the research on effect of radiation intensity on the removals efficiency. This is important for improving the removals efficiency of the method.
- Applying the same method on other recalcitrant wastewater like pesticide and synthetic fragrance.
- Executing the treatment process under the direct sunlight without using the SolSim photoreactor.

REFERENCES

Alexy, R., Kümpel, T. & Kümmerer, K, Assessment of degradation of 18 antibiotics in the closed bottle test; Chemosphere 57 (6) (2004): 505-512.

Alaton, I. & Dogruel, S., Pretreatment of penicillin formulation effluent by advanced oxidation processes; Journal of Hazardous materials 112 (1-2) (2004): 105-113.

Anderson, P. D., D'Aco, V. J., Shanahan, P., Chapra, S. C., Buzby, M. E., Cunningham, V. L., Duplessie, B. M., Hayes, E. P., Mastrocco, F. J., Parke, N. J., Rader, J. C., Samuelian, J. H. & Schwab, B. W., Screening analysis of human pharmaceutical compounds in US surface waters; Environmental Science & Technology 38 (1) (2004): 838-849.

Andersson, D. I., Persistence of antibiotic resistant bacteria; Current Opinion in Microbiology 6 (5) (2003): 452-456.

Ashnagar, A. & Naseri, N. G., Analysis of Three Penicillin Antibiotics (Ampicillin, Amoxicillin and Cloxacillin) of Several Iranian Pharmaceutical Companies by HPLC; E-Journal of Chemistry 4 (4) (2007): 536-545.

Auerbach, E. A., Seyfried, E. E. & McMahon, K. D., Tetracycline resistance genes in activated sludge wastewater treatment plants; Water Resource 41 (5) (2007): 1143-1151.

Banik, K.K., Hossain, S., Pharmaceuticals in drinking water: a future water quality threat; Indian Journal Environment Protection 26 (10) (2006): 926-932.

Bennett, P. M., Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria; British Journal of Pharmacology 153 (1) (2008): 347-357.

Boxall, A. B. A., Kolpin, D. W., Sorensen, B. H. & Tolls, J., Are veterinary medicines causing environmental risks?; Environmental Science & Technology 37 (15) (2003): 286A-294A.

Brooks, J. P., Maxwell, S. L., Rensing, C., Gerba, C. P. & Pepper, I. L., Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of Biosolids; Canadian Journal of Microbiology 53 (5) (2007): 616-622.

Carballa, M., Omil, F., Lema, J. M., Llompart, M., Garcia-Jares, C., Rodriguez, I., Gomez, M. & Ternes, T., Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant; Water Research 38 (12) (2004): 2918-2926.

Chamarro, E., Marco, A. & Esplugas, S., Use of fenton reagent to improve organic chemical biodegradability; Water Resources 35 (4) (2001): 1047-1051.

Chaudhuri, M. & Wei, T. Y., Decolourisation of Reactive Dyes by Modified Photo-Fenton Process Under IrradiationWith Sunlight; Nature Environment & Pollution Technology 8 (2) (2009): 359-363.

Chee-Sanford, J. C., Aminov, R. I., Krapac, I. J., Garrigues-Jeanjean, N. & Mackie, R. I., Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities; Applied & Environmental Microbiology 67 (4) (2001): 494-502.

Choi, K., Kim, S-G. & Kim, S-H., Removal of antibiotics by coagulation and granular activated carbon filtration; Journal Hazardous Material 151 (1) (2008): 38-43.

Dang, H., Zhang, X., Song, L., Chang, Y. & Yang, G., Molecular characterizations of oxytetracycline resistant bacteria and their resistance genes from mariculture waters in China; Marine Pollution Bulletin 52 (11) (2006): 1494-1503.

Daughton, C. G. & Ternes, T. A., Pharmaceuticals and personal care products in the environment: agents of subtle change?; Environment Health Perspective 107 (6) (1999): 907-938.

Elmolla, E. & Chaudhuri, M., Optimization of Fenton process for treatment of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution; Journal of Hazardous Materials 170 (2-3) (2009a): 666-672.

Elmolla, E. & Chaudhuri, M., Degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-Fenton process; Journal of Hazardous Materials 172 (2-3) (2009b): 1476-1481.

Elmolla, E. & Chaudhuri, M., Degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution by the UV/ZnO photocatalytic process; Journal of Hazardous Materials 173 (1-3) (2010a): 445-449.

Elmolla, E. & Chaudhuri, M., Photocatalytic degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution using UV/TiO_2 and UV/H_2O_2/TiO_2 photocatalysis; Desalination 252 (1-3) (2010b): 46-52.

Gallert, C., Fund, K. & Winter, J., Antibiotic resistance of bacteria in raw and biologically treated sewage and in groundwater below leaking sewers; Applied Microbiology & Biotechnology 69 (1) (2005): 106-112.

Ghafari, S., Aziz, H.A., Isa, M.H. and Zinatizadeh, A.A. (2009). Application of response surface methodology (RSM) to optimize coagulation flocculation treatment of leachate using poly-aluminium chloride (PAC) and alum. *Journal of Hazardous Materials*, 163, 650-656.

Giger, W., Alder, A. C., Golet, E. M., Kohler, H.-P. E., McArdell, C. S., Molnar, E., Siegrist, H. & Su, M. J.-F., Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludge, and surface waters; CHIMIA International Journal for Chemistry 57 (9) (2003): 485-491.

Goswami, D.Y., Engineering of the solar photocatalytic detoxification & disinfection process; Advances in Solar Energy 10 (1995): 165-209.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K. L., Occurrence of antibiotics in the aquatic environment; Science Total Environment 225 (1-2) (1999): 109-118.

Ikehata, K., Naghashkar, N. J. & Ei-Din, M. G., Degradation of aqueous pharmaceuticals by ozonation & advanced oxidation processes; A review of Ozone Science & Engineering 28 (6) (2006): 353-414.

Iwane, T., Urase, T. & Yamamoto, K., Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water; Water Science & Technology 43 (2) (2001): 91-99.

JETACAR, The use of antibiotics in food-producing animals: Antibiotic-resistant bacteria in animals and humans; Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR), A Division of the CDHAC and CDAFF, Canberra (1999).

Karthikeyan, K. G. & Meyer, M. T., Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA; Science of the Total Environment 361 (1-3) (2006): 196-207.

Khuri, A. I. & Cornell, J. A., Response Surfaces: Designs and Analyses; Marcel Dekker Inc., New York 2nd edition (1996).

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. & Buxton, H. T. Pharmaceuticals, hormones & other organic wastewater contaminants in U.S. streams, 1999-2000; Environmental Science & Technology 36 (6) (2002): 1202-1211.

Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C. G., Irfan, S., Krishnan, P., Kumar, A. V., Maharjan, S., Mushtaq, S., Noorie, T., Paterson, D. L., Pearson, A., Perry, C., Pike, R., Rao, B., Ray, U., Sarma, J. B., Sharma, M., Sheridan, E., Thirunarayan, M. A., Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore, D. M. & Woodford, N., Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK; a molecular, biological, and epidemiological study; Lancet Infectious Diseases 10 (9) (2010): 597-602.

Li, D., Yang, M., Hu, J. Y., Zhang, J., Liu, R. Y., Gu, X., Zhang, Y. & Wang, Z. Y., Antibiotic-resistance profile in environmental bacteria isolated from penicillin production wastewater treatment plant and the receiving water; Environmental Microbiology 11 (6) (2009): 1506-1517.

Mara, D. & Horan, N., Handbook of Water and Wastewater Microbiology; Academic Press, London 1st edition (2003).

Martinez, J. L., Environmental pollution by antibiotics and by antibiotic resistance determinants; Environmental Pollution 157 (11) (2009): 2893-2902.

Martı´nez, J. L., Antibiotics and antibiotic resistance genes in natural environments; Science 321 (5887) (2008): 365-367.

Meirelles-Pereira, F., Santos-Pereira, A. M., Gomes de Silva, M. C., Gonealves, V. D., Brum, P. B., De Castro, A., Pereira, A. A. & Esteves Pereira, J. A. A., Ecological aspects of the antimicrobial resistance in bacteria of importance to human infection; Brazilian Journal of Microbiology 33 (4) (2002): 287-293.

Mezrioui, N. & Baleux, B. Resistance Patterns of E. Coli Strains Isolated from Domestic Sewage Before and After Treatment in Both Aerobic Lagoon and Activated Sludge; Water Resources 28 (11) (1994): 2399-2406.

Morse, A. N., Fate and effect of amoxicillin in space and terrestrial water reclamation systems; PhD Dissertation, Graduate Faculty of Texas Tech University (2003).

Nikolaou, A., Meric, S. & Fatta, D., Occurrence patterns of pharmaceuticals in water and wastewater environments; Analytical & Bioanalytical Chemistry 387 (4) (2007): 1225-1234.

Noordin, M. Y., Venkatesh, V. C., Sharif, S., Elting, S. & Abdullah, A., Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel; Journal of Materials Processing Technology 145 (1) (2004): 46-58.

Ollis, D. F., Pelizzetti, E. & Serpone, N., Photocatalyzed destruction of water contaminants; Environmental Science & Technology 25 (9) (1991): 1522-1529.

Park, J. C., Lee, J. C., Oh, J. Y., Jeong, Y. W., Cho, J. W., Joo, H. S., Le, W. K. & Lee, W. B., Antibiotic selective pressure for the maintenance of antibiotic genes in coliform bacteria isolated from the aquatic environment; Water Science & Technology 47 (3) (2003): 249-253.

Pasieczna-Patkowska, S., Czech, B., Ryczkowski, J. & Patkowski, J., Removal of recalcitrant pollutants from wastewater; Applied Surface Science 256 (17) (2010): 5434-5438.

Pera-Titus, M., Garcla-Molina, V., Baños, M. A., Giménez, J. & Esplugas, S., Degradation of chlorophenols by means of advanced oxidation processes; Applied Catalysis B: Environmental 47 (4) (2004): 219-256.

Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., Antibiotic resistance genes as emerging contaminants; Studies in Northern Colorado; Environmental Science & Technology 40 (23) (2006): 7445-7450.

Rabiet, M., Togola, A., Brissaud, F., Seidel, J. L., Budzinski, H. & Elbaz-Poulichet, F. Consequences of treated water recycling as regards pharmaceuticals and drugs in surface and ground waters of a medium-sized Mediterranean catchment; Environmental Science & Technology 40 (17) (2006): 5282-5288.

Reinthaler, F. F., Posch, J., Feierl, G.,Wust, G., Haas, D., Ruckenbauer, G., Mascher, F. & Marth, E., Antibiotic resistance of E. coli in sewage and sludge; Water Resources 37 (8) (2003): 1685-1690.

Safarzadeh-Amiri, A., Bolton, J. R. & Cater, S. R., Ferrioxalate-mediated photodegradation of organic pollutants in contaminated water; Water Resources 31 (4) (1997): 787-798.

Sapkota, A. R., Curriero, F. C., Gibson, K. E. & Schwab, K. J., Antibiotic-resistant Enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation; Environmental Health Perspectives 115 (7) (2007): 1040-1045.

Se′veno, N. A., Kallifidas, D., Smalla, K., van Elsas, J. D., Collard, J. M., Karagouni, A.D. & Wellington, E. M. H., Occurrence and reservoirs of antibiotic resistance genes in the environment; Reviews in Medical Microbiology 13 (1) (2002): 15-27.

Stackelberg, P. E., Furlong, E. T., Meyer, M. T., Zaugg, S. D., Henderson, A. K. & Reissman, D. B. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant; Science of the Total Environment 329 (1-3) (2004): 99-113.

Schwartz, T., Kohnen, W., Jansen, B. & Obst, U., Detection of antibiotic resistant and their resistance genes in wastewater, surface water and drinking water biofilms; FEMS Microbiology Ecology 43 (3) (2003): 325-335.

Tripathi, P. & Chaudhuri, M., Decolourisation of complex azo dyes and treatment of a dyehouse waste by modified photo-Fenton (UV-vis/ferrioxalate/H_2O_2) process; Engineering & material sciences 11 (6) (2004): 499-504.

Walter, M. V. & Vennes, J.W., Occurrence of multiple-antibiotic resistant enteric bacteria in domestic sewage and oxidative lagoons; Applied & Environmental Microbiology 50 (4) (1985): 930-933.

Westerhoff, P., Yoon, Y., Snyder, S. A. & Wert, E., Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes; Environmental Science & Technology 39 (17) (2005): 6649-6663.

WHO, The World Health Report - A Safer Future-Global: Public Health Security in the 21st Century; World Health Organization, Geneva (2007).

Yang, L., Yu, L. E. & Ray, M. B., Degradation of paracetamol in aqueous solutions by TiO2 photocatalysis; Water Research 42 (3) (2008): 3480-3488.

Yaziz, M. I., The Occurrence of Antibiotic Resistant Salmonellas in Sewage and the Effect of Primary Sedimentation on their Numbers; Pertanika 4 (1) (1981): 39-42.

Zhang, N., Huang, J. & Zheng, W., Research on photo degradation of aniline wastewater by dynamic UV-vis/H2O2/ferrioxalate complexes process; Chemical Industry & Engineering (China) 53 (1) (2002): 36-39.

Zhang, T., Zhang, M., Zhang, X. & Fang, H. H., Tetracycline resistance genes and tetracycline resistant lactose-fermenting Enterobacteriaceae in activated sludge of sewage treatment plants; Environmental Science & Technology 43 (10) (2009a): 3455-3460.

Zhang, Y., Marrs, C. F., Simon, C. & Xi, C., Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter app.; Science of the Total Environment 407 (12) (2009b): 3702-3706.