**DOKUZ EYLÜL UNIVERSITY**
**GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

# ADVANCED OXIDATION TREATMENT OF ANTIBIOTIC CONTAINING WATER

**by**
**Filiz AY**

**December, 2009**
**İZMİR**

# ADVANCED OXIDATION TREATMENT OF ANTIBIOTIC CONTAINING WATER

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**by**
**Filiz AY**

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**M.Sc THESIS EXAMINATION RESULT FORM**

We have read the thesis entitled **"ADVANCED OXIDATION TREATMENT OF ANTIBIOTIC CONTAINING WATER"** completed by **FİLİZ AY** under supervision of **PROF. DR. FİKRET KARGI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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**ADVANCED OXIDATION TREATMENT OF ANTIBIOTIC CONTAINING WATER**

## ABSTRACT

In advanced oxidation experiments, Amoxicillin was selected as the pollutant, and was used in form of Amoxicillin trihydrate. Studying degradation and mineralization of Amoxicillin in aqueous solution by using advanced oxidation methods, namely the Fenton and photo-Fenton treatments were the major objectives of this thesis. Various concentrations of Amoxicillin containing synthetic wastewater were prepared and used in experimental studies.

Antibiotic (Amoxicillin) and TOC measurements were carried out to determine the most effective catalyst, oxidant and antibiotic concentration combinations and reaction time for advanced oxidation of Amoxicillin by Fenton and photo-Fenton and to compare the tested methods and conditions to select the most suitable method and conditions. Box-Behnken statistical experiment design method was used to determine the effects of reagent concentrations on degradation and mineralization of amoxicillin.

Advanced oxidation experiments were carried out with synthetic medium containing Amoxicillin. The most suitable dosages yielding the highest Amoxicillin degradation and mineralization were determined using the Fenton and photo-Fenton treatments. In oxidation experiments; hydrogen peroxide (35 percent) was used as oxidant. The catalyst was ferrous sulphate. Sulfuric acid was used for pH adjustment. Advanced oxidation methods were compared in terms of removal performances. In advanced oxidation of antibiotic containing synthetic wastewater by Fenton's reagent, maximum antibiotic and TOC removal efficiencies were 100 percent and 37.08 percent, respectively. In photo-Fenton oxidation, maximum antibiotic and TOC removal efficiencies were 100 percent and 50.25 percent, respectively.

Both methods were proven to be effective for Amoxicillin removal. However photo-Fenton oxidation was more effective for TOC removal or mineralization.

**Keywords:** Advanced oxidation, Amoxicillin, Amoxicillin Trihydrate, Box-Behnken

**ADVANCED OXIDATION TREATMENT OF ANTIBIOTIC CONTAINING WATER**

## ÖZ

İleri oksidasyon deneyleri için kirletici madde olarak Amoksisilin seçilmiş, Amoksisillin trihidrat formunda kullanılmıştır. Bu tezin ana amacı Fenton ve foto-Fenton işlemleri ile sulu çözeltilerde ileri oksidasyon metodları kullanarak Amoksisillinin bozunma ve mineralizasyonunu incelemektir. Çeşitli kontsantrasyonlarda Amoksisilin içerikli sentetik atık sular hazırlanarak deneylerde kullanılmıştır.

Antibiyotik (Amoksisillin) ve Toplam Organic Karbon (TOK) ölçümleri, Amoksisillin'in Fenton ve foto-Fenton ileri oksidasyon yöntemleri ile arıtılmasında en etkili katalizör, oksidant ve antibiyotik konsantrasyonlarının kombinasyonlarının ve reaksiyon süresinin belirlenmesi, test edilen metodların koşullarının karşılaştırılması ve en etkili yöntemin seçilmesi için yapılmıştır. Reaktif konsantrasyonlarının Amoksisillinin parçalanması ve mineralizasyonu üzerindeki etkilerini belirlemek için Box-Behnken istatistiksel deney tasarım yöntemi kullanılmıştır.

Amoksisillin içeren sentetik çözeltide ileri oksidasyon deneyleri yapılmıştır. Fenton ve foto-Fenton işlemleri kullanılarak en yüksek Amoksisillin bozunması ve mineralizyonu sağlayan dozlar belirlenmiştir.

İleri oksidasyon deneylerinde, oksidant olarak hidrojen peroksit (yüzde 35'lik), katalizör olarak demir sülfat, pH ayarlamaları için sülfürik asit kullanılmıştır. İleri oksidasyon yöntemlerinin antibiyotik giderim ve mineralizasyon performansları karşılaştırılmıştır. Amoksisillin içeren sentetik atıksuların ileri oksidasyonunda Fenton reaktifi kullanılarak yüzde 100 antibiyotik ve yüzde 37.08 TOK giderimi sağlanmıştır. Foto-Fenton yöntemi kullanıldığında yüzde 100 antibiyotik ve yüzde 50.25 TOK giderimi saglanmıştır.

Antibiyotik giderim yüzdeleri açısından kullanılan iki yöntem de verimlidir. Ancak TOK gideriminde foto-Fenton yöntemi daha etkilidir.

**Anahtar Kelimeler:** İleri oksidasyon, Amoksisillin, Amoksisillin Trihidrat, Box-Behnken

**CONTENTS**

Page

# CHAPTER ONE
# INTRODUCTION

## 1.1 General Information About Antibiotics

The term antibiotic originally referred to any agent with biological activity against living organisms; however, ‘‘antibiotic” now refers to substances with antibacterial, anti-fungal, or anti-parasitical activity (Kümmerer and Henninger, 2003). Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases in humans, animals and plants. Over the years, this definition has been expanded to include synthetic and semi-synthetic produces (Kümmerer, 2009).

Antibiotics can be grouped by either their chemical structure or mechanism of action. They are a diverse group of chemicals that can be divided into different sub-groups such as ß-lactams, quinolones, tetracyclines, macrolides, sulphonamides and others. There are currently about 250 different chemical entities registered for use in medicine and veterinary medicine (Kümmerer and Henninger, 2003). In the same molecule antibiotics may contain different functionalities. Because of that they are often complex molecules.

### *1.1.1 History And Background*

The first antibiotics were of natural origin, e.g. penicillins produced by fungi in the genus Penicillium, or streptomycin from bacteria of the genus Streptomyces. Currently, antibiotics are obtained by chemical synthesis, such as the sulfa drugs (e.g. sulfamethoxazole), or by chemical modification of compounds of natural origin (Kümmerer, 2009).

Work by Alexander Fleming (1881-1955), Howard Florey (1898-1968) and Ernst Chain (1906-1979), penicillin was first produced on a large scale for human use in 1943. At this time, the development of a pill that could reliably kill bacteria was a

remarkable development and many lives were saved during World War II because this medication was available. But quickly, it became obvious that this new "wonder drug" could bear improvement. For example:

• Penicillin is not well absorbed from the intestinal tract meaning that at least 70% of an oral dose is wasted.

• Penicillin is also a short-acting medication, with half of the amount circulating being removed from the body every half hour.

• Not all bacteria have the type of cell wall which is susceptible to destruction by Penicillin. (Bacteria are classified as Gram negative or Gram positive, depending on the cell wall characteristics. Penicillin is able to punch holes through the Gram positive cell wall but is not very effective against the Gram negative cell wall.)

• *Staphylococci* (an important group of bacteria) have developed an enzyme to break the Penicillin molecule apart and are thus rarely susceptible to Penicillin (Amoxicillin, n.d.).

Recently, the attention of many researchers working in the environmental field was focused on the presence in the environment (and more specifically inwaters) of pharmaceuticals as a new class of pollutants (Kümmerer, 2001; Heberer, 2002).

Human and veterinary drugs represent more than 4000 molecules and 10000 specialized products and are the main sources of pharmaceutical contamination in natural water systems (Beausse, 2004; Bendz et al., 2005). Waterways contamination by pharmaceuticals is widely documented: hormones, beta-lactamides, anti-inflammatories, analgesics, lipid regulators, anti-depressants, antibiotics, cytostatic agents have been found in small creeks, lakes, rivers, estuaries and, rarely, in groundwater, drinking water and marine water (Kümmerer, 2001; Heberer, 2002; Beausse, 2004; Fent et al., 2006; Ikehata et al., 2006) . In the aquatic environment, ecological risk of antibiotics should not be underestimated.

Environmental pollution because of the presence of drugs may also be generated by manufacturing residues, agriculture, where large amounts of pharmaceutical agents are applied in veterinary medical care and also medical substances used by humans. In addition, sewage sludge and manure applied as fertilizer may also contribute to groundwater contamination. Problem that may be created by the presence of antibiotics at low concentrations in the environment is the development of antibiotic resistant bacteria (Walter and Vennes, 1985).

In recent years, this emerging pollution issue in aquatic environment caused by pharmaceutically active compounds (PhACs) has been researched (Heberer, 2002). Fig. 1.1 (Haaling-Sørensen et al., 1998) shows possible sources and anticipated exposure routes for occurance of different type of PhAC residues in the environment.

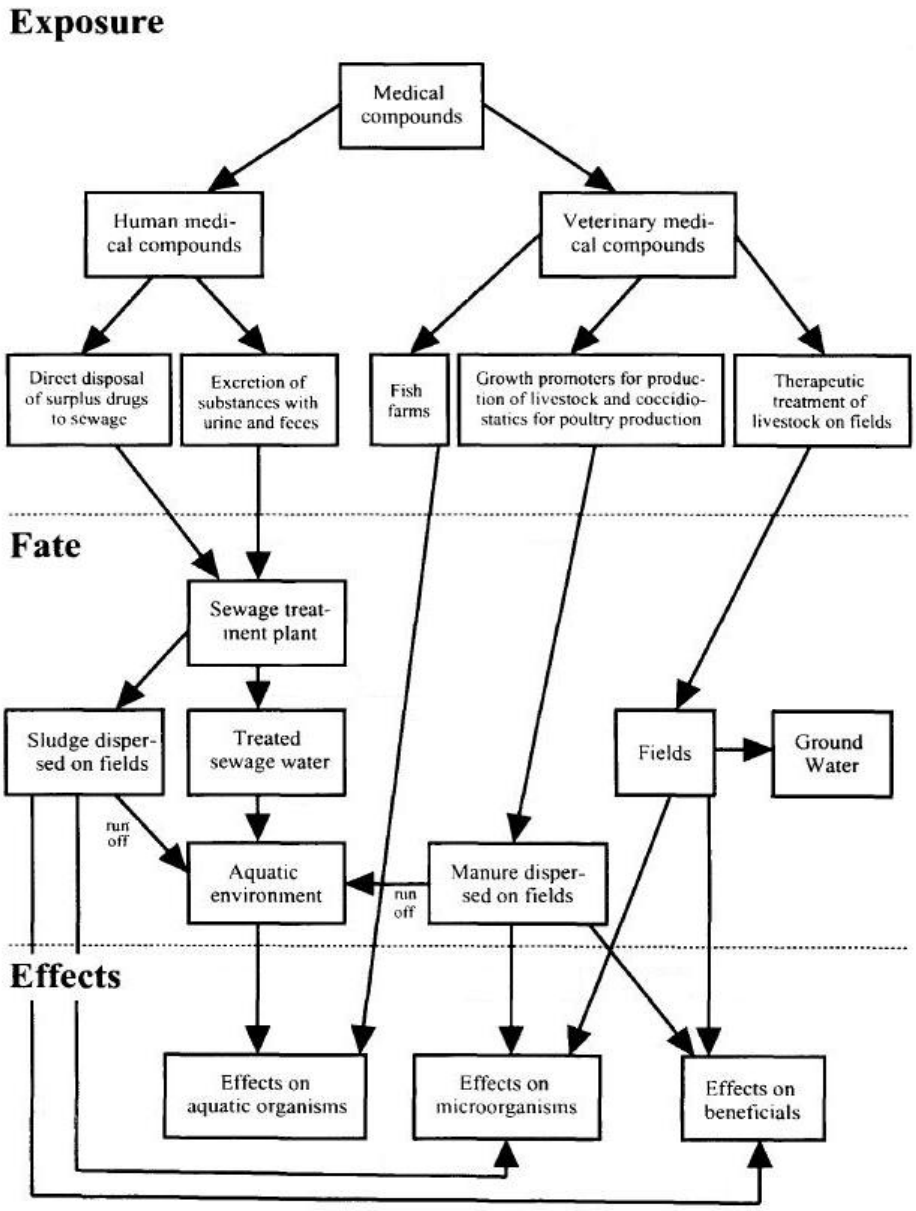



Figure 1.1 Possible sources and anticipated exposure routes for occurance of different type of PhAC residues in the environment (Haaling-Sørensen et al., 1998).

### *1.1.2 General Information About Amoxicillin*

Amoxicillin is one of the widely used human and veterinary medicine of environmental concern. Amoxicillin is a semi-synthetic penicilin obtaining its antimicrobial properties from the presence of a beta-lactam ring which is active against gram-positive cocci, including non-penicillin resistant streptococcal, staphylococcal and enterococcal species. Amoxicillin represents a synthetic

improvement upon the original penicillin molecule and is more resistant to damages from stomach acid yielding less waste of the oral dose. While Amoxicillin is still susceptible to destruction by Staphylococcal enzymes, it does have a much broader spectrum against the Gram negative cell wall and is able to last a bit longer (Amoxicillin, n.d.). Inhibition of bacterial cell wall synthesis depends on binding to one or more of the penicillin-binding proteins (e.g., carboxypeptidases, endopeptidases, transpeptidases) in the cytoplasmic membrane. This attachment inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls. Bacterial cell death occurs due to the action of autolytic enzymes (autolysins and murein hydrolases) (Castle, 2008). In addition to gram-negative organisms, it has activity against some gram-positive anaerobic organisms and gram-negative anaerobic organisms.

*1.1.2.1 Uses of Amoxicillin*

Amoxicillin is usually given by mouth, as the Amoxicillin Trihydrate.

Amoxicillin trihydrate is a white odorless crystalline powder. It has been used as an alternative to chloramphenicol in the treatment of infections caused by Salmonella. The usual dose is 250–500 mg three times daily. It is usually used for moderate infections, but 1 g may be given in every 6 h for severe infections (Basker and Sutherland, 1977; Strausbaugh et al., 1978).

Amoxicillin is especially helpful in anaerobic infections (those which grow without the benefit of oxygen). Typical uses might include:

- Infected bite wounds,
- Upper respiratory infections,
- Infected teeth,
- Bladder infections (Amoxicillin, n.d.).

A new formulation of the antibiotics Amoxicillin and clavulanate can be used to deliver a double-dose of Amoxicillin without increasing the side effects in children with ear infections. This is the claim of studies reported at the American Academy of Pediatrics annual meeting (17–21 October 1998, San Francisco, CA, USA).

Daniel Burch, Group Director, Clinical Antiinfectives at SmithKline Beecham pharmaceuticals (Brentford, UK), presented the results of a clinical trial performed on 453 children, aged from three months to 12 years. He explained that the new formulation was as effective as the original, and had shown no increased incidence of adverse effects such as diarrhoea.

*1.1.2.2 Interactions With Other Drugs*

When the organism in a serious infection cannot be isolated, a common strategy is to attempt to "cover" for all possible bacteria. Amoxicillin is frequently used in combination with other antibiotics for this purpose.

Clavulanic acid may be added to Amoxicillin to increase Amoxicillin's spectrum against Staphylococcal bacteria (Bradley, 1999).

*1.1.2.3 Side Effects*

Some individuals experience nausea with this medication. Giving the medication with food seems to reduce this effect (Bradley, 1999).

*1.1.2.4 Special Cautions*

The oral suspension should be refrigerated, though if it is mistakenly left out of the refrigerator, this is not a problem. The oral suspension should be discarded after 2 weeks.

Amoxicillin may be given with or without food.

Amoxicillin will cross the placenta in a pregnant patient, but is felt to be safe for use during pregnancy (Bradley, 1999).

## 1.2 Literature Review

A number of studies on removal of antibiotics by advanced oxidation are reported in literature.

Fenton treatment was found to be effective in treatment of an aqueous solution of Amoxicillin, ampicillin and cloxacillin. Under optimum operating conditions (COD/$H_2O_2$/$Fe^{2+}$ molar ratio 1:3:0.30, pH 3), for an aqueous solution of Amoxicillin (104 mg/L), ampicillin (105 mg/L) and cloxacillin (103 mg/L), complete degradation of the antibiotics occurred in 2 min (Elmolla and Chaudhuri., 2009).

In another experimental study, relatively higher COD and TOC removal rates were obtained with the dark $Fe^{2+}$/$H_2O_2$/pH=3 process when compared with dark Fenton-like ($Fe^{3+}$/$H_2O_2$/pH=3) reactions as a direct consequence of Fenton's chemistry. The presence of UV-C light only slightly improved the treatment performance. Highest removal efficiency in terms of TOC could be achieved via photo-Fenton's reagent, whereas COD removal was higher for the photo-Fenton-like process. Separate experimental studies conducted with the penicillin active substance Amoxicillin trihydrate indicated that the aqueous antibiotic substance can be completely eliminated after 40 min advanced oxidation applying photo-Fenton's reagent (pH = 3; $Fe^{2+}$:$H_2O_2$ molar ratio = 1:20) and alkaline ozonation (at pH = 11.5), respectively (Alaton and Dogruel, 2004).

Photodegradation of the pharmaceuticals Amoxicillin (AMX), bezafibrate (BZF) and paracetamol (PCT) in aqueous solutions via the photo-Fenton process was investigated under black-light and solar irradiation. The results presented in this work demonstrate that the photo-Fenton process could be successfully applied to the degradation of AMX, BZF and PCT even when present in complex samples, such as

STP (sewage treatment plant) effluent, where they are often encountered (Trovó et al., 2008).

Removal of 28 human and veterinary antibiotics was assessed in a conventional (activated sludge) and advanced (microfiltration/reverse osmosis) wastewater treatment plant (WWTP) in Brisbane, Australia. The dominant antibiotics detected in wastewater influents were cephalexin, ciprofloxacin, cefaclor, sulphamethoxazole and trimethoprim. Results indicated that both treatment plants significantly reduced antibiotic concentrations with an average removal efficiency of 92%. However, antibiotics were still detected in both effluents from the low-to-mid ng $L^{-1}$ range. Antibiotics detected in effluent from the activated sludge WWTP included ciprofloxacin, sulphamethoxazole, lincomycin and trimethoprim. Antibiotics identified in microfiltration/reverse osmosis product water included naladixic acid, enrofloxacin, roxithromycin, norfloxacin, oleandomycin, trimethoprim, tylosin and lincomycin. Certain traditional parameters, including nitrate concentration, conductivity and turbidity of the effluent were assessed as predictors of total antibiotic concentration (Watkinson et al., 2007).

Rizzo et al. (2009), investigated that degradation kinetics and mineralization of an urban wastewater treatment plant effluent contaminated with a mixture of pharmaceutical compounds composed of Amoxicillin (10 mg/L), carbamazepine (5 mg/L) and diclofenac (2.5 mg/L) by $TiO_2$ photocatalysis. The process efficiency was evaluated through UV absorbance and TOC measurements. The photocatalytic effect was investigated using both spiked distilled water and actual wastewater solutions. A pseudo-first order kinetic model was found to fit well the experimental data. The mineralization rate (evaluated in terms of TOC measurements) in wastewater contaminated with pharmaceuticals was found to be really slow ($t_{1/2}$=86.6 min) compared to that of the same pharmaceutical mixture in distilled water ($t_{1/2}$=46.5 min), probably because of the interference of radicals scavengers such as carbonates which typically occur in high concentrations in wastewaters (Rizzo et al., 2009).

Zerovalent iron powder (ZVI or $Fe^0$) and nanoparticulate ZVI (nZVI or $nFe^0$) are proposed as cost-effective materials for the removal of aqueous antibiotics. Results showed complete removal of Amoxicillin (AMX) and Ampicillin (AMP) upon contact with $Fe^0$ and $nFe^0$. Kinetic studies demonstrated that AMP and AMX (20 mg/L) undergo first-order decay with half-lives of about 60.3±3.1 and 43.5±2.1 min respectively after contact with ZVI under oxic conditions. In contrast, reactions under anoxic conditions demonstrated better degradation with $t_{1/2}$ of about 11.5±0.6 and 11.2±0.6 min for AMP and AMX respectively. NaCl additions accelerated $Fe^0$ consumption, shortening the service life of $Fe^0$ treatment systems (Ghauch et al., 2009).

In a work by Travó et al. (2009) the photocatalytic degradation of the antibiotic sulfamethoxazole (SMX) by solar photo-Fenton at pilot plant scale was evaluated in distilled water (DW) and in seawater (SW). The influence of $H_2O_2$ and iron concentration on the efficiency of the photocatalytic process was evaluated. An increase in iron concentration from 2.6 to 10.4 mg/L showed only a slight improvement in SMX degradation and mineralization. However, an increase in $H_2O_2$ concentration up to 120 mg/L during photo-Fenton in DW decreased SMX solution toxicity from 85% to 20%, according to results of *Daphnia magnia* bioassays. The same behaviour was not observed after photo-Fenton treatment in SW. Despite 45% mineralization in SW, toxicity increased from 16% to 86% as shown by *Vibrio fischeri* bioassays, which suggests that the intermediates generated in SW are different from those in DW.

Another experimental study examined the results of the performance of photo-induced oxidation, heterogeneous photocatalysis, ozonation and peroxone in degrading the fluoroquinolone antimicrobial ciprofloxacin (CIP) in a hospital effluent. The real samples were collected from the treatment system of the University Hospital of Santa Maria (HUSM). Both heterogeneous photocatalysis and peroxone led to almost complete CIP degradation after 60 min treatment. Ozonation showed the best performance: total degradation after 30 min treatment (Vasconcelos et al., 2009).

In another study the degradation of the worldwide Non-Steroidal Anti-Inflammatory Drug (NSAID) ibuprofen (IBP) by photo-Fenton reaction by use of solar artificial irradiation was carried out. Non-photocatalytic experiments (complex formation, photolysis and UV/Vis- $H_2O_2$ oxidation) were executed to evaluate the isolated effects and additional differentiated degradation pathways of IBP. The degradation pathway can be described as an interconnected and successive principal decarboxylation and hydroxylation steps. TOC depletion of 40% was observed in photo-Fenton degradation. Both decarboxylation and hydroxylation mechanisms, as individual or parallel process are responsible for IBP removal in Fenton and photo-Fenton systems. An increase in the biodegradability of the final effluent after photo-Fenton treatment was observed. Final $BOD_5$ of 25 mg/L was reached in contrast to the initial $BOD_5$ shown by the untreated IBP solution (BOD5 <1 mg/L). The increase in the biodegradability of the photo-Fenton degradation byproducts opens the possibility for a complete remediation with a final post-biological treatment (Mendez-Arriaga et al., 2009).

Ben et al. (2009), investigated the degradation of six selected antibiotics, including five sulfonamides and one macrolide, by Fenton's reagent in swine wastewater pretreated with sequencing batch reactor (SBR). The studied antibiotics were purchased from the following sources: sulfathiazole (STZ, 99%), sulfamethoxazole (SMX), sulfamethizole (SML), sulfadimethoxine (SDM) from Sigma–Aldrich (St. Louis, MO, USA); sulfamethazine (SMN, 99%) from Acros (New Jersey, USA); and tiamulin fumarate (TIA, 98%) from Dr. Ehrenstorfer (Augsburg, Germany).The results indicate that the optimal conditions for Fenton's reagent with respect to practical application were as follows: batch dosing mode, 1.5:1 molar ratio of $[H_2O_2]/[Fe^{2+}]$, initial pH 5.0. Under the optimal conditions, Fenton's reagent could effectively degrade all the selected antibiotics and was resistant to the variations in the background COD (0–419 mg/L) and SS (0–250 mg/L) of the SBR effluent. Besides, Fenton's reagent helped not only to remove total organic carbon (TOC), heavy metals (As, Cu and Pb) and total phosphorus (TP), but also inactivated bacteria and reduced wastewater toxicity. This work demonstrated

that the integrated process combining SBR with Fenton's reagent could provide comprehensive treatment to swine wastewater.

### *1.2.1 Some Applications of Box-Behnken Design (BBD)*

Matthews et al. (1981), used BBD for the optimization of an enzymatic procedure for the determination of arsenic in aqueous solutions.

Rorigues et al. (2007), developed a method to detect the most important factors affecting formation of the four trihalomethanes (THM) (chloroform, bromodichloromethane, chlorodibromomethane and bromoform) in water disinfection processes using chlorine. BBD was used during the optimization step.

Petz and Lamar (2007), developed a receptor protein microplate assay for the detection and determination of penicillins and cephalosporins with intact beta-lactam in milk, bovine and porcine muscle juice, honey and egg samples. The optimization step was performed using BBD.

BBD was employed for the optimization of an electrochemical process using reticulated vitreous carbon-supported-onpolyaniline cathodes for the reduction of hexavalent chromium of industrial wastewater samples (Ruotolo and Gubulin, 2005).

Fu et al. (2007), developed a photoelectrocatalytic oxidation system using a $Ti/TiO_2$ electrode for the degradation of fulvic acid (FA). The optimization step was carried out using BBD.

The use of the Box–Behnken experimental design in the optimisation and robustness testing of a capillary electrophoresis method for the analysis of ethambutol hydrochloride in a harmaceutical formulation (Ragonese et al., 2002).

Aslan and Cebeci (2007) used Box–Behnken experimental design and response surface methodology for modeling some Turkish coals.

Advanced oxidation of an azo-dye, Direct Red 28 (DR 28) by photo-Fenton treatment was investigated in batch experiments using Box–Behnken statistical experiment design and the response surface analysis. Dyestuff (DR 28), $H_2O_2$ and Fe(II) concentrations were selected as independent variables in Box–Behnken design while color and total organic carbon (TOC) removal (mineralization) were considered as the response functions (Ay et al., 2009).

Advanced oxidation of Direct Red 28 (DR 28) in aqueous solution by Fenton's reagent using $FeSO_4$ as source of Fe (II) was investigated. Effects of the dyestuff and the reagent concentrations ($H_2O_2$ and Fe (II)) on oxidation of the azo dye were investigated by using a Box-Behnken statistical experiment design and the surface response analysis. Degradation and mineralization (conversion to $CO_2$ and $H_2O$) of the azo dye by Fenton treatment was evaluated following total organic carbon (TOC) and color removal (Ay et al., 2008)

In analytical chemistry, multivariate techniques have been applied to the optimization of chemical factors during the development of analytical strategies involving pre-concentration systems using solid phase extraction (Barbosa et al., 2007; Penteado et al., 2006) cloud point extraction (Lamos et al., 2007; Bezerra et al., 2004), liquid–liquid extraction (Baranda et al., 2005; Ebrahimzadeh et al., 2007) and coprecipitation (Saracoglu et al., 2006); procedures for sample digestion (Soriano et al., 2007; Jalbani et al., 2006); sampling systems (Conde et al., 2004); chromatographic methods (Garcia-Villar et al., 2006; Carasek et al., 2007); capillary electrophoresis (Mamani et al., 2006) methods employing flow injection analysis (del Campo et al., 2006) and sequential injection analysis (da Silva et al., 2004; Idrıs et al., 2006); electroanalytical methods (Teofilo et al., 2003; Zarei et al., 2006) and thermogravimetry (Felsner et al., 2004).

Other applications include the optimization of instrumental parameters of equipment for analysis by graphite furnace atomic absorption spectrometry (GF AAS) (Pereira-Filho et al., 2002; de Amorim et al., 2006), inductively coupled plasma optical emission spectrometry (ICP OES) (Villaneuva et al., 2000; Trevizon et al., 2005) and inductively coupled plasma mass spectrometry (ICP-MS) (Woller et al., 1998). Several review papers have been published on this subject (Ferreira et al., 2004; Ferreira et al., 2007).

### 1.3 Objectives and Scope

The major objective of this thesis is to study degradation and mineralization of Amoxicillin in aqueous solution by using advanced oxidation methods, namely the Fenton and photo-Fenton treatments.

- To determine the most effective catalyst, oxidant and antibiotic concentration combinations and reaction time for advanced oxidation of Amoxicillin by Fenton oxidation.

- To determine the most effective catalyst, oxidant and antibiotic concentration combinations and reaction time for advanced oxidation of Amoxicillin by photo-Fenton treatment.

- To compare the tested methods and conditions and to select the most suitable method and conditions.

Two different advanced oxidation methods were used for treatment of Amoxicillin containing water. Antibiotic and TOC removals were quantified in order to evaluate and compare the performance of the selected advanced oxidation methods.

In the first part of the experimental studies, calibration curves were prepared for TOC and antibiotic. Different chemical dosages were tried and the most suitable upper and lower concentrations were obtained.

In the second part of experimental studies, advanced oxidation experiments were carried out with synthetic medium containing Amoxicillin. The most suitable dosages yielding the highest Amoxicillin degradation and mineralization were determined using the Fenton and photo-Fenton treatments.

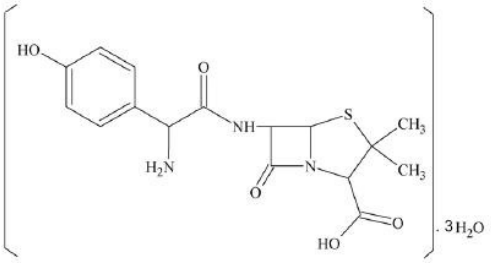
At the end of the experimental studies, advanced oxidation methods were compared in terms of removal performances.

# CHAPTER TWO
# MATERIALS AND METHODS

## 2.1 Materials

In advanced oxidation experiments, Amoxicillin was selected as the pollutant, and was used in form of Amoxicillin trihydrate. Amoxicillin trihydrate was supplied from Bilim Pharmaceuticals in İstanbul, Turkey. Synthetic wastewaters containing various concentrations of Amoxicillin were prepared. Some basic characteristics of Amoxicillin trihydrate are summarized in Table 2.1.

Table 2.1 Basic characteristics of Amoxicilin trihydrate

| | |
|---|---|
| *Name of the Clinical Form* | Amoxicillin trihydrate |
| *Molecular Formula* | $C_{16}H_{19}N_3O_5S.3H_2O$ |
| *Chemical Structure* (Travó et al., 2008) |  |
| *Comments* | Amoxicillin is a semi-synthetic penicillin. The free amino group on this molecule enhances activity against gram-negative bacteria in comparison with natural penicillins, such as penicillin G (McEvoy, 2001). |
| *Molecular Weight* | 419.408 g |
| *Experimental Solubility* | 3430 mg/L, soluble in water (Showing Drug Card of Amoxicillin (DB01060), n.d.) |

In oxidation experiments, hydrogen peroxide (35%, w/w solution) was used. The catalyst was ferrous sulphate ($FeSO_4.7H_2O$). Sulfuric acid ($H_2SO_4$) was used for pH adjustment. All chemicals were from Merck.

Concentrated stock solution of Fe(II) (5000 mg/L), stock solution of $H_2O_2$ (10000 mg/L) and Sulfuric acid solution (1 N) were prepared for further dilution to obtain solutions of desired concentrations. Fe(II) stock solution was stored in the dark to prevent oxidation of Fe(II).

HPLC-grade acetonitrile, methanol and $KH_2PO_4$ (Merck) were used for HPLC analyses. For the TOC measurements, potassium phythalate solution was used as calibration standards.

Water used in all experiments and for chemical solutions was purified using a Mili-Q system (milipore filtration). All glassware was first rinsed with acid solution, secondly with tap water and then with distilled water before use.

## 2.2 Methods

### *2.2.1 Experimental System*

#### *2.2.1.1 Fenton Experiments*

A jar test apparatus consisting of four beakers of 1 liters each were used as the experimental system. The beakers were filled with 1 liter of the Amoxicillin solution and predetermined amounts of oxidants ($H_2O_2$ and Fe (II)) were injected into the agitated reactors (185 rpm) containing antibiotic solution at the beginning of each experiment. The iron salt was mixed well with aqueous antibiotic solution before the addition of hydrogen peroxide solution. The beakers were open to the atmosphere at room temperature (23–25°C). Temperature changes during reactions were negligible.

#### *2.2.1.2 Photo-Fenton Experiments*

Figure 2.1 (Ay et al, 2009) depicts a schematic diagram of the laboratory-scale photochemical reactor used in the experimental studies at constant temperature and stirring. All photo-oxidation experiments were performed in the completely mixed,

cylindrical photo-reactor made of glass with a total volume of 2.2 L operated in batch mode. The reactor was covered with an aluminum foil to avoid any light leakage to the outside. The reactor was placed on a magnetic stirrer for mixing of aqueous solution and had inlets for feeding reactants, and ports for measuring temperature and withdrawing samples. The UV irradiation source placed in a quartz tube was a 16W low-pressure mercury vapor lamp with maximum emission at 254 nm. The intensity of the UV radiation was measured using the ferrioxalate actinometry method and found to be $4.98\times10^{-6}$ Einstein $s^{-1}$. The lamp was surrounded with a water-cooling jacket in order to control temperature. The reaction chamber was filled with synthetic Amoxicillin solution, which was placed between the reactor walls and UV lamp system.

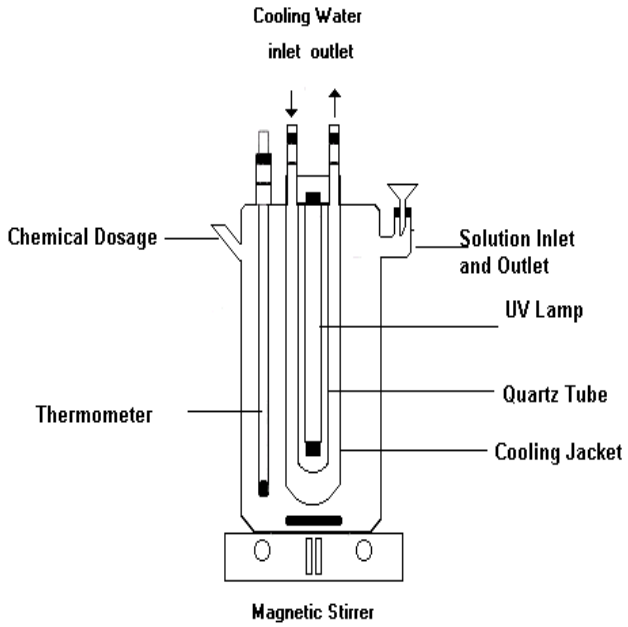



Figure 2.1 A schematic diagram of the experimental set-up used for photo-Fenton treatment

### *2.2.2 Design of Experiments*

In recent years, chemometric tools have been frequently applied to the optimization of analytical methods, considering their advantages such as a reduction

in the number of experiments that need to be executed resulting in lower reagent consumption and considerably less laboratory work (Ferreira et al., 2007).

Response surface methodology (RSM) is used when only several significant factors are involved in optimization. Different types of RSM designs include 3-level factorial design, central composite design (CCD) (Box and Wilson, 1951; Boza et al., 2000), Box-Behnken design (Singh et al., 1995), and D-optimal design (Sanchez-Lafuente et al., 2002).

Among all the response surface methodology (RSM) designs, the Box-Behnken design requires fewer runs than the others (e.g, 15 runs for a 3-factor experimental design) (Ay et al., 2008). Table 2.2 contains the coded values of the factor levels for BBD on three variables.

Table 2.2 A Coded factor levels for a Box-Behnken design of a three-variable system

| *Number of Experiment* | $X_1$ | $X_2$ | $X_3$ |
|---|---|---|---|
| 1 | +1 | -1 | 0 |
| 2 | -1 | -1 | 0 |
| 3 | -1 | +1 | 0 |
| 4 | +1 | +1 | 0 |
| 5 | +1 | 0 | -1 |
| 6 | +1 | 0 | +1 |
| 7 | -1 | 0 | -1 |
| 8 | -1 | 0 | +1 |
| 9 | 0 | -1 | -1 |
| 10 | 0 | -1 | +1 |
| 11 | 0 | +1 | -1 |
| 12 | 0 | +1 | +1 |
| 13 (C) | 0 | 0 | 0 |
| 14 (C) | 0 | 0 | 0 |
| 15 (C) | 0 | 0 | 0 |

A multiple regression analysis is carried out to obtain the coefficients of the response functions. The experimental design method used in this study was Box–Behnken, a fractional factorial design for three independent variables. It is applicable once the critical variables have been identified (Khajeh, 2009).

Box–Behnken design is a spherical, revolving design consisting of a central point and the central points of the edges of the cube circumscribed on the sphere (Evans, 2003). It is a three level fractional factorial design consisting of a full $2^2$ factorial seeded into a balanced incomplete block design. The B-B design consists of three interlocking $2^2$ factorial designs having points, all lying on the surface of a sphere surrounding the center of the design. The method has been applied for optimization of several chemical and physical processes; and the number of experiments are decided accordingly (Kumar et al., 2007).

The Box-Behnken is a good design for response surface methodology because the method permits: (i) estimation of the parameters of the quadratic model; (ii) building of sequential designs; (iii) detection of lack of fit of the model; and (iv) use of blocks (Ferreira et al., 2007).

Box-Behnken designs (BBD) (Box and Behnken, 1960) are a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. For three factors its graphical representation can be seen in two forms:

- A cube that consists of the central point and the central points of the edges, as can be observed in Figure 2.2.
- A figure of three interlocking $2^2$ factorial designs and a central point, as shown in Figure 2.3.

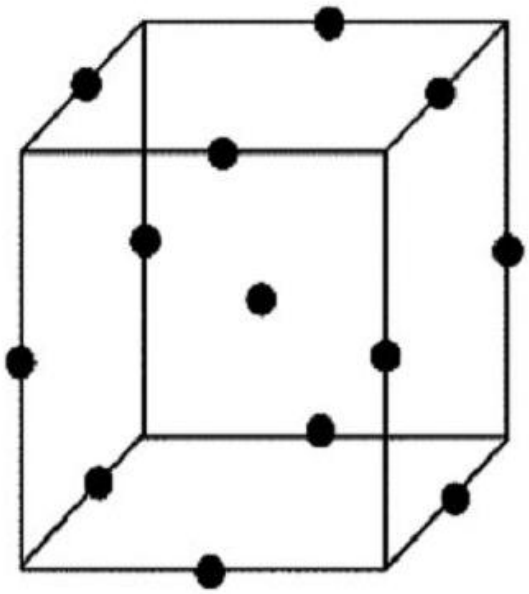


Figure 2.2 The design, as derived from a cube.

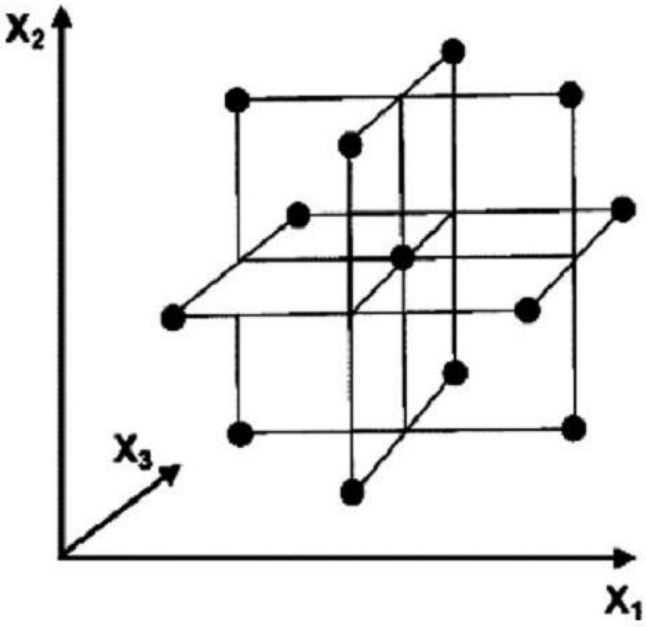



Figure 2.3 Representation of interlocking $2^2$ factorial experiments.

A comparison between the BBD and other response surface designs (central composite, Doehlert matrix and three-level full factorial design) has demonstrated that the BBD and Doehlert matrix are slightly more efficient than the central composite design but much more efficient than the three-level full factorial designs

where the efficiency of one experimental design is defined as the number of coefficients in the estimated model divided by the number of experiments (Ferreira et al., 2007).

Advantage of the BBD is that it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. So these designs are useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results might occur. Conversely, they are not indicated for situations in which we would like to know the responses at the extremes, that is, at the vertices of the cube (Ferreira et al., 2007).

The optimization procedure involves studying the response of the statistically designed combinations, estimating the coefficients by fitting the experimental data to the response functions, predicting the response of the fitted model, and checking the adequacy of the model (Ay et al., 2008). Three experimental parameters, or factors, were varied at three levels: the doses of Amoxicillin (mg/L) ($X_1$), hydrogen peroxide (mg/L) ($X_2$) and ferrous ion (mg/L) ($X_3$). These parameters were chosen as they were considered to have the most significant effect on the antibiotic and TOC removal efficiency. The levels were selected based on knowledge of the system acquired from initial experimental trials.

The low, center, and high levels of each variable are designated as -1, 0, and +1, respectively, as shown in Table 2.3.

Table 2.3 The level of variables chosen for the Box-Behnken design.

| *Variable* | *Symbol* | *Coded variable level* | | |
|---|---|---|---|---|
| | | *Low* | *Center* | *High* |
| | | *-1* | *0* | *+1* |
| Antibiotic Conc. (mg/L) | $X_1$ | 10 | 105 | 200 |
| $H_2O_2$ Dose (mg/L) | $X_2$ | 10 | 255 | 500 |
| Fe(II) Dose (mg/L) | $X_3$ | 0 | 25 | 50 |

For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response. For the three factors, the equation is:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

**(Equation 1)**

Where $Y$ is the predicted response; $b_0$ is model constant; $X_1$, $X_2$ and $X_3$ are independent variables; $b_1$, $b_2$ and $b_3$ are linear coefficients; $b_{12}$, $b_{13}$ and $b_{23}$ are cross-product coefficients; and $b_{11}$, $b_{22}$ and $b_{33}$ are the quadratic coefficients. The quality of fit of the polynomial model equation was expressed by the coefficient of determination $R^2$ (Dong et al., 2009).

### *2.2.3 Analytical Methods*

#### *2.2.3.1 Sampling*

Samples withdrawn from the system at certain time intervals were analyzed immediately to avoid further reactions. Samples (20 ml) of raw and treated synthetic wastewater solutions were analyzed for TOC and antibiotic removal after filtration using Millipore filter paper with 0.45 μm pore size.

#### *2.2.3.2 Antibiotic Analysis*

Antibiotic analysis were carried out using Agilent 1100 Series High Performance Liquid Chromotography (HPLC). For antibiotic analyses Prevail C18 Column (150x4.6 mm, 5μm) was used in HPLC. The mobile phase used was 40% acetonitril at pH=3, and 60% 25 mM of $KH_2PO_4$ solution with a flow rate of 1ml/min. Under these conditions, Amoxicillin retention time was 5.5 min. For antibiotic measurements, Amoxicillin solution was used as calibration standards with concentrations between 0 and 500 mg/L.

*2.2.3.3 TOC Analysis*

TOC analysis were carried out using Apollo 9000 Combustion TOC Analyzer, Teledyne Tekmar. For the TOC measurements, potassium phthalate solution was used as calibration standard with the concentrations between 0 and 25, 10 and 100, 100 and 500 mg/L.

*2.2.3.4 Final Calculations*

After experimental studies and determination of the response function coefficients, optimal conditions for Amoxicillin and TOC removals were determined. Analysis of variance (ANOVA) was used for evaluation of the statistical method. Design Expert 7.0 program was used for this purpose.

### 2.2.4 Advanced Oxidation Processes (AOPs)

The chemical-oxidation processes called advanced oxidation processes (AOPs), are characterized by the generation of hydroxyl radicals. Besides fluorine, the hydroxyl radical is the strongest known oxidant. Therefore, hydroxyl radicals can oxidize and mineralize almost every organic molecule to $CO_2$ and inorganic ions. Rate constants for most of the reactions involving hydroxyl radicals in aqueous solution are usually in the order of $10^6$ to $10^9$ mol/L.s (Haag and Yao, 1992; Buxton et al., 1988). Reaction rate constant for ozone and hydroxyl radicals are compared in Table 2.4.

Table 2.4 Reaction rate constant (k, in l/samples) of ozone vs. hydroxyl radical (Legrini et all., 1993)

| *Compounds* | *$O_3$* | *•OH* |
|---|---|---|
| Chlorinated alkenes | $10^{-1}$ to $10^{3}$ | $10^{9}$ to $10^{11}$ |
| Phenols | $10^{3}$ | $10^{9}$ to $10^{10}$ |
| N-containing Organics | 10 to $10^{2}$ | $10^{8}$ to $10^{10}$ |
| Aromatics | 1 to $10^{2}$ | $10^{8}$ to $10^{10}$ |
| Ketones | 1 | $10^{9}$ to $10^{10}$ |
| Alcohols | *$10^{-2}$ to 1* | $10^{8}$ to $10^{9}$ |
| Alkanes | *$10^{-2}$* | $10^{6}$ to $10^{9}$ |

The environmental applications of AOPs are numerous, including water and wastewater treatment (i.e. removal of organic and inorganic pollutants and pathogens), air pollution abatement and soil remediation. AOPs are applied for the abatement of pollution caused by the presence of residual pharmaceuticals in waters for the last decade. The effectiveness of various AOPs for pharmaceutical removal from aqueous systems are summarized in a recent review (Klavarioti et al., 2009).

In advanced oxidation experiments; two of numerous advanced oxidation techniques were applied to antibiotic containing synthetic wastewater. These methods were Fenton ($H_2O_2/Fe^{+2}$) and photo-Fenton treatments ($UV/H_2O_2/Fe^{+2}$).

*2.2.4.1 Advanced Oxidation Experiments Using Fenton Process*

Its inventor H.J.H. Fenton first observed the reactivity of this system in 1894, but its utility was not recognized until the 1930's when the mechanisms were identified. By 1900's Fenton published several more complete studies describing that ferrous iron in the presence of hydrogen peroxide, yielded a solution with powerful and extraordinary oxidizing capabilities (Fenton, 1876). Some researchers all reported enhanced degradation of hydrogen peroxide in the presence of ferrous salts (George, 1948). The critical support for the pure system ferric mechanism is the evaluation of the oxygen. Iron catalyzed decomposition of $H_2O_2$ has recently been noted to slowly occur under alkaline conditions. This process is only effective at acidic pH level of

about 2.8 (Pignatello, 1992) or acidic conditions (Bishop et al., 1968; Walling, 1975). The hydroxyl radicals with high oxidation potential (2.8 V) completely destroys the pollutants in Fenton treatment. Oxidation potentials of some oxidants are shown in Table 2.5.

Table 2.5 Oxidation potentials of oxidants (www.$H_2O_2$.com)

| *Oxidant* | *Oxidation Potential, V* |
|---|---|
| Fluorine | 3.0 |
| Hydroxyl radical | 2.8 |
| Ozone | 2.1 |
| Hydrogen peroxide | 1.8 |
| Potassium permanganate | 1.7 |
| Chlorine dioxide | 1.5 |
| Chlorine | 1.4 |

Fenton's reagent, a reaction system consisting of $H_2O_2$ and $Fe^{2+}$ ion, is one of the most effective advanced oxidation processes with respect to degrading recalcitrant organic compounds. The following mechanism, Reaction (1) and (2), for the independent Fenton's Reagent activity has been accepted (Bishop et al., 1968).

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

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

Due to high oxidation potential of $\bullet OH$ , Fenton's reagent has been commonly applied either as a pre-treatment process to increase wastewater biodegradability or as a polishing process to further remove recalcitrant pollutants escaping from the foregoing biological unit (Zazo et al., 2005; Catalkaya and Kargi, 2007). The effectiveness of Fenton's reagent, assisted with UV radiation, on decomposition of antibiotics such as tetracycline and sulfamethoxazole in distilled water has been investigated recently (Bautitz and Nogueira, 2007; González et al., 2007).

Fenton systems are easy to handle and operate, Fenton reactions may conveniently be employed to treat micro-pollution caused by residual pharmaceuticals in surface waters as well as industrial effluents (e.g. hazardous hospital wastes or from drug manufacturing) with increased organic loading (Klavarioti et al., 2009).

Besides, Fenton's reagent can be used not only for total organic carbon (TOC) , heavy metals (As, Cu and Pb) and total phosphorus (TP) removals, but also to inactivate bacteria and reduce wastewater toxicity (Ben et al., 2009).

*2.2.4.2 Advanced Oxidation Experiments Using Photo-Fenton Process*

The AOPs are based on the formation of hydroxyl radicals ($\bullet OH$) by the combination of oxidants such as ozone or hydrogen peroxide with ultraviolet or visible irradiation and catalysts such as metal ions or semiconductors. Moreover, efficiency may be enhanced in the presence of UV irradiation as more hydroxyl radicals are produced in the so-called photo-Fenton reaction (Klavarioti , 2009). Among the AOPs, the photo-Fenton process has gained increasing attention due to its simplicity and the possibility of using sunlight for reduced operating costs (Malato et al., 2007; Nogueira et al., 2007).

Photo enhancement of reaction rates is because of photo oxidation of $Fe^{+2}$ to $Fe^{+3}$ photo-decarboxylation of ferric carboxylate complexes; and photolysis of $H_2O_2$.

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

The ferrous iron ($Fe^{+2}$) initiates and catalyzes the decomposition of $H_2O_2$, resulting in the generation of hydroxyl radicals. The generation of these radicals involves a complex reaction sequence in an aqueous solution (Pignatello, 1992).

$$Fe(OH)^2 + h\nu \rightarrow Fe^{2+} + \bullet OH \quad (4)$$

$$OH^- + Fe^{2+} \rightarrow 4OH^- + Fe^{3+} \quad (5)$$

$$Fe^{3+} + H_2O_2 \leftrightarrow Fe - OOH^{2+} + H^+ \quad (6)$$

$$Fe - OOH^{2+} \rightarrow 4HO\bullet_2 + Fe^{2+} \quad (7)$$

$$Fe^{2+} + HO\bullet_2 \rightarrow 4Fe^{3+} + \bullet OH_2 \quad (8)$$

$$Fe^{3+} + HO\bullet_2 \rightarrow 4Fe^{2+} + O_2 + H^+ \quad (9)$$

$$OH\bullet + H_2O_2 \rightarrow 4H_2O + HO\bullet_2 \quad (10)$$

$$Fe^{2+} + O_2 + H^+ \rightarrow Fe^{3+} + HO^-{}_2 \quad (11)$$

$$R + O_2 \rightarrow RO_2\bullet \quad (12)$$

The $H_2O_2$ was depleted to about 90% at the peak of each cycle and completely at the end of the cycles. As seen in Reaction (10), $H_2O_2$ can act as a •OH scavenger as well as an initiator. Due to formation of ($Fe^{+3}$) during the reaction, the Fenton reaction is normally accompanied by the precipitation of Fe $(OH)_3$. Ferrous ion is continuously recycled by irradiation and therefore is not depleted during the course of oxidation, as stated by Zepp et al. (1992).

# CHAPTER THREE
# RESULTS AND DISCUSSION

In this thesis, advanced oxidation techniques (Fenton and pohoto-Fenton) were used for degradation and mineralization of Amoxicillin in aqueous medium. Box-Behnken statistical design method was used for designing the experiments. Variables and the intervals were decided on the basis of literature reports. Amoxicillin, peroxide and Fe(II) concentrations were considered as independent variables. Percent Amoxicillin and TOC removals were response functions.

The required reaction time for each advanced oxidation method was determined at the central point of the experimental design (Ant.: 105 mg/L; $H_2O_2$: 255 mg/L; $Fe^{2+}$:25 mg/L).

Variations of percent TOC and antibiotic removals with time for Fenton and photo-Fenton oxidations are depicted in Figures 3.1 and 3.2, respectively. In these experiments Amoxicillin removals reached 100% within the first 2.5 minutes.

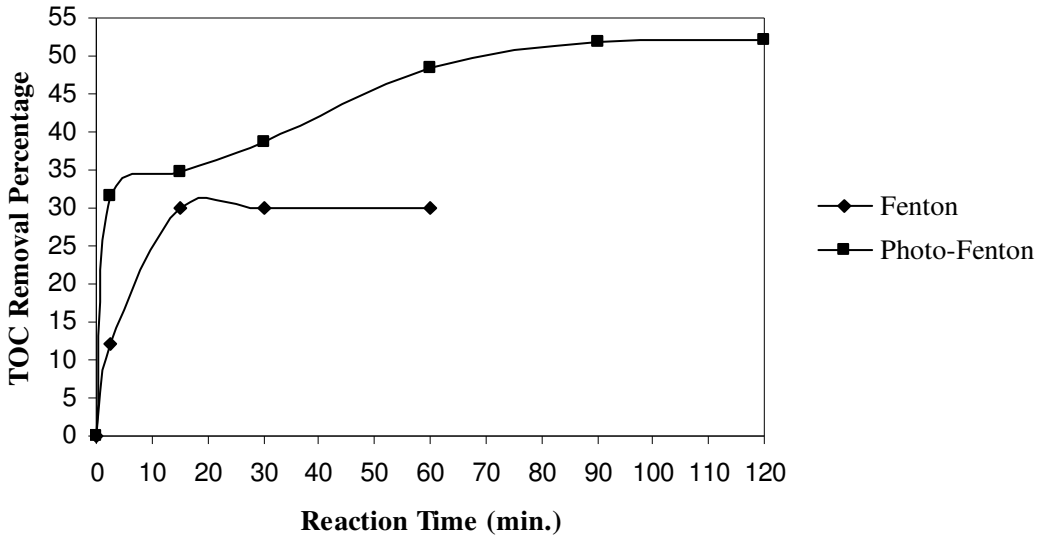



Figure 3.1 Variation of percent TOC removal with time in Fenton and photo-Fenton oxidation of Amoxicillin. Peroxide: 255 mg/L; Fe(II): 25 mg/L; Amoxicillin: 105 mg/L; pH : 3-3.5; UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

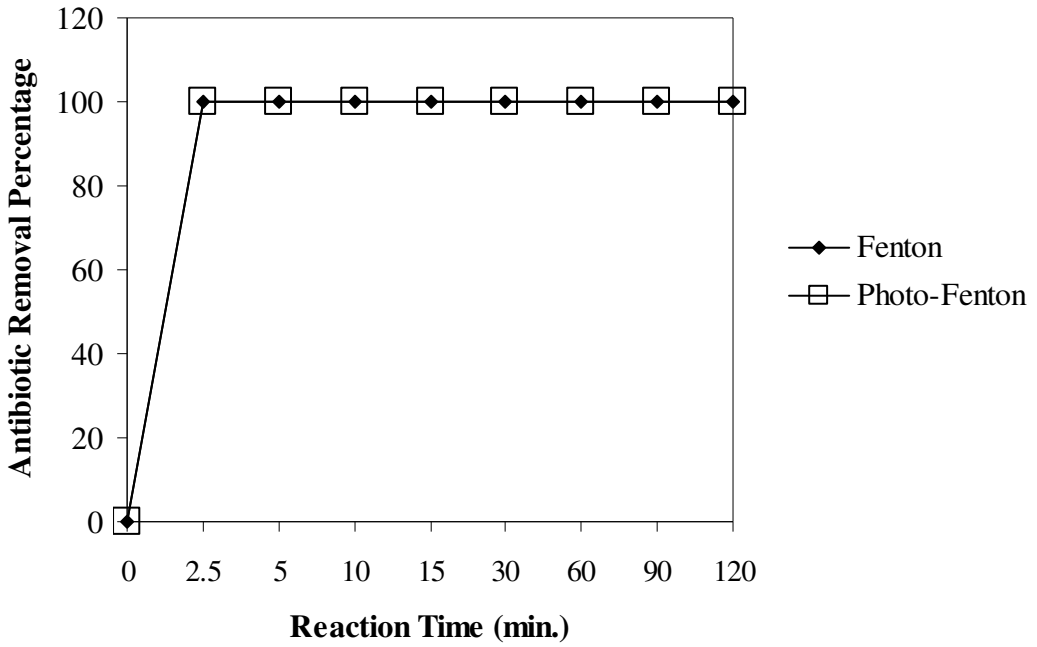



Figure 3.2 Variation of percent antibiotic removal with time in Fenton and photo-Fenton oxidation of Amoxicillin. Peroxide: 255 mg/L; Fe(II): 25 mg/L; Amoxicillin: 105 mg/L; pH : 3-3.5; UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

As it is seen in Figure 3.1 TOC removal reached the highest level within 15 minutes with Fenton oxidation. Therefore, 15 min reaction time was used in further experiments. Similarly, 60 min. was selected as the reaction time for photo-Fenton oxidation experiments.

The results of the experiments at the Box-Behnken experimental design points are presented in Table 3.1.

Table 3.1 The experimental results at Box-Behnken experimental design points

| *Run No* | *Actual and coded levels of variables* | | | *Experimental percent removal (%)* | | | |
|---|---|---|---|---|---|---|---|
| | | | | *Antibiotic* | | *TOC* | |
| | *$X_1$ Antibiotic (mg/L)* | *$X_2$ $H_2O_2$ (mg/L)* | *$X_3$ Fe(II) (mg/L)* | *Fenton* | *Photo-Fenton* | *Fenton* | *Photo-Fenton* |
| 1 | 200 **(+1)** | 10 **(-1)** | 25 **(0)** | 1.97 | 1.15 | 2.51 | 0.55 |
| 2 | 10 **(-1)** | 10 **(-1)** | 25 **(0)** | 16.18 | 25.25 | 14.62 | 22.86 |
| 3 | 10 **(-1)** | 500 **(+1)** | 25 **(0)** | 100 | 100 | 24.82 | 13.61 |
| 4 | 200 **(+1)** | 500 **(+1)** | 25 **(0)** | 90 | 100 | 21.45 | 46.97 |
| 5 | 200 **(+1)** | 255 **(0)** | 0 **(-1)** | 35 | 73.07 | 7 | 5.24 |
| 6 | 200 **(+1)** | 255 **(0)** | 50 **(+1)** | 44.61 | 100 | 25.93 | 27.14 |
| 7 | 10 **(-1)** | 255 **(0)** | 0 **(-1)** | 90 | 100 | 5 | 19.38 |
| 8 | 10 **(-1)** | 255 **(0)** | 50 **(+1)** | 85 | 100 | 35 | 3.75 |
| 9 | 105 **(0)** | 10 **(-1)** | 0 **(-1)** | 8.18 | 1.25 | 2.75 | 6.10 |
| 10 | 105 **(0)** | 10 **(-1)** | 50 **(+1)** | 11.52 | 18.57 | 5 | 0.92 |
| 11 | 105 **(0)** | 500 **(+1)** | 0 **(-1)** | 100 | 100 | 14.56 | 9.18 |
| 12 | 105 **(0)** | 500 **(+1)** | 50 **(+1)** | 100 | 100 | 37.08 | 50.25 |
| 13 | 105 **(0)** | 255 **(0)** | 25 **(0)** | 100 | 100 | 30 | 46 |
| 14 | 105 **(0)** | 255 **(0)** | 25 **(0)** | 100 | 100 | 30 | 48.40 |
| 15 | 105 **(0)** | 255 **(0)** | 25 **(0)** | 100 | 100 | 30 | 45.38 |

### 3.1 Regression Model

Response Surface Methodology (RSM) establishes a relationship between the dependent and independent variables. Mathematical relation between dependent (Y) variable and independent (X) variables can be represented as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^{\,2} + b_{22}X_2^{\,2} + b_{33}X_3^{\,2}$$

**(Equation 1)**

Equation 1 includes a constant (bo), three linear, three quadratic and three interaction terms. The constants were determined by using the experimental results and the Stat-Ease statistical program. The response functions with determined

coefficients are are presented in eqns 2 to 5 for Fenton and photo-Fenton processes. Analysis of Variance (ANOVA) test results are presented in Tables Table 3.2 to 3.5.

Table 3.2 ANOVA test for response function $Y_1$ (% antibiotic removal) of Fenton experiments

| *Source* | *Sum of squares* | *Df* | *Mean square* | *F ratio* | *P value Prob>F* |
|---|---|---|---|---|---|
| Model | 22114.39 | 9 | 2457.15 | 19.12 | 0.0023 |
| A-Antibiotic (mg/L) | 1788.02 | 1 | 1788.02 | 13.92 | 0.0136 |
| B-$H_2O_2$ (mg/L) | 15501.20 | 1 | 15501.20 | 120.64 | 0.0001 |
| C-$Fe^{2+}$ (mg/L) | 7.90 | 1 | 7.90 | 0.061 | 0.8140 |
| AB | 4.43 | 1 | 4.43 | 0.034 | 0.8600 |
| AC | 53.36 | 1 | 53.36 | 0.42 | 0.5477 |
| BC | 2.79 | 1 | 2.79 | 0.022 | 0.8886 |
| $A^2$ | 1420.97 | 1 | 1420.97 | 11.06 | 0.0209 |
| $B^2$ | 2966.54 | 1 | 2966.54 | 23.09 | 0.0049 |
| $C^2$ | 1033.45 | 1 | 1033.45 | 8.04 | 0.0364 |
| Residual | 642.45 | 5 | 128.49 | | |
| *Lack of Fit* | 642.45 | 3 | 214.15 | | |
| *Pure Error* | 0.000 | 2 | 0.000 | | |
| Cor Total | 22756.84 | 14 | | | |

The Model F-value of 19.12 implies the model is significant. Values greater than 0.1000 indicate the model terms are not significant and values of "Prob>F" less than 0.0500 indicate the model terms are significant.

"Adeq Precision" measures the signal to noise ratio. A ratio grater than 4 is desirable. Our's ratio of 12.743 indicates an adequate signal. This model can be used to navigate the design space.

There is only a 0.23% chance that a "Model F-Value" this large could occur due to noise. In this case $X_1$, $X_2$, $X_1^2$, $X_2^2$ and $X_3^2$ are determined as significant model terms.

Response function of antibiotic removal ($Y_1$) efficiency for Fenton experiments:

$$Y_1 = 2.692 + 0.249 * X_1 + 0.419 * X_2 + 1.251 * X_3 + 4.522 * 10^{-5} * X_1X_2 + 1.536 * 10^{-3} * X_1X_3 - 1.363 * 10^{-4} * X_2X_3 - 2.174 * 10^{-3} * X_1^2 - 4.722 * 10^{-4} * X_2^2 - 0.027 * X_3^2$$

**(Equation 2) - R-squared (adjusted for df)=**0.9210 – **Std. Dev.:** 11.34

Table 3.3 ANOVA test for response function $Y_2$ (% TOC removal) of Fenton experiments

| *Source* | *Sum of squares* | *Df* | *Mean square* | *F ratio* | *P value Prob>F* |
|---|---|---|---|---|---|
| Model | 2035.63 | 9 | 226.18 | 10.38 | 0.0095 |
| A-Antibiotic (mg/L) | 63.56 | 1 | 63.56 | 2.92 | 0.1484 |
| B-$H_2O_2$ (mg/L) | 666.67 | 1 | 666.67 | 30.58 | 0.0027 |
| C-$Fe^{2+}$ (mg/L) | 678.96 | 1 | 678.96 | 31.15 | 0.0025 |
| AB | 19.10 | 1 | 19.10 | 0.88 | 0.3923 |
| AC | 30.64 | 1 | 30.64 | 1.41 | 0.2891 |
| BC | 102.72 | 1 | 102.72 | 4.71 | 0.0821 |
| $A^2$ | 106.97 | 1 | 106.97 | 4.91 | 0.0776 |
| $B^2$ | 283.82 | 1 | 283.82 | 13.02 | 0.0154 |
| $C^2$ | 150.53 | 1 | 150.53 | 6.91 | 0.0467 |
| Residual | 109.00 | 5 | 21.80 | | |
| *Lack of Fit* | 109.00 | 3 | 36.33 | | |
| *Pure Error* | 0.000 | 2 | 0.000 | | |
| Cor Total | 2144.63 | 14 | | | |

The Model F-value of 10.38 implies the model is significant. Values grater than 0.1000 indicate the model terms are not significant and values of "Prob>F" less than 0.0500 indicate model terms are significant.

"Adeq Precision" measures the signal to noise ratio. A ratio grater than 4 is desirable. Our's ratio of 9.622 indicates an adequate signal. This model can be used to navigate the design space.

There is only a 0.95% chance that a "Model F-Value" this large coud occur due to noise. In this case $X_2$, $X_3$, $X_2^2$ and $X_3^2$ are determined as significant model terms.

Response function of TOC removal ($Y_2$) efficiency for Fenton experiments:

$$Y_2 = -3.327 + 0.101 * X_1 + 0.081 * X_2 + 0.791 * X_3 + 9.388 * 10^{-5} * X_1 X_2 - 1.165 * 10^{-3} * X_1 X_3 + 8.273 * 10^{-4} * X_2 X_3 - 5.964 * 10^{-4} * X_1^{\,2} - 1.460 * 10^{-4} * X_2^{\,2} - 0.010 * X_3^{\,2}$$

**(Equation 3) - R-squared (adjusted for df)=**0.8577 **– Std. Dev.:** 4.67

Table 3.4 ANOVA test for response function $Y_1$ (% antibiotic removal) of photo-Fenton experiments

| *Source* | *Sum of squares* | *Df* | *Mean square* | *F ratio* | *P value Prob>F* |
|---|---|---|---|---|---|
| Model | 24916.04 | 9 | 2768.45 | 83.02 | < 0.0001 |
| A-Antibiotic (mg/L) | 324.23 | 1 | 324.23 | 9.72 | 0.0263 |
| B-$H_2O_2$ (mg/L) | 17254.32 | 1 | 17254.32 | 517.39 | < 0.0001 |
| C-$Fe^{2+}$ (mg/L) | 88.44 | 1 | 88.44 | 2.65 | 0.1643 |
| AB | 144.00 | 1 | 144.00 | 4.32 | 0.0923 |
| AC | 181.31 | 1 | 181.31 | 5.44 | 0.0671 |
| BC | 0.027 | 1 | 0.027 | 8.164E-004 | 0.9783 |
| $A^2$ | 0.45 | 1 | 0.45 | 0.014 | 0.9118 |
| $B^2$ | 6850.91 | 1 | 6850.91 | 205.43 | < 0.0001 |
| $C^2$ | 150.41 | 1 | 150.41 | 4.51 | 0.0871 |
| Residual | 166.74 | 5 | 33.35 | | |
| *Lack of Fit* | 166.74 | 3 | 55.58 | | |
| *Pure Error* | 0.000 | 2 | 0.000 | | |
| Cor Total | 25082.79 | 14 | | | |

The Model F-value of 83.02 implies the model is significant. Values grater than 0.1000 indicate the model terms are not significant and values of "Prob>F" less than 0.0500 indicate model terms are significant.

"Adeq Precision" measures the signal to noise ratio. A ratio grater than 4 is desirable. Our's ratio of 22.399 indicates an adequate signal. This model can be used to navigate the design space.

There is only a 0.01% chance that a "Model F-Value" this large coud occur due to noise. In this case $X_1$, $X_2$ and $X_2^2$ are determined as significant model terms.

Response function of antibiotic removal ($Y_1$) efficiency for photo-Fenton experiments:

$$Y_1 = 16.331 - 0.195 * X_1 + 0.528 * X_2 + 0.342 * X_3 + 2.578 * 10^{-4} * X_1X_2 + 2.835 * 10^{-3} * X_1X_3 + 1.347 * 10^{-5} * X_2X_3 - 3.878 * 10^{-5} * X_1{}^2 - 7.176 * 10^{-4} * X_2{}^2 - 0.010 * X_3{}^2$$

**(Equation 4) - R-squared (adjusted for df)=**0.9814 – **Std. Dev.:** 5.77

Table 3.5 ANOVA test for response function $Y_2$ (% TOC removal) of photo-Fenton experiments

| *Source* | *Sum of squares* | *Df* | *Mean square* | *F ratio* | *P value Prob>F* |
|---|---|---|---|---|---|
| Model | 4488.75 | 9 | 498.75 | 8.73 | 0.0140 |
| A-Antibiotic (mg/L) | 51.51 | 1 | 51.51 | 0.90 | 0.3858 |
| B-$H_2O_2$ (mg/L) | 646.74 | 1 | 646.74 | 11.33 | 0.0200 |
| C-$Fe^{2+}$ (mg/L) | 447.15 | 1 | 447.15 | 7.83 | 0.0381 |
| AB | 774.79 | 1 | 774.79 | 13.57 | 0.0142 |
| AC | 352.13 | 1 | 352.13 | 6.17 | 0.0556 |
| BC | 204.49 | 1 | 204.49 | 3.58 | 0.1170 |
| $A^2$ | 989.65 | 1 | 989.65 | 17.33 | 0.0088 |
| $B^2$ | 314.16 | 1 | 314.16 | 5.50 | 0.0659 |
| $C^2$ | 986.33 | 1 | 986.33 | 17.27 | 0.0089 |
| Residual | 285.53 | 5 | 57.11 | | |
| *Lack of Fit* | 280.44 | 3 | 93.48 | 36.74 | 0.0266 |
| *Pure Error* | 5.09 | 2 | 2.54 | | |
| Cor Total | 4774.27 | 14 | | | |

The Model F-value of 8.73 implies the model is significant. Values grater than 0.1000 indicate the model terms are not significant and values of "Prob>F" less than 0.0500 indicate model terms are significant.

"Adeq Precision" measures the signal to noise ratio. A ratio grater than 4 is desirable. Our's ratio of 7.623 indicates an adequate signal. This model can be used to navigate the design space.

There is only a 1.4% chance that a "Model F-Value" this large coud occur due to noise. In this case $X_2$, $X_3$, $X_1X_2$, $X_1^2$ and $X_3^2$ are determined as significant model terms.

Response function of TOC removal ($Y_2$) efficiency for photo-Fenton experiments:

$$Y_2 = 14.440 + 1.156 * X_1 + 0.023 * X_2 + 0.894 * X_3 + 5.980 * 10^{-4} * X_1X_2 + 3.951 * 10^{-3} * X_1X_3 + 1.167 * 10^{-3} * X_2X_3 - 1.814 * 10^{-3} * X_1^2 - 1.537 * 10^{-4} * X_2^2 - 0.026 * X_3^2$$

**(Equation 5) - R-squared (adjusted for df)=**0.8325 **– Std. Dev.:** 7.56

### 3.2 Fenton Experiments ($H_2O_2/Fe^{2+}$)

Fenton's reagent, a reaction system consisting of $H_2O_2$ and $Fe^{2+}$ ion, is one of the most effective advanced oxidation processes with respect to degrading recalcitrant organic compounds. The following mechanism, Reaction (1) and (2), for the independent Fenton's Reagent activity has been accepted (Bishop et al., 1968).

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

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

In advanced oxidation with Fenton's reagent 15 min. was selected as the reaction time for TOC removal. By using Design Expert 7.0 program, predicted efficiencies were obtained. The predicted and observed experimental result are presented in Table 3.6.

Table 3.6 Observed and predicted percent removals for response functions of Fenton experiments

| *Run No* | *Predicted percent removals (%)* | | *Observed percent removals (%)* | |
|---|---|---|---|---|
| | $Y_1$ *Antibiotic* | $Y_2$ *TOC* | $Y_1$ *Antibiotic* | $Y_2$ *TOC* |
| 1 | 0.00 | 1.72 | 1.97 | 2.51 |
| 2 | 24.02 | 11.72 | 16.18 | 14.62 |
| 3 | 100.00 | 25.61 | 100.00 | 24.82 |
| 4 | 82.16 | 24.35 | 90.00 | 21.45 |
| 5 | 44.06 | 8.97 | 35.00 | 7.00 |
| 6 | 53.35 | 21.86 | 44.61 | 25.93 |
| 7 | 81.26 | 9.07 | 90.00 | 5.00 |
| 8 | 75.94 | 33.03 | 85.00 | 35.00 |
| 9 | 9.08 | 1.57 | 8.18 | 2.75 |
| 10 | 12.73 | 9.86 | 11.52 | 5.00 |
| 11 | 98.79 | 9.70 | 100.00 | 14.56 |
| 12 | 99.19 | 38.26 | 100.00 | 37.08 |
| 13 | 100.00 | 30.00 | 100.00 | 30.00 |
| 14 | 100.00 | 30.00 | 100.00 | 30.00 |
| 15 | 100.00 | 30.00 | 100.00 | 30.00 |

### *3.2.1 Antibiotic Removal*

3D surface graphics of observed antibiotic removal efficiencies by using Fenton's reagent are shown in Figure 3.3 for constant antibiotic concentration (central point: 105 mg/L), Figure 3.4 for constant $H_2O_2$ concentration (central point: 255 mg/L) and Figure 3.5 for constant Fe(II) concentration (central point: 25 mg/L).

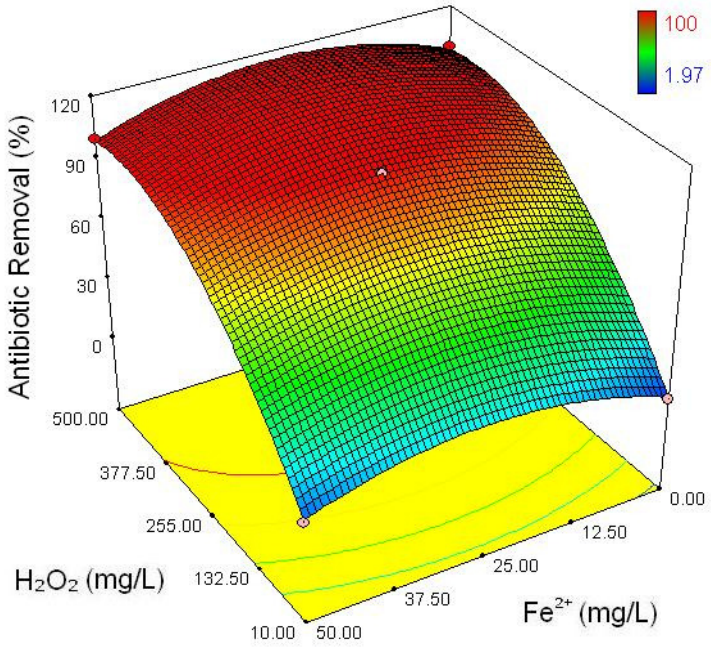



Figure 3.3 Antibiotic removal efficiencies by using Fenton's reagent for constant antibiotic concentration (central point: 105 mg/L), pH=3-3.5

As it's seen in Figure 3.3, Fe(II) ($X_3$) is not one of major factors in antibiotic removal efficiency at the central point concentration of the antibiotic. Removal percentages were independent from Fe(II) concentration. As it's said before, according to Equation 2, interaction of Fe(II) by itself ($X_3^2$) can be one of the factors which can affect antibiotic removal efficiency. The main factor is $H_2O_2$ concentration because of being the source of hydroxyl radicals. Amoxicillin removal efficiency increased with increasing peroxide concentration.

Antibiotic removal efficiency did not change at constant $H_2O_2$ concentration with variable $Fe^{+2}$ concentration. For example; for 132.5 mg/L $H_2O_2$ concentration there is no significant difference in Amoxicillin removal for Fe(II) concentrations of 0 and 50 mg/L.

At a constant Amoxicillin concentration of 105 mg/L, high treatment efficiencies can be observed with minimum 25 mg/L Fe(II) and $H_2O_2$ concentration of 255 mg/L.

However, as shown in Figure 3.3, the highest removals were obtained at $H_2O_2$ concentration of 400 mg/L.

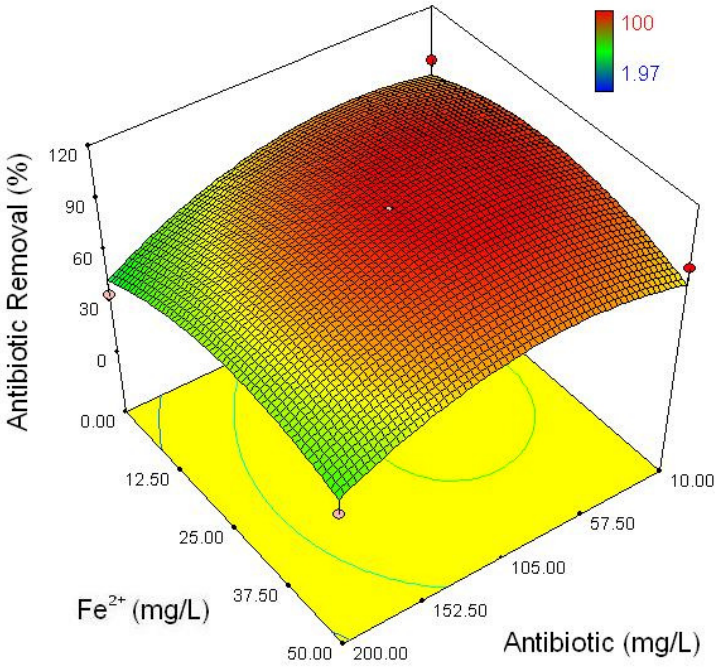



Figure 3.4 Antibiotic removal efficiencies by using Fenton's reagent for constant $H_2O_2$ concentration (central point: 255 mg/L), pH=3-3.5

As shown in Figure 3.4, at constant $H_2O_2$ concentration of 255 mg/L, Fe(II) was not one of the major factors affecting the antibiotic removal. Fe(II) concentration was not sufficient at high antibiotic concentrations to obtain high antibiotic removal efficiencies. Dependency of removal efficiency on Fe(II) concentration is low. For this graphic, antibiotic concentration was the most effective factor affecting treatment performance.

For the most effective antibiotic removal by the Fenton reagent 25 mg/L Fe(II) and nearly 60 mg/L antibiotic concentrations are the optimum dosages.

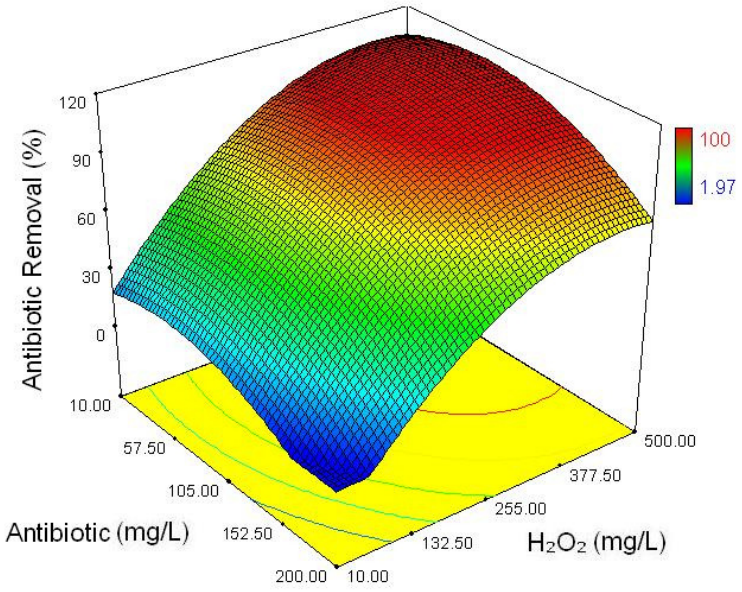



Figure 3.5 Antibiotic removal efficiencies by using Fenton's reagent for constant Fe(II) concentration (central point: 25 mg/L), pH=3-3.5

As shown in Figure 3.5, low levels of $H_2O_2$ were not sufficient for high levels of antibiotic removals. Percent antibiotic removal increased with increasing peroxide concentration. High antibiotic concentrations resulted in low levels of antibiotic removals due to insufficient oxidant and the catalyst doses. Peroxide concentrations should be high and Amoxicillin should be low in order to obtain high levels of antibiotic removals. Optimum levels of antibiotic and peroxide concentrations were found to be 60 mg/L and 400 mg/l, respectively at Fe/II) dose of 25 mg/L.

Figure 3.6, 3.7 and 3.8 show variations of percent Amoxicillin removal with different concentration combinations of Amoxicillin, $H_2O_2$ and Fe(II) at pH=3-3.5.

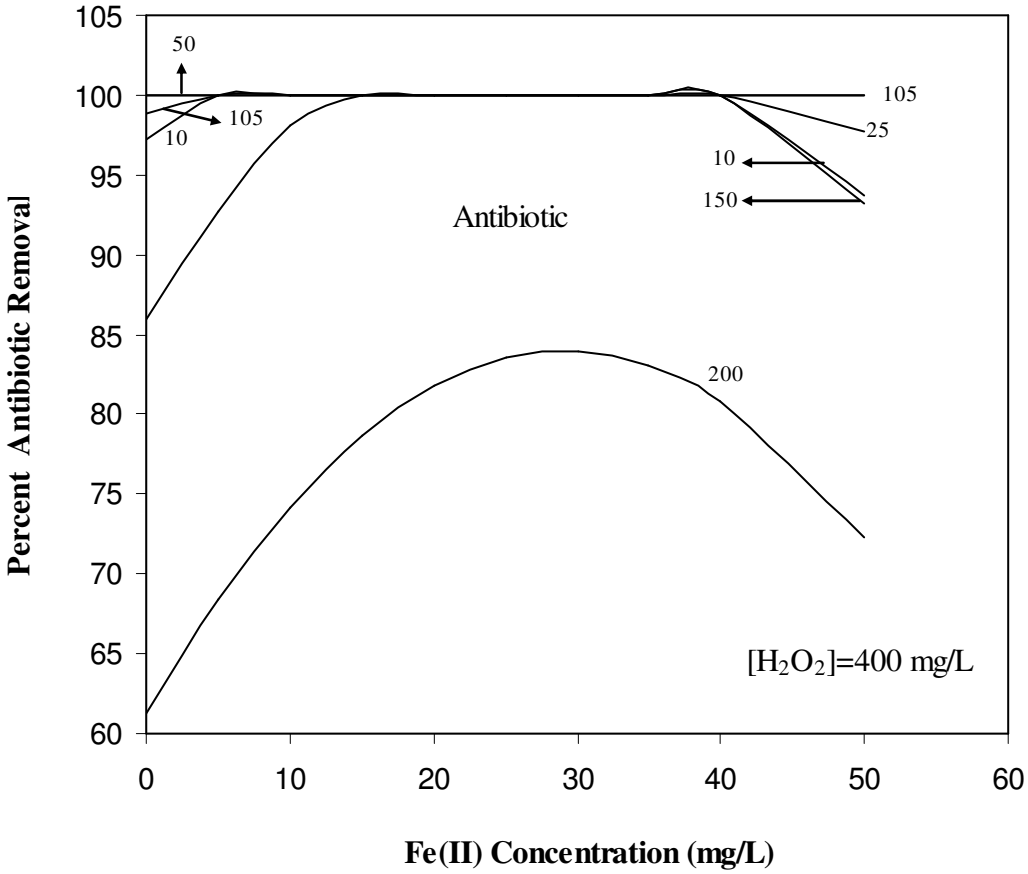



Figure 3.6 Variation of percent Amoxicillin removal using Fenton process with Fe(II) concentration at different antibiotic doses and constant $H_2O_2$ dose of 400 mg/L, pH=3-3.5

Variation of percent antibiotic removal with Fe(II) concentration at different antibiotic concentrations and constant peroxide concentration of 400 mg/L is depicted in Fig 3.6. For all antibiotic doses, antibiotic removal increased with increasing Fe(II) concentration up to 25 mg/L and then decreased with further increases in Fe(II) dose. Apparently, high Fe(II) doses caused scavenging effect on hydroxyl ions. Antibiotic removals were nearly complete for antibiotic concentrations up to 150 mg/L at peroxide and Fe(II) doses of 400 mg/L and 25 mg/L, respectively. At high antibiotic concentrations of 200 mg/L antibiotic removal decreased to 83% for optimum Fe(II) and peroxide doses.

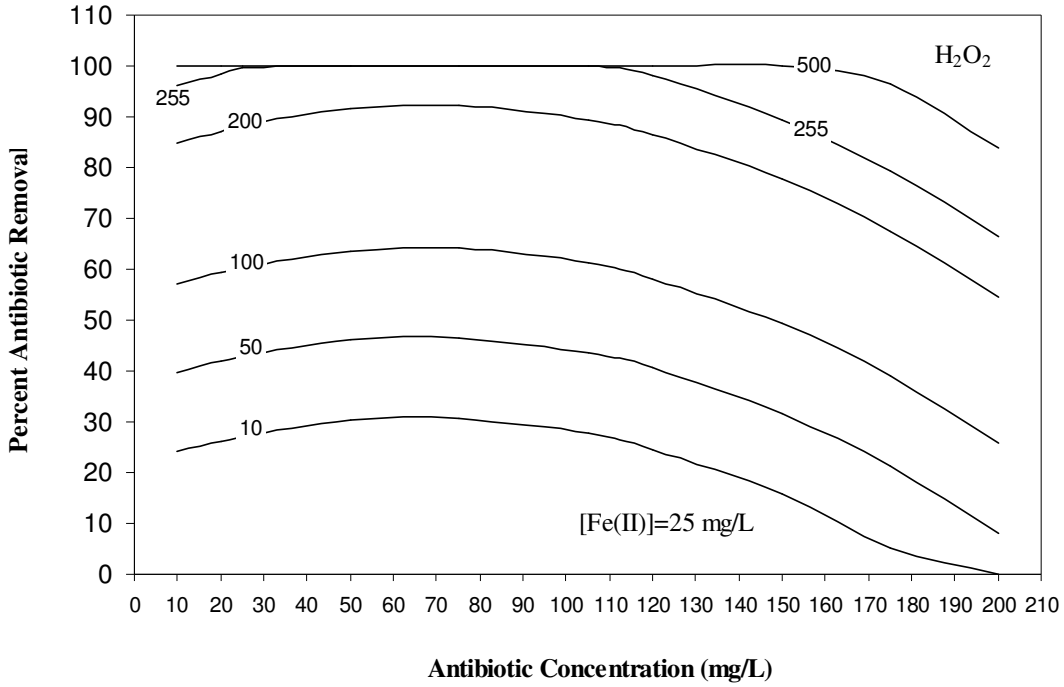



Figure 3.7 Variation of percent Amoxicillin removal using Fenton process with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 25 mg/L, pH=3-3.5

Figure 3.7 depicts variation of percent Amoxicillin removal with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 25 mg/L at pH=3-3.5. Percent antibiotic removal increased with increasing peroxide dose up to 255 mg/L and remained constant for higher peroxide doses. The results indicated peroxide limitations for peroxide doses below 255 mg/L. Antibiotic removal also increased with antibiotic concentration up to 60 mg/L indicating antibiotic limitations. Further increases in antibiotic concentration above 60 mg/L resulted in decreases in percent antibiotic removal due to limitations by other reactants of peroxide and Fe(II). High antibiotic concentrations required high peroxide doses.

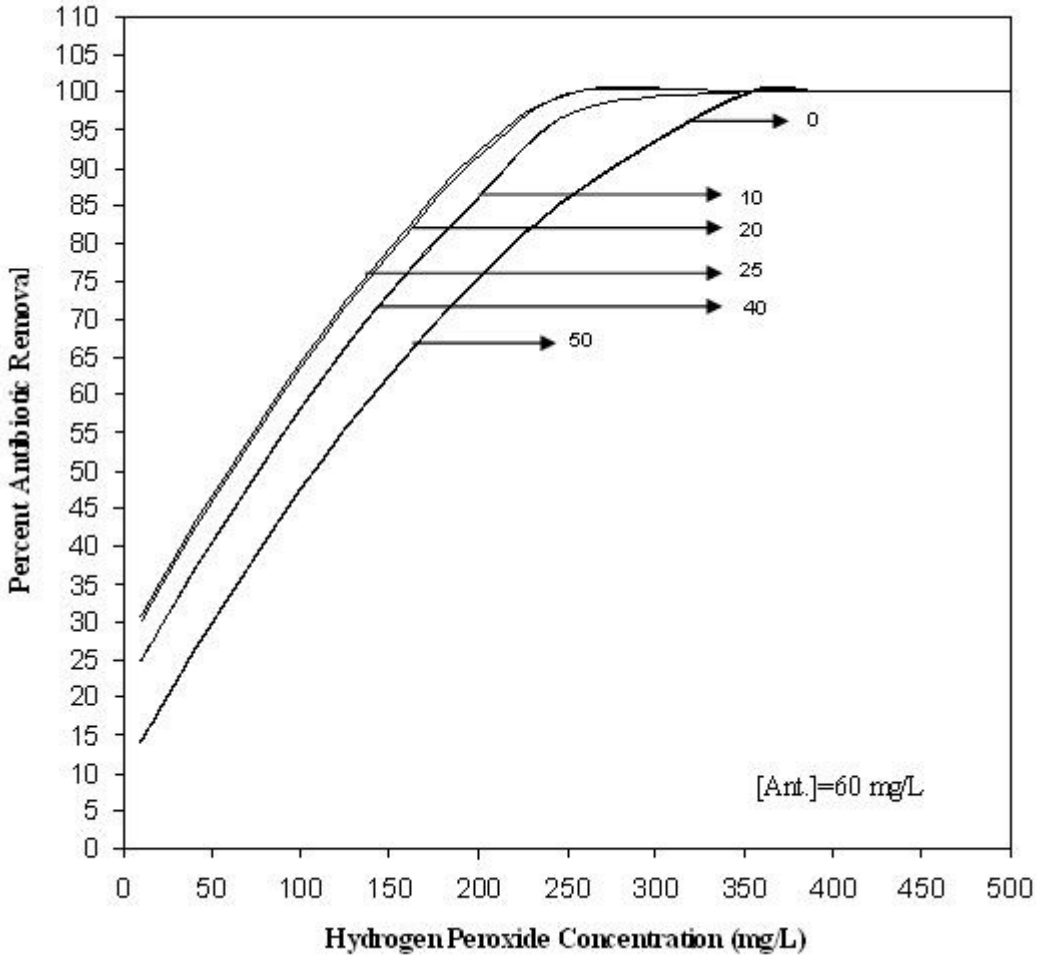



Figure 3.8 Variation of percent Amoxicillin removal with $H_2O_2$ concentration at different Fe(II) doses and constant Amoxicillin dose of 60 mg/L, pH=3-3.5

Variation of percent Amoxicillin removal with $H_2O_2$ concentration at different Fe(II) doses and constant Amoxicillin dose of 60 mg/L is depicted in Fig 3.8. Antibiotic removal increased with increasing peroxide dose up to 400 mg/L peroxide indicating limitations by oxidant concentration at low peroxide doses. The optimum peroxide dose varied with Fe(II) doses. Low Fe(II) doses required low peroxide doses for maximum antibiotic removal. Antibiotic removal also increased with Fe(II) doses up to 25 mg/L indicating limitations by Fe(II) ions at low concentrations. Further increases in Fe(II) doses above 25 mg/L resulted in decreases in antibiotic removal probably due to hydroxyl ion scavenging effects

excess Fe(II) doses. In the absence of Fe(II), antibiotic removal by only peroxide oxidation was lower than that of the Fenton oxidation.

### *3.2.2 TOC Removal*

Variation of percent TOC removals with the reagent doses are depicted in Figure 3.9 in form of 3D surface plot for constant antibiotic concentration of 105 mg/L. Figure 3.10 is a similar plot for constant $H_2O_2$ concentration of 255 mg/L . Figure 3.11 depicts TOC removal for constant Fe(II) concentration of 25 mg/L.

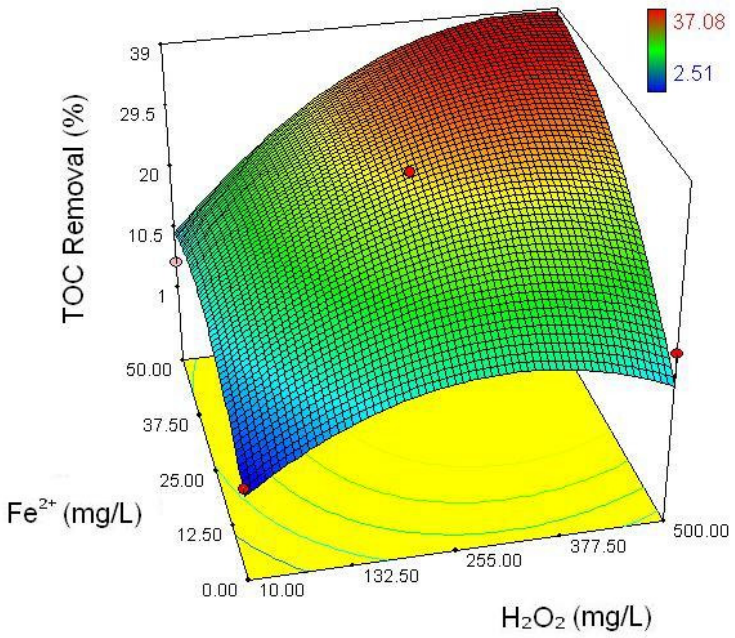



Figure 3.9 TOC removal efficiencies by using Fenton's reagent for constant antibiotic concentration (central point: 105 mg/L), pH=3-3.5

At a constant antibiotic concentration of 105 mg/L the highest percent TOC removal (37.08%) was obtained with Fe(II) = 50 mg/L and peroxide = 500 mg/L concentrations (Fig 3.9).

At low Fe(II) and peroxide concentrations TOC removal was very low. TOC removal increased with increasing peroxide concentration up to 255 mg/L indicating peroxide limitations at low peroxide concentrations. Further increases in peroxide

concentration resulted in lower TOC removals due to hydroxyl ion scavenging effects of peroxide at high concentrations.

At low peroxide concentrations TOC removals were not affected from increases in Fe(II) concentration. TOC removal increased with increasing Fe(II) at high peroxide concentrations.

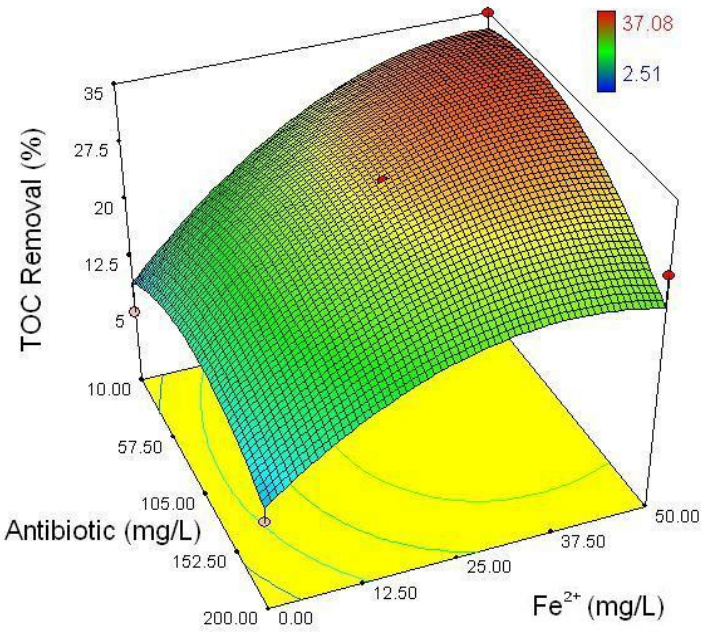



Figure 3.10 TOC removal efficiencies by using Fenton's reagent for constant $H_2O_2$ concentration (central point: 255 mg/L), pH=3-3.5

At a constant peroxide dose of 255 mg/L, TOC removal increased with increasing Fe(II) dose up to Fe(II) = 25 mg/L at all antibiotic concentrations indicating limitations by the catalyst (Fig 3.10). Further increases in Fe(II) dose resulted in decreases in TOC removal due to hydroxyl ion scavenging effects of excess Fe(II) concentration. TOC removal also increased with increasing antibiotic dose up to a certain level and then decreased with further increases indicating limitations by the antibiotic dose at low antibiotic concentrations. The highest TOC removal (37%) was obtained with Fe(II) = 50 mg/L at 10 mg/L antibiotic and 255 mg/L peroxide doses.

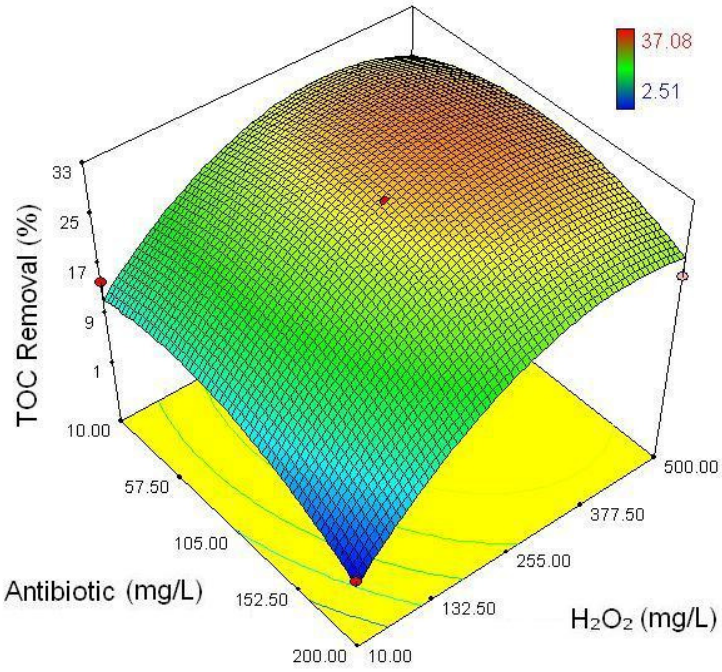



Figure 3.11 TOC removal efficiencies by using Fenton's reagent for constant Fe(II) concentration (central point: 25 mg/L), pH=3-3.5

For a constant Fe(II) dose of 25 mg/L, TOC removal increased with increasing peroxide and decreased with increasing antibiotic doses (Fig 3.11). Low peroxide and high antibiotic doses resulted in low TOC removals. At high antibiotic doses over 100 mg/L, high peroxide doses above 400 mg/L should be used for high TOC removals. High TOC removals at high antibiotic concentrations require high peroxide doses.

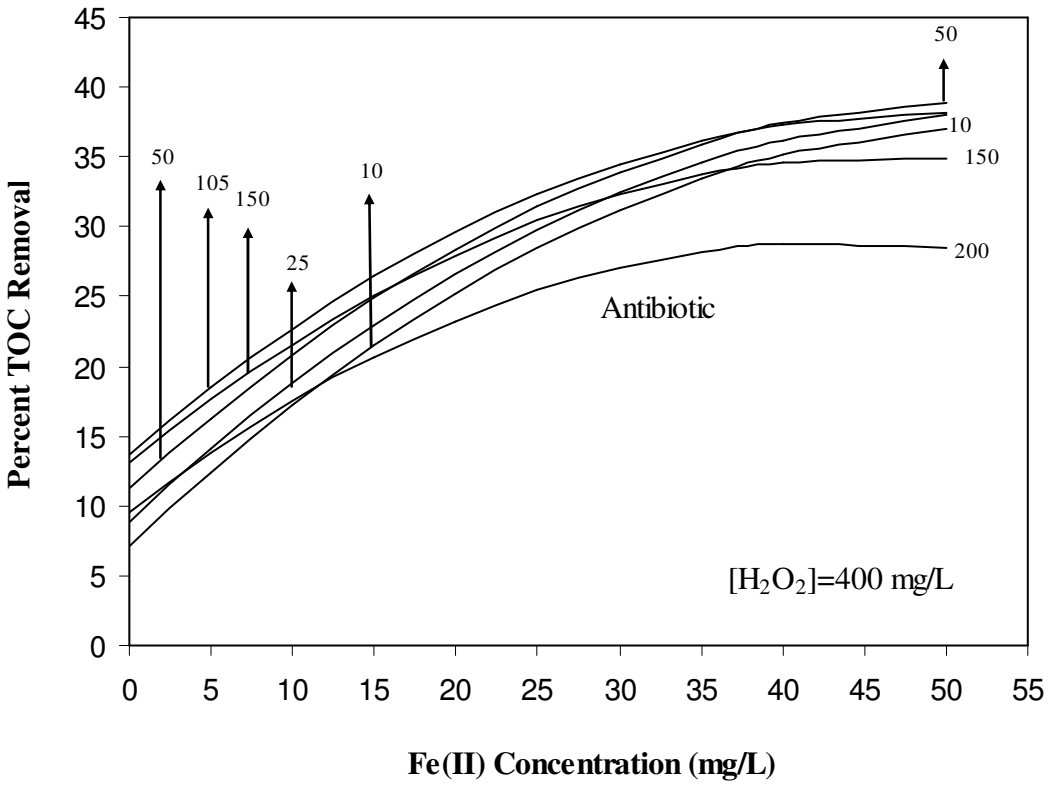



Figure 3.12 Variation of percent TOC removal using Fenton process with Fe(II) concentration at different Amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L, pH=3-3.5

Figure 3.12 depicts variation of percent TOC removal with Fe(II) concentration at different Amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L at pH=3-3.5. Percent TOC removal increased with increasing Fe(II) dose up to 50 mg/L due to limitations by the catalyst concentration at high peroxide dose of 400 mg/L. Increases in antibiotic concentration resulted in slight increases in TOC removal up to 105 mg/L antibiotic dose due to limitations by the antibiotic concentration. However, TOC removal decreased at high antibiotic doses above 105 mg/L due to limitations by the other reagents. At 200 mg/L antibiotic dose, TOC removal was nearly 25% at peroxide and Fe(II) doses of 400 mg/L and 50 mg/L, respectively. The highest TOC removal was 37% at 50 mg/L antibiotic, 50 mg/L Fe(II) and 400 mg/L peroxide doses.

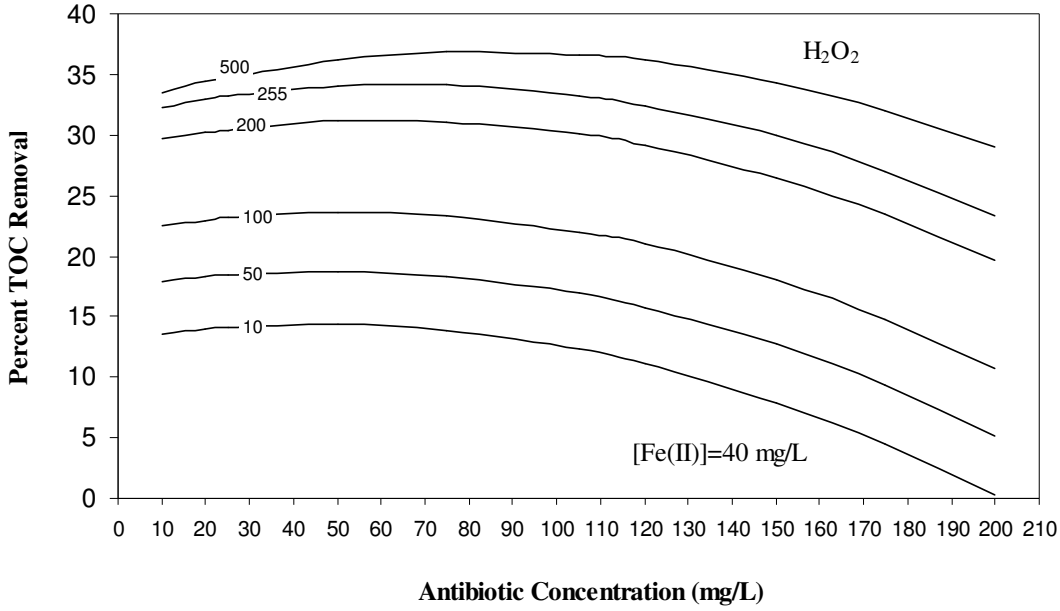



Figure 3.13 Variation of percent TOC removal using Fenton process with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 40 mg/L, pH=3-3.5

Variation of percent TOC removal with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 40 mg/L is depicted in Figure 3.13. TOC removal slightly increased up to 60 mg/L antibiotic concentration and then decreased with increasing antibiotic doses above 60 mg/L for all peroxide doses between 10 and 500 mg/L. Increases in peroxide dose resulted in significant increases in percent TOC removal due to limitations by the peroxide doses. At an antibiotic dose of 60 mg/L, and Fe(II) = 40 mg/L, an increase in peroxide dose from 10 to 255 mg/L resulted in a significant increase in percent TOC removal from 14% to 33%. Further increases in peroxide doses to 500 mg/L resulted in slight increases (from 33% to 35%) in percent TOC removal. High antibiotic concentrations yielded low TOC removals due to limitations by the other reagents. Optimum antibiotic dose was found to be 60 mg/L for a peroxide dose of above 255 mmg/L and Fe(II) dose of 40 mg/L.

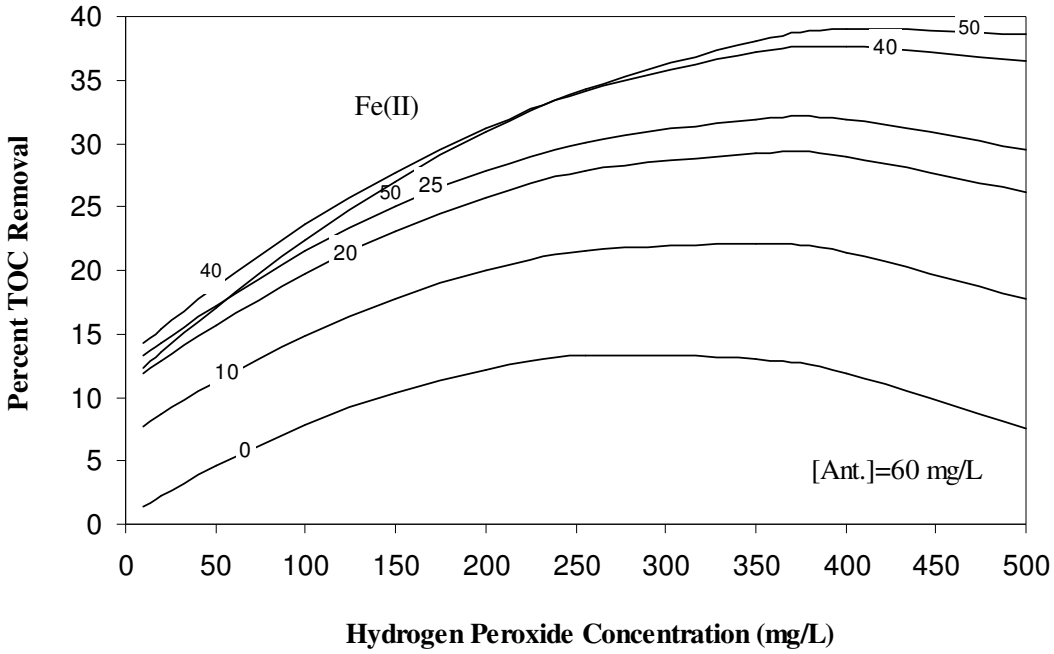



Figure 3.14 Variation of percent TOC removal using Fenton process with peroxide concentration at different Fe(II) doses and constant Amoxicillin dose of 60 mg/L, pH=3-3.5

Figure 3.14 depicts variation of percent TOC removal with peroxide concentration at different Fe(II) doses and constant Amoxicillin dose of 60 mg/L. Percent TOC removal increased with peroxide concentration up to 400 mg/L due to limitations by the peroxide dose at low peroxide levels. Further increases in peroxide doses resulted in decreases in TOC removal due to hydroxyl ion scavenging effect of peroxide at high concentrations. The optimum peroxide dose varied depending on Fe(II) dose. Optimum peroxide dose was around 250 mg/L at low Fe(II) doses below 20 mg/L which increased with increasing Fe(II) dose and reached nearly 400 mg/L level for Fe(II) doses above 40 mg/L. TOC removal in the absence of the catalyst (Fe(II)) was less than 10% indicating negligible TOC removals by peroxide alone. The highest TOC removal (37%) at an antibiotic dose of 60 mg/L was obtained with 400 mg/L peroxide and 50 mg/L Fe(II) doses. Difference between the TOC removals with 40 and 50 mg/L Fe(II) doses was negligible.

### 3.3 Photo-Fenton Oxidation Experiments ($UV/H_2O_2/Fe^{2+}$)

The AOPs are based on the formation of hydroxyl radicals ($\bullet OH$) by the combination of oxidants such as ozone or hydrogen peroxide with ultraviolet or visible irradiation and catalysts such as metal ions or semiconductors. Moreover, efficiency may be enhanced in the presence of UV irradiation as more hydroxyl radicals are produced in the photo-Fenton reaction (Klavarioti , 2009).

Photo enhancement of reaction rates is likely because of photo oxidation of $Fe^{+2}$ to $Fe^{+3}$ photo-decarboxylation of ferric carboxylate complexes; and photolysis of $H_2O_2$.

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

The ferrous iron ($Fe^{+2}$) initiates and catalyzes the decomposition of $H_2O_2$, resulting in the generation of hydroxyl radicals. The generation of these radicals involves a complex reaction sequence in an aqueous solution (Pignatello, 1992).

$$Fe(OH)^2 + h\nu \rightarrow Fe^{2+} + \bullet OH \qquad (4)$$
$$OH^- + Fe^{2+} \rightarrow 4OH^- + Fe^{3+} \qquad (5)$$
$$Fe^{3+} + H_2O_2 \leftrightarrow Fe - OOH^{2+} + H^+ \qquad (6)$$
$$Fe - OOH^{2+} \rightarrow 4HO\bullet_2 + Fe^{2+} \qquad (7)$$
$$Fe^{2+} + HO\bullet_2 \rightarrow 4Fe^{3+} + \bullet OH_2 \qquad (8)$$
$$Fe^{3+} + HO\bullet_2 \rightarrow 4Fe^{2+} + O_2 + H^+ \qquad (9)$$
$$OH\bullet + H_2O_2 \rightarrow 4H_2O + HO\bullet_2 \qquad (10)$$
$$Fe^{2+} + O_2 + H^+ \rightarrow Fe^{3+} + HO^-_2 \qquad (11)$$
$$R + O_2 \rightarrow RO_2\bullet \qquad (12)$$

Peroxide was depleted to about 90% at the peak of each cycle and completely at the end of the cycles. As seen in equation (10), $H_2O_2$ can act as a •OH scavenger as well as an initiator. Due to the formation of ($Fe^{+3}$) during the reaction, the Fenton reaction is normally accompanied by the precipitation of Fe $(OH)_3$. Ferrous ion is

continuously recycled by irradiation and therefore it is not depleted during the course of the oxidation, as stated by Zepp et al. (1992).

In advanced oxidation with photo-Fenton process, 60 min. was selected as the reaction time for TOC removal since TOC removal did not change with reaction time after 60 minutes. Experimental data were correlated with the response functions using regression analysis and the coefficients were determined. Response functions with determined coefficients were used for model predictions of the percent antibiotic and TOC removals. Design Expert 7.0 program was used for this purpose.The experimental results are presented in Table 3.7 along with the model predictions.

Table 3.7 Observed and predicted percent removals for response functions of photo-Fenton experiments

| *Run No* | *Predicted percent removals (%)* | | *Observed percent removals (%)* | |
|---|---|---|---|---|
| | $Y_1$ *Antibiotic* | $Y_2$ *TOC* | $Y_1$ *Antibiotic* | $Y_2$ *TOC* |
| 1 | 0.00 | 0.63 | 1.15 | 0.55 |
| 2 | 22.50 | 23.39 | 25.15 | 22.86 |
| 3 | 100.00 | 13.53 | 100.00 | 13.61 |
| 4 | 100.00 | 46.44 | 100.00 | 46.97 |
| 5 | 76.84 | 0.00 | 73.07 | 5.24 |
| 6 | 96.96 | 33.27 | 100.00 | 27.14 |
| 7 | 100.00 | 13.25 | 100.00 | 19.38 |
| 8 | 96.23 | 9.43 | 100.00 | 3.75 |
| 9 | 0.86 | 11.71 | 1.25 | 6.10 |
| 10 | 7.34 | 12.36 | 0.92 | 18.57 |
| 11 | 93.58 | 15.39 | 100.00 | 9.18 |
| 12 | 100.00 | 44.64 | 100.00 | 50.25 |
| 13 | 100.00 | 46.59 | 100.00 | 46.00 |
| 14 | 100.00 | 46.59 | 100.00 | 48.40 |
| 15 | 100.00 | 46.59 | 100.00 | 45.38 |

### *3.3.1 Antibiotic Removal*

3D surface graphics for percent antibiotic removals are shown in Figures 3.15 to 3.17. Figure 3.15 is for constant antibiotic concentration of 105 mg/L, while Figures

3.16 and 3.17 are for constant peroxide concentration of 255 mg/L and for constant Fe(II) concentration of 25 mg/L.

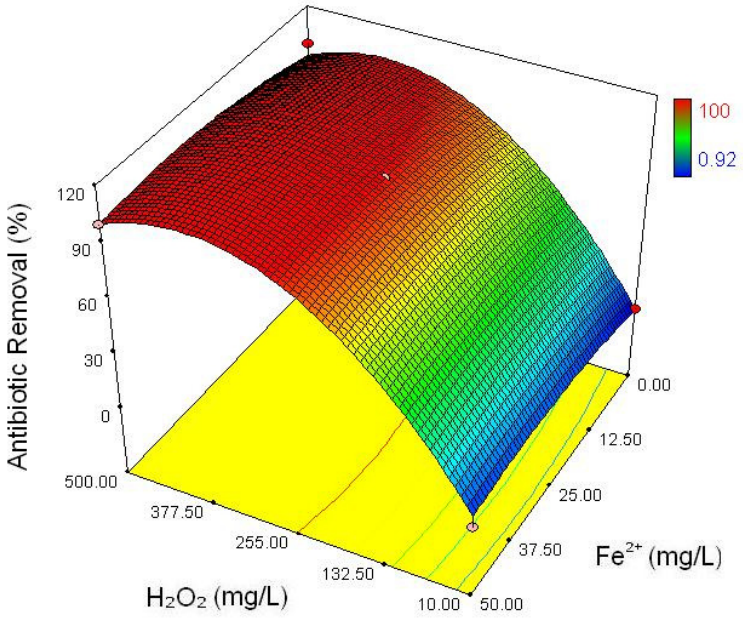



Figure 3.15 Antibiotic removal efficiencies by using photo-Fenton process for constant antibiotic concentration (central point: 105 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.15 depicts variation of percent antibiotic removal with peroxide and Fe(II) doses at a constant antibiotic dose of 105 mg/L in form of a 3D surface plot. Peroxide concentration has a pronounced effect on antibiotic removal while Fe(II) concentration has rather limited effect. Complete antibiotic removals can be obtained at peroxide doses above 400 mg/L at a Fe(II) dose of 25 mg/L. Hydrogen peroxide dose is the main factor affecting antibiotic removal in photo-Fenton treatment.

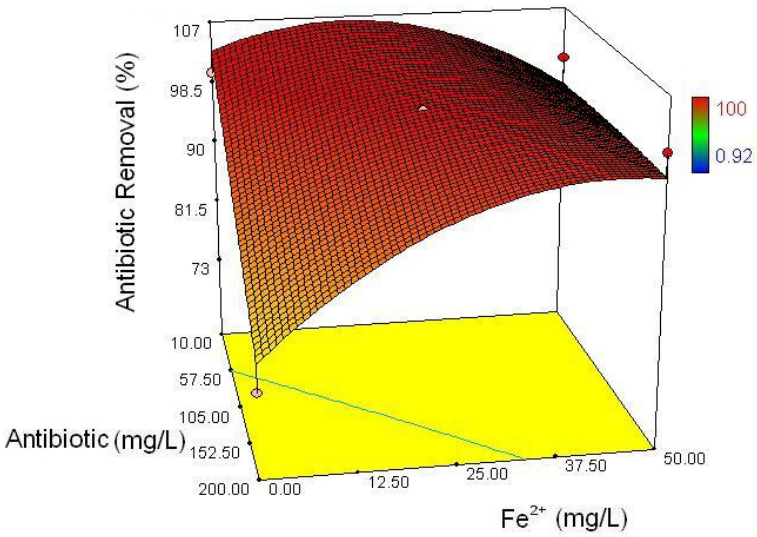



Figure 3.16 Antibiotic removal efficiencies by using photo-Fenton process for constant $H_2O_2$ concentration (central point: 255 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

A 3D surface plot for antibiotic removal is depicted in Figure 3.16 for constant peroxide dose of 255 mg/L. Both antibiotic and Fe(II) doses have considerable effects on percent antibiotic removal. Increases in Fe(II) dose and decreases in antibiotic dose resulted in increases in antibiotic removals. Complete removal of antibiotic was possible at low antibiotic doses and at a high Fe(II) dose of 40 or 50 mg/L when peroxide was 255 mg/L. Negligible antibiotic removals were obtained in the absence of Fe(II) ions (ie peroxide oxidation alone). Fe(II) ions were necessary for effective antibiotic degradation by the photo-Fenton treatment and the removal efficiency increased with Fe(II) ion concentration. High initial antibiotic doses required high peroxide and Fe(II) doses for effective antibiotic removal.

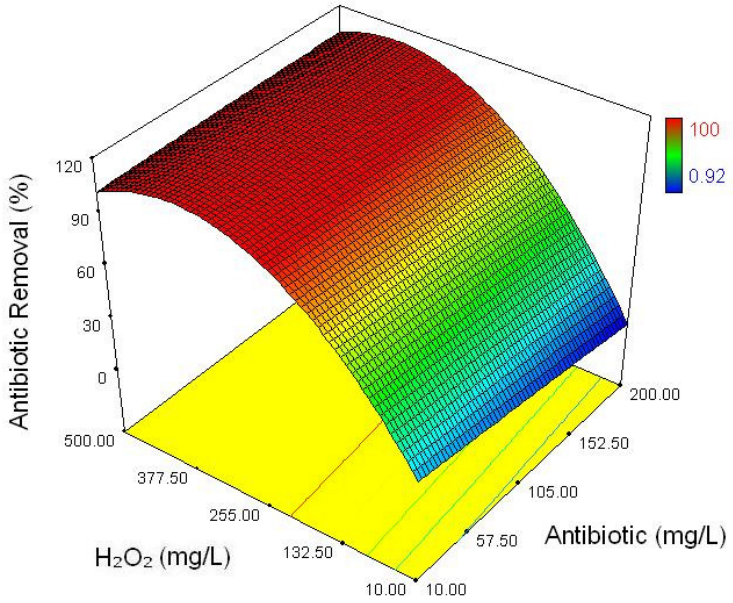



Figure 3.17 Antibiotic removal efficiencies by using photo-Fenton process reagent for constant Fe(II) concentration (central point: 25 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.17 depicts a 3D surface plot for antibiotic removal at constant Fe(II) dose of 25 mg/L. Peroxide concentration affected antibiotic removal considerably yielding increases in antibiotic removal with increases in peroxide dose. Complete antibiotic removals were obtained for peroxide doses above 400 mg/L at almost all antibiotic concentrations when Fe(II) was 25 mg/L. As stated before, hydrogen peroxide concentration was the main factor affecting antibiotic removals in photo-Fenton treatment. Antibiotic concentration also affected percent antibiotic removal, but to a lesser extent as compared to peroxide effect.

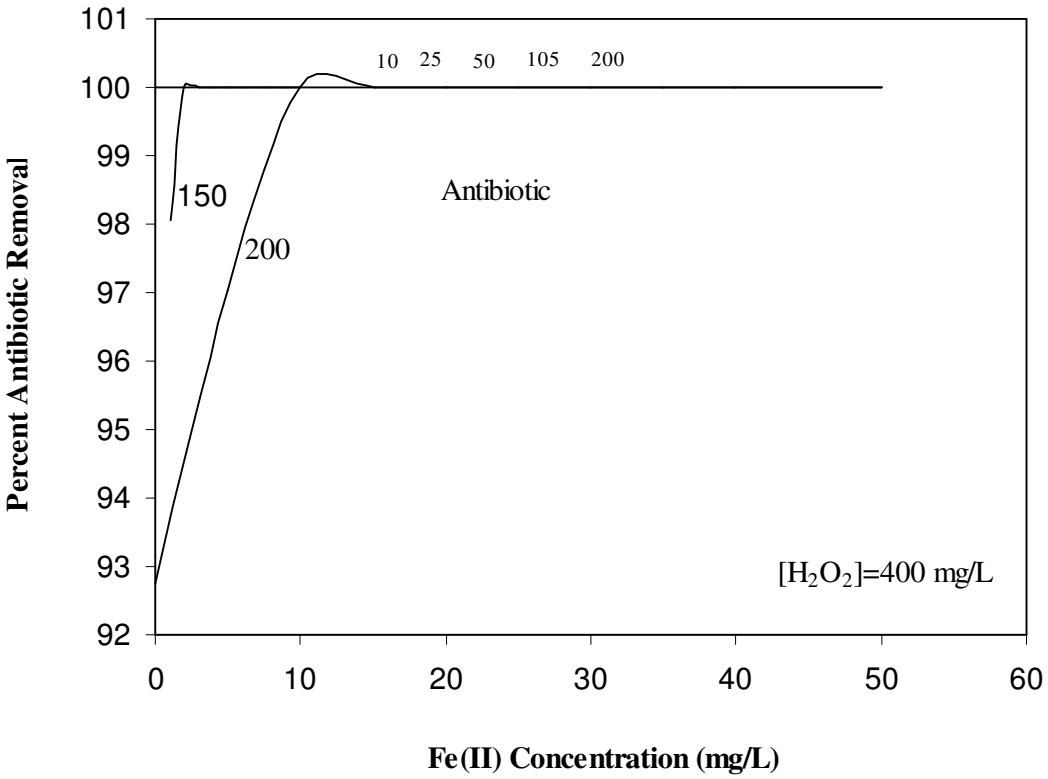



Figure 3.18 Variation of percent antibiotic removal using photo-Fenton process with Fe(II) concentration at different Amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Variation of percent antibiotic removal with Fe(II) concentration at different amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L is depicted in Figure 3.18 for photo-Fenton treatment. At low antibiotic concentrations below 105 mg/L, antibiotic removal was 100% for all Fe(II) doses at peroxide dose of 400 mg/L. However, at high antibiotic concentrations above 150 mg/L, antibiotic removal increased with increasing Fe(II) doses up to 20 mg/L. Complete antibiotic removal can be obtained even at high antibiotic concentrations (200 mg/L) using high peroxide (400 mg/L) and low Fe(II) doses of 20 mg/L. However, antibiotic degradation yielded some intermediates which ought to be removed for complete mineralization.

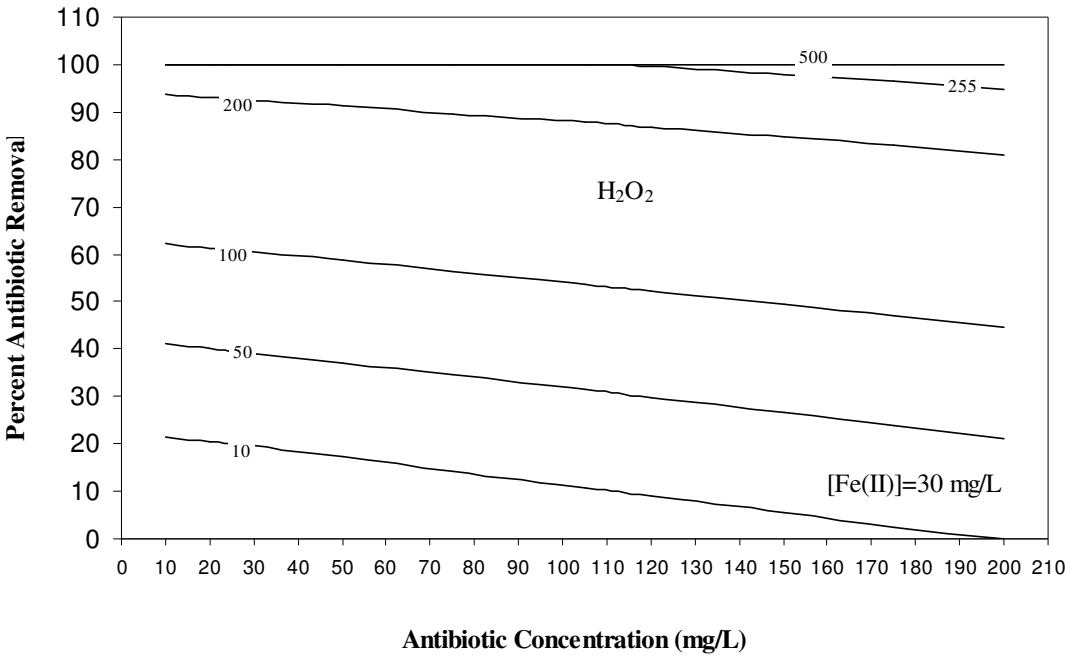



Figure 3.19 Variation of percent antibiotic removal using photo-Fenton with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 30 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.19 depicts variation of percent antibiotic removal with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 30 mg/L. As clearly shown in the figure, antibiotic removal decreased with increasing initial antibiotic concentration due to limitations by other reagents at high antibiotic doses. This decrease was more severe at low peroxide doses due to limitations by peroxide dose. At high peroxide doses above 255 mg/L nearly complete antibiotic removals were obtained even at high antibiotic concentrations of 200 mg/L due to elimination of peroxide limitations. Percent antibiotic removal increased with increasing peroxide doses at all antibiotic concentrations. Fe(II) dose of 30 mg/L was sufficient for complete antibiotic removal. The limiting factor was peroxide dose which was eliminated by increasing the dose obove 255 mg/L.

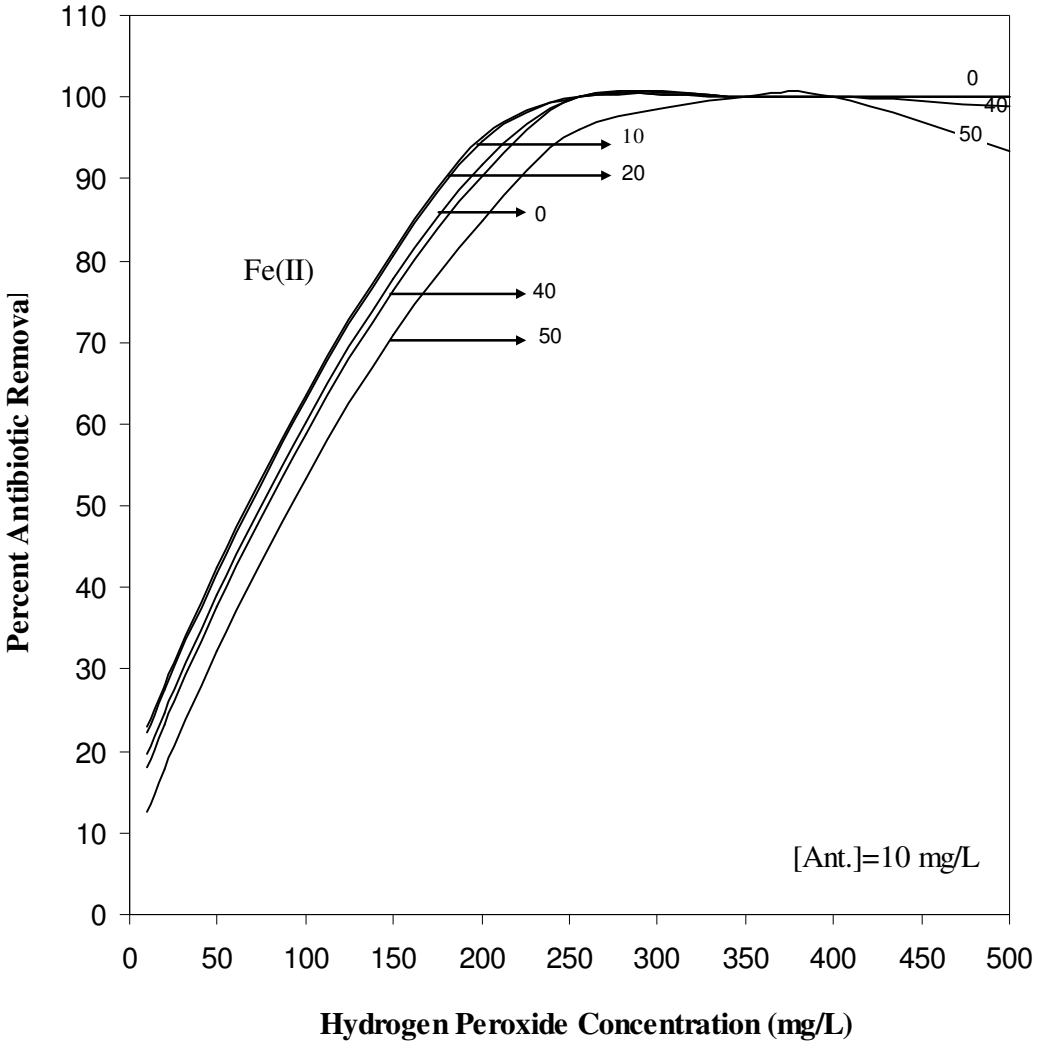



Figure 3.20 Variation of percent antibiotic removal using photo-Fenton with $H_2O_2$ concentration at different Fe(II) doses and constant Amoxicillin dose of 10 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Effects of peroxide concentration on percent antibiotic removal at different concentrations of Fe(II) and constant antibiotic dose of 10 mg/L is depicted in Figure 3.20. Antibiotic removal increased with increasing peroxide dose very steeply indicating pronounced effect of peroxide dose. At a low antibiotic concentration of 10 mg/L, complete antibiotic removal was obtained at 250 mg/L peroxide dose for all Fe(II) doses. Unlike peroxide, Fe(II) dose did not affect antibiotic removal considerably. Percent antibiotic removal increased with Fe(II) dose slightly up to 20 mg/L Fe(II) dose due to limitations by the catalyst. Further increases in Fe(II) dose to 40 or 50 mg/L resulted in decreases in antibiotic removal probably due to hydoxyl

ion scavenging effect of high Fe(II) doses. Even in the absence of Fe(II) ions, only peroxide oxidation was sufficient for complete antibiotic removal at low doses of antibiotic when peroxide dose was 250 mg/L.

In summary it can be said that complete antibiotic removal by photo-Fenton oxidation can be achieved. The required doses of peroxide and Fe(II) for complete degradation varied depending on initial antibiotic concentration. High antibitic cconcentrations required high peroxide doses above 255 mg/L and Fe(II) dose of 25 mg/L for complete mineralization.

### *3.3.2 TOC Removal*

Variation of percent TOC removals with the reagent doses are depicted in Figures 3.21 to 3.23 in form of 3D surface plots. Antibiotic concentration was constant at 105 mg/L in Figure 3.21 while peroxide and Fe(II) doses were constant at 255 mg/L and 25 mg/L in Figures 3.22 and 3.23, respectively.

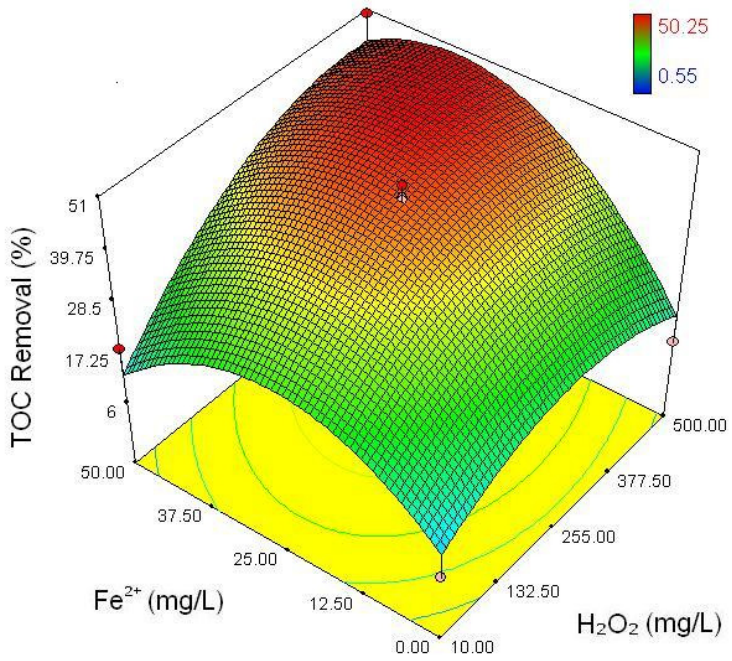



Figure 3.21 TOC removal efficiencies by using photo-Fenton process for constant antibiotic concentration (central point: 105 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.21 depicts TOC removals as function of peroxide and Fe(II) doses in form of 3D surface plot in photo-Fenton oxidation at constant antibiotic dose of 105 mg/L. Unlike Fenton oxidation the highest TOC removal in photo-Fenton oxidation was 50 %. This is a significant improvement over 37% TOC removal in Fenton oxidation. Percent TOC removal increased with increasing Fe(II) dose up to 25 mg/L due to Fe(II) ion limitations at low Fe(II) doses. Further increases in Fe(II) dose above 25 mg/L caused decreases in TOC removal due to hydroxyl ion scavenging effect of high Fe(II) doses. Similar behaviour was observed with the peroxide dose too. The optimal peroxide dose was 255 mg/L below which peroxide was limiting and above which peroxide caused hydroxyl ion scavenging. Apparently, UV irradiation improved TOC removal or percent mineralization as compared to Fenton oxidation.

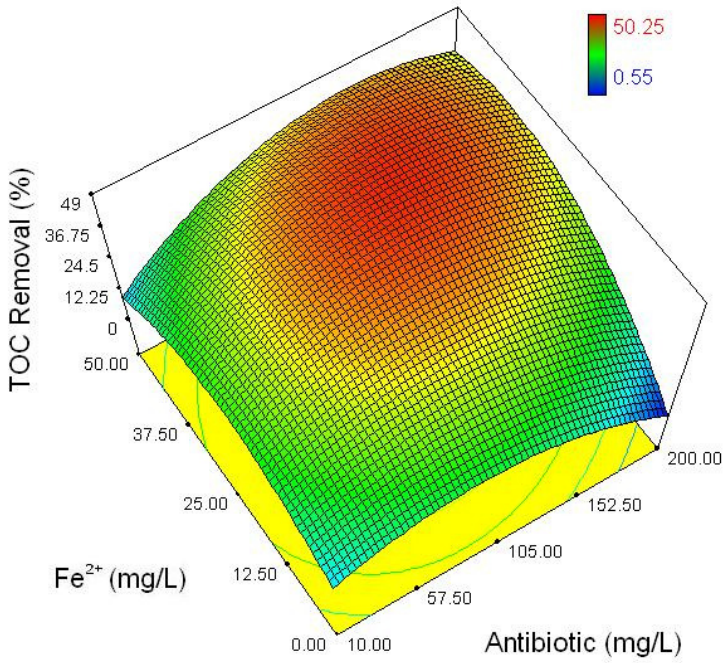



Figure 3.22 TOC removal efficiencies by using photo-Fenton process for constant $H_2O_2$ concentration (central point: 255 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

A similar 3D surface plot is depicted in Figure 3.22 for constant peroxide dose of 255 mg/L. The highest TOC removal (50%) was obtained at antibiotic and Fe(II)

doses of 105 mg/L and 25 mg/L when peroxide was 255 mg/L. Percent TOC removal increased with antibiotic dose up to 105 mg/L due to antibiotic limitations at low antibiotic doses. High antibiotic doses above 105 mg/L resulted in decreases in TOC removal due to limitations by the other reagents. The optimum Fe(II) dose was again 25 mg/L, below which Fe(II) dose was limiting and above which Fe(II) ions caused hydroxyl ion scavenging reducing percent TOC removal. Almost no TOC removal was observed in the absence of Fe(II) ions.The optimal doses of antibiotic and Fe(II) were 105 mg/L and 25 mg/L, respectively when peroxide dose was 255 mg/L.

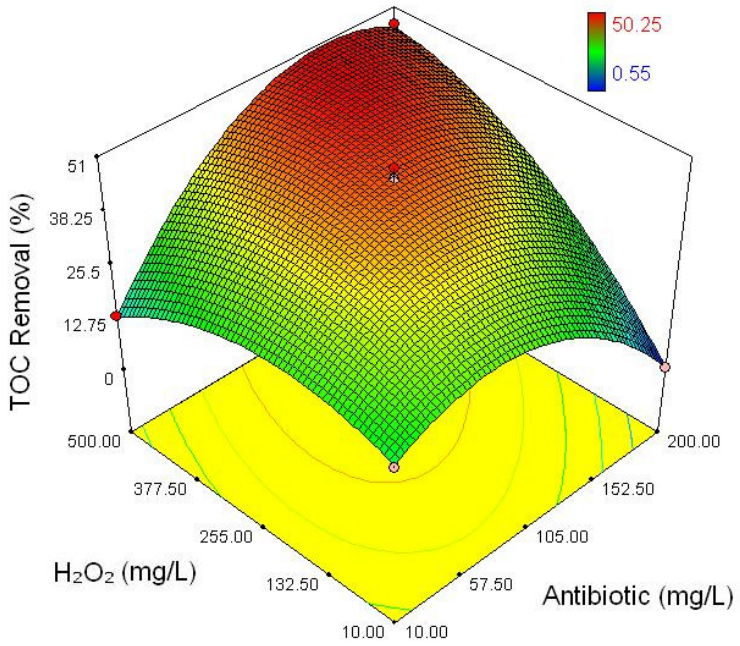



Figure 3.23 TOC removal efficiencies by using photo-Fenton process for constant Fe(II) concentration (central point: 25 mg/L), pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.23 is a 3D surface plot depicting variation of TOC removal with antibiotic and peroxide concentrations at constant Fe(II) dose of 25 mg/L. The optimum antibiotic and peroxide doses were 105 mg/L and 255 mg/L at a Fe(II) dose of 25 mg/L. At low peroxide doses below 255 mg/L TOC removal was limited by peroxide dose. High peroxide doses above 255 mg/L caused hydoxyl ion scavenging yielding low TOC removals. High concentrations of antibiotic requires high peroxıde

doses for effective TOC removal. The highest TOC removal was 50% which is considerably higher than that obtained with the fenton oxidation (37%).

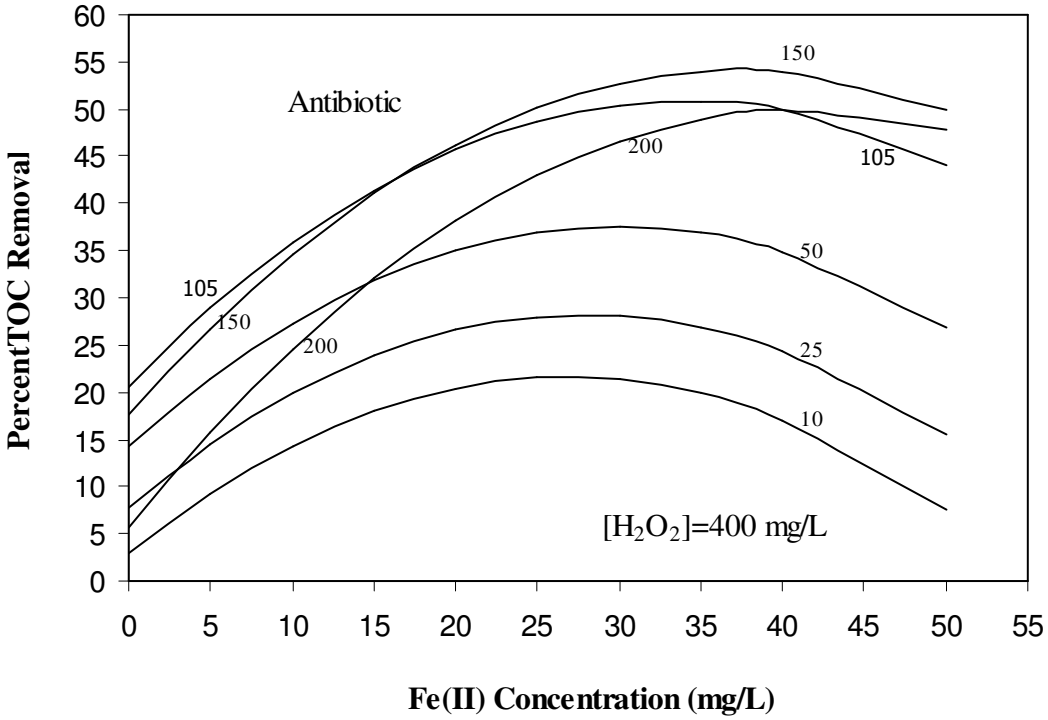



Figure 3.24 Variation of percent TOC removal using photo-Fenton process with Fe(II) concentration at different Amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Variation of percent TOC removal with Fe(II) concentration at different Amoxicillin doses and constant $H_2O_2$ dose of 400 mg/L is depicted in Figure 3.24 in photo-Fenton oxidation. At constant peroxide dose, percent TOC removal increased with Fe(II) dose up to certain Fe(II) dose due to limitations by the catalyst at low doses. The optimum Fe(II) dose varied depending on the antibiotic concentration. For antibiotic concentrations below 105 mg/L, the optimum Fe(II) dose yielding the highest percent TOC removal was 25 to 30 mg/L. However at higher antibiotic doses the optimum Fe(II) dose became 40 mg/L. That is high antibitic concentrations require high Fe(II) doses for effective TOC removal. The highest TOC removal (55%) was obtained with 150 mg/L antibiotic dose requiring 40 mg/L Fe(II) and 400

mg/L peroxide doses. Increases in initial antibiotic dose from 10 to 150 mg/L resulted in increases in percent TOC removal due to antibiotic limitations at low antibiotic doses. Furher increases in antibiotic doses above 150 mg/L yielded lower TOC removals due to limitations by the other reagents. Up to 105 mg/L antibiotic concentrations 30 mg/L Fe(II) and 400 mg/L peroxide doses were sufficient for the highest percent TOC removal of 50%.

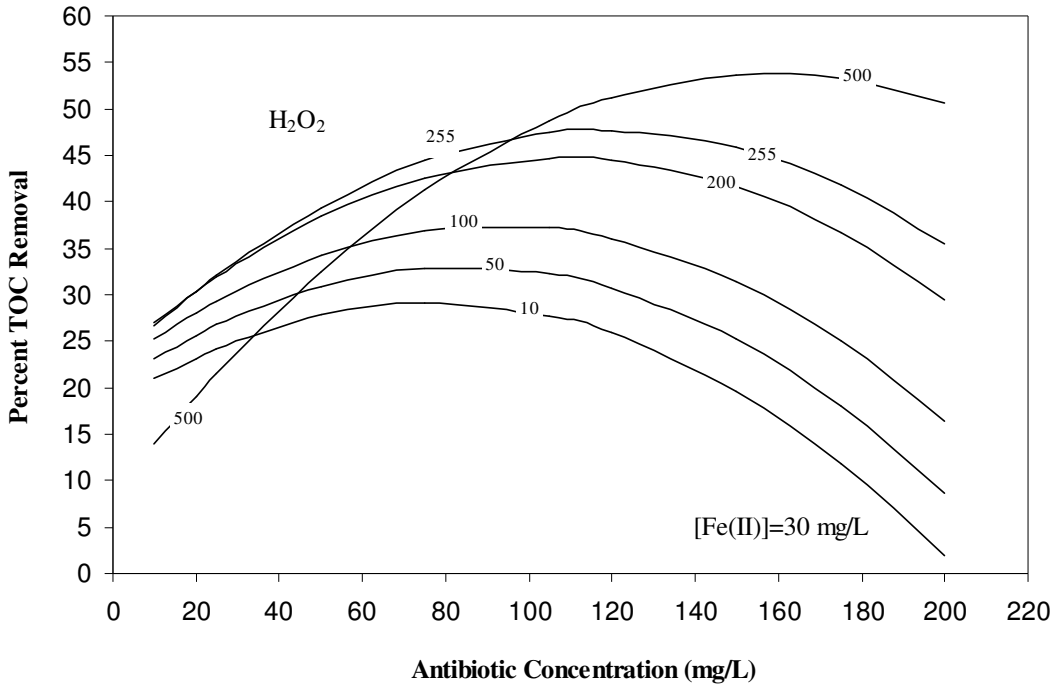



Figure 3.25 Variation of percent TOC removal using photo-Fenton process with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 30 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Figure 3.25 depicts variation of percent TOC removal with Amoxicillin concentration at different $H_2O_2$ doses and constant Fe(II) dose of 30 mg/L. Both peroxide and antibiotic doses had pronounced effects on percent TOC removal. TOC removal increased with increasing antibiotic dose up to certain level above which further increases caused decreases in TOC removals. The optimum antibiotic dose varied depending on peroxide concentration. Up to peroxide dose of 255 mg/L the optimum antibiotic dose was around 100-110 mg/L. However at higher peroxide doses such as 500 mg/L, the optimum peroxide dose became 150 mg/L. That is, at high peroxide doses high antibiotic doses can be used in photo-Fenton oxidation. The

optimum peroxide/Fe/ antibiotic dose was 255/30/105 mg/L up to 105 mg/L antibiotic dose. Increases in peroxide dose resulted in considerable increases in TOC removals at all antibiotic doses. This effect became more pronounced at high antibiotic concentrations due to requirement for high peroxide doses. TOC removal increased from 26% to 47% when peroxide dose was increased from 10 to 255 mg/L at antibiotic and Fe(II) doses of 105 mg/L and 30 mg/L.

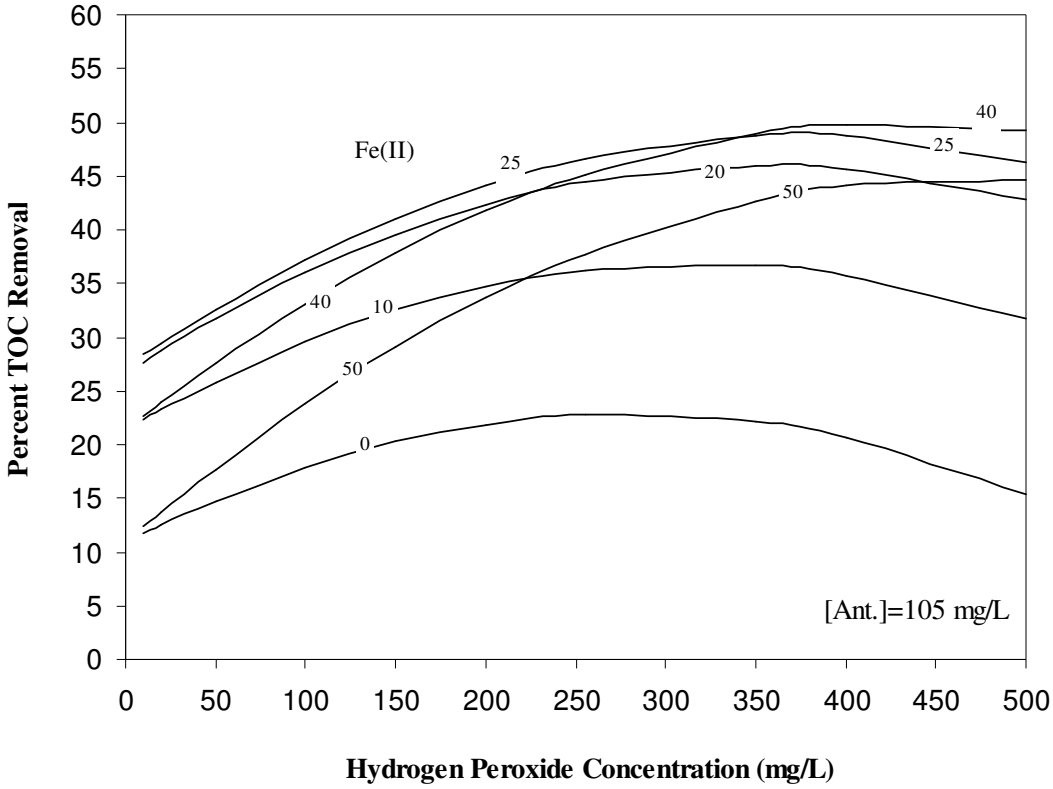



Figure 3.26 Variation of percent TOC removal using photo-Fenton process with $H_2O_2$ concentration at different Fe(II) doses and constant Amoxicillin dose of 105 mg/L, pH=3-3.5, UV irradiation source: 16W low-pressure mercury vapor lamp with maximum emission at 254 nm.

Variation of percent TOC removal with $H_2O_2$ concentration at different Fe(II) doses and constant amoxicillin dose of 105 mg/L is depicted in Figure 3.26. At low Fe(II) doses below 20 mg/L, percent TOC removal increased with peroxide dose up to 250 mg/L and reached the maximum level at this dose. The optimum peroxide dose shifted towards higher levels at high Fe(II) doses above 20 mg/L due to requirement for high peroxide doses at high Fe(II) doses. Fe(II) dose of 40 mg/L

required nearly 400 mg/L peroxide for the highest TOC removal of 50%. The optimum peroxide/Fe/antibiotic ratio yielding the highest level of TOC removal was 100/40/105. Increases in Fe(II) dose resulted in increases in TOC removal up to 40 mg/L due to Fe(II ) limitations at low Fe(II) doses. Further increases in Fe(II) dose to 50 mg/L resulted in decreases in TOC removal due to hydroxyl ion scavenging effect of high Fe(II) doses.

Antibiotic and TOC removal performances of Fenton and photo-Fenton oxidations are compared in Table 3.8 at different antibiotic concentrations. Complete antibiotic removals were achieved by both methods. However, photo-Fenton method required lower reagent concentrations as compared to the Fenton oxidation. This is due to formation of extra hydroxyl radicals by UV irradiation in photo-Fenton treatment. Photo-Fenton treatment resulted in higher TOC removals due to mineralization of degradation intermediates by UV light. The highest TOC removal of the photo-Fenton oxidation was 53% while Fenton oxidation resulted in 38% TOC removal. Photo-Fenton treatment is more advantageous especially at high initial antibiotic concentrations (200 mg/L) yielding complete antibiotic and 53% mineralization.

Table 3.8 Comparison of Fenton and photo-Fenton oxidation performances for antibiotic and TOC removal

| *Ant. Conc. (mg/L)* | *FENTON* | | | | *PHOTO-FENTON* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Required Concentrations (mg/L)* | | *Max. Removal (%)* | | *Required Concentrations (mg/L)* | | *Max. Removal (%)* | |
| | $H_2O_2$ | $Fe^{2+}$ | *Antibiotic* | *TOC* | $H_2O_2$ | $Fe^{2+}$ | *Antibiotic* | *TOC* |
| 10 | 456.29 | 44.32 | 98.92 | 35.89 | 203.75 | 16.56 | 96.10 | 27.60 |
| 105 | 477.40 | 49.40 | 100 | 38.46 | 442.18 | 35.80 | 10050.62 | 50.62 |
| 200 | 448.01 | 37.01 | 81.25 | 28.86 | 462.78 | 46.43 | 10053.19 | 53.19 |

Table 3.9 Some important results of the literature studies as compared to this study

| *Reference* | *Target Drug /Initial Concentration* | *AOP Method* | *Summary of Results* |
|---|---|---|---|
| Andreozzi et al., 2005 | Amoxicillin $5*10^{-4}$ M | $1.6*10^{-4}$ M $O_3$ at pH=2–7 | 90% drug removal and 18% mineralization after 4 and 20 min respectively. |
| Elmolla and Chaudhuri, 2009 | Amoxicillin (104 mg/L), Ampicillin (105 mg/L) and Cloxacillin (103 mg/L) | $COD/H_2O_2/Fe^{2+}$ molar ratio 1:3:0.30 and pH=3 | Complete degradation of the antibiotics occurred in 2 min |
| Rizzo et al., 2009 | Mixture of pharm. compounds composed of Amoxicillin (10 mg/L), Carbamazepine (5 mg/L) and Diclofenac (2.5 mg/L) | Catalyst loading range and irradiation time, 0.2-0.8 $gTiO_2/L$ and 120 min respectively | No significant variation in terms of TOC and UV absorbance after 120 min exposure to UV light alone was observed. The experiments under dark conditions (adsorption on $TiO_2$ in the absence of UV light) showed a quite low removal at 0.8 $gTiO_2/L$ (<15% as TOC) after 120 min. |
| Alaton and Dogruel, 2004 | Amoxicillin trihydrate (<400 mg/L) | $O_3/OH^-$ (at pH=11.5), $H_2O_2/UV$, $Fe^{2+}/H_2O_2$, $Fe^{3+}/H_2O_2$, $Fe^{2+}/H_2O_2/UV$ (pH=3; $Fe^{2+}$:$H_2O_2$ molar ratio=1:20) and $Fe^{3+}/H_2O_2/UV$ | Alkaline ozonation and the photo-Fenton's reagents both appeared to be the most promising AOPs in terms of COD (49–66%) and TOC (42–52%) abatement rates. Antibiotic substance can be completely eliminated after 40 min advanced oxidation applying photo-Fenton's reagent and alkaline ozonation. |
| Trovó et al., 2008 | Amoxicillin (AMX), 210 mg/L | Solar irradiation | AMX degradation is favored in the presence of $FeO_x$, reaching 84% of oxidation after 1 min while 62% was observed using $Fe(NO_3)_3$. |
| This study | Amoxicillin 10, 15 and 200 mg/L | Fenton and photo-Fenton oxidation | Complete antibiotic removal and nearly 53% TOC removals were achieved in our studies using photo-Fenton oxidation within 60 min. |

There are limited number of studies on advanced oxidation of Amoxicillin (Andreozzi et al., 2005; Elmolla and Chaudhuri, 2009; Rizzo et al., 2009; Alaton and Dogruel, 2004; Trovó et al., 2008). Table 3.9 summarizes some important results of the literature studies as compared to our study. Different AOPs were used by different investigators for removal of Amoxicillin. Andreozzi et al (2005) used ozonation for Amoxicillin removal and obtained nearly 90% drug and 18 % TOC removal within 20 minutes. Elmolla and Chaudhuri (2009) used Fenton oxidation for removal of a mixture of antibiotics and reported complete degradation of antibiotics in 2 min and nearly 81% COD removal within 60 minutes. Rizzo et al (2009), used $TiO_2$ and UV treatment for removal of a mixture of antibiotics and toxicity from wastewater. More than 90% TOC removal was achieved within 120 min. However, toxicity removal was not complete. Alkaline ozonation and photo-Fenton methods were reported to be effective for TOC and COD removal from Amoxicillin containing water ( Alaton and Dogruel, 2004). A mixture of antibiotics were subjected to photo-Fenton treatment by using solar and black light. Nearly 84% antibiotic degradation was obtained within 1 min using solar light. As compared to the literature studies, complete antibiotic removal and nearly 53% TOC removals were achieved in our studies using photo-Fenton oxidation. The extent of antibiotic and TOC removals obtained in this study are higher than some of the literature studies and are comparable to the others within the same time span of 60 minutes.

# CHAPTER FOUR
# CONCLUSIONS AND RECOMMENDATIONS

Advanced oxidation of Amoxicillin was achieved in aqueous medium by using the Fenton and photo-Fenton treatments. Antibiotic and TOC removals were quantified at different experimental conditions. Box-Behnken statistical experiment design was used by considering the antibiotic, peroxide and Fe(II) concentrations as independent variables. Objective functions were percent antibiotic and TOC removals (mineralization). The reagent doses were varied within certain limits.

Initial experiments conducted at the central point indicated that 2.5 min was sufficient reaction time for antibiotic degradation while TOC removal (mineralization) took nearly 15 minutes for Fenton oxidation. That is antibiotic degradation was faster than mineralization.

The intermediates formed after 2.5 minutes of degradation was mineralized within 15 minutes in Fenton oxidation. In photo-Fenton oxidation, mineralization took nearly 60 minutes and was not complete.

In Fenton oxidation experiments complete antibiotic degradation was obtained at four experimental points. Antibiotic removal increased with increasing peroxide and Fe(II) doses at high antibiotic concentrations indicating limitations by the oxidant and the catalyst at low concentrations. High concentrations of peroxide and Fe(II) resulted in lower antibiotic removals due to hydroxyl ion scavenging effects of high peroxide and Fe(II) doses. The optimum peroxide/Fe/antibiotic ratio resulting in complete antibiotic removal was 255/ 25/ 105 mg/L.

Unlike complete antibiotic removal, TOC removal or mineralization was not complete in Fenton oxidation due to presence of degradation intermediates in the medium. Apparently some of the intermediate compounds were resistant to advanced oxidation by Fenton reagent. The highest TOC removal was 37% at all antibiotic concentrations. Peroxide and Fe(II) requirements increased with increasing antibiotic

concentration. For example, 105 mg/L antibiotic required 255 mg/L peroxide and 25 mg/L Fe(II) for he highest degree of mineralization, while the peroxide and Fe(II) requirements for 200 mg/L antibiotic were 400 mg/L and 30 mg/L, respectively.

Photo-Fenton oxidation experiments yielded higher degree of antibiotic degradation and mineralization as compared to the Fenton oxidation experiments at all data points. This is due to extra hydroxyl ion formation by UV irradiation. Nearly complete antibiotic removals were obtained at eight experimental conditions. Antibiotic removal increased with increasing peroxide and Fe(II) doses up to optimum levels of the reagents. Further increases in reagent concentrations above optimum levels resulted in decreases in antibiotic removal due to hydroxyl ion scavenging effects of high peroxide and Fe(II) doses. The optimum reagent doses varied with the antibiotic dose. High antibiotic doses required high peroxide and Fe(II) doses.

Percent mineralization or TOC removal with the photo-Fenton treatment was considerably higher than that obtained with the Fenton oxidation. The highest percent TOC removal was 50% with photo-Fenton treatment as compared to 37% removal with the Fenton oxidation. Percent TOC removal also increased with increasing peroxide and Fe(II) doses up to optimum levels of the reagents and then decreased with further increases. At low concentrations of reagents the limiting factor was the reagent doses. At high reagent doses hydroxyl ion scavenging effects of peroxide and Fe(II) caused low TOC removals. The optimum reagent doses varied depending on the initial antibiotic concentration. High antibiotic concentrations required high peroxide and Fe(II) doses for effective mineralization. For the central antibiotic concentration of 105 mg/L the optimum peroxide/Fe(II)/antibiotic ratio was 255/30/105 mg/L.

Fenton and photo-Fenton methods yielded complete removal of Amoxicillin. Therefore, degradation of the antibiotic was not a problem, no matter which method was used. However some intermediates were produced from degradation of the antibiotic which could not be removed by the methods tested in this study. In other

words mineralization of the antibiotic or TOC removal was not complete in Fenton and photo-Fenton oxidations. However, photo-Fenton method has improved the TOC removal as compared to the Fenton treatment. Future studies should be directed to improve TOC removal or percent mineralization.

Recommended studies for future research on the same topic can be summarized as follows:

1. Intensity of the UV light can be increased for more effective photo-Fenton treatment.

2. AOP studies with ozone can be performed as an alternative for the Fenton and photo-Fenton oxidations.

3. AOPs by peroxone (peroxide-ozone) treatment can be investigated for comparison.

4. AOPs utilizing $TiO_2$ and UV light can be used for further improvements on TOC removal.

5. A combination of the tested methods such as photo-Fenton followed by peroxone treatment can be used.

**REFERENCES**

*Amoxicillin*, (n.d.). Retrieved August 5, 2009, from http://www.marvistavet.com/html/body_Amoxicillin.html

Andreozzi, R., Canterino, M., Marotta, R., Paxeus, N. (2005). Antibiotic removal from wastewaters: the ozonation of Amoxicillin. *Journal of Hazardous Materials, 122*, 243-250.

Arslan-Alaton, I., Dogruel, S. (2004). Pre-treatment of penicillin formulation effluent by advanced oxidation processes. *Journal of Hazardous Materials, 112* (1-2), 105-113.

Aslan, N., Cebeci, Y. (2007). Application of Box-Behnken design and response surface methodology for modeling of some Turkish coals. *Fuel, 86*, 90-97.

Ay, F., Çatalkaya, E.C., Kargı, F. (2008). Advanced oxidation of Direct Red (DR 28) by Fenton treatment. *Environmental Engineering Science, 25 (10)*, 1455-1462.

Ay, F., Çatalkaya, E.C., Kargı, F. (2009). A statistical experiment design approach for advanced oxidation of Direct Red azo-dye by photo-Fenton treatment. *Journal of Hazardous Materials, 162*, 230-236.

Baranda, A.B., Etexbarria, N., Jimenez, R.M., Alonso, R.M. (2005). Deveopment of a liquid-liquid extraction procedure for five 1,4-dihydropyridines calcium channel antagonistics from human plasma using experimental design. *Talanta, 67* (5), 933-941.

Barbosa, A.F., Segatelli, M.G., Pereira, A.C., Santos, A.S., Kubota, L.T., Luccas, P.O., Tarley, C.R.T. (2007). Solid-phase extraction system for Pb (II) ions enrichment based on multiwall carbon nanotubes coupled on-line to flame atomic absorption spectrometry. *Talanta, 71*, (4), 1512-1519

Basker, M.J., Sutherland, R. (1977). Activity of Amoxicillin, alone, and in combination with aminoglycoside antibiotics aginst atreptococci associated with bacterial endocarditis. *Journal of Antimicrobial Chemotherapy, 3*, 273-282

Bautitz, I.R., Nogueira, R.F.P. (2007). Degradation of tetracycline by photo-Fenton process-Solar irradiation and matrix effects. *Journal of Photochemistry and Photobiology A, 187*, 33–39.

Beausse, J. (2004). Selected drugs in solid matrices: A review of environmental determination, occurrence and properties of principal substances. *Trends in Analytical Chemistry, 23*, 753–761.

Ben, W., Qiang, Z., Pan, X., Chen, M. 2009. Removal ofveterinary antibiotics from sequencing btch reactor (SBR) pretreated swine wastewater by Fenton's reagent. *Water Research, 43*, 4392-4402.

Bendz, D., Paxéus, N.A., Ginn, T.R., Loge, F.J. (2005). Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. *Journal of Hazardous Materials, 122*, 195–204.

Bezerra, M.D., Conceição¸ A.L.B., Ferreira, S.L.C. (2004). Doehlert matrix for optimization of procedure for determination of nickel in saline oil-refinery effluents by use a flame atımic absorption spectrometry after preconcentration by cloud-point extraction. *Analytical and Bioanalytical Chemistry, 378,* 798-803.

Bishop, D.F., Stern, G., Fleischman, M., Marshall L.S. (1968). Hydrogen peroxide catalytic oxidation of refractory organics in municipal wastewater. *Industrial & Engineering Chemistry, Process Design & Development, 7*, 110-117.

Box, G.E.P., Behnken, D.W. (1960). *Technometrics 2* (195).

Box, G.E.P., Wilson, K.B. (1951). On the experimental attainment of optimum multifactorial conditions. *Royal Statistics Society 13*, 1–12.

Boza, A., De la Cruz, Y., Jordan, G., Jauregui-Haza, U., Aleman, A., and Caraballo, I. (2000). Statistical optimization of a sustained-release matrix tablet of lobenzarit disodium. *Drug Development and Industrial Pharmacy. 26*, 1303–1307.

Bradley, D. (1999). Double-dosed Amoxicillin formulation proves efficacious and safe. *Pharmaceutical Science & Technology Today, 2* (1), 6.

Buxton, G.U., Greenstock, C.L., Helman, W.P., Ross, A.B. (1988). Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (OH•/O•–) in aqueous solution, *Journal of Physical and Chemical Reference Data, 17*, 513–886.

Carasek, E., Cudjoe, E., Pawliszyn, J. (2007). Fast and sensitive method to determine chloroanisoles in cork using an internally cooled solid-phase microextraction fiber. *Journal of Chromatography A, 1138* (1-2), 10-17.

Castle, S.S. (2008). Amoxicillin. *xPharm: The Comprehensive Pharmacology Reference*, 1–6.

Catalkaya, E.C., Kargi, F. (2007). Effects of operating parameters on advanced oxidation of diuron by the Fenton's reagent: a statistical design approach. *Chemosphere, 69*, 485–492.

Conde, F.J., Ayala, J.H., Afonso, A.M., Gonzalez, V. (2004). Optimization of sampling method to determine polycyclic aromatic hydrocarbons in smoke from incomplete biomass combustion. *Analytica Chimica Acta, 524* (1-2), 287.

da Silva, J.E., Pimentel, M.F., da Silva, V.L., B.S.M. Montenegro, M.D.C., Araújo, A.N. (2004). Simultaneous determination of pH, chloride and nickel in electroplating baths using sequential injection analysis. *Analytica Chimica Acta, 506* (2), 197-202.

de Amorim, F.V., Bof, C., Franco, M.B., da Silva, J.B.B., Nascentes, C.C. (2006). Comparatie study of conventional and multivariate methods for aluminum determination in soft drinks by graphite furnace atomic absorption spectromtry. *Microchemical Journal, 82* (2), 168-173.

del Campo, G., Gallego, B., Berregi, I. (2006). Fluorimetric determination pf histamine in wine and cider by using an anion-exchange column-FIA system and factorial design study. *Talanta, 68* (4), 1126-1134.

Dong, C.-H., Xie, X.-Q., Wang, X.-L., Zhan, Y., Yao, Y.-J. (2009). Application of Box-Behnken design in optimisation for polysaccharides extraction from cultured mycelium of Cordyceps sinensis. *Food and Bioproducts Processing, 87*, 139–144.

Ebrahimzadeh, H., Yamini, Y., Kamarei, F., Shariati, S. (2007). Homogeneous liquid-liquid extraction of trace amounts of mononitrotoluenes from waste water samples. *Analytica Chimica Acta, 594* (1), 93-100.

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

Evans, M. (2003). *Optimization of Manufacturing Processes: A Response Surface Approach*. London: Carlton House Terrace,

Felsner, M.L., Cano, C.B., Matos, J.R., de Almeida-Muradian, L.B., Bruns, R.E. (2004). Optimization of thermogravimetric analysis of ash content in honey. *Journal of the Brazilian Chemical Society, 15* (6), 797-802.

Fent, K., Weston, A., Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology, 76*, 122–159.

Fenton, H.J.H. (1894). Oxidation of tartaric acid in presence of iron. *Journal of The Chemical Society, 65*, 899.

Ferreira, S.L.C., Bruns, R.E., da Silva, E.G.P., dos Santos, W.N.L., Quintella, C.M., David, J.M., de Andrade, J.B., Breitkreitz, M.C., Jardim, I.C.S.F., Neto, B.B. (2007). Statistical design and response surface techniques for the optimization of chromatographic systems. *Journal of Chromatography A, 1158* (1-2), 2-14.

Ferreira, S.L.C., Bruns, R.E., Ferreira, H.S., Matos, G.D., David, J.M., Brandão, G.C., da Silva, E.G.P., Portugal, L.A., dos Reis, P.S., Souza, A.S., dos Santos, W.N.L. (2007). Box-Behnken design: An alternative for the optimization of analytical methods. *Analytica Chimica Acta, 597*, 179-186.

Ferreira, S.L.C., dos Santos, W.N.L., Quintella, C.M., Neto, B.B., Bosque-Sendra, J.M. (2004). Doehlert matrix: a chemometric tool for analytical chemistry-review. *Talanta, 63* (4), 1061-1067.

Fu, J., Zhao, Y., Wu, Q. (2007). Optimising photoelectrocatalytic oxidation of fulvic acid using response surface methodology. *Journal of Hazardous Materials, 144* (1-2), 499-505.

García-Villar, N., Saurina, J., Hernández-Cassou, S. (2006). High-performance liquid chromotographic determination of biogenic amines in wines with an experimental design optimization procedure. *Analytica Chimica Acta, 575* (1), 97-105.

George, P. (1948). A comparison of the decomposition of hydrogen peroxide by catalase, ferrous and ferric ions, heamin and ferrous phtalocyanine. *Biochemical Journal, 43,* 287-295.

Ghauch, A., Tuqan, A., Assi, H.A. (2009). Antbiotic removal from water: Elimination of Amoxicillin and ampicillin by microscale and nanoscale iron particles. *Environmental Pollution, 157*, 1626-1635.

González, O., Sans, C., Esplugas, S. (2007). Sulfamethoxazole abatement by photo-Fenton toxicity, inhibition and biodegradability assessment of intermediates. *Journal of Hazardous Materials, 146*, 459–464.

Haag, W.R., Yao, C.D. (1992). Rate constant for reaction of hydroxyl radicals with several drinking water contaminants. *Environmental Science and Technology, 26*, 1005–1013.

Haaling-Sørensen, B., Nors Nielsen, S., Lanzsky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E. (1998). Occurence, fate and effects of pharmaceutical substances in the environment-A review. *Chemosphere, 36* (2), 357-393.

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. *Toxicology Letters, 131*, 5–17.

Idris, A.M., Assubaie, F.N., Sultan, S.M. (2006). Chemometric optimization of a SIA promethazine hydrocloride assay method. *Microchemical Journal, 83* (1), 7-13.

Ikehata, K., Naghashkar, N.J., Gamal El-Din, M. (2006). Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: A review. *Ozone Science and Engineering, 28*, 353–414.

Jalbani, N., Kazi, T.G., Arain, B.M., Jamali, M.K., Afridi, H.I., Sarfraz, R.A. (2006). Application of factorial design in optimizationof ultrasonic-assistedextraction of aluminum in juices and soft drinks. *Talanta, 70* (2), 307-314.

Kang, S.F., Liao, C.H., Po, S.T. (2000). Decolorization of textile wastewater by photo-Fenton oxidation technology. *Chemosphere, 41*, 1287–1297.

Khajeh, M. (2009). Application of Box–Behnken design in the optimization of a magnetic nanoparticle procedure for zinc determination in analytical samples by inductively coupled plasma optical emission spectrometry. *Journal of Hazardous Materials, 172,* 385–389.

Klavarioti, M., Mantzavinos, D., Kassinos, D. (2009). Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environment International, 35*, 402-417.

Kumar, A., Prasad, B., Mishra, I.M. (2007). Process parametric study for ethene carboxylic acid removal onto powder activated carbon using Box–behnken design. *Chemical Engineering Technology, 30* (7), 932–937.

Kümmerer, K. (2001). Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – A review. *Chemosphere, 45*, 957–969.

Kümmerer, K. (2009). Antibiotics in the aquatic environment – A review – Part I. *Chemosphere, 75*, 417–434.

Kümmerer, K., Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluents. *Clinical Microbiology and Infection, 9*, 1203–1214.

Legrini, O., Olivers, E., Braun, A.M. (1993). Photochemical process for water treatment. *Chemical Reviews, 93,* 671-698.

Lemos, V.A., Santos, M.S., dos Santos, M.J.S., Vieira, D.R., Novaes, C.G. (2007). Determination of copper in water samples by atomic absorption spectrometry after cloud point extraction. *Microchimica Acta, 157*, 215-222.

Malato, S., Blanco, J., Alarcón, D.C., Maldonado, M.I., Fernández-Ibáñez, P., Gernjak, W., 2007. Photocatalytic decontamination and disinfection of water with solar collectors. *Catalysis Today, 122*, 137–149.

Mamani, M.C.V., Farfán, J.A., Reyes, F.G.R., Rath, S. (2006). Simultaneous determination of tetracyclines in pharmaceuticals by CZE using experimental design. *Talanta, 70* (15), 236-243.

Matthews, R.C., Goode, S.R., Morgan, S.L. (1981). Characterization of an enzymatic determination of arsenic(V) based on response surface methodology. *Analytica Chimica Acta, 133* (2), 169-182.

McEvoy, G. (Ed.) (2001). *Drug Monographs*. American Hospital Formulary System, 364–382, American Society of Health-System Pharmacists, Inc., Bethesda, Maryland.

Mendez-Arriaga, F., Esplugas, S., Giménez, J. (2009). Degradation of the emerging contaminant ibuprofen in water by photo-Fenton. *Water Research*, *in press.*

Nogueira, R.F.P., Trovó , A.G., Silva, M.R.A., Villa, R.D., Oliveira, M.C., 2007. Fundaments and environmental applications of Fenton and photo-Fenton processes. *Quimica Nova, 30*, 400–408.

Penteado, J.C., Bruns, R.E., de Carvalho, L.R.F. (2006). Factorial design optimization of solid phase microextraction condition for gas chromotography-mass spectrometry (GC-MS) analysis of linear alkylbenzenes (LABs) in detergents. *Analytica Chimica Acta, 562* (2), 152-157.

Pereira-Filho, E.R., Poppi, R.J., Arruda, M.A.Z. (2002). Employment of factorial design for optimization of pirolysis and atomization temperatures for Al, Cd, Mo and Pb determination by ETAAS. *Química Nova, 25* (2), 246-253.

Petz, M., Lamar, J. (2007). Development of receptor-based microplate assat for the detection of beta-lactam antibiotics in different food matrices. *Analytica Chimica Acta, 586* (1-2), 296-303.

Pignatello, J.J. (1992). Dark & photoassisted $Fe^{3+}$ catalyzed degradation of chlorophenoxy herbicides by hydrogen peroxide. *Environmental Science and Technology, 26,* 944-951.

Ragonese, R., Macka, M., Hughes, J., Petocz, P. (2002). The use of the Box–Behnken experimental design in the optimisation and robustness testing of a capillary electrophoresis method for the analysis of ethambutol hydrochloride in a pharmaceutical formulation. *Journal of Pharmaceutical and Biomedical Analysis, 27*, 995–1007.

Rizzo, L., Meric, S., Guida, M., Kassinos, D., Belgiorno, V. (2009). Heterogenous photocatalytic degradation kinetics and detoxification of an urban wastewater treatment plant effluent contaminated with pharmaceuticas. *Water Research, 43*, 4070-4078

Rodrigues, P.M.S.M., Silva, J.C.G.E., Antunes, M.C.G. (2007). Factorial analysis of the trihalomethanes formation in water disinfection using chlorine. *Analytica Chimica Acta, 595* (1-2), 266-274.

Ruotolo, L.A.M, Gubulin, J.C. (2005). A factorial-design study of the variables affecting the electrochemical reduction of Cr(VI) at polyaniline-modified electrodes. *Chemical Engineering Journal, 110* (1-3), 113-121.

Sanchez-Lafuente, C., Furlanetto, S., and Fernandez-Arevalo, M. (2002). Didanosine extended-release matrix tablets: optimization of formulation variables using statistical experimental design. *International Journal of Pharmaceutics. 237*, 107–118.

Saracoglu, S., Soylak, M., Peker, D.S.K., Elci, L., dos Santos, W.N.L., Lemos, V.A., Ferreira, S.L.C. (2006). A pre-concentration procedure using coprecipitation for determination of lead and iron in several samples using flame atomic absorption spectrometry. *Analytica Chimica Acta, 575* (1), 133-137.

*Showing Drug Card of Amoxicillin (DB01060)*, (n.d.). Retrieved December 31, 2009, from http://www.drugbank.ca/drugs/DB01060

Singh, S.K., Dodge, J., Durrani, M.J., and Khan, M.A. (1995). Optimization and characterization of controlled release pellets coated with an experimental latex: I. Anionic drug. *International Journal of Pharmaceutics 125*, 243–255.

Soriano, S., Netto, A.D.P., Cassella, R.J. (2007). Multivariate optimization of a microwave-assisted leaching procedure using dilute acid solutions, for FAAS determination of Cu, Fe, Mn, and Zn in multivitamin/multimineral supplements. *Analytical and Bioanalytical Chemistry, 387*, 1113-1320.

Strausbaugh, L.J., Girgis, N.I., Mikhail, I.A., Edman, D.C., Miner, W.F., Yassin, M.W. (1978). Penetration of Amoxicillin into celebrespinal fluid. *Antimicrobial Agents and Chemotherapy, 14* (6)*,* 899-902.

Teofilo, R.F., Reis, E.L., Reis, C., da Silva, G.A., Kubota, L.T. (2004). Experimental design employed to square wave voltammetry response optimization for the gyphosate determination. *Journal of the Brazilian Chemical Society, 15* (6), 865-871.

Tombesi, N.B., Freije, R.H., Augusto, F. (2004). Factorial experimental design optimization of solid phase microextraction (SPME) conditions for analysis of butylated hydroxytoluene (BHT) in bottleed water. *Journal of the Brazilian Chemical Society*, 15, 658-663.

Travó, A.G., Melo, A.S.S., Nogueira, R.F.P. (2008). Photodegradation of the pharmaceuticals Amoxicillin, bezafibrate and paracetamol by the photo-Fenton process-Application to sewage treatment plant effluent. *Journal of Photochemistry and Photobiology A: Chemistry, 198*, 215-220.

Travó, A.G., Nogueira, R.F.P., Agüera, A., Fernandez-Alba, A.R., Sirtori, C., Malato, S. (2009). Degradation of sulfomethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation. *Water Research, 43*, 3922-3931.

Trevizan, L.C., Vieira, E.C., Nogueira, A.R.A., Nóbrega, J.A. (2005). Use of factorial design for evaluation of plasma conditions and comparison of two liquid sample introduction systems for an axially viewed inductively coupled plasma optical emission spectrometer. *Spectrochimica Acta Part:B, 60* (5), 575-581.

Vasconcelos, T.G., Kümmerer, K., Henriques, D.M., Martins, A.F. (2009). Ciprofloxacin in hospital effluent: Degredation by ozone and photoprocesses. *Journal o Hazardous Materials, 169*, 1154-1158.

Villaneuva, M., Catasus, M., Salin, E.D., Pomares, M. (2000). Study of mixed-matrix effect induced by Ca and Mg in ICP-AES. *Journal of Analytical Atomic Spectrometry, 15*, 877-882.

Walling, C. (1975). Fenton's Reagent Revisited. *Accounts of Chemical Research, 8,* 125-131.

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

Watkinson, A.J., Murby, E.J., Costanzo, S.D. (2007). Removal of antibiotics in conventional and advanced wastewater treatment: Inplications for environmental discharge and wastewater recycling. *Water Research, 41*, 4164-4176.

Woller, A., Garraud, H., Boisson, J., Dorthe, A.M., Fodor, P., Donard, O.F.X. (1998). Simultaneous speciation of redox species of arsenic and selenium by anion exchange microbore coloumn hyphenated to micro concentric nebulization and inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 13*, 141-149.

Zarei, K., Atabati, M., Ilkhani, H. (2006). Catalytic adsoptive stripping voltammetry determination of ultra trace amount of molybdenum using factorial design for optimization. *Talanta, 69* (4), 816-821.

Zazo, J.A., Casas, J.A., Mohedano, A.F., Gilarranz, M.A., Rodríguez, J.J. (2005). Chemical pathway and kinetics of phenol oxidation by Fenton's reagent. *Environmental Science and Technology, 39*, 9295–9320.

Zepp, R.G., Faust, B.C., Hoigne, J. (1992). Hydroxyl radical formation in aqueous reactions (pH 3–8) of iron(II) with hydrogen-peroxide - the photo-Fenton reaction. *Environmental Science and Technology, 26*, 313–319.

## APPENDICES

Raw data on antibiotic and TOC removals for Fenton and photo-Fenton oxidations are summarized in Tables A.1 to A.4.

Table A.1 Raw data for Fenton's Reagent oxidation of Amoxicillin: Antibiotic removal

| *Run No* | *Concentrations* | | | *Antibiotic Concentrations (mg/L)* | | | *Antibiotic Removal (%) (15 min)* |
|---|---|---|---|---|---|---|---|
| | $X_1$ *Ant. (mg/L)* | $X_2$ $H_2O_2$ *(mg/L)* | $X_3$ *Fe(II) (mg/L)* | *Raw* | *2.5min.* | *15min.* | |
| 1 | 200 | 10 | 25 | 200.90 | 199.65 | 196.94 | 1.97 |
| 2 | 10 | 10 | 25 | 11.11 | 10.64 | 9.32 | 16.18 |
| 3 | 10 | 500 | 25 | 9.57 | 0 | 0 | 100 |
| 4 | 200 | 500 | 25 | 200.30 | 120.18 | 20.03 | 90 |
| 5 | 200 | 255 | 0 | 203.21 | 191.36 | 132.09 | 35 |
| 6 | 200 | 255 | 50 | 202.09 | 187.06 | 111.94 | 44.61 |
| 7 | 10 | 255 | 0 | 11.32 | 9.62 | 1.13 | 90 |
| 8 | 10 | 255 | 50 | 10.35 | 8.89 | 1.55 | 85 |
| 9 | 105 | 10 | 0 | 105.72 | 104.28 | 97.08 | 8.18 |
| 10 | 105 | 10 | 50 | 103.12 | 100.75 | 91.24 | 11.52 |
| 11 | 105 | 500 | 0 | 104.40 | 0 | 0 | 100 |
| 12 | 105 | 500 | 50 | 105.32 | 0 | 0 | 100 |
| 13 | 105 | 255 | 25 | 105.51 | 0 | 0 | 100 |
| 14 | 105 | 255 | 25 | 104.98 | 0 | 0 | 100 |
| 15 | 105 | 255 | 25 | 104.86 | 0 | 0 | 100 |

Table A.2 Raw data for photo-Fenton oxidation of Amoxicillin: Antibiotic removal

| *Run No* | *Concentrations* | | | *Antibiotic Concentrations (mg/L)* | | | | *Ant. Removal (%) (60 min)* |
|---|---|---|---|---|---|---|---|---|
| | *$X_1$ Ant. (mg/L)* | *$X_2$ $H_2O_2$ (mg/L)* | *$X_3$ Fe(II) (mg/L)* | *Raw* | *15 min* | *30 min* | *60 min.* | |
| 1 | 200 | 10 | 25 | 201.97 | 201.39 | 200.81 | 199.65 | 1.15 |
| 2 | 10 | 10 | 25 | 11.41 | 10.69 | 9.97 | 8.53 | 25.25 |
| 3 | 10 | 500 | 25 | 10.98 | 0 | 0 | 0 | 100 |
| 4 | 200 | 500 | 25 | 202.90 | 0 | 0 | 0 | 100 |
| 5 | 200 | 255 | 0 | 201.67 | 164.83 | 127.99 | 54.31 | 73.07 |
| 6 | 200 | 255 | 50 | 200.99 | 0 | 0 | 0 | 100 |
| 7 | 10 | 255 | 0 | 10.41 | 0 | 0 | 0 | 100 |
| 8 | 10 | 255 | 50 | 10.74 | 0 | 0 | 0 | 100 |
| 9 | 105 | 10 | 0 | 103.77 | 103.44 | 103.12 | 102.47 | 1.25 |
| 10 | 105 | 10 | 50 | 105.76 | 105.52 | 105.28 | 104.79 | 0.92 |
| 11 | 105 | 500 | 0 | 104.74 | 0 | 0 | 0 | 100 |
| 12 | 105 | 500 | 50 | 104.92 | 0 | 0 | 0 | 100 |
| 13 | 105 | 255 | 25 | 105.82 | 0 | 0 | 0 | 100 |
| 14 | 105 | 255 | 25 | 107.97 | 0 | 0 | 0 | 100 |
| 15 | 105 | 255 | 25 | 106.69 | 0 | 0 | 0 | 100 |

Table A.3 Raw data for Fenton's Reagent oxidation of Amoxicillin: TOC removal

| *Run No* | *Concentrations* | | | *TOC Concentrations (mg/L)* | | | | *TOC Removal (%)* |
|---|---|---|---|---|---|---|---|---|
| | $X_1$ *Ant. (mg/L)* | $X_2$ $H_2O_2$ *(mg/L)* | $X_3$ *Fe(II) (mg/L)* | *Raw* | *2.5min.* | *10 min.* | *15min.* | |
| 1 | 200 | 10 | 25 | 117.27 | 116.78 | 115.31 | 114.33 | 2.51 |
| 2 | 10 | 10 | 25 | 6.49 | 6.33 | 5.86 | 5.54 | 14.62 |
| 3 | 10 | 500 | 25 | 5.59 | 5.36 | 4.66 | 4.20 | 24.82 |
| 4 | 200 | 500 | 25 | 116.92 | 112.74 | 100.20 | 91.84 | 21.45 |
| 5 | 200 | 255 | 0 | 118.62 | 117.24 | 113.09 | 110.32 | 7 |
| 6 | 200 | 255 | 50 | 117.97 | 112.87 | 97.57 | 87.38 | 25.93 |
| 7 | 10 | 255 | 0 | 6.61 | 6.55 | 6.39 | 6.28 | 5 |
| 8 | 10 | 255 | 50 | 6.04 | 5.69 | 4.63 | 3.93 | 35 |
| 9 | 105 | 10 | 0 | 61.72 | 61.43 | 60.58 | 60.02 | 2.75 |
| 10 | 105 | 10 | 50 | 60.19 | 59.69 | 58.19 | 57.18 | 5 |
| 11 | 105 | 500 | 0 | 60.94 | 59.46 | 55.03 | 52.07 | 14.56 |
| 12 | 105 | 500 | 50 | 61.48 | 57.68 | 46.28 | 38.68 | 37.08 |
| 13 | 105 | 255 | 25 | 61.59 | 58.51 | 49.27 | 43.11 | 30 |
| 14 | 105 | 255 | 25 | 61.28 | 58.22 | 49.03 | 42.90 | 30 |
| 15 | 105 | 255 | 25 | 61.21 | 58.15 | 48.97 | 42.85 | 30 |

Table A.4 Raw data for photo-Fenton oxidation of Amoxicillin: TOC removal

| *Run No* | *Concentrations* | | | *TOC Concentrations (mg/L)* | | | | *TOC Removal (%)* |
|---|---|---|---|---|---|---|---|---|
| | *$X_1$ Ant. (mg/L)* | *$X_2$ $H_2O_2$ (mg/L)* | *$X_3$ Fe(II) (mg/L)* | *Raw* | *15 min.* | *30 min.* | *60 min.* | |
| 1 | 200 | 10 | 25 | 117.90 | 117.74 | 117.57 | 117.25 | 0.55 |
| 2 | 10 | 10 | 25 | 6.66 | 6.28 | 5.90 | 5.14 | 22.86 |
| 3 | 10 | 500 | 25 | 6.41 | 6.19 | 5.97 | 5.54 | 13.61 |
| 4 | 200 | 500 | 25 | 118.44 | 104.53 | 90.63 | 62.81 | 46.97 |
| 5 | 200 | 255 | 0 | 117.72 | 116.18 | 114.64 | 111.56 | 5.24 |
| 6 | 200 | 255 | 50 | 117.32 | 109.36 | 101.40 | 85.48 | 27.14 |
| 7 | 10 | 255 | 0 | 6.08 | 5.78 | 5.49 | 4.90 | 19.38 |
| 8 | 10 | 255 | 50 | 6.26 | 6.21 | 6.15 | 6.03 | 3.67 |
| 9 | 105 | 10 | 0 | 60.57 | 59.65 | 58.72 | 56.88 | 6.10 |
| 10 | 105 | 10 | 50 | 61.74 | 58.87 | 56.00 | 50.27 | 18.57 |
| 11 | 105 | 500 | 0 | 61.14 | 59.74 | 58.33 | 55.53 | 9.18 |
| 12 | 105 | 500 | 50 | 61.25 | 53.55 | 45.86 | 30.47 | 50.25 |
| 13 | 105 | 255 | 25 | 60.05 | 53.14 | 46.24 | 32.43 | 46.00 |
| 14 | 105 | 255 | 25 | 63.99 | 56.25 | 48.51 | 33.02 | 48.40 |
| 15 | 105 | 255 | 25 | 62.28 | 55.22 | 48.15 | 34.02 | 45.38 |

## LIST OF TABLES

**LIST OF FIGURES**