AMOXICILLIN AND AMPICILLIN REMOVAL FROM WASTEWATER BY FENTON AND PHOTO-FENTON PROCESSES

FENTON VE FOTO-FENTON PROSESLERİ KULLANARAK ATIK SUDAN AMOKSİSİLİN VE AMPİSİLLİN ANTİBİYOTİKLERİN GİDERİMİ

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ETHICAL STATEMENT

In this thesis study, which is prepared in accordance with the spelling rules of institute of graduate school of science and engineering of Hacettepe University,

I declared that

- All the information and documents have been obtained in the base of the academic rules
- All audio-visual and written information and results have been presented according to the rules of scientific ethics
- In case of using others work, related studies have been cited in accordance with the scientific standards
- All cited studies have been fully referenced
- I did not do any distortion in the data set

And any part of this thesis has not been presented as another thesis study at this or any other university

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ABSTRACT

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In this study, degradation of amoxicillin and ampicillin (penicillin groups) were investigated using Fenton and Photo-Fenton oxidation. For this purpose, effect of pH, Fe(II), H_2O_2, initial antibiotic concentrations and temperature were studied to find the optimum condition. Antibiotics and COD measurements were also carried out to determine the most effective conditions. Optimum conditions for pH , Fe^{2+}, H_2O_2 at 50 mg/L concentration of amoxicillin was 3, 30 mg/L and 300 mg/L, respectively. Under these conditions the removal of COD and amoxicillin was determined as 76.8% and 100% for Fenton reactions and 84.81% and 100% for photo-Fenton reactions, respectively. Optimum condition for the concentration of 50 mg/L ampicillin was found to be pH 3, Fe^{2+} 25 mg/L, H_2O_2 300 mg/L. Under these conditions the removal of COD and ampicillin was determined as 72.67% and 100% for Fenton reaction and 78.71% and 100% for photo-Fenton reaction, respectively. Under these optimized conditions complete removal of amoxicillin achieve in 5 min for both Fenton and photo-Fenton process. In addition, under best conditions for ampicillin, complete removal of ampicillin occurs in 15 and 5 min of reaction for Fenton and photo-Fenton reaction, respectively. Both methods are proven to be effective for amoxicillin and ampicillin degradation. However photo-Fenton process is most effective for COD degradation and photo-Fenton process indicated higher rate constant than Fenton process.

Keywords: Advance Oxidation, Amoxicillin, Ampicillin, COD Removal, Fenton, Photo-Fenton, Fe_2O_3 Particles

ÖZET

AMOKSİSİLİN VE AMPİSİLLİN ANTİBİYOTİKLERİNİN FENTON VE FOTO-FENTON KULLANARAK ATIK SUDAN GİDERİLMESİ

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Yüksek Lisans, Çevre Mühendisliği Bölümü

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Bu çalışmada Amoksisilin ve Ampisilin (penesilin gurubundan) giderimi için fenton ve fotofenton ileri oksidasyon prosesleri kullanılmıştır. Bu amaca yönelik olarak pH, Fe(II), H_2O_2, antibiyotik derişimi ve sıcaklık etkilerini belirlemek için farklı koşullarda deneyler yapılmıştır. Deneyler sürecinde KOİ ve antibiyotik giderimi farklı koşullar altında incelenmiştir. Yapılan deneyler sonucunda; Fenton ve Foto-Fenton oksidasyonu için optimum koşullar amoksisilin antibiyotik türünde 50 mg/L antibiyotik derişiminde, pH 3, Fe^{2+} 30 mg/L, H_2O_2 300 mg/L bulunmuştur. Bu koşullarda fenton oksidasyonu ile %100 amoksisilin ve %76.8 KOİ giderimi elde edilmiştir. Foto-fenton oksidasyonu ile %100 amoksisilin ve %84.81 KOİ giderimi elde edilmiştir. Ampisilin antibiyotik türünde ise 50 mg/L antibiyotik derişiminde, pH 3, Fe^{2+} 25 mg/L, H_2O_2 300 mg/L, optimum giderim koşulu olarak bulunmuştur.

Bu çalışma koşullarında Fenton oksidasyonu ile %100 Ampisilin ve %72.67 KOİ giderimi elde edilmiştir. Foto-fenton oksidasyonu ile %100 Ampisilin giderimi ve %78.71 KOİ giderimi elde edilmiştir. Optimum koşullar altında Fenton ve Foto-Fenton oksidasyonu oksidasyonu ile amoksisilin giderimi 5 dakika içinde gerçekleşmektedir. Optimum şartlar altında Fenton ve Foto-Fenton oksidasyonu kullanarak Ampisilinin tamamen giderilmesi sırasıyla 15 ve 5 dakikada gerçekleşmektedir. kullanılan iki yöntem de amoksisilin ve Ampisilin giderim yüzdeleri açısından verimlidir. Ancak KOİ gideriminde Foto-Fenton yöntemi daha etkilidir ve Fenton yöntemine göre reaksiyon hızı daha yüksektir.

Anahtar Kelimeler: İleri oksidasyon, Amoksisilin, Ampisilin, KOİ Giderimi, Fenton, Foto-Fenton, Fe_2O_3 partikülleri

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1. INTRODUCTION

Water is essential ingredient to human life more than we can imagine. As the water resources are diminishing day by day, its importance to improve the quality of life is increasing. Protection of water resources is a must for the survival of future generations.

The quality of the water has diminished due to various sources of pollution. Increase in population numbers, solid, liquid and gaseous wastes resulting from residential, commercial, and industrial sources has disturbed the biodiversity and threatened the human life. These wastes and discharges needs to be controlled in order to reduce the environmental pollution risk which is growing day by day. Additionally; pesticides and fertilizers used in agriculture and animal husbandry are important pollution sources deteriorating ting the water quality. The increase in the discharges of medicine, food, chemicals, textile and dye, pharmaceuticals and food wastes cause pollution in both surface water and ground water, if not treated in advance.

These wastes are also known as priority pollutants [1]. Pharmaceutical industry uses numerous raw materials but produced a little number of products comparatively. The waste water resulting from the processes contain significant amount of toxic pollutants. When this wastewater is release into the environment, it disrupts the ecological balance.

Today, antibiotics are used to treat human and animal disease and for the industrial scale production of aquatic organisms such as fish, preservation of food in food industry, in hospitals, in pharmaceutical industry and scientific research activities. However, as a result of uncontrolled use and release of antibiotics, environmental problems do occur and due to transport mechanisms of food chain, health problems are seen in several living organisms. Antibiotics can persist in ecological cycle for a long time without degradation and they can affect soil, water resources, as well as living organisms. Research in the past suggests that even very small concentrations of antibiotics have been found in soil and water resources. Therefore, in recent years scientists have started working on eliminating antibiotic pollution [2].

Today pollution prevention of antibiotics is managed by the conventional treatment process based on biological treatment. Antibiotics contain complex organic interactions in their structures. Therefore biologically treatment cannot be effective alone under these conditions. Also the problems caused by the formation of sludge and its disposal increase the cost of the processes. So, for complex structure and biological treatment it is necessary to use advance treatment or a combination of physical, biological and chemical methods [3].

Advance oxidation processes are among the relatively inexpensive methods which are alternative to conventional treatment methods. These processes have a high oxidation potential and generate the hydroxyl radicals (OH^-) and these radicals react with the organic compounds in the environment. These reactions may results in many organic components and at the end final products could be water (H_2O) and carbon dioxide (CO_2), if the oxidation process is completed. This provides high removal efficiency at the end of treatment more than any other processes.

During this thesis, the most consumed antibiotics, ampicillin and amoxicillin from the group of penicillin, were selected and antibiotic removal efficiencies of these antibiotics in solutions were examined by Fenton reaction (Fe^{2+}/H_2O_2) and photo Fenton reaction ($UV/Fe^{2+}/H_2O_2$), which are described as advanced oxidation processes.

1.1. ANTIBIOTICS

Antibiotics, also known as antibacterials, are the medications that destroy or slow down the growth of bacteria. They are not effective against infections caused by viruses; for example, common cold or flu. Antibiotics are being used since 1928; for the treatment of infection in human beings, animals and plants. Before that time there were afew ways of destroying infections caused by microorganisms.

The most weighty years in discovery and development of antimicrobial drugs were between 1928 and 1940. 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 [4].

Antibiotics have shown their effect by [5]:

1. Inhibiting cell wall synthesis
2. Increasing the cytoplasmic membrane permeability
3. Preventing the synthesis of nucleic acid
4. Disrupting middle metabolism
5. Inhibiting protein synthesis in ribosomes of bacteria

1.2. USES OF ANTIBIOTICS

In 1928, Alexander Fleming discovered penicillin and from that time a lot of antibiotics are used for different purposes. Commonly used antibiotics in Turkey; penicillin (45%), cephalosporin (20%), macrolides (17.5%), quinolones (17%) and tetracycliens (4%) [6].

Antibiotics are used for human and animal therapy, animal husbandry, the pharmaceutical industry, hospitals and for growth of organisms such as fish. Human and animal antibiotics are continuously discharged in environment by manufacturing processes, as unused or outdated products or by urine and feces. The impact of antibiotics on the environment and living organisms are increasing day by day.

It is preferred to use antibiotics in veterinary services because of ecomonical concerns and antibiotics are easily accessible and inexpensive. For animal husbandry, especially tetracycline group are used in Turkey [7]. A study determined that up to %90 of the antibiotics that used for this purpose were excreted in feces. In this study the amount of tetracycline and sulfonamide was reported to be 46 mg/kg and 91 mg/kg, respectively [8].

Preventive use of antibiotics in the treatment of bacterial diseases in fish is also common practice. The antibiotic is mixed to the fish food and some parts of them are not consumed and fell down. Fish and selfish take drugs with waste feeding and by accumulating in their body they reach high concentration and this could affect food chain, water ecosystem and living organisms negatively.

Antibiotics, due to their chemical and physically properties reach to soil and sediment and so they reach groundwater. Furthermore, antibiotics can be found in the output of wastewater treatment and it may be a pollution factor for surface waters [9] [10]. Antibiotics contain complex organic compounds in their structure and because of this, it is difficult to break down them biologically into simple final products and they persist in the environment for months without degradation. In this case, for organisms living in water and soil the antibiotic concentration between µg/L–mg/L can make toxic and negatively affect and disrupt the ecological balance [9].

Possible sources and anticipated exposure routes for medication substance used for human beings and animals is shown in figure 1.1.

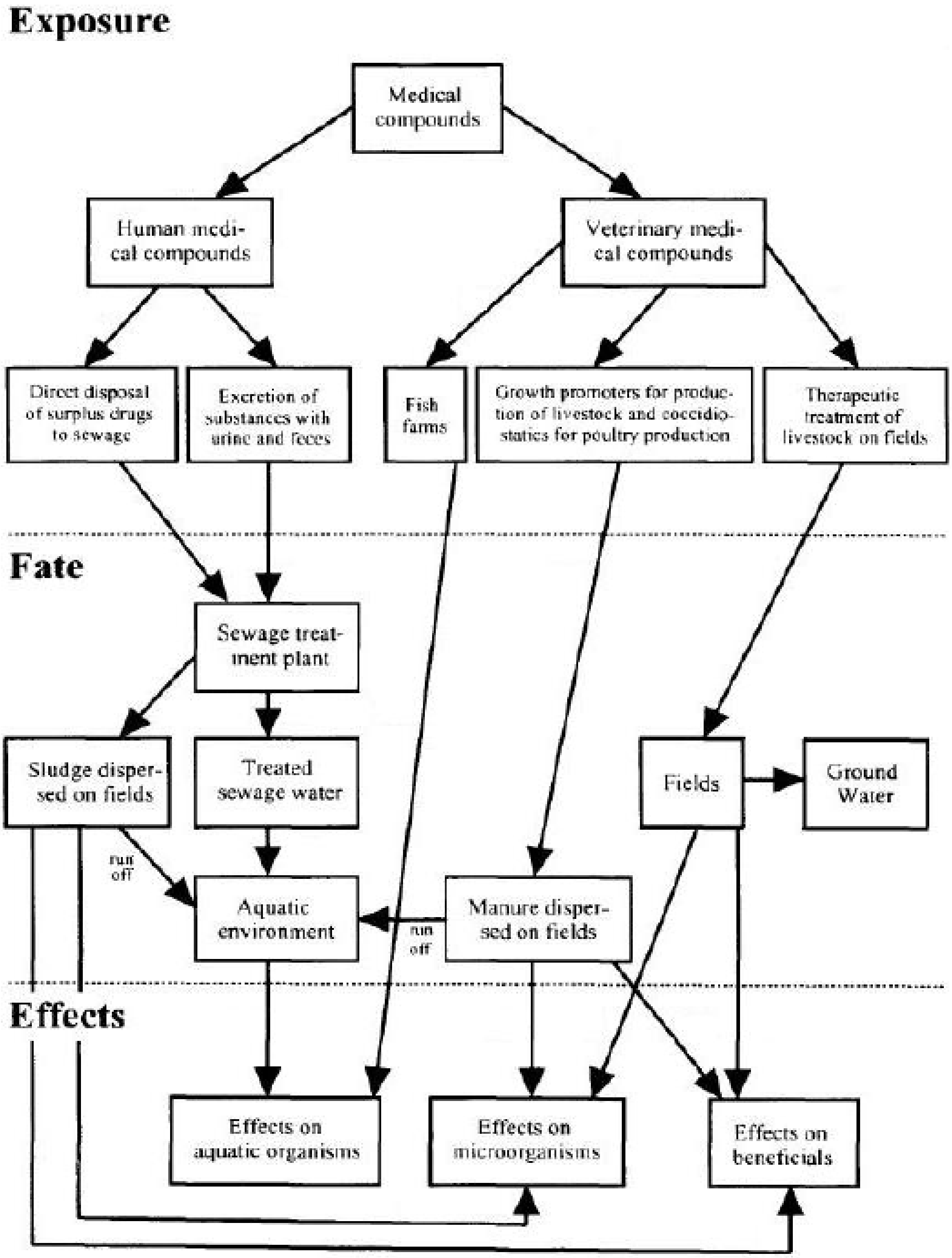

Figure 1.1. Possible sources and anticipated exposure routes for occurrence of different type of PhAC residues in the environment [9].

Table 1-1- average amount of antibiotics in different water streams [11]

SUBSTANCE	AMOUNT	LOCATION	REFERENCES
Ampicillin	20 – 80 µg/L	Hospital Wastewater	Kümmerer, 2001
Ciprofloxacin	0,70 – 124,5 µg/L 249 – 405 ng/L 3 – 87 µg/L	Hospital wastewater Output of treatment plant Hospital wastewater	Kümmerer, 2003 Heberer, 2002
Chloramphenicol	max. 0,06 µg/L	Surface water	Hirsch et al., 1999
Erythromycin-H_2O	max. 1,70 µg/L	Surface water	Hirsch et al., 1999
Erythromycin	max. 6 µg/L ~ 1 µg/L 0,70 – 17,4 ng/L 10 – 630 ng/L 47,40 ng/L 4,50 ng/L	Residential treatment plant River water River water River sediment Residential treatment plant	Hirsch et al., 1999 Halling –Sorensen et al., 1998; Zuccato et al., 2000 Zuccato et al., 2005
Clarithromycin	max. 0,24 µg/L max. 0,26 µg/L 18,10 ng/L	Residential treatment plant Surface water Residential treatment plant	Hirsch et al., 1999 Zuccato et al., 2005
Oxytetracycline	0,10 – 11 µg/g	Sediment	Halling – Sorensen et al., 1998
Penicillin Group	> 25 ng/L > 10 ng/L	River water Potable water	Halling – Sorensen et al., 1998

1.3. TYPE OF ANTIBIOTICS

Antibiotics, according to their mechanism of action and chemical structure are classified in seven main groups [3]:

- penicillins
- cephalosporins
- fluoroquinolones
- aminoglycosides
- tetracyclines
- macrolides
- polypeptides

1.3.1. Penicillins

Penicillins are obtained from various fungal and they have bactericidal effects. Penicillins are produced naturally or semi-synthetic methods. The basic structure of all penicillins are 6-aminopenicillanic acid (6-APA). Penicillins, show their effects by disrupting the structure of cell walls of bacteria. General chemical structure of penicillin is given in figure 1.2.

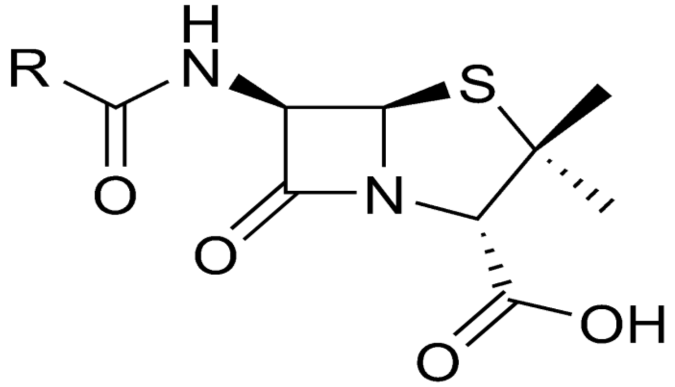

Figure 1.2. Chemical structure of penicillin group

1.4. GENERAL INFORMATION ABOUT AMOXICILLIN

Amoxicillin (AMOX) is a broad-spectrum β-lactam antibiotic that belongs to the penicillin class and is used in veterinary and human medicine, representing one of the most prescribed antibiotics in Europe and in the United States [12] [13]. It is produced semi-synthetic and is kind of broad spectrum antibiotic in penicillin group. Its color is off-white and it has slightly aromatic odor. Amoxicillin is generally found in crystalline powder form and has bitter taste. Its chemical formula is "$C_{16}H_{19}N_3O_5S$" and has molecular weight of 365.4 g / mol. Its half-life in the body is 61.3 minutes. Amoxicillin Trihydrate is used as the drug. Anhydrous forms of amoxicillin may also be prepared. It can produce semi-synthetic by (+)-6-aminopenicillanic acid (6-APA) or by chemical synthesis. Amoxicillin is slightly soluble in water and alcohol and it is insoluble in chloroform and ether. It is effective against gram positive and gram negative bacteria. Amoxicillin inhibits the synthesis of bacterial cell walls. They are resistant to stomach acid. The chemical structure of amoxicillin is schematically shown in Figure 1.3.

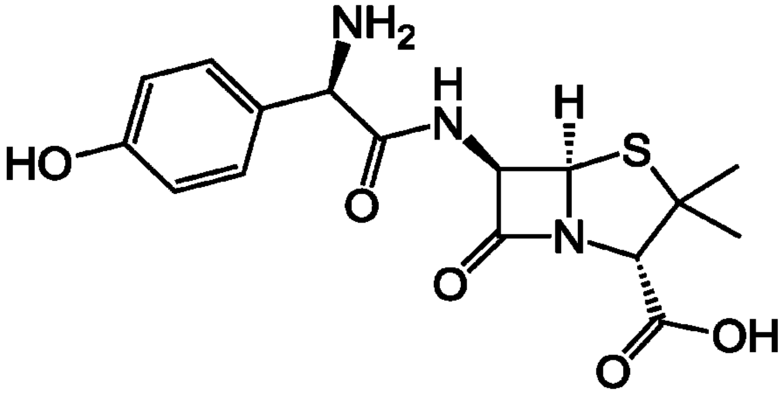

Figure 1.3. Chemical structure of Amoxicillin tri-hydrate

1.4.1. Use and Side Effect of Amoxicillin

Amoxicillin is generally used as Amoxicillin Trihydrate. It can be dispensed in some forms to be taken like dry tablet, chewable tablet, capsule, a suspension or drops for children. Amoxicillin is usually taken either twice a day (every 12 hours) or three times a day (every 8 hours), depending on the doctor's instruction. The normal dose is 250-500 mg three times daily. It is generally used for moderate infections, but 1 g may be given in every 6 h for severe infections [14] [15].

Amoxicillin is especially helpful to treat certain infections that have been caused by bacteria. The amoxicillin can be prescribed to treat different infections. Table 1-2 shows the use and side effect of amoxicillin [16] [17].

Table 1-2- Use and side effects of amoxicillin

Use of Amoxicillin	Side Effect of Amoxicillin
Ear infections Gonorrhea Bronchitis Throat infection Skin infection Lyme diseases Typhoid Pneumonia Urinary tract infection	Agitation Confusion Nausea and vomiting Black tongue Dizziness Diarrhea Insomnia White patches in throat, tongue and mouse

Amoxicillin is also useful in anaerobic infections (those which grow without the benefit of oxygen). It might be used in these situations [16]:

- Infected bite wounds

- Upper respiratory infections
- Infected teeth

Amoxicillin can also be used in combination with another antibiotic called clarithromycin in order to treat stomach ulcers caused by *H. pylori* bacterial infection. These drugs can also be augmented with the use of a stomach acid reducer called lansoprazole. Clavulanic acid may be added to Amoxicillin to increase Amoxicillin's spectrum against Staphylococcal bacteria [16].

1.5. GENERAL INFORMATION ABOUT AMPICILLIN

Ampicillin is one of antibiotics in penicillin group of drug. It is used to treat a wide variety of bacterial infections. It works by stopping the growth of bacteria. Its chemical formula is "$C_{16}H_{19}N_3O_4S$" and has molecular weight of 349.41 g/mol. Ampicillin is a beta-lactam antibiotic that is part of amino penicillin family and it is active against gram-positive and gram-negative bacteria. Ampicillin sodium salt is semi synthetic penicillin derived from the basic nucleus, 6-aminopenicillanic acid. The chemical structure of ampicillin is schematically shown in Figure 1.4.

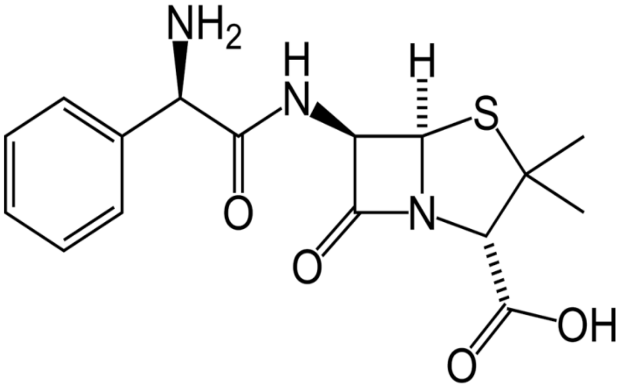

Figure 1.4. Chemical structure of ampicillin sodium

1.5.1. Use and Side effect of Ampicillin

Ampicillin treats only bacterial infections. It can be dispensed in some forms to be taken like dry tablet, capsule or suspension. Ampicillin is usually taken by mouth and the dosage is based on medical condition and response to therapy. Table 1-3 shows the use and side effect of antibiotic ampicillin [18].

Table 1-3- Use and Side effects of ampicillin

Use of Ampicillin	Side Effect of Ampicillin
Ear infections	
Throat infections	Nausea
Food poising	Vomiting
Skin infections	Diarrhea
Typhoid fever	Mouth or tongue sores
Chronic Bronchitis	Agitation
Acute sinus infections	

1.6. ANTIBIOTIC REMOVAL FROM WATER

Over the past, the removal of antibiotics form water became an important environmental research subject and scientists began to conduct experiments on this new topic [19]. Presence of high concentrations of antibiotics in the environment, have toxic effects on microorganisms and leads to disruption of the ecological balance. On the other hand, low concentrations of antibiotics in ecological reservoirs cause the pathogenic and non-pathogenic bacteria to acquire antibiotic resistance. These observations indicate that removal of antibiotics is imperative at every concentration [19].

Currently, biological removal-processes may be economically more feasible in wastewater treatment but conventional treatment processes (aerobic – anaerobic biological treatment processes) are inadequate and time consuming to remove the antibiotics from water [19]. In addition toxic and persistent feature of pesticides limit the efficiency of biological process during treatment process. In these (waste) waters; biochemical oxygen demand (BOD) is generally low. Because of these reasons, in recent years advance treatment techniques are applied to the treatment of antibiotics in water and wastewater processes. Removal of antibiotics is achieved using membrane filtration (nanofiltration, reverse osmosis, etc.), adsorption, chemical oxidation and electrocoagulation. Although physicochemical methods for treatment of antibiotic contaminated wastewaters (coagulation-flocculation, filtration, activated carbon adsorption, stripping with air) are effective, they are not preferred because pollutants are transferred from one environmental reservoir to another which out degradation. For this purpose, especially before biological treatment, advance oxidation techniques are implemented to reduce the organic load and toxicity [19].

1.7. ADVANCE OXIDATION PROCESSES

Advance oxidation process (AOP) is based on a higher electrochemical oxidation potential and formation of hydroxyl radical. Hydroxyl radicals ($OH^{\cdot}$) in the medium react with all organics and end products such as CO_2 and H_2O have been produced. The hydroxyl radical reacts more rapidly than ozone and hydrogen peroxide, so economical benefits in operational costs and in size of treatment system is achieved. Also OH radical is a powerful chemical oxidant [20].

The effectiveness of AOP depends on pH, physicochemical parameters such as the initial oxidant dosage, contact time and irradiation conditions. AOP are divided into homogeneous and heterogeneous processes, according to their presence of catalyst.

1.7.1. Homogeneous Processes

The homogeneous processes are defined as the systems that all reagents are dissolved (e.i. all compounds are < 45 μm).

Homogeneous process produces hydroxyl radical are given below:

- H_2O_2 / Fe^{2+} (Fenton)
- H_2O_2 / Fe^{3+} (Like – Fenton)
- H_2O_2 / Fe^{2+} (Fe^{3+}) / UV (Photo-Fenton)
- O_3 / H_2O_2 (Peroxone)
- O_3 / UV
- H_2O_2 / UV
- O_3 / H_2O_2 / UV

1.7.1.1. Fenton and Fenton Like processes

Formation of hydroxyl radical in aqueous solution is a complex reaction chain. Fe (II) initiates the decomposition of H_2O_2; catalysis and hydroxyl radical are formed. Fe (III) ions, which are the product of former reaction, reacts with H_2O_2 also, this sequential process is called Fenton– like process. Fenton process is based on reaction of Fe^{+3} / Fe^{+2} with hydrogen peroxide under acidic conditions. Hydroxyl radicals are formed as a result of this reaction. Reaction steps and rate constants are given below.

The Fenton process is carried out in four steps:

1. pH adjustment
2. oxidation reaction
3. neutralization-coagulation
4. precipitation

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$ $\quad k = 63\ M^{-1}\ s^{-1}$

$OH^{\cdot} + Fe^{2+} \rightarrow OH^{-} + Fe^{3+}$ $\quad K = (2.5\text{-}5) \times 10^{8}\ L\ mol^{-1}\ S^{-1}$

$Fe^{3+} + H_2O_2 \leftrightarrow Fe\text{-}OOH^{2+} + H^{+}$ $\quad K = (1\text{-}2) \times 10^{-2}\ L\ mol^{-1}\ S^{-1}$

$Fe\text{-}OOH^{2+} \leftrightarrow HO_2^{\cdot} + Fe^{2+}$

$Fe^{2+} + HO_2^{\cdot} \rightarrow Fe^{2+} + O_2 + H^{+}$

$OH^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot}$

Hydroxyl radicals are oxidized organic matters by removing the protons and produce very good reactive organic radicals [21].

$RH + OH^{\cdot} \rightarrow H_2O + R^{\cdot}$ $\quad k = 10^{7}$ to $10^{10}\ M^{-1}\ s^{-1}$

Organic free radicals can be oxidized with Fe (II) and be reduced with Fe (III).

$R^{\cdot} + Fe^{3+}$ – oxidation $\rightarrow$ $R^{+} + Fe^{2+}$

$R^{\cdot} + Fe^{2+}$ – reduction $\rightarrow$ $R^{-} + Fe^{3+}$

Fenton process has different treatment functions depending on H_2O_2/Fe^{2+} ratio. If Fe (II) concentration is more than H_2O_2 chemical coagulation is more effective instead of oxidation in treatment.

Advantages of Fenton process are [19]:

- Iron and hydrogen peroxide are inexpensive chemicals
- The absence of mass transfer limitations due to the homogeneous catalytic structure
- Process is simple as technology

1.7.1.2. Photo – Fenton Processes

Combination of Fe (II), H_2O_2 and light-radiation (usually UV radiation is used) is called photo – Fenton reaction. Photo – Fenton process produces more hydroxyl radicals than Fenton process and increase the speed of decomposition and mineralization of organic pollutants. As photochemical, because of iron ions cycle, reduction of Fe (II) and again oxidation of Fe (III) in Fenton reaction Fe(II) / Fe(III) ion is required [21].

$Fe(OH)^{2+} + hv \rightarrow [Fe(OH)^{2+}]^{\cdot} \rightarrow Fe^{2+} + OH^{\cdot}$

Photo – Fenton reaction may use photons that are close to 400 nm wavelength [19].

1.7.1.3. Electro – Fenton Processes

Electrochemical processes have two oxidation activities namely: direct and indirect activity. Direct oxidation occurs in anionic surfaces and indirect oxidation carried out in with strong oxidants formed in electrochemical processes [22].

In the electro – Fenton process, hydrogen peroxide is generated by electrochemical reduction of the cationic oxygen and by addition of Fe (II) ions oxidation is performed.

$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$

This reaction contains the formation of anionic superoxide radical $(O_2)^{\cdot}$ and hydro peroxide radical $HOO^{\cdot}$ and this is a complex reaction. One of advantage of electro – Fenton reaction is controlling the production of hydroxyl radicals better than other chemical processes.

Due to continuous production of hydrogen peroxide and catalytic reduction of Fe (III) ions, Fe (II) ions formed together and this triggers production of more hydroxyl radical. Minimizing amount of reagents provides a reduction in operating costs and increases the economic feasibility of the technology [23].

1.7.1.4. Ozonation

The complete reaction of ozone and $OH^{\cdot}$ is as following:

$3O_3 + H_2O \xrightarrow{OH^-} 2OH^{\cdot} + 4\,O_2$

The mechanism of ozonation is effective at certain pH values [24]. Hydroxyl radical forms produced as a result of complex chain reaction. This process occurs by decomposition of organic content of bicarbonate, carbonate and humic substances, beginning the chain reaction with ozone and hydroxide ions, formation of radical-radical processes and capture of hydroxyl radicals. In the reactions with ozone to be associated with the presence of various contaminants at neutral pH, the contaminant is able to react with ozone and hydroxyl radical simultaneously.

1.7.1.5. Ozone / Hydrogen Peroxide Process (O_3/H_2O_2)

This reaction begins in the presence of hydrogen peroxide and ozone and as a result hydroxyl radical is formed [25]. H_2O_2 mainly used in reaction, can also show the effect of destroying the reaction at higher doses.

The fundamental reactions of this process are:

$H_2O_2 \leftrightarrow HO_2^- + H^+$

$HO_2^- + O_3 \rightarrow HO_2^\cdot + O_3^-$

$H_2O_2 + {}^\cdot OH \rightarrow O_2^- + H_2O + H^+$

$HO_2^- + {}^\cdot OH \rightarrow OH^- + HO_2^\cdot$

Overall reaction of this process can be given as:

$2O_3 + H_2O_2 \rightarrow 2\,{}^\cdot OH + 3O_2$

1.7.2. Heterogeneous Processes

Oxidation process can also occur at the catalyst attached solid surfaces. In this type of processes, metal oxide semiconductor or Fe ions that are attached to a surface are used for catalytic oxidation. If oxidation occurs in an environment where the photons of catalytic reaction are used in the system, when it is defined as photo catalytic reaction.

The sun light rays categorise into three parts: visible light, infrared radiation (IR) and ultraviolet rays. UV radiation forms the part in the short wavelength of electromagnetic spectrum (200-800 nm). This range is divided into three wavelength parts known as UV-A, UV-B and UV-C, respectively [26].

UV-A: wavelength between 315-400 nm

UV-B: wavelength between 280-315 nm

UV-C: wavelength is less than 280 nm.

1.7.2.1. Hydrogen peroxide / UV Processes (H_2O_2 / UV*)*

Light is one of the necessary parameters for photochemical reactions, the other basic parameter required for this process is the agent that can form radicals or convert to radicals with exposure to light. Hydroxyl radicals which form in the ultrasonic process to compare with other radicals are more effective in degradation [27]. General wavelength range of photochemical reactions is between 100-1000 nm. When the energy of photons that their wavelength is higher than 1000 nm is absorbed, it is too low for a chemical change and the energy of photons in the low wavelength (less than 100 nm) is high enough to cause ionization and radiation. As the energy of the system increase, the degradation potential of system increase too. The application of high frequency radicals provides oxidation in environment [28].

The maximum light absorbance of H_2O_2 occurs at 220 nm. When low pressure mercury lamps are used, sufficient production of $OH^{\bullet}$ is necessary due to low molar absorption capacity of lamps to increase the amount of H_2O_2.

1.7.2.2. Ozone/UV Processes (O_3/UV)

Photolysis of hydrogen peroxide in the water causes the formation of ozone or UV radiation reacts with ozone and the formation of hydroxyl radical is provided.

The reactions that occur during this process:

$O_3 + hv + H_2O \rightarrow H_2O_2 + O_2$

$H_2O_2 + hv \rightarrow 2\ {}^{\cdot}OH$

$$2O_3 + H_2O_2 \rightarrow 2\ {}^{\cdot}OH + 3O_2$$

1.7.2.3. Ozone/Hydrogen Peroxide/UV Processes ($O_3/H_2O_2/UV$)

In this process UV light makes a contribution to the formation of hydroxyl radicals. This process is better as a result of addition of hydrogen peroxide to the medium [29].

Basic reaction steps of the process are as follows:

$$H_2O_2 \overset{-H^+}{\Leftrightarrow} HO_2^- \xrightarrow{O_3} HO_2^{\cdot} + O_3^{\cdot -}$$

In case of addition UV light to ozone in medium, superoxide ion in ozone is decomposed and by the action of water hydrogen peroxide is comprised.

$$O_3 \xrightarrow{UV} O_2 + O\ (1D) \xrightarrow{H_2O} H_2O_2 + O_2$$

1.7.2.4. UV/Tio$_2$ Processes

Radical sources in these reactions are semiconductors. Semiconductors are solid materials having electrical conductivity between conductors and insulators. Semiconductors are as effective as photo catalysts under irradiation in aqueous media. Semiconductors are characterized into two different energy bands, one of them is full of electrons and another is containing empty energy levels. Electrons are filling up the energy levels from the lowest energy, but some energy levels due to the wave properties of atoms are prohibited. Unlike the conductors, semiconductors and insulators have a band between the conduction and valance band that is called forbidden energy gap. Typical energy gap of semiconductors is around 1 eV, insulators energy gap is much larger than the electron thermal energy. Forbidden energy gap in semiconductors depends on chemical bond type and structure of atoms. In different semiconductors forbidden energy gap vary from 0.1 eV to 5 eV.

Hydroxyl radicals in semiconductors is formed in two ways; the H_2O that adsorbed the valance band of cavity or as reaction with surface OH^- groups.

The semiconductors used in the environment applications are TiO_2, ZnO and strontium titanium trioxide. TiO_2 are usually preferred in photochemical oxidation technology due to their easy accessibility, low toxicity, low price and having high photo conductivity [30] [31].

1.8. PARAMETERS AFFECT FENTON PROCESS

Factors that affect the Fenton process are; concentration of Fe (II), Fe (III) and H_2O_2, pH, temperature, the amount of organic and inorganic pollutants. These parameters determine the reaction efficiency [19].

1.8.1. pH

In Fenton process pH is an effective parameter in degradation of pollutants [22]. In the studies of Fenton process, generally pH 3 is found as optimum pH [32]. In lower pH the formation of $[Fe^{2+} (H_2O)]^{2+}$ occurs and because of that less hydroxyl radical produced . In case of pH>4 due to formation of Fe (II) complexes the rate of process decreases.

1.8.2. Concentration of Fe (II)

In case of increasing the concentration of iron ions, the disintegration speed of reaction will increase. However, above the certain concentration degradation rate will decrease significantly. Due to use of more iron ions the amount of dissolved or suspended iron will increase in output.

1.8.3. Concentration of H_2O_2

Decomposition rate of contaminants generally increase with increasing the concentration of hydrogen peroxide [33]. But in case of increasing the concentration of hydrogen peroxide, they will react with hydroxyl radicals and it is not recommended. Also, increasing the concentration of hydrogen peroxide leads to increase the amount of COD.

1.8.4. Temperature

In one study the optimal temperature was found to be 30^0c [22]. In case of increasing temperature of 10^0c to 40^0c the decomposition efficiency did not change; to increase more than 40^0c due to increasing decomposition of hydrogen peroxide to water and oxygen, the cooling process should be performed [34].

1.8.5. Pollutant Concentration

The pollutant concentration is one of the factors that affect the Fenton reaction efficiency. Sometimes in industrial wastewater before oxidation dilution is required.

1.9. LITERATURE REVIEW

Fenton oxidation method is used to treat variety of water pollutants in recent days. For example, it is used for removal of phenols, formaldehydes, BTEX, pesticides, rubber chemicals and several other pollutants. The use of Fenton process may different purposes: toxicity reduction, improvement of biodegradability, BOD/COD removal, odor and color removal, destruction of organic pollutants in general [35].

Application of Fenton oxidation process for destruction of hazardous organics was used since 1960s. As literature shows Fenton reaction is effective in treating various industrial wastewater components including pesticides [36] [37] [38], different types of dyes [39] [40] [41], surfactants [42] [43] [44], aromatic amines [45] and explosives [43]. Also, Fenton oxidation method has been used to treat different type of wastes such as textile industry wastes, refinery and fuel terminals wastes, chemical manufacturing wastes, engine and metal cleaning wastes and etc [41] [46]. The Fenton reagent can also effectively be used for the destruction of toxic wastes and non-biodegradable effluents to render them more suitable for secondary biological treatment [47]. In addition, the importance of Fenton chemistry has long been recognized among others in food chemistry and material ageing [48].

There are some studies on removal of different pollutant by Fenton reagent; some of them are as below:

In one of experimental study, Fenton, photo-Fenton and UV/H_2O_2 methods have been used to removal of cosmetic from wastewater. Before using these methods the cosmetic wastewater were coagulated by $FeCl_3$. They achieved

74% COD removal by photo-Fenton method and it was the most effective method at pH 3 and H_2O_2/Fe^{2+} molar ratio was 1000/100 mg/L. The COD removal for Fenton process was 72% when H_2O_2/Fe^{2+} molar ratio was 1200/500 mg/L and for UV/H2O2 the COD removal was 47% at the same condition [49].

Another experimental study examined the results of performance of Fenton, photo-Fenton and Fenton-like reagent for removal of phenol from aqueous solutions. The effect of some parameters such as pH, hydrogen peroxide concentration, catalyst and time were investigated to determine the phenol removal efficiency. As results shows photo-Fenton method is most effective than others with efficiency of 97.5% phenol removal. Fenton and Fenton-like processes removal efficiency of phenol were 78.7% and 82.5%, respectively. The optimum conditions determined for these processes were pH 3, H_2O_2 3mM, Fe^{2+} 0.1 mM, phenol concentration 100 mg/L at 60 min of time reaction [50].

In one study the effect of Gamma Radiation on the destruction of aqueous halogenated hydrocarbons has been examined. An increase in chloride ion yield in oxygenated aqueous chloroform solutions has been observed [51].

In another study, photo-Fenton like process was used to removal of diesel fuel from aqueous wastewater. The effect of various parameters such as pH, hydrogen peroxide concentration, initial concentration of total petroleum hydrocarbons, nZVI concentration and reaction time were investigated to determine the removal of total petroleum hydrocarbons. The optimal conditions for these experiments were found for these parameters such as: nZVI concentration 20mg/L, H_2O_2 concentration 5mmol/L, initial total petroleum hydrocarbons concentration 0.7 mg/L and pH 3. Under these optimum conditions the removal of petroleum hydrocarbons for 60 min reaction time was 98.5% by photo-Fenton like process [52].

Degradation of some pesticides such as chloropiryphos, metribuzyne, and metriam using Fenton process under UV irradiation and strong halogen light is also investigated. The UV lamp was used in experiments had 350nm and the strong halogen lamp was 400w. In conducted experiment, the past sell-by date chemicals were degraded in stages into simpler compound. However, these processes were occurring slowly so they cannot use in practical technology [53].

Removal performance of Fenton and Photo-Fenton was compared in a study where hetero bi-functional reactive dye Sumifix Supra Yellow 3RF (CI Reactive Yellow 145) was used as model pollutants. Optimal conditions of temperature, Fe(II) and H_2O_2 concentrations were determined to removal of dye. In the optimization, the correlation coefficients for the model (R^2) were 0.948 and 0.965 for Fenton and photo-Fenton treatments, respectively. The optimal conditions were found as pH 3, temperature 298 K, H_2O_2 11.765 mM and Fe(II) 1.075 mM for 60min reaction time, the COD removals were 79% and 92% for Fenton and photo-Fenton processes, respectively [54].

There are a number of studies on removal of antibiotics using advance oxidation in literature; some of them are as below:

In one of experimental study, relatively higher COD and TOC removal rates were obtained with the dark Fenton process (Fe^{2+}/H_2O_2) and pH=3 when compared with dark Fenton-like (Fe^{3+}/H_2O_2/pH=3) reactions as a direct consequence of Fenton's chemistry. Highest removal efficiency in terms of COD is achieved by photo-Fenton-like reaction, whereas TOC removal was higher for the photo Fenton's process. The studies conducted with Amoxicillin trihydrate indicated that the aqueous antibiotic solution can be completely eliminated after 40 min for photo-Fenton reagent (pH = 3; $Fe^{2+}:H_2O_2$ molar ratio = 1:20) and alkaline ozonation (at pH=11.5) [55].

Elmolla and Chaudhuri have been investigated the removal of amoxicillin, ampicillin and cloxacillin aqueous solution by oxidation technique. They used photo-Fenton process in their experiments. Optimum conditions for this study is reported as pH=3, H_2O_2/COD=1.5mM, H_2O_2/ Fe^{2+}=20mM. In 50min reaction time BOD/COD ratio increased from 0 to 0.4 and 80.8% removal for COD and 58.4% removal for dissolved organic carbon (DOC) was observed [56].

In another experimental study, photo degradation of some pharmaceuticals was examined under black-light and solar irradiation. In this study amoxicillin (AMX), bezafibrate (BZF) and paracetamol (PCT) was investigated via the photo-Fenton process. The results presented in this work indicate that photo-Fenton process could be successfully applied to the degradation of AMX, BZF and PCT even in complex samples, such as sewage treatment plant effluent, where they are often encountered [57].

Another experimental study examined the results of performance of Fenton reagent for degradation of six selected antibiotics, including five sulfonamids and one macrolide in swine wastewater pretreated with sequencing bath reactor (SBR). The optimal conditions of Fenton reagent in this work were as follows: 1:1.5 molar ratio of $[Fe^{2+}]$ / $[H_2O_2]$ at pH=5. All the selected antibiotics could effectively degrade by Fenton reagent under optimal conditions. Fenton reagent is not suitable only to remove total organic carbon (TOC), total phosphate (TP) and heavy metals (As, Cu and Pb), but also inactivated bacteria and reduced wastewater toxicity. This study indicated that the integrated process combining SBR with Fenton's reagent could provide comprehensive treatment to swine wastewater [58].

Kuixiao Li studied the effect of different pH on degradation of antibiotic oxytetracycline. They investigated 100 mg/L and 200 mg/L concentrations of oxytetracycline at pH 3, 7 and 11 in term of ozonation method. Accordingly, after 60 min ozonation for oxytetracycline solutions of 100 mg/L and 200 mg/L concentration at pH 11, the results indicated that BOD_5/COD rations are 0.69 and 0.52, respectively. In this study the COD removal rate, depending on ozon decomposition at high pH increased with increasing pH [59].

In this work the chemical and biological treatability of pharmaceutical wastewater have been examined. The Fenton oxidation was tried in this study to remove high COD of the wastewater. At various pH values, different amount of catalyst ($FeSO_4.7H_2O$) and hydrogen peroxide (H_2O_2) has been tested and optimal values are obtained by considering the cost of examinations. At pH 4 with %5 H_2O_2 and 20g/L of $FeSO_4.7H_2O$ was found as best alternative to use. 75% COD removal efficiency was obtained by Fenton oxidation and after that anaerobic treatment has been applied to achieve the desired discharged value. Up to 95% efficiency was obtained as a result of anaerobic treatment [60].

In another study, for removal of aqueous antibiotics zerovalent iron powder (ZVI or Fe^0) and nanoparticulate ZVI (nZVI or nFe^0) are proposed as cost-effective materials. Amoxicillin (AMX) and Ampicillin (AMP) were used as antibiotics in this work. Results indicated the total removal of AMX and AMP upon contact with Fe^0 and nFe^0. Under oxic conditions and after contact with ZVI, kinetics studies showed that AMP and AMX (both at 20 mg/L) undergo first-order decay with half-lives of about 60.3±3.1 and 43.5±2.1 min respectively. But, reactions under anoxic conditions indicated better degradation with half life 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 [61].

Removal of three antibiotics amoxicillin, ampicillin and cloxacillin was examined in a study. Fenton reagent was found to be effective in treatment of these antibiotics. Optimum operating conditions was reported as ($COD/H_2O_2/Fe^{2+}$ 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). Results demonstrated that complete degradation of antibiotics occurs in 2 min [62].

Removal of synthetic wastewater containing oxytetracycline antibiotic was examined under laboratory conditions with combination systems like UV, US, O_3, H_2O_2 and US/UV, US/O_3, US/H_2O_2. Accordingly, effect of some parameters such as pH, temperature, initial concentration of antibiotic, volume and time were investigated to determine the efficiency removal of oxytetracycline. Consequently, optimum conditions were determined for this process and 55-96% efficiency was obtained for advance oxidation process [5].

Photo-induced oxidation, heterogeneous photocatalysis, ozonation and peroxone methods were examined in a study in degrading the fluoroquinolone antimicrobial ciprofloxacin (CIP) in a hospital effluent. The samples that were used in this work were collected from the treatment system of the University Hospital of Santa Maria (HUSM). Complete degradation of CIP was shown after 60 min treatment in both heterogeneous photocatalysis and peroxone methods. Results indicated that ozonation had best performance: total degradation after 30 min treatment [63].

Photo-Fenton process was used for degradation of Sulfamethazine antibiotic. They used sunlight (400-580nm) for these experiments. The optimal conditions were pH 3, sulfamethazine concentration 50mg/L, Fe^{2+} 50mg/L and H_2O_2 600 mg/L. Under these optimum conditions 60% TOC removal were achieved. Total degradation of sulfamethazine was occurred after 2min of reaction [64].

In this study the degradation of sulfamethoxazol was investigated in distilled water and seawater by solar photo-Fenton process. The operating conditions were pH 2.5-9, Fe^{2+} 2.6-10.4mg/L, H_2O_2 30-210mg/L and lamp at 290nm for 50mg/L sulfamethoxazol. The removal of sulfamethoxazol in seawater was hindered compared to distilled water. The results indicated that DOC removal for distilled water and seawater were 80% and 50%, respectively [2].

Removal of three antibiotics amoxicillin, ampicillin and cloxacillin was examined in this study by semiconductor photocatalysis process. The operating conditions were UV light (6W) at 365 nm, 0.5-2.0 g/L TiO_2, 50-300mg/L, H_2O_2 pH 3-11. No significant degradation occurred by 300min of UV irradiation. At pH 5 and 1.0g/L TiO2, 50% degradation was achieved for all compounds (81% DOC removal). At pH 5 and 0.1 g/L TiO_2, by addition of 100mg/L H_2O_2 complete degradation was achieved after 30min reaction and 40% mineralization after 24h [65].

In this work the removal of ampicillin was investigated by Fenton and photo-Fenton processes. The optimal conditions were pH 3.7, Fe^{2+} 87 μM, H_2O_2 373 μM and pH 3.5, Fe^{2+} 87 μM, H_2O_2 457 μM for Fenton and photo-Fenton processes, respectively. Under these optimum conditions 20 mg/L ampicillin removal was achieved. 50 % TOC removal by photo-Fenton and 20% TOC removal by Fenton process was achieved [66].

In another study removal of three antibiotics amoxicillin, penicillin, cefadroxil were investigated by Chlorination method. The concentrations of amoxicillin, penicillin, cefadroxil were 1.60, 25 and 1.89 mg/L, respectively. Total removal of penicillin was occurred after 2 hours. The degradation of amoxicillin and cefadroxil was achieved after 1min of reaction [67].

Removal of amoxicillin and cloxacillin response surface methodology was used in Fenton oxidation study. The optimal conditions were pH 3, H_2O_2/COD molar ratio 2, H_2O_2/Fe^{2+} molar ratio 76. For 90 min reaction time 78.98, 72.96, and 81.18% removal of COD, total organic carbon, and ammonia–nitrogen were achieved, respectively [68].

Central composite factorial design methodology was also applied during Fenton processes. During the study removal of amoxicillin was examined by Fenton reagent. For 450 µg/L amoxicillin at pH 3.5, H_2O_2 3.50–4.28 mg/L, Fe^{2+} 254–350 µg/L and temperature 20–30 °C, total degradation of amoxicillin were achieved [69].

2. MATERIALS AND METHODS

2.1. MATERIALS

Antibiotics used in this study ampicillin and amoxicillin, were in the form of crystal powder. Amoxicillin trihydrate was supplied from Sigma-Aldrich (A8523) an ampicillin sodium salt was purchased commercially. In oxidation experiments, hydrogen peroxide (35%, w/w solution) was used. The catalyst used in this study was ferrous sulphate ($FeSO_4.7H_2O$). Sulfuric acid (H_2SO_4) was used for pH adjustment, and all chemicals were from Merck. Sodium hydroxide (NaOH) was used for inhibition of Fenton oxidation, were from Carlo ERBA.

Potassium dichromate ($K_2Cr_2O_7$), Mercury Sulfate ($HgSO_4$), Sulfuric acid (H_2SO_4), silver Sulfate (Ag_2SO_4) and Potassium hydrogen phthalate (KHP) ($HOOCC_6H_4COOK$) were used for COD test. For the COD measurements, potassium phythalate solution was used as calibration standards. All chemicals were in analytical grade from Merck. All chemicals used in this study were in analytical grade or higher.

The devices used in this study:

- Magnetic stirrer (ARE Heating-Magnetic Stirrer)
- pH meter (Fisher Scientific)
- Centrifuge (NF 200 nüve)
- UV/VIS spectrophotometer (Beckman DU 530)
- COD reactor (HACH DRB 200)
- HPLC (Agilent 1100 Series High Performance Liquid Chromotography)
- Scanning electron microscope (SEM), ZEISS EVO 50

Concentrated stock solution of Fe (II) (5000 mg/L), stock solution of H_2O_2 (10000 mg/L), Sulfuric acid solution (1 N) and Sodium hydroxide 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).

Deionized water used in all experiments and for chemical solutions was purified using a Milli-Q system (millipore filtration). All glassware was first rinsed with acid solution, followed by them with tap water and then with distilled water before use.

2.2. METHODS

2.2.1. Preparation of Antibiotic Solutions

Model contaminants of this study, Amoxicillin and ampicillin, were prepared as 500 mg/L amoxicillin and 500 mg/L ampicillin concentrations in distilled water. All of prepared solutions were stored in +4° C and prepared weekly. During the experiments, prepared solutions were diluted to desired concentrations.

2.2.2. Determination of Antibiotics Wavelength

Amoxicillin and ampicillin antibiotics that used in this study were scanned from 200 to 800 nm wavelength rage to determine their spectra signal with UV/VIS spectrophotometer. Amoxicillin and ampicillin showed maximum absorbance at 230 nm and 233 nm wavelengths, respectively. Figure 2.1 and Figure 2.2 show the spectrum of amoxicillin and ampicillin, respectively. For both antibiotics standards were prepared and calibration curves of them were drawn.

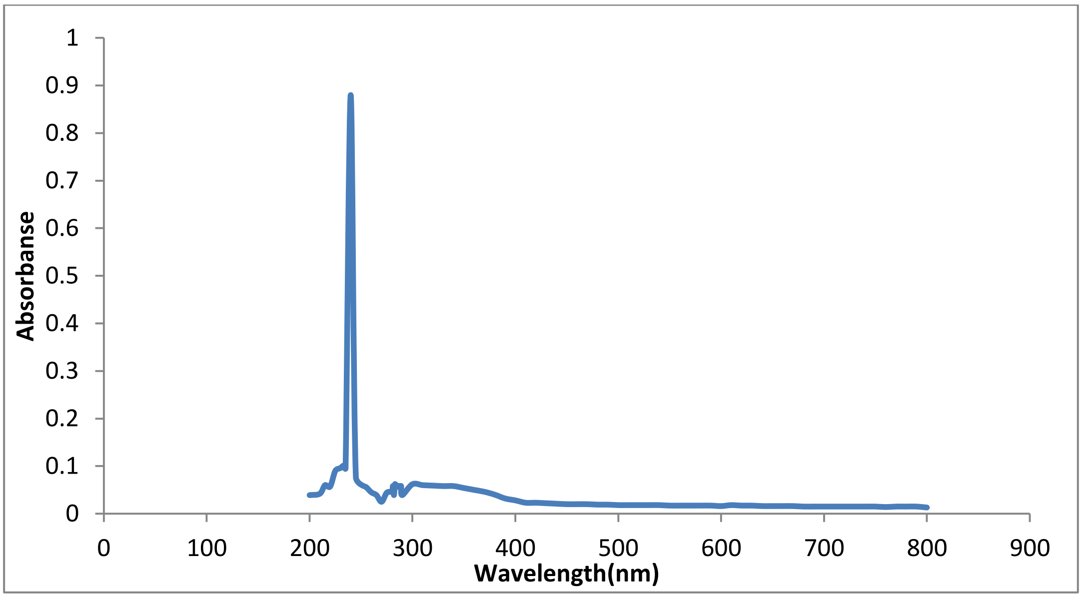

Figure 2.1. UV/VIS Spectrum of AMX obtained using a spectrophotometer during the study

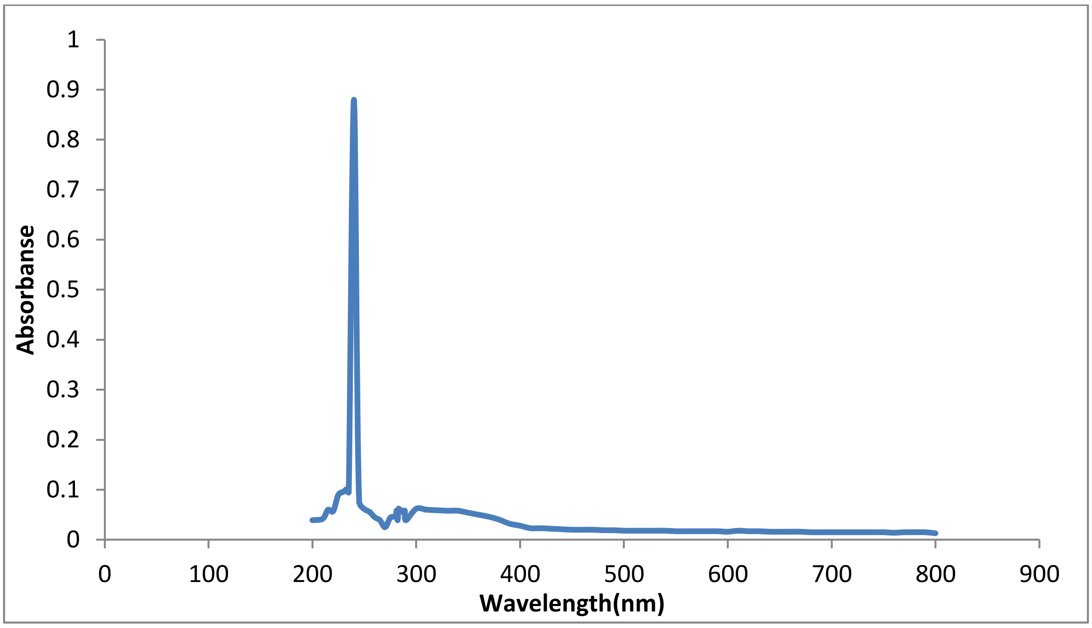

Figure 2.2. UV/VIS Spectrum of AMP obtained by using a spectrophotometer during the study

2.2.3. Preparation of Fe_2O_3 Magnetite Nanoparticles

Fe_2O_3 magnetite particles were used as catalyst in Fenton and photo-Fenton oxidations. The experiments were carried out under optimum conditions that were found earlier for amoxicillin and ampicillin by Fenton and Photo-Fenton oxidations.

The Fe_2O_3 magnetite particles that were investigated in experiments were synthesized by co-precipitation method. A volume of 100 mL of ferric chloride (0.5 M) was added to 200 mL of ferrous chloride solution (0.5 M) and 150 mL of ammonium hydroxide (1 M). 300 mL of deionized water was deoxygenated by bubbling N_2 gas for 30 min in a 1000 mL flask and then added to the solution. Subsequently, 50 mL of ammonium chloride was added and the mixture was stirred magnetically for 10 min under a nitrogen atmosphere. Afterwards, 50 mL of ferrous chloride 0.5 M and 50 mL of ferric chloride 0.5 M were added and then the resultants were aged for 10 min before being separated. At last, the Fe_3O_4 product was separated by a centrifugal pump and washed twice with deionized water and ethanol. The obtained fine Fe_3O_4 particles were dried at 60

˚C for 8 hour. The size of these particles has found to be approximately 500 nm. Figure 2.3 and Figure 2.4 show the scanning electron microscopy (SEM) images of Fe_2O_3 synthesized particles.

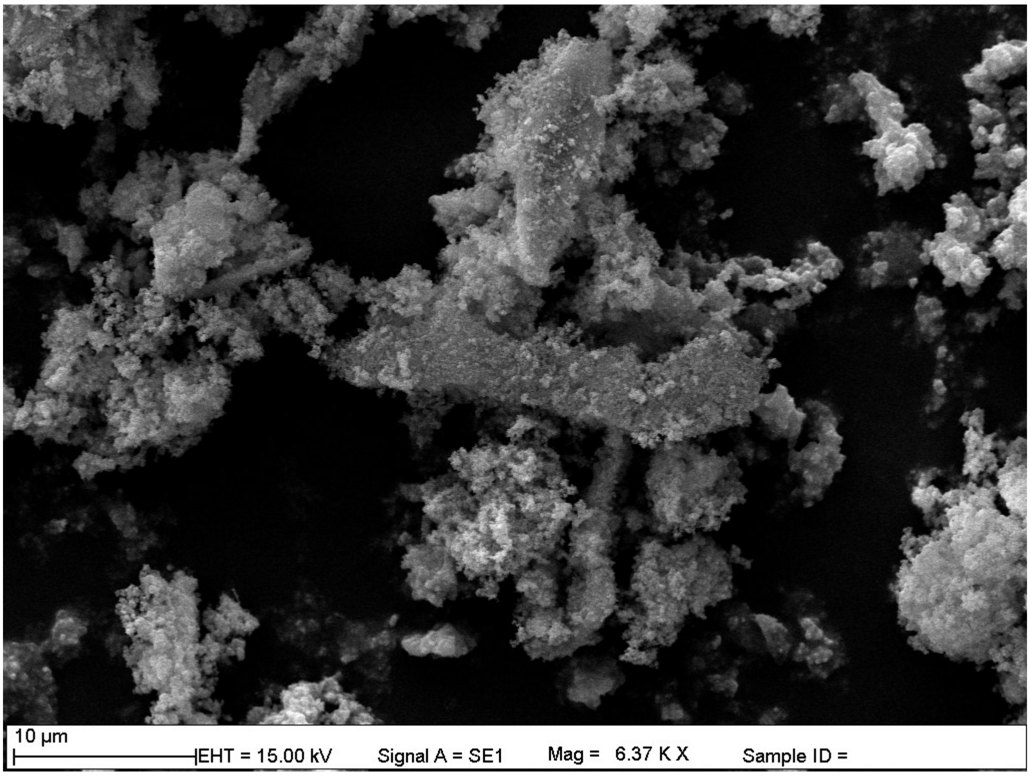

Figure 2.3. SEM image of Fe_2O_3 synthesized particles

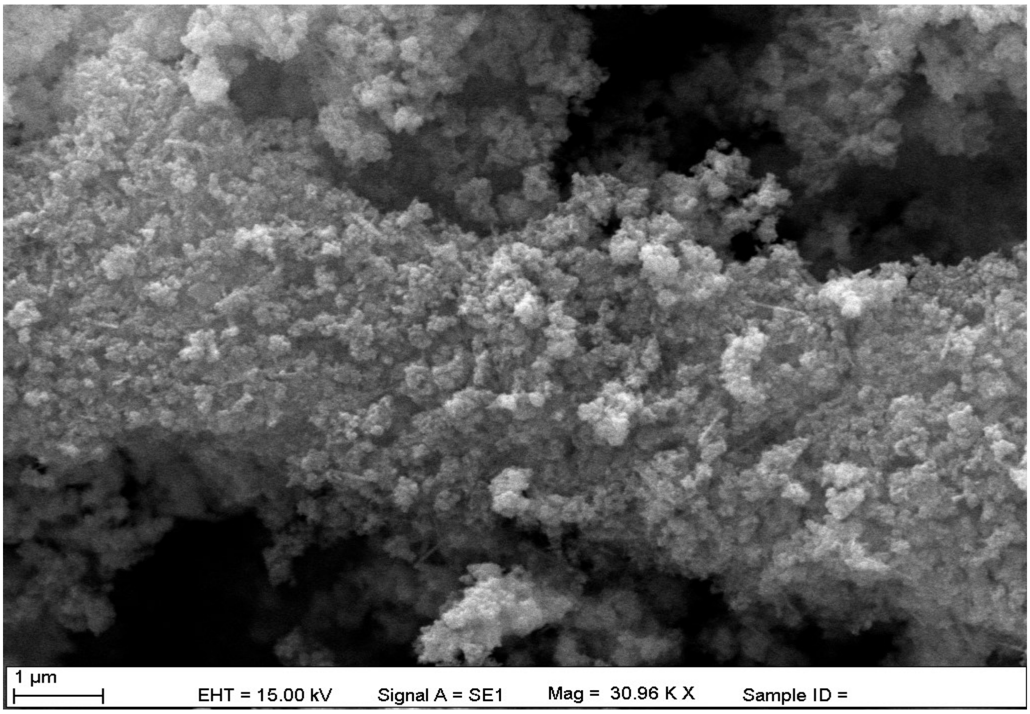

Figure 2.4. SEM image of Fe_2O_3 synthesized particles

2.3. ANALYSIS of ANTIBIOTICS USING HPLC

Selected samples of the study were used to calibrate and verify the spectral system by HPLC. For this purpose, Agilent 1100 Series High Performance Liquid Chromotography (HPLC) was used. 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. Figure 2.5 shows the HPLC Chromotography of amoxicillin and ampicillin. HPLC analysis was conducted at Ankara University Faculty of Pharmaceuticals.

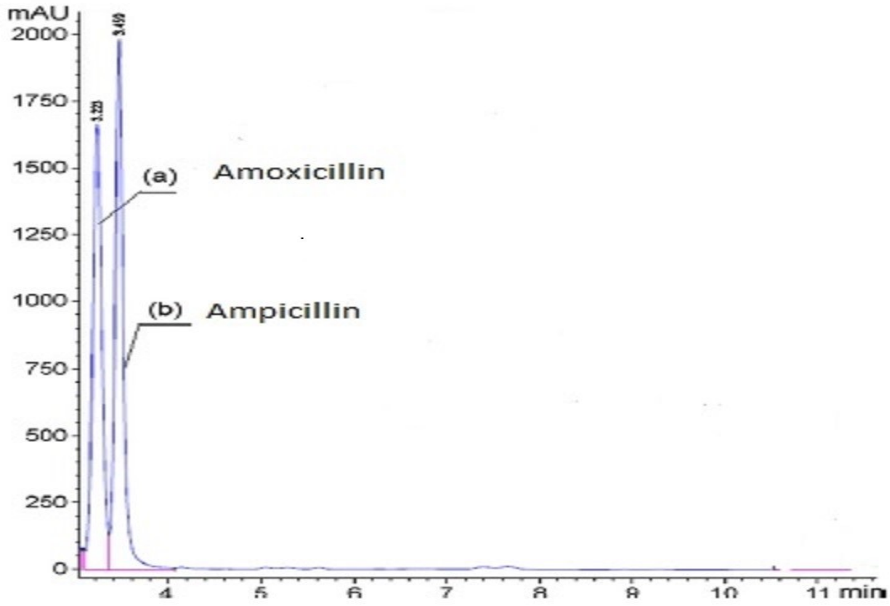

Figure 2.5. HPLC chromatograph of the antibiotics (a) amoxicillin, (b) ampicillin

2.3.1. Fenton Oxidation Experiments

Batch experiments were performed in a 500 ml pyrex reactor with 250 ml of the antibiotics aqueous solution. The predetermined amount of iron in the form of $FeSO_4.7H_2O$ was added to the aqueous solution. To ensure complete homogeneity during reaction, aqueous solution mixed by a magnetic stirrer. Thereafter, required amount of hydrogen peroxide was added to the mixture. pH adjusted to the required value by using H_2SO_4 or NaOH. When hydrogen peroxide was added to the solution was considered as beginning of the experiments.

To determine the maximum yield of Fenton oxidation; different pH values, different concentrations of Fe (II), H_2O_2 and initial concentration of antibiotics was applied to investigated the effect of the removal efficiencies and found optimal values of these parameters.

The steps of Fenton processes used in this study are conducted in the flowing sequence:

First, in order to optimize the pH, 1 N H_2SO_4 was used. After detection of optimum pH, different amounts of Fe (II) were added to the antibiotics solutions. Aqueous solutions mixed by a magnetic stirrer to had a complete homogeneity. By adding H_2O_2, the reaction began and hydroxyl radicals were generated. After determination of optimum Fe (II), to find optimal concentration of H_2O_2, different experiments were carried out. After 60 minutes stirring (185 rpm), 1 N NaOH was added to the solution for neutralization and pH adjusted to optimal pH (9.5 to 11) that $Fe(OH)_3$ flocks could be able to precipitate. After neutralization, 60 minutes was waiting for complete precipitation of $Fe(OH)_3$ flocks; at the end all of the samples were centrifuged for 15 min at 5000 rpm. Chemical oxygen demand analyses (COD) were performed through a Standard Method, 1989, 5220 D. The removal of antibiotics, were determined by UV/VIS spectrometer.

2.3.2. Photo-Fenton Experiments

The source of UV light was an UV lamp (low pressure mercury vapor lamp) with nominal power of 16W, emitting radiations at wave lengths ≈ 254nm and it is placed above the reactor. All photo-oxidation experiments were performed in a completely mix reactor, made of glass with a total volume of 500 ml with a 250 ml of the antibiotics aqueous solutions, operated in bath mode. The reactor was placed on a magnetic stirrer for mixing of aqueous solution. The samples were taken at pre-selected time intervals using a syringe; then chemical oxygen demand analyses and removal of antibiotics were determined. All of the experiments were tested for three times. Experimental design of Fenton oxidation and photo-Fenton oxidation for antibiotic solutions are given in table 2. All experiments have at least one triplicate data set.

Table 2-1- Experiment set of Fenton and photo-Fenton oxidation for antibiotic solutions

parameter	**Set I**	**set II**	**Set III**	**Set IV**	**Set V**
pH	variable	Optimum pH (constant)	Optimum pH (constant)	Optimum pH (constant)	Optimum pH (constant)
Fe^{2+} (mg/L)	constant	variable	Optimum Fe^{2+} (constant)	Optimum Fe^{2+} (constant)	Optimum Fe^{2+} (constant)
H_2O_2 (mg/L)	constant	constant	variable	Optimum H_2O_2 (constant)	Optimum H_2O_2 (constant)
Antibiotic concentration (mg/L)	constant	constant	constant	variable	Optimum antibiotic concentration (constant)
Temperature (◦C)	constant	constant	constant	constant	variable

2.3.3. Chemical Oxygen Demand (COD) Analysis

Chemical oxygen demand analyses (COD) were performed through a Standard Method, 1989, 5220 D.

Preparation of reagents and standard:

1. Potassium dichromate - Mercury Sulfate Solution: 33.3 gr $HgSO_4$ was dissolved in 700 ml pure water and 167 ml of H_2SO_4 (184 g/ml). The solution was cooled and then 10.216 gr $K_2Cr_2O_7$ (dried for 2 hour at 105°C) was added. The solution was diluted with 1 liter distilled water.
2. Sulfuric Acid - Silver Sulfate Solution: 10.12 gr Ag_2SO_4 was dissolved in 1 liter H_2SO_4 (184 g/ml). Solution should be prepared 1 day before using.
3. Potassium hydrogen phthalate (KHP) (HOOCC6H4COOK) Stock: 0.425 gr KHP was dissolved in distilled water and diluted to 500 ml. Theoretically, 1 mg KHP = 1,176 mg O_2 / mg.
4. Preparation of COD standards: standards made from COD stock solution in 10 ml tubes by dilutions in the range of 10-1000.

Standard solutions mixed with reagents as follows:

- 2.5 ml standard solution (0, 100, 200, 400, 800, 1000 mg / L)
- 3.5 mL of Sulfuric Acid - Silver Sulfate Mixture Solution
- 1.5 ml of potassium dichromate - Mercury Sulfate Solution

The tubes were heated at 148 °c for two hours by COD reactor (HACH DRB 200). After 2 hours, the concentration of standards was measured at 600 nm by spectrophotometer. Calibration curve was obtained and now we could determine COD concentration of our samples.

Chemical oxygen demand (COD) was measured according this standard method. When the sample contained H_2O_2, to reduce interference in COD determination 0.02 g MnO_2 was added to decompose H_2O_2 to oxygen and water [70]. Samples were taken at pre-selected time and filtered through a 0.45 μm for COD measurement.

2.3.4. Determination of Antibiotic Removal Efficiency

Removal of antibiotic concentration was measured by UV/VIS spectrophotometer.

Percentage removal of antibiotics was calculated as follows:

$$\eta\,(\%) = \frac{C_o - C_v}{C_o} \times \mathbf{100}$$

η: removal efficiency

C_o: the initial antibiotic concentration

C_v: the final antibiotic concentration

Two methods (spectroscopic and chromatographic) used to monitor antibiotic concentrations are also correlated and correlation results are given in results section.

Statistical analysis ANOVA test were performed for Fenton and Photo-Fenton data set by Statgraphics program. Also, degradation kinetic for both amoxicillin and ampicillin were performed for zero, first and second order kinetics. The results of statistical analysis and kinetic degradation were given in results section.

3. RESULTS AND DISCUSSION

In this study, removal efficiencies of advanced oxidation techniques, Fenton and photo-Fenton were investigated to degrade amoxicillin and ampicillin in aqueous solutions, which represents an industrial wastewater.

3.1. Fenton Experiments Results

Amoxicillin, ampicillin, Fe (II) and hydrogen peroxide concentrations, PH, temperature and time are considered as independent variables. Percent amoxicillin, ampicillin and COD removals were response functions. In a different set of experiments iron is also provided as nanoparticles into the system. Blank tests of the experiments were also conducted.

3.1.1. Effect of pH

Removal of amoxicillin and ampicillin in aqueous solutions by Fenton oxidation was initiated with determination of optimum pH value. For this, different experiments were performed at various pH values. In Fenton oxidation $FeSO_4.7H_2O$ was used as catalyst and H_2O_2 as oxidant.

3.1.1.1. Effect of pH on Removal of COD

To determine the optimal pH value, experiments were conducted by varying pH in the range of 2 to 7. Figure 3.1 and Figure 3.2 show the effect of pH on COD degradation for amoxicillin and ampicillin, respectively. COD degradation percent after 60 min reaction time was 68.12, 74.28, 64.01, 57.32, 49.73 and 38.94 at pH 2, 3, 4, 5, 6 and 7, respectively for amoxicillin (Figure 3.3). In addition, COD degradation percent after 60 min reaction time was 65.54, 68.22, 62.81, 58.91, 52.11 and 47.21 at pH 2, 3, 4, 5, 6 and 7, respectively for ampicillin (Figure 3.2).

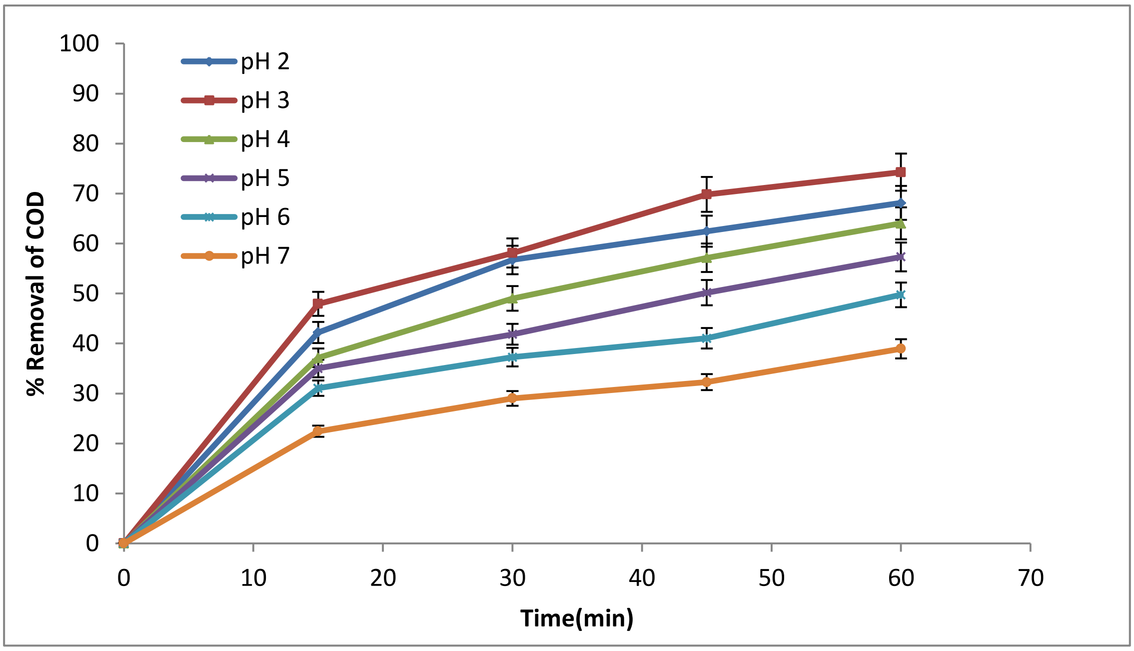

Figure 3.1. Effect of pH on removal of COD using Fenton reaction from solutions which contain amoxicillin

(conditions: Amx 100 mg/L; Fe^{2+} 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

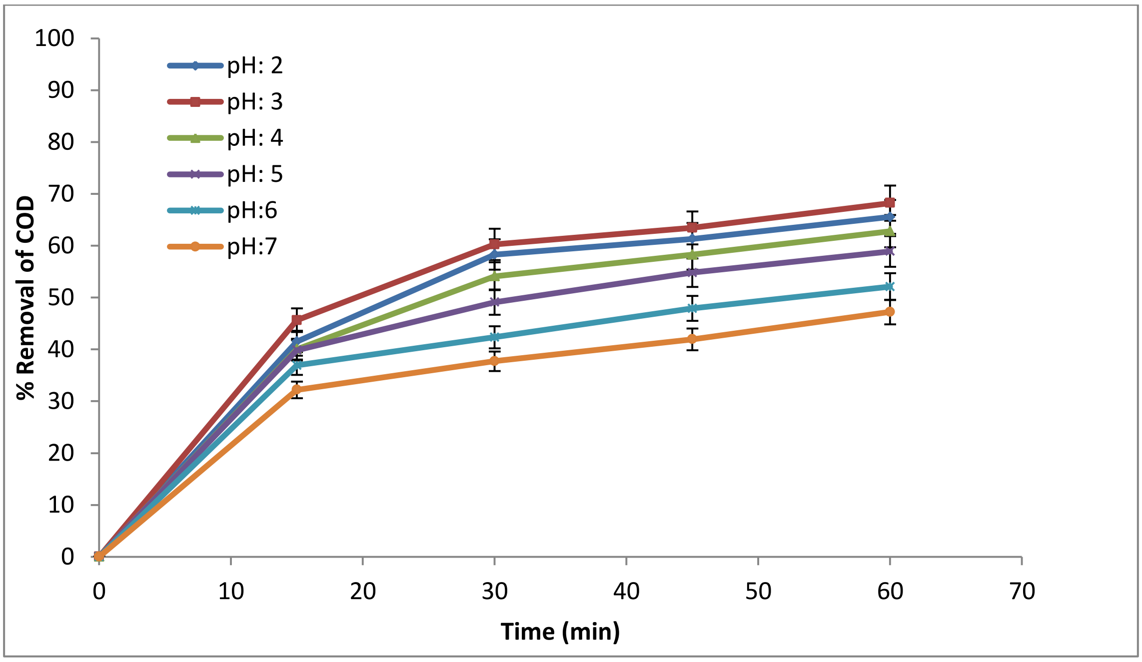

Figure 3.2. Effect of pH on removal of COD using Fenton reaction from solutions which contain ampicillin

(conditions: Amp 100 mg/L; Fe^{2+} 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7

The pH value affects the generation of hydroxyl radicals and hence the oxidation efficiency [62]. The results indicate that, COD degradation was significantly affected by the pH of the reactor. According to the results, the optimum pH of the wastewater is found to be 3 for the removal of COD from AMX and AMP. The results reveal that the overall reduction of COD, decrease for pH values higher than 3. The reason of decreasing the degradation of COD at pH higher than 3, may be because of the decrease in dissolved iron (II) and also decrease in the production rate of hydroxyl radicals. The dissociation and auto-decomposition of hydrogen peroxide decrease the rate of oxidation [71]. In addition, H_2O_2 is stable at low pH perhaps it solvates a proton to compose an oxonium ion (H_3O^+). An oxonium ion makes H_2O_2 electrophilic to improve its stability; so reduce the reactivity with ferrous ions at pH lower than 3 [72] [73]. The results agree well with the reported results such as Homem et al., (2011) and Elmolla and Chaudhuri (2009) [62] [56] [74]. Therefore the remaining experiments of the study were conducted at pH 3.

3.1.1.2. Effect of pH on Antibiotics Removal

The effect of pH on the removal efficiency of the antibiotics were determined and the results were given as percent removal in Figure 3.3 and Figure 3.4 for amoxicillin and ampicillin, respectively. Amoxicillin degradation percent was 86.65, 92.23, 86.12, 72.42, 69.31and 60.41 at pH 2, 3, 4, 5, 6 and 7, respectively (Figure 3.3). Also, ampicillin degradation was 85.45, 91.87, 80.32, 73.42, 70.31 and 64.42 at pH 2, 3, 4, 5, 6 and 7, respectively (Figure 3.4).

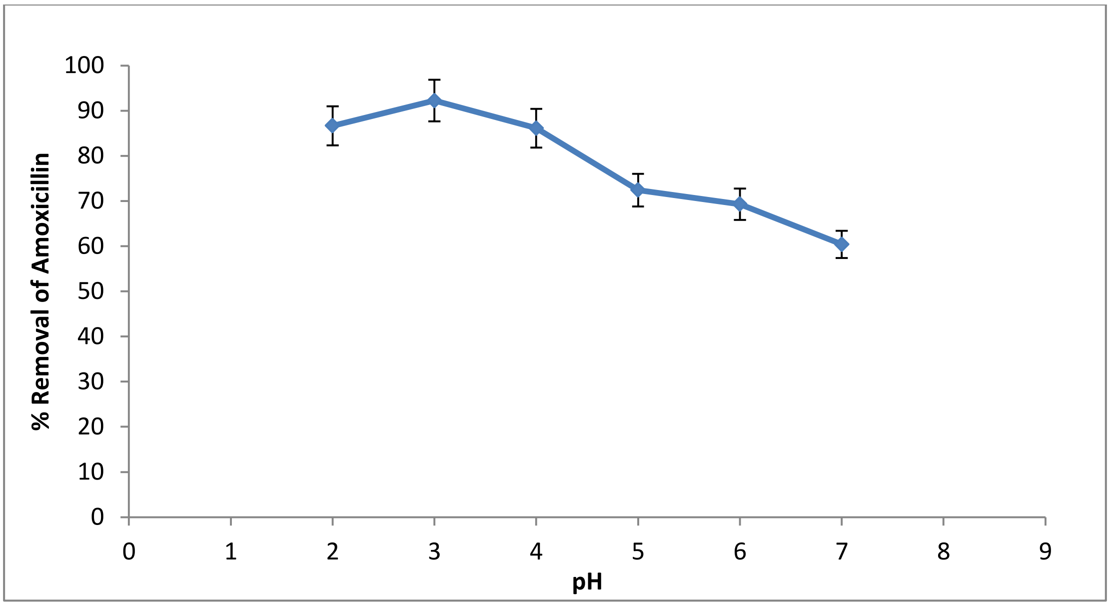

Figure 3.3. Effect of pH on removal of amoxicillin using Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; Fe^{2+} 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

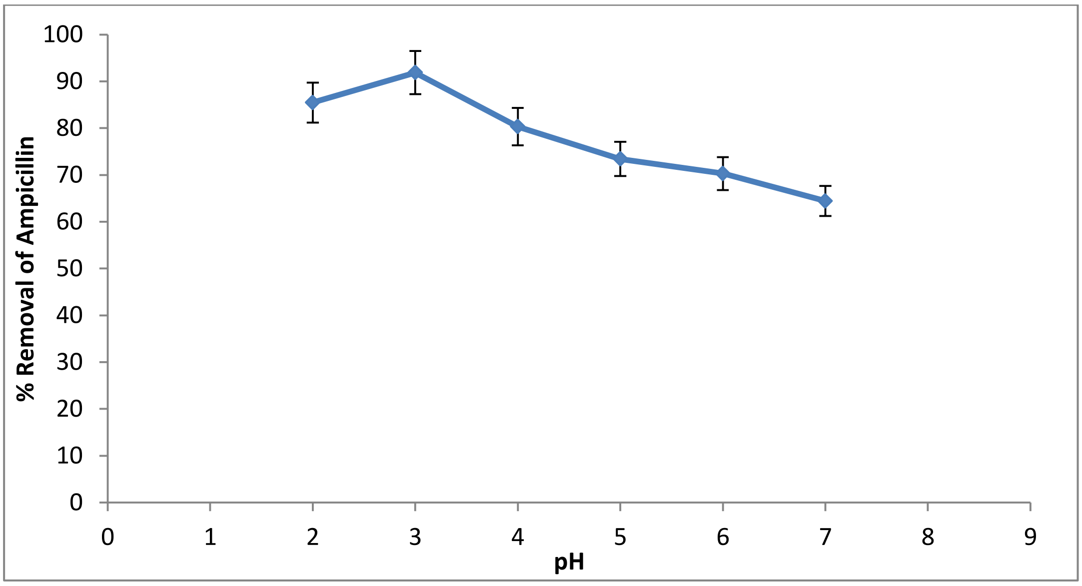

Figure 3.4. Effect of pH on removal of ampicilin using Fenton reaction at equilibrium time

(conditions: Amp 100 mg/L; Fe^{2+} 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

As shown in Figure 3.3 and Figure 3.4, percent antibiotics removal decreased with increasing pH value. The pH values play a major role in oxidative degradation of antibiotics. The maximum removals were occurred at pH 3 for both amoxicillin and ampicillin antibiotics. Increasing the pH value higher than 3 cause to decrease degradation of antibiotic due to decrease in dissolved iron and decrease in oxidation rate of hydroxyl radicals [71] [72].

3.1.2. Effect of Fe (II)

Removal of amoxicillin and ampicillin in aqueous solutions by Fenton oxidation was continued by determination of optimum iron ion concentration. For this, different experiments were performed at various Fe (II) concentrations. The pH value was fixed at 3.

3.1.2.1. Effect of Fe (II) on Removal of COD

Variation of percent COD removals with changing Fe (II) concentrations are depicted in Figure 3.5 and Figure 3.6 for amoxicillin and ampicillin respectively. COD degradation percent for amoxicillin after 60 min reaction was 37.87, 43.39, 52.12, 65.31, 75.28, 70.3 and 62.6 at Fe (II) concentrations of 2, 5, 10, 15, 30, 60 and 150 mg/L, respectively (Figure 3.5). As results show in Figure 3.6, COD degradation percent for ampicillin was 32.77, 40.49, 48.22, 60.61, 71.28, 64.13 and 56.06 at Fe (II) concentrations of 2, 5, 10, 15, 25, 50 and 100 mg/L, respectively.

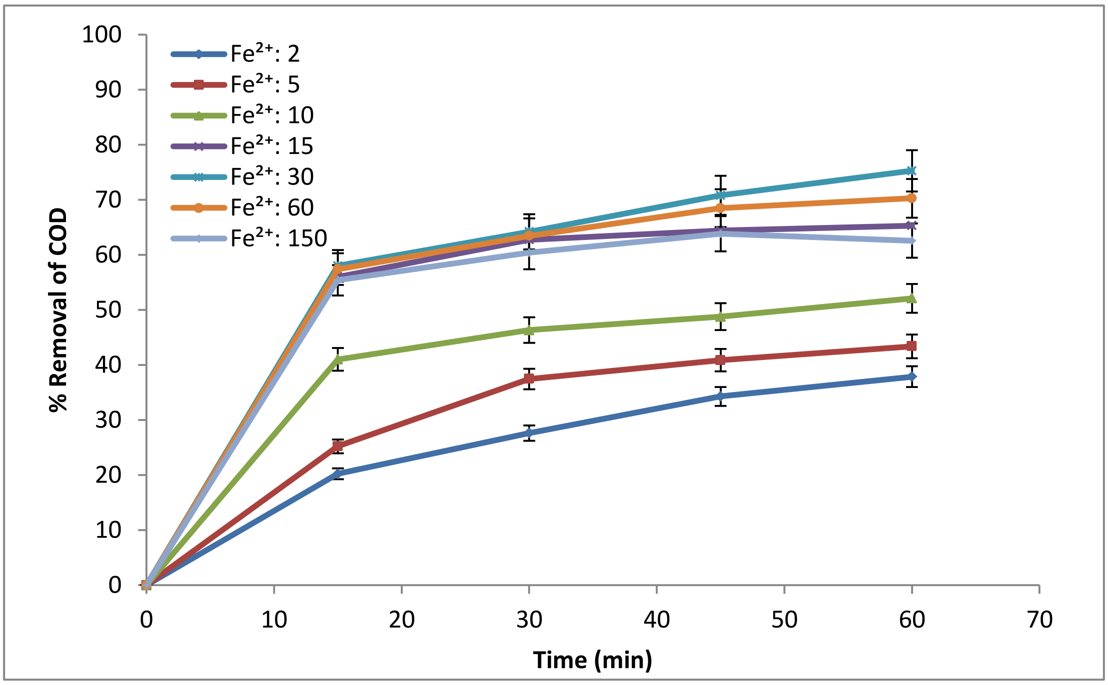

Figure 3.5. Effect of Fe(II) on removal of COD using Fenton reaction from solutions which contain amoxicillin

(conditions: Amx 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) conc. 2-150 mg/L)

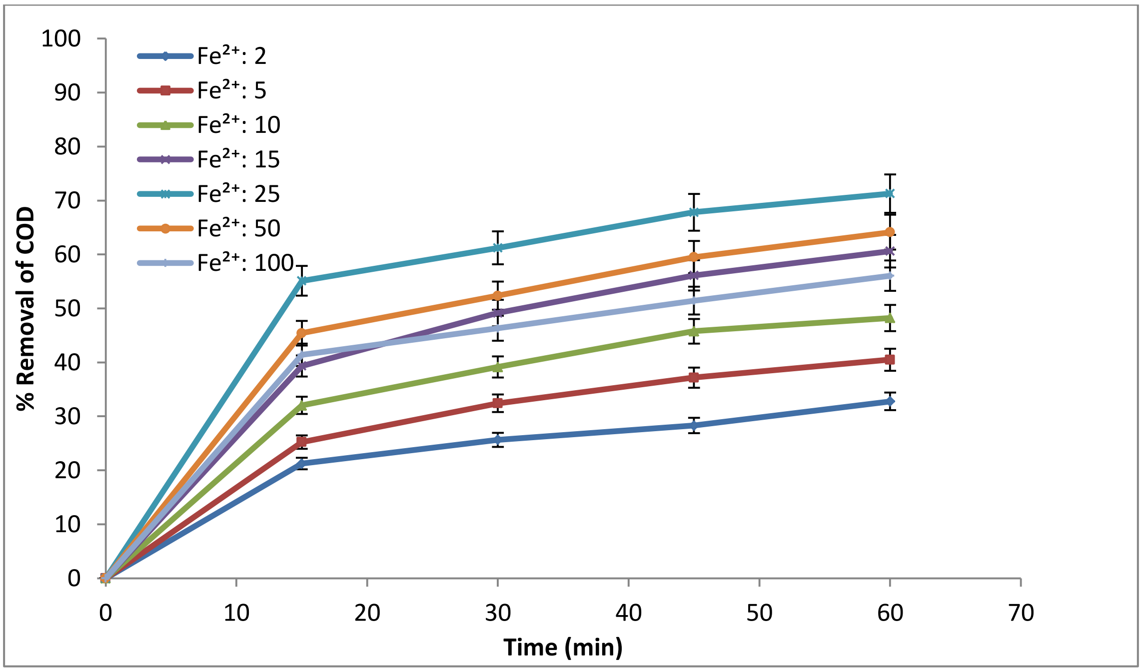

Figure 3.6. Effect of Fe(II) on removal of COD using Fenton reaction from solution which contain ampicillin

(conditions: Amp 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) conc.2-100 mg/L)

The results indicate that for different Fe(II) doses, COD degradation increased with increasing Fe(II) concentration up to 30 mg/L and 25 mg/L for amoxicillin and ampicillin, respectively and then decrease with the increase of iron(II) doses. Apparently, high Fe(II) doses caused scavenging effect on hydroxyl ions. As results show the COD degradation percent decrease with the increase of Fe(II) concentrations, this may be due to direct reaction of $OH^{\cdot}$ radical with metal ions in high doses of Fe(II) [75] as shown in reaction:

$Fe^{2+} + HO^{\cdot} \rightarrow Fe^{3+} + HO^{-}$ $\quad K = (2.5\text{-}5) \times 10^{8}\ L\ mol^{-1}\ S^{-1}$

The results agree well with Elmolla and Chaudhuri (2009) and Kavitha et al., (2005) [62] [73].

3.1.2.2. Effect of Fe (II) on Antibiotics Removal

Figure 3.7 and Figure 3.8 show variations of percent amoxicillin and ampicillin removal with different Fe(II) concentrations, respectively. Amoxicillin degradation percent was 55.87, 61.39, 72.13, 87.31, 92.18, 86.6 and 77.3 at Fe (II) concentrations of 2, 5, 10, 15, 30, 60 and 150 mg/L, respectively (Figure 3.7). Also, ampicillin degradation was 56.77, 60.69, 75.13, 86.41, 92.88, 87.5 and 74.39 at Fe(II) concentrations of 2, 5, 10, 15, 25, 50 and 100 mg/L, respectively (Figure 3.8).

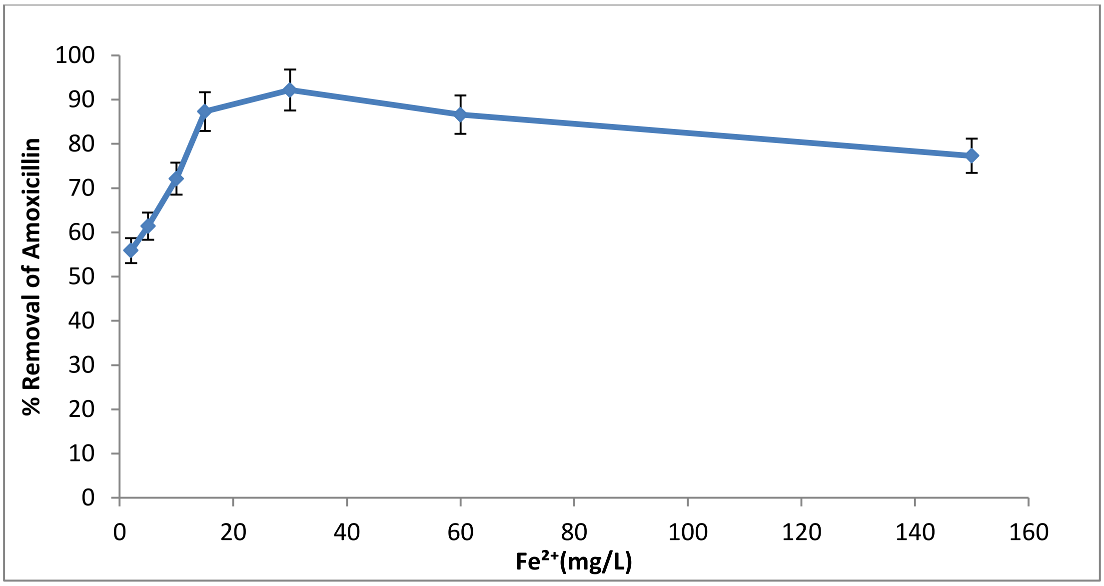

Figure 3.7. Effect of Fe(II) on removal of amoxicillin using Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) conc. 2-150 mg/L)

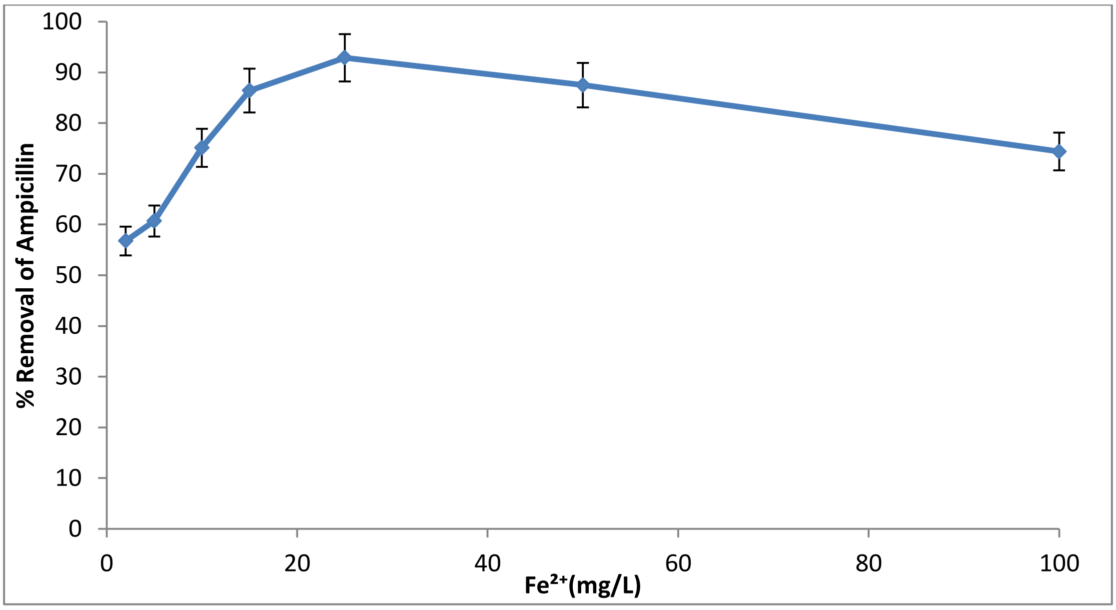

Figure 3.8. Effect of Fe(II) on removal of ampicillin using Fenton reaction at equilibrium time

(conditions: Amp 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) conc. 2-100 mg/L)

Effect of iron ions concentrations on percent antibiotics removal are depicted in Figure 3.7 and Figure 3.8. As results indicate percent antibiotic removal increased with Fe(II) dose up to 30 mg/L and 25 mg/L for amoxicillin and ampicillin respectively. “Further increases in Fe(II) dose to 50 or 100 mg/L resulted in decreases in antibiotic removal probably due to hydroxyl ion scavenging effect of high Fe(II) doses” [76].

3.1.3. Effect of Hydrogen Peroxide Concentration

To examine the effect of hydrogen peroxide on COD and antibiotic degradations, initial H_2O_2 concentration was varied and the other operating conditions were kept constant.

3.1.3.1. Effect of H_2O_2 on Removal of COD

Variation of percent COD removals with H_2O_2 doses are depicted in Figure 3.9 and Figure 3.10 for amoxicillin and ampicillin, respectively. COD decrease, presented as removal percent for amoxicillin after 60 min reaction was 21.02, 42.11, 57.37, 62.43, 73.44 and 67.14 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.9). As results show in Figure 3.10, COD degradation percent for ampicillin was 16.72, 38.11, 50.37, 58.43, 70.46 and 62.04 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively.

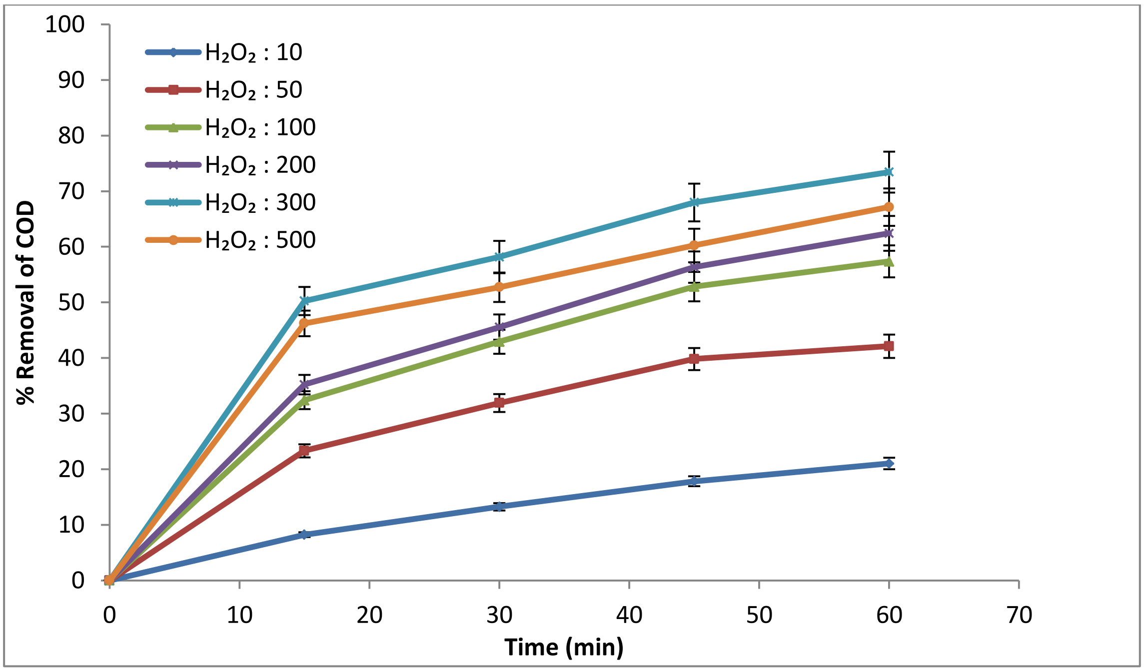

Figure 3.9. Effect of H_2O_2 on removal of COD using Fenton reaction from solution which contain amoxicillin

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

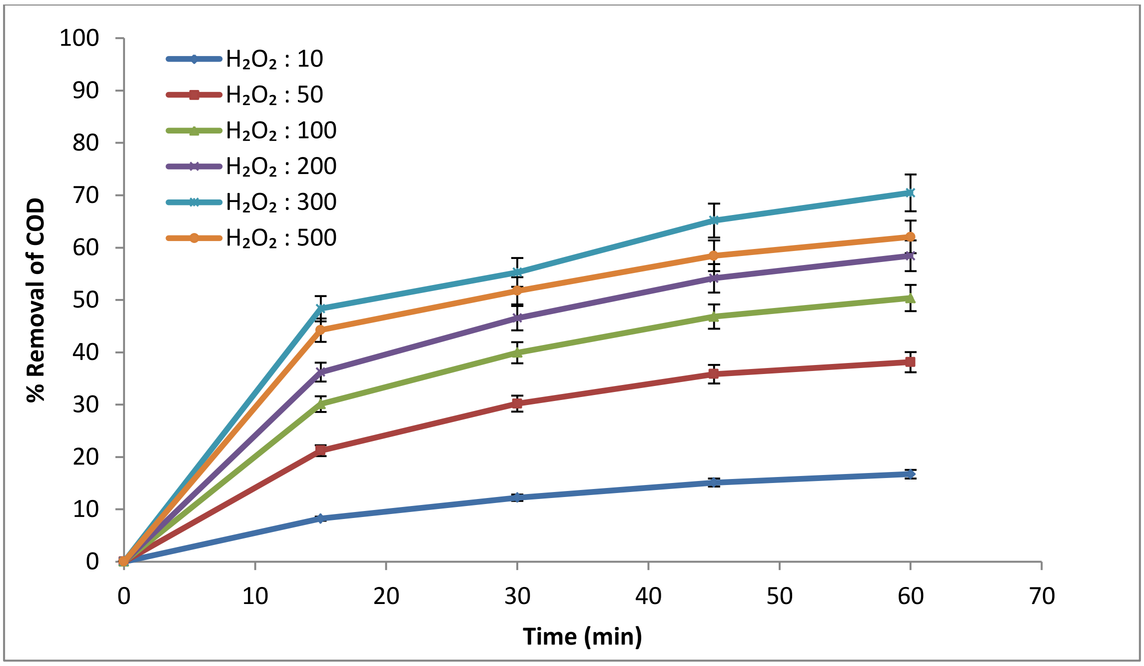

Figure 3.10. Effect of H_2O_2 on removal of COD using Fenton reaction from solution which contain ampicillin

(conditions: Amp 100 mg/L; Fe(II) 25 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

Hydroxyl radicals are the major parameter that influences oxidation efficiency. Results indicate that percent COD degradation increased with increasing peroxide dose for both amoxicillin and ampicillin. Further increasing in hydrogen peroxide concentration did not improve the removal. “This may due to auto-decomposition of H_2O_2 to oxygen and water and scavenging of $OH^{\cdot}$ by H_2O_2” [77] according to these reactions:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

$H_2O_2 + OH^{\cdot} \rightarrow H_2O + HO^{\cdot}_2$ K= (1.7-4.5) ×10^7 L mol^{-1} S^{-1}

“Moreover, the excess H_2O_2 reacts with ferric ions to form hydroperoxyl radical” [73] as in this reaction:

$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO^{\cdot}_2 + H^+$ K= (1-2) ×10^{-2} L mol^{-1} S^{-1}

The same results were also reported by Sun et al. (2007) and Lucas and Peres (2006) [54] [78].

3.1.3.2. Effect of H_2O_2 Concentration on Removal of Antibiotic

Figure 3.11 and Figure 3.12 show variations of percent amoxicillin and ampicillin removal with different H_2O_2 concentrations, respectively. Amoxicillin degradation percent was 32.19, 62.54, 78.37, 83.54, 92.14 and 88.14 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.11). Also, ampicillin degradation was 30.49, 59.64, 74.17, 80.64, 90.79 and 86.04 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.12).

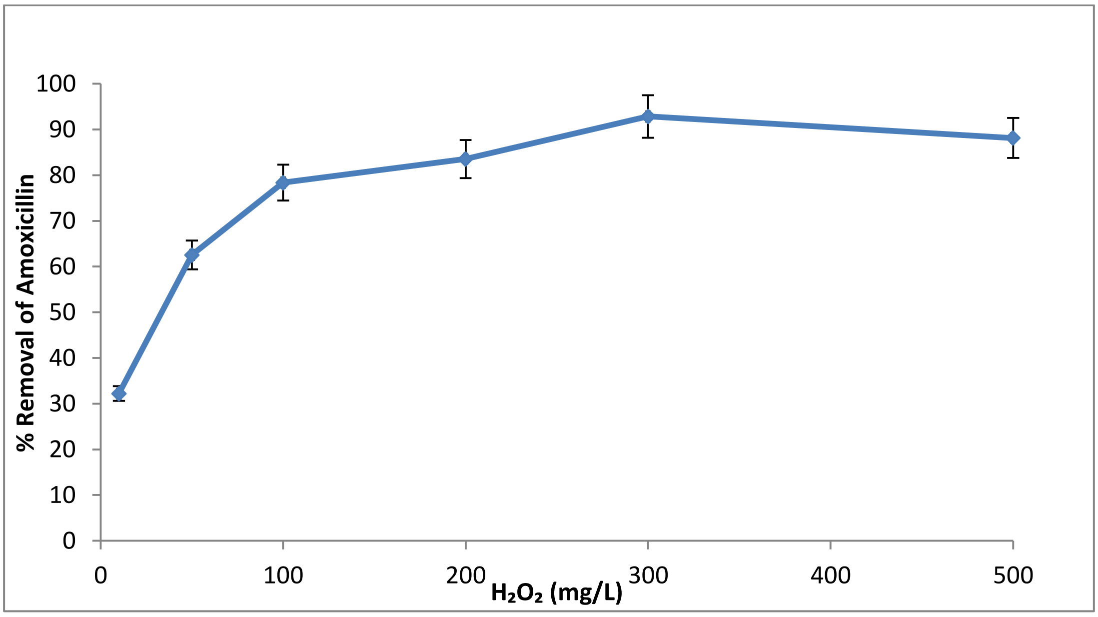

Figure 3.11. Effect of H_2O_2 on removal of amoxicillin using Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

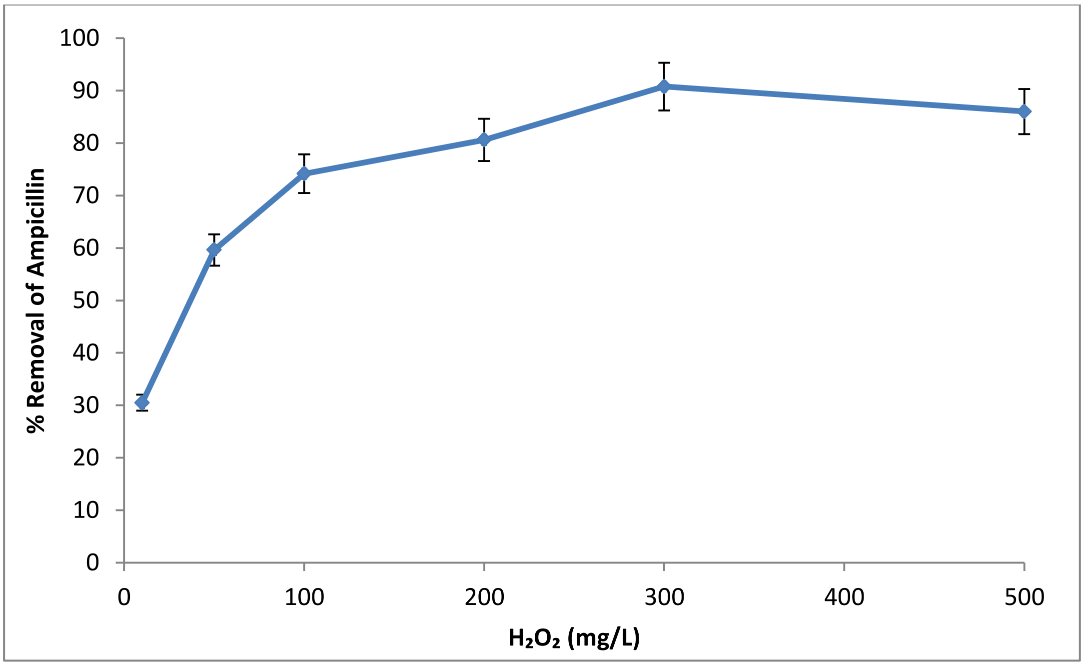

Figure 3.12. Effect of H_2O_2 on removal of ampicillin using Fenton reaction at equilibrium time

(conditions: Amp 100 mg/L; Fe(II) 25 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

As shown in Figure 3.11 and Figure 3.12, percent antibiotics removal increased with increasing H_2O_2 concentration. The H_2O_2 concentration, play a major role in degradation of antibiotics because it is the source of hydroxyl radicals. The maximum removals were occurred at high concentration of H_2O_2 for both amoxicillin and ampicillin antibiotics. Further increase in amount of H_2O_2 cause to decrease degradation of antibiotic due to auto-decomposition of H_2O_2 to oxygen and water and scavenging of $OH^{\cdot}$ by H_2O_2 [77]. The results were in good agreement with reported results at literature [62] [56].

3.1.4. Effect of Initial Antibiotics Concentration

The effect of antibiotic concentration on the removal efficiency, were investigated at different concentrations of amoxicillin and ampicillin.

3.1.4.1. Effect of Initial Antibiotic Concentration on Removal of COD

Figure 3.13 and Figure 3.14 show variations of percent COD removal with different concentrations of amoxicillin and ampicillin. COD degradation percent for amoxicillin after 60 min reaction was 60.4, 66.97, 76.81, 73.87, 53.21 and 40.67 at amoxicillin concentrations of 10, 25, 50, 100, 200 and 500 mg/L, respectively (Figure 3.14). As results show in Figure 3.15, COD degradation percent for ampicillin was 58.8, 64.27, 73.81, 71.37, 51.03 and 40.17 at ampicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively.

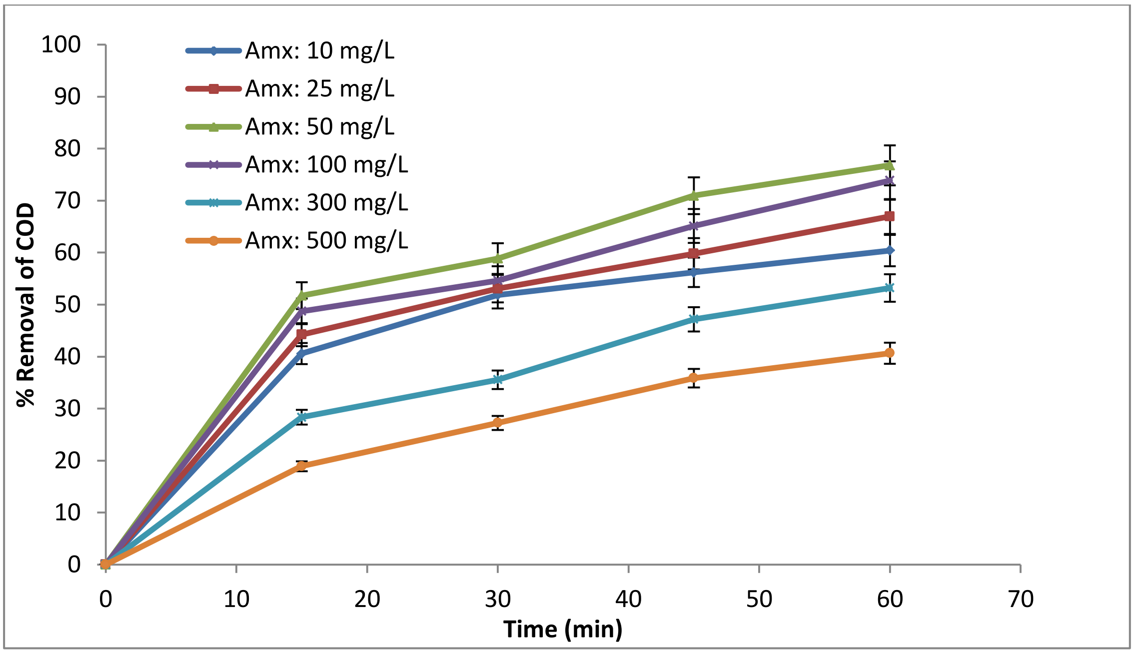

Figure 3.13. Effect of initial antibiotic concentration on removal of COD using Fenton reaction from solution which contain amoxicillin

(conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amx 10-500 mg/L)

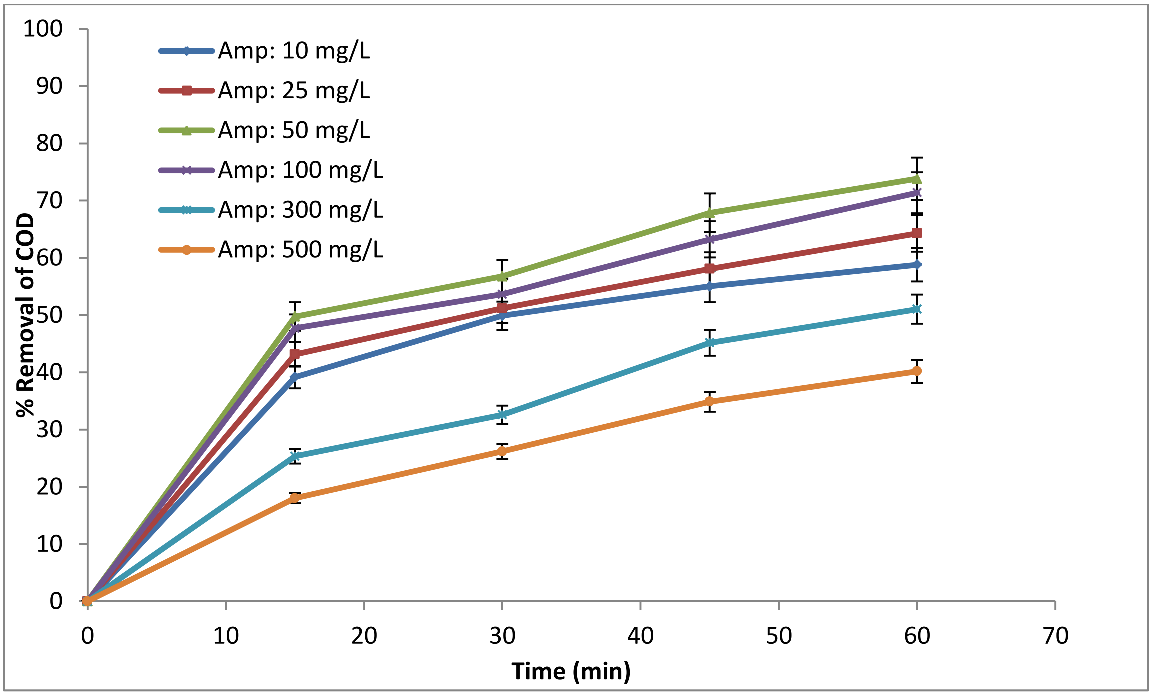

Figure 3.14. Effect of initial antibiotic concentration on removal of COD using Fenton reaction from solution which contain ampicillin

(conditions: pH 3; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amp 10-500 mg/L)

Effect of initial antibiotics concentrations on COD removal are presented in Figure 3.13 and Figure 3.14. The results indicate that COD degradation increase with antibiotic concentration up to 50 mg/L for both amoxicillin and ampicillin, further increase in antibiotic concentration above 50 mg/L resulted in decrease in COD degradation. The reason of this may be due to limitations by other reactants of H_2O_2 and iron ions [76]. High antibiotic concentrations required high peroxide dose [76].

3.1.4.2. Effect of Initial Antibiotic Concentration on Removal of Antibiotic

Figure 3.15 and Figure 3.16 show variations of percent amoxicillin and ampicillin removal with different antibiotic concentrations, respectively. Amoxicillin degradation percent was 85.54, 88.79, 94.32, 91.86, 81.21 and 77.68 at amoxicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively (Figure 3.15). Also, ampicillin degradation was 84.34, 88.19, 93.82, 91.06 80.11

and 74.68 at ampicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively (Figure 3.16).

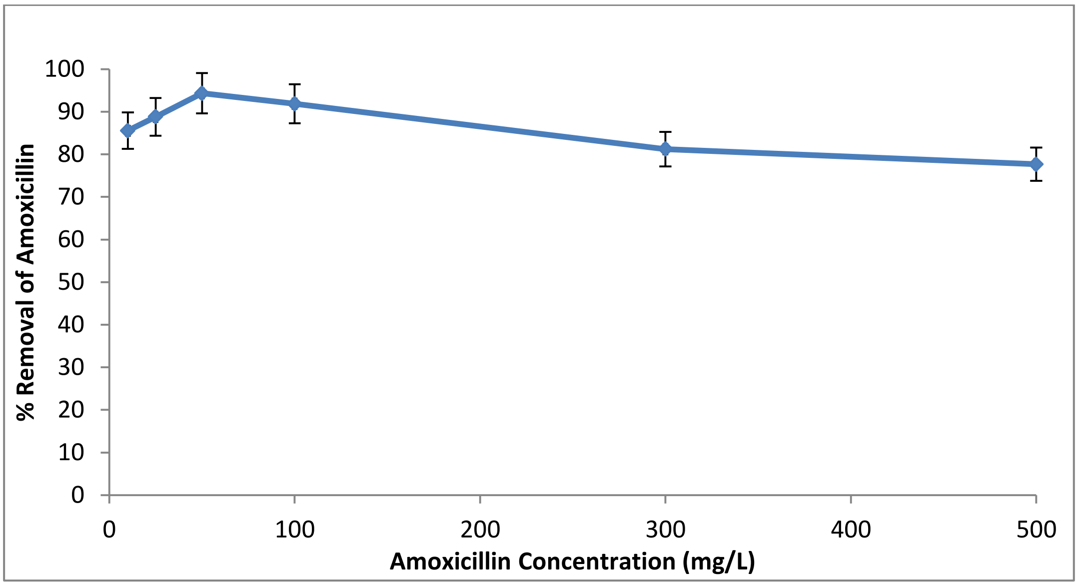

Figure 3.15. Effect of initial amoxicillin concentration using Fenton reaction at equilibrium time

(conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; amoxicillin 10-500 mg/L)

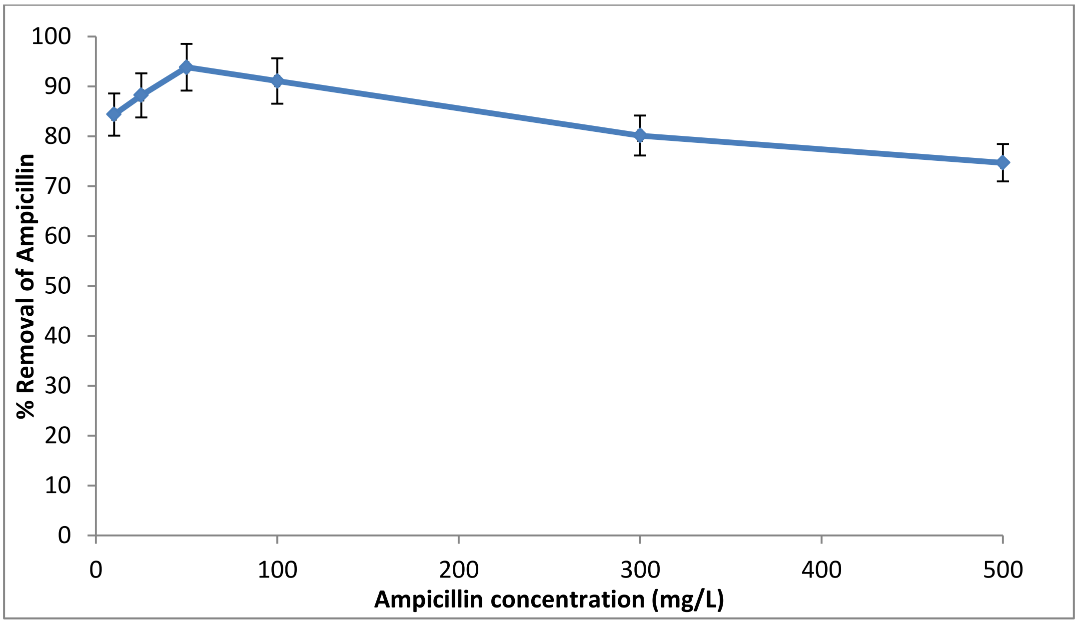

Figure 3.16. Effect of initial ampicillin concentration using Fenton reaction at equilibrium time

(conditions: pH 3; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; ampicillin 10-500 mg/L)

Variation of percent amoxicillin and ampicillin removal at different antibiotic doses, are depicted in Figure 3.15 and Figure 3.16. Antibiotic removal also increased with antibiotic concentration up to 50 mg/L for both amoxicillin and ampicillin. Further increases in antibiotic concentration above 50 mg/L resulted in decreases in percent antibiotic removal due to limitations by other reactants of peroxide and Fe(II) [76]. When the initial concentrations of the antibiotics increased, the hydroxyl radical concentrations remain constant for all antibiotic molecules and hence the removal rate decreases [78] [79].

3.1.5. Effect of Temperature

To determine the effect of temperature on COD and antibiotic degradations, temperature was varied in range of 25 to 60 ˚C and the other operating conditions were constant.

3.1.5.1. Effect of Temperature on Removal of COD

Variation of percent COD removals with temperature are depicted in figure 3.17 and Figure 3.18 for amoxicillin and ampicillin, respectively. COD degradation percent for amoxicillin after 60 min reaction was 73.61, 73.95, 74.93, 75.81 and 75.83 at temperature of 25, 30, 40, 50 and 60 ˚C, respectively (Figure 3.17). As results show in Figure 3.18, COD degradation percent for ampicillin was 73.61, 73.95, 74.04, 73.81 and 74.63 at temperature of 25, 30, 40, 50 and 60 ˚C, respectively.

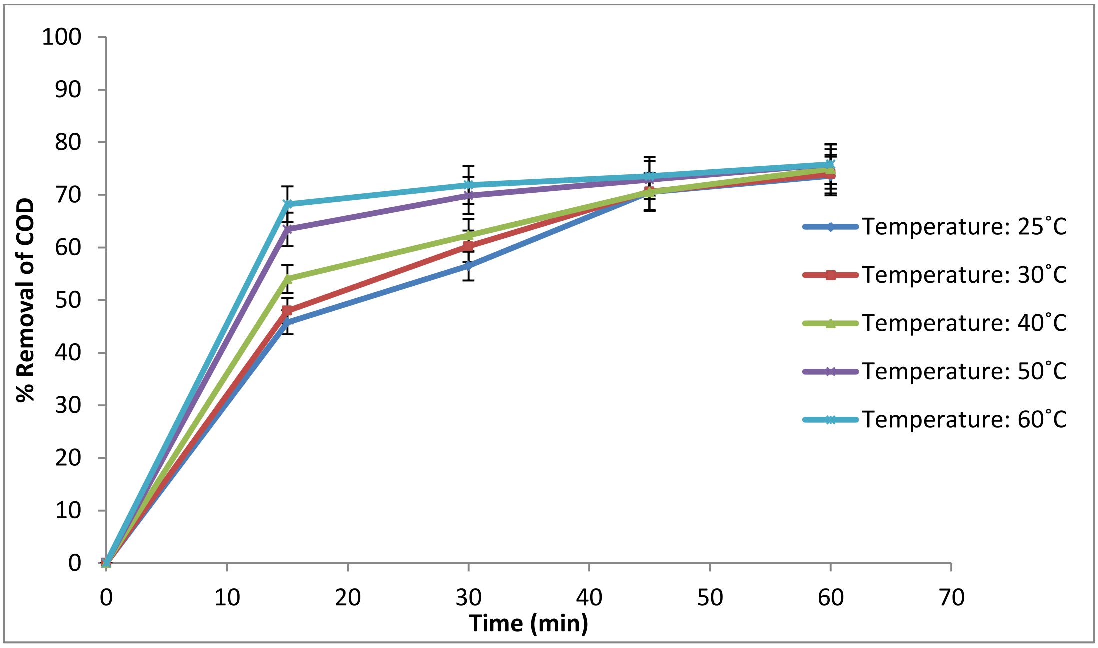

Figure 3.17. Effect of temperature on removal of COD using Fenton reaction from solution which contain amoxicillin

(conditions: Amx 50 mg/L; Fe(II) 30 mg/L; H_2O_2 300; pH 3; temperature 25-60 ˚C)

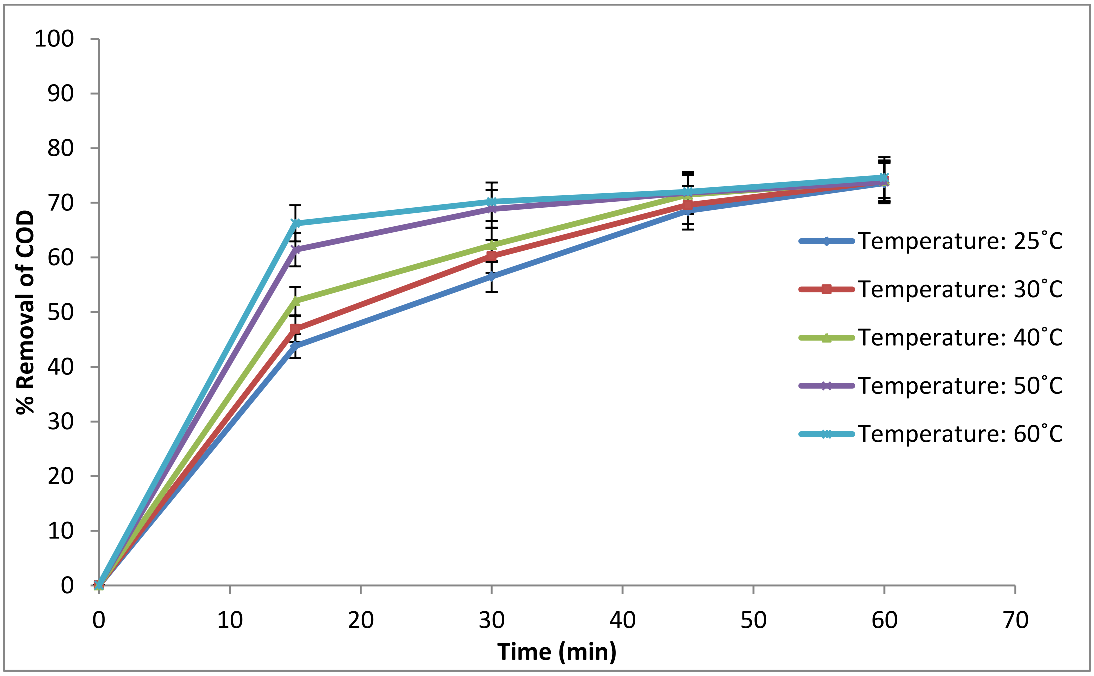

Figure 3.18. Effect of temperature on removal of COD using Fenton reaction from solution which contain ampicillin

(conditions: Amp 50 mg/L; Fe(II) 25 mg/L; H_2O_2 300; pH 3; temperature 25 - 60 ˚C)

As results indicated, no significant differences were observed in COD removal for tested temperatures. The reported results show that the temperature of wastewater nearly does not affect the efficiency of COD degradation in Fenton reaction [80]. The results are in line with Tekin et al., findings [81].

3.1.5.2. Effect of Temperature on Removal of Antibiotic

Figure 3.19 and Figure 3.20 indicate variations of percent amoxicillin and ampicillin removal at different temperatures, respectively. Amoxicillin degradation percent was 92.34, 92.21, 91.98, 92.65 and 92.43 at temperature 25, 30, 40, 50 and 60 ˚C, respectively (Figure 3.19). Also, ampicillin degradation was 92.44, 92.52, 91.98, 92.25 and 92.83 at temperature 25, 30, 40, 50 and 60 ˚C, respectively (Figure 3.20).

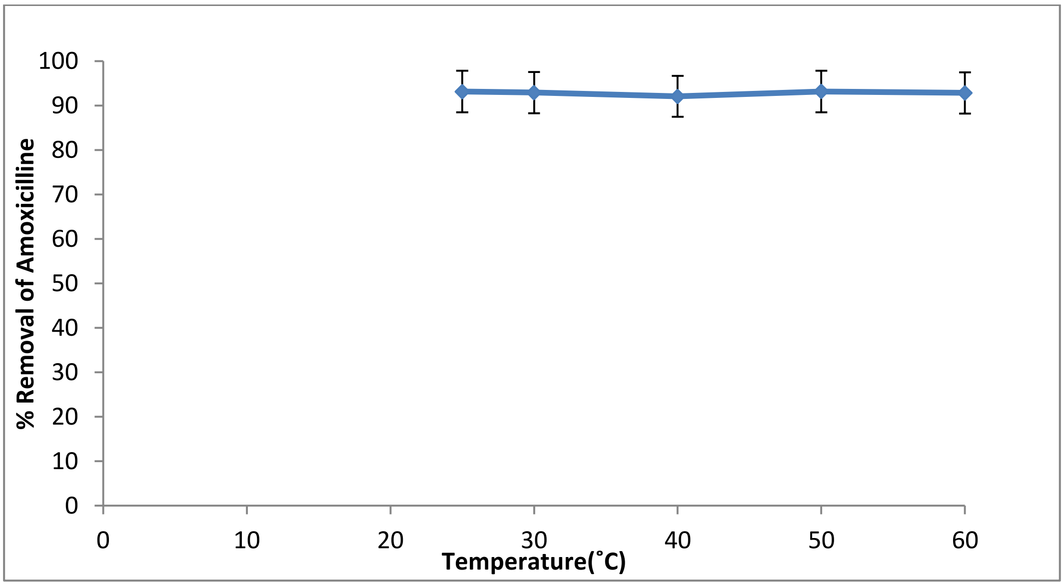

Figure 3.19. Effect of temperature on removal of amoxicillin using Fenton reaction at equilibrium time

(conditions: Amx 50 mg/L; Fe(II) 30 mg/L; H_2O_2 300; pH 3; temperature 25-60 ˚C)

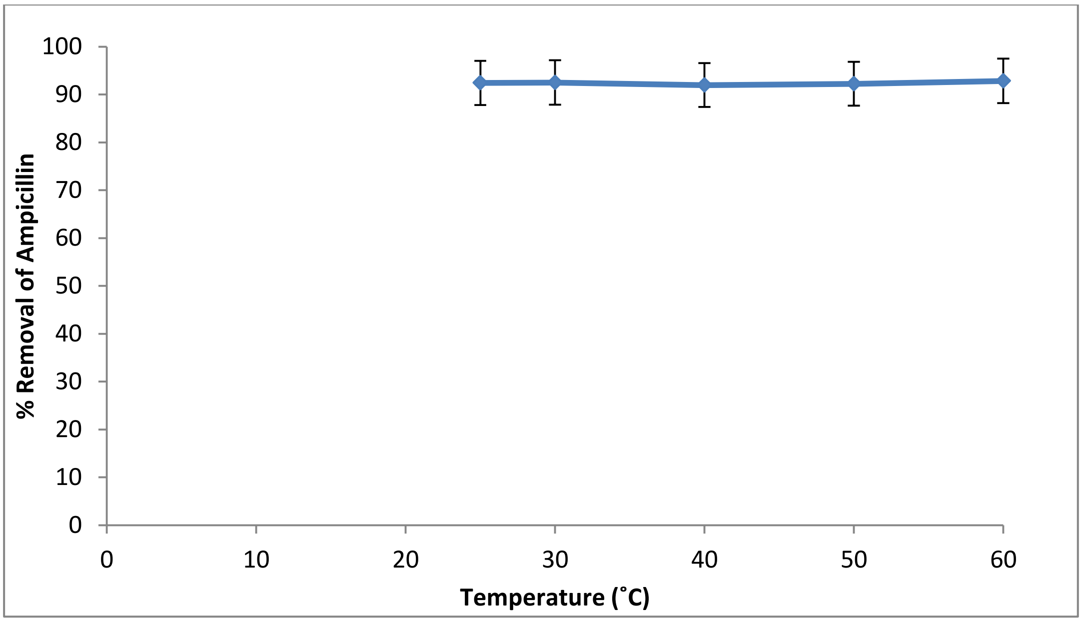

Figure 3.20. Effect of temperature on removal of amoxicillin using Fenton reaction at equilibrium time

(conditions: Amp 50 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25-60 ˚C)

As results indicated, no significant differences were observed in antibiotic removal for tested temperatures for both amoxicillin and ampicillin. The reported results show that the temperature of wastewater nearly does not affect the efficiency of degradation in Fenton reaction [80]. The finding results are in good agreement with Tekin et al., findings [81].

3.2. STATISTICAL ANALYSIS FOR FENTON OXIDATION

Table 3-1- One-way ANOVA for COD removal of Amoxicillin by Fenton oxidation

Factor	Average		Standard deviation	F ratio	P value
pH	2	67.8067	0.427239	**3808.42**	**0.000**
	3	73.7933	0.602107		
	4	64.0733	0.221886		
	5	57.2133	0.304357		
	6	49.5267	0.210317		
	7	38.88	0.20664		
Fe^{2+} (mg/L)	2	37.5067	0.338575	**4306.73**	**0.008**
	5	43.4667	0.479618		
	10	52.08	0.153948		
	15	65.2567	0.215019		
	30	75.11	0.17		
	60	70.0033	0.122202		
	150	61.9333	0.702377		
H_2O_2 (mg/L)	10	21.1033	0.967695	**773.80**	**0.0000**
	50	41.9167	1.40005		
	100	56.9733	1.47555		
	200	62.29	0.90813		
	300	74.96	1.37011		
	500	65.9833	1.0053		
Antibiotic (mg/L)	10	60.0267	1.42711	**339.78**	**0.007**
	25	66.6533	0.765789		
	50	76.5167	1.06086		
	100	72.2567	1.78444		
	300	51.6267	1.39851		
	500	40.56	0.840417		
Temperature(˚C)	25	72.9867	0.78143	**3.48**	**0.0503**
	30	72.4733	1.07593		
	40	73.4267	0.941931		
	50	74.63	1.03595		
	60	75.2867	1.46712		

Table 3-1 shows the Analysis of Variance (ANOVA) test results for removal of COD by Fenton oxidation for amoxicillin. ANOVA analysis performed on the results presented that, COD removal was significantly affected by pH, Fe(II) concentration, H_2O_2 concentration and initial antibiotic concentration, because the P-value is less than 0.05 at the 95% confidence level. The results are in agreement with Elmolla et.al; [62] and Filiz.Ay et.al; [76]. A statistical analysis (one-way ANOVA) for temperature results indicated that, COD removal was not significantly affected by temperature because the P-value is greater than 0.05 at the 95% confidence level.

Table 3-2- One-way ANOVA for COD removal of Ampicillin by Fenton oxidation

Factor	Average		Standard Deviation	F ratio	P value
pH	2	63.4733	1.87836	**125.64**	**0.0053**
	3	68.8467	0.615332		
	4	61.19	1.40471		
	5	57.4933	1.2772		
	6	51.1533	0.905336		
	7	45.6967	1.37027		
Fe^{2+} (mg/L)	2	33.0367	1.9537	**310.74**	**0.0000**
	5	40.1	0.937923		
	10	48.5467	1.62482		
	15	60.72	1.34837		
	25	71.3567	0.94733		
	50	63.2333	0.954481		
	100	56.1333	1.17172		
H_2O_2 (mg/L)	10	16.4433	1.0429	**568.36**	**0.0087**
	50	38.0567	1.75061		
	100	50.3067	1.06641		
	200	59.8033	1.2548		
	300	70.01	1.40993		
	500	62.38	1.81406		
Antibiotic (mg/L)	10	56.77	1.78429	**164.03**	**0.0000**
	25	63.9767	1.6398		
	50	72.12	1.81006		
	100	69.6	1.55913		
	300	51.6067	1.52887		
	500	39.9867	1.4537		
Temperature(˚C)	25	72.0533	1.71803	**1.54**	**0.2649**
	30	72.5367	1.28769		
	40	73.1967	0.799396		
	50	73.6067	1.17823		
	60	74.3033	0.991228		

Table 3-1 shows the Analysis of Variance (ANOVA) test results for removal of COD by Fenton oxidation for ampicillin. ANOVA analysis performed on the results presented that, COD removal was significantly affected by pH, Fe(II) concentration, H_2O_2 concentration and initial antibiotic concentration, because the P-value is less than 0.05 at the 95% confidence level. The results are in agreement with Elmolla et.al; [62]. A statistical analysis (one-way ANOVA) for temperature results indicated that, COD removal was not significantly affected by temperature because the P-value is greater than 0.05 at the 95% confidence level.

3.3. PHOTO FENTON EXPERIMENTS RESULTS

Photo Fenton oxidation ($UV/H_2O_2/Fe^{2+}$) was used to degradation of amoxicillin and ampicillin. Removal efficiency may be enhanced in the presence of UV irradiation as more hydroxyl radicals are produced in the photo-Fenton reaction [82].

3.3.1. Effect of UV irradiation

The effect of UV irradiation for removal of amoxicillin and ampicillin were investigated. The source of UV light was an UV lamp (low pressure mercury vapor lamp) with nominal power of 16W, emitting radiations at wave lengths ≈ 254nm and it is placed above the reactor. The experimental conditions were initial amoxicillin and ampicillin concentration of 100 mg/L for both antibiotics at pH 3. Degradation of amoxicillin and ampicillin were 2.3% and 3.1% after 2 hour irradiation. As results show the removal of amoxicillin and ampicillin by photo-Fenton method will be mainly due to production of hydroxyl radical from the photo-Fenton reaction, as the below reactions.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH^{\cdot} + H^{+}$$

3.3.2. Effect of pH

Removal of amoxicillin and ampicillin in aqueous solutions by Photo-Fenton oxidation was initiated with determination of optimum pH value. For this, different experiments were performed at various pH values. In Photo-Fenton oxidation $FeSO_4.7H_2O$ was used as catalyst and H_2O_2 as oxidant with UV irradiation.

3.3.2.1. Effect of pH on Removal of COD

Effect of pH on the Fenton reactions, were determined in the pH range of 2 to 7. Figure 3.21 and Figure 3.22 show the effect of pH on COD degradation for amoxicillin and ampicillin respectively. COD degradation percent after 60 min irradiation time was 63.17, 74.18, 68.16, 59.82, 53.11 and 43.32 at pH 2, 3, 4, 5, 6 and 7, respectively for amoxicillin (Figure 3.21). In addition, COD degradation percent after 60 min irradiation time was 63.17, 74.18, 68.16, 59.82, 57.11 and 50.32 at pH 2, 3, 4, 5, 6 and 7, respectively for ampicillin (Figure 3.22).

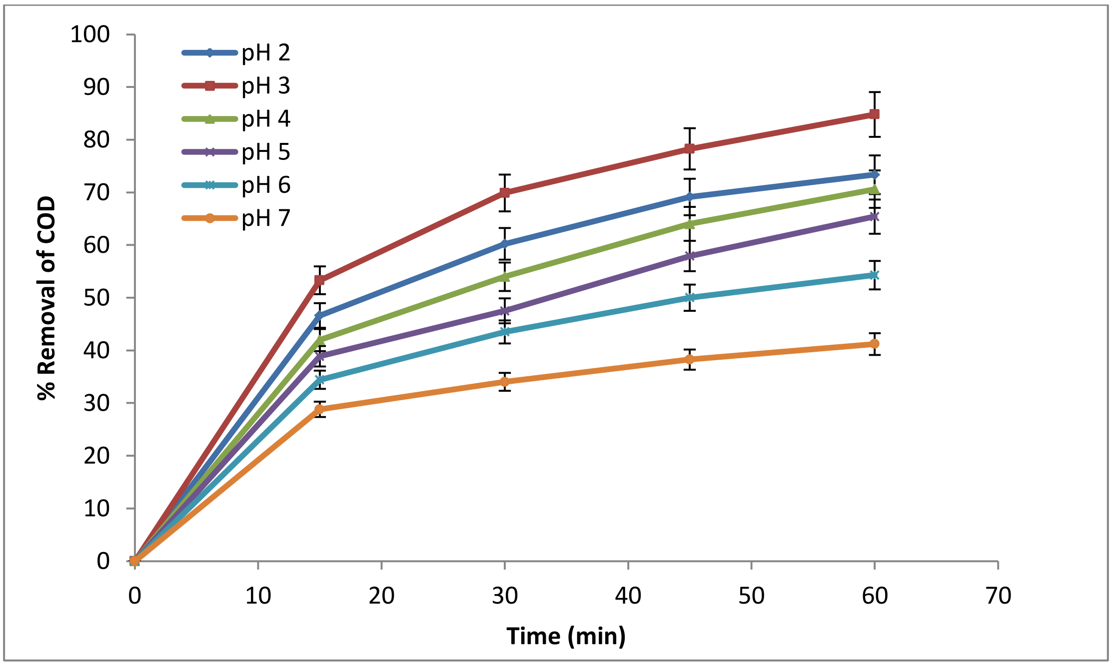

Figure 3.21. Effect of pH on removal of COD using Photo-Fenton reaction from solution which contain amoxicillin

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

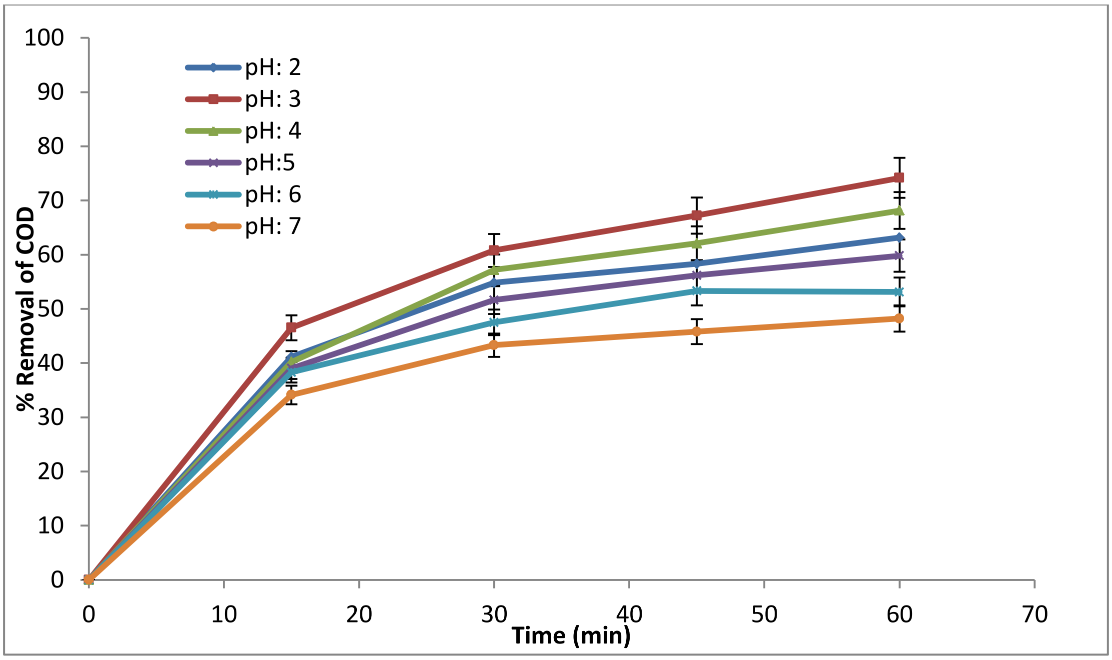

Figure 3.22. Effect of pH on removal of COD using Photo-Fenton reaction from solution which contain ampicillin

(conditions: Amx 100 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

The pH value affects the generation of hydroxyl radicals and hence the oxidation efficiency [62]. The results indicate that, COD degradation was significantly affected by pH. According to the results, the optimum pH for removal of COD for AMX and AMP from wastewater by photo-Fenton oxidation is 3. The results reveal that the degradation of COD, decrease at pH higher than 3. The reason of decreasing the degradation of COD at pH higher than 3 may be because of the dissociation and auto-decomposition of hydrogen peroxide decrease the rate of oxidation [71]. Also, iron precipitated as hydroxide at high pH value, which resulted in a reduction in the transmission of radiation [83]. In addition, H_2O_2 is stable at low pH perhaps it solvates a proton to compose an oxonium ion (H_3O^+). An oxonium ion makes H_2O_2 electrophilic to improve its stability; so reduce the reactivity with ferrous ions at pH lower than 3 [72] [73]. Moreover, the oxidation potential of hydroxyl radicals decreases with increasing pH value. The results agree well with the reported results such as Homem et al., (2011) and Elmolla and Chaudhuri (2009) [62] [56] [74]. The remaining experiments were carried out at pH 3.

3.3.2.2. Effect of pH on the Removal of Antibiotics

Figure 3.23 and Figure 3.24 show variations of percent amoxicillin and ampicillin removal with different pH values, respectively. Amoxicillin degradation percent was 90.25, 93.27, 89.32, 76.42, 72.31 and 65.42 at pH 2, 3, 4, 5, 6 and 7, respectively (Figure 3.23). Also, ampicillin degradation was 89.25, 94.14, 90.32, 72.42, 70.31 and 65.42 at pH 2, 3, 4, 5, 6 and 7, respectively (Figure 3.24).

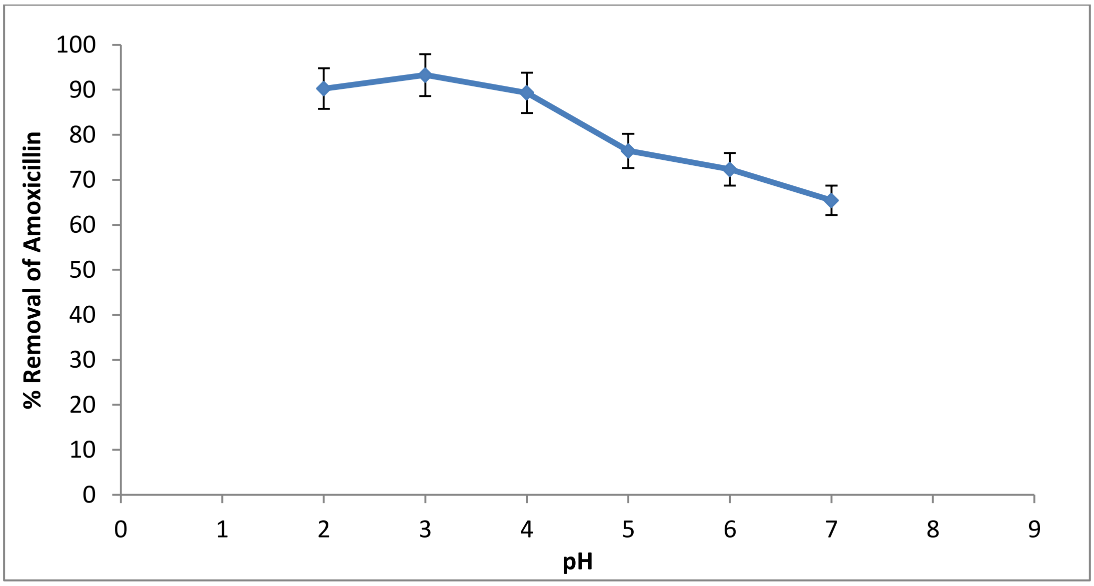

Figure 3.23. Effect of pH on removal of amoxicillin using Photo-Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

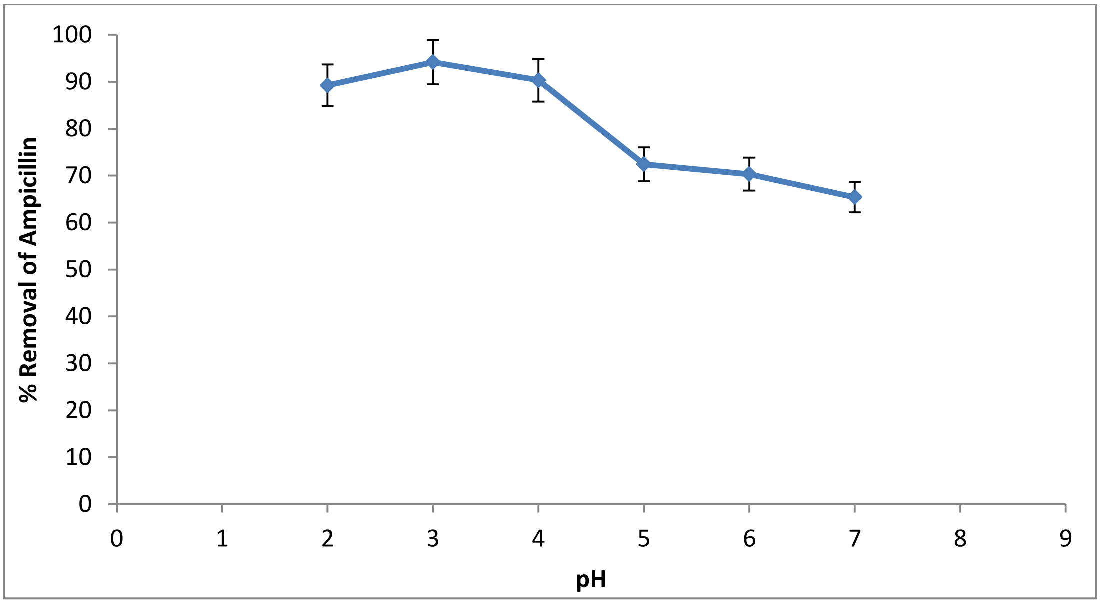

Figure 3.24. Effect of pH on removal of amoxicillin using Photo-Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; pH 2-7)

As shown in Figure 3.23 and Figure 3.24, percent antibiotics removal decreased with increasing pH value. The pH values play a major role in degradation of antibiotics. The maximum removals were occurred at pH 3 for both amoxicillin and ampicillin antibiotics. Increasing the pH value higher than 3 cause to decrease degradation of antibiotic due to decrease in dissolved iron and decrease in oxidation rate of hydroxyl radicals [71] [72].

3.3.3. Effect of Fe(II)

Removal of amoxicillin and ampicillin in aqueous solutions by photo-Fenton oxidation was continued by determination of optimum iron ion concentration. For this, different experiments were performed at various Fe (II) concentrations. The pH value was fixed at 3.

3.3.3.1. Effect of Fe(II) on Removal of COD

Variations of percent COD removal with different Fe (II) concentrations are depicted in Figure 3.25 and Figure 3.26 for amoxicillin and ampicillin respectively. COD degradation percent for amoxicillin after 60 min irradiation was 40.03, 45.61, 55.39, 70.04, 84.32, 74.43 and 65.88 at Fe (II) concentrations of 2, 5, 10, 15, 30, 60 and 150 mg/L, respectively (Figure 3.25). As results show in Figure 3.26, COD degradation percent for ampicillin was 32.77, 40.49, 48.22, 60.61, 71.28, 64.13 and 56.06 at Fe (II) concentrations of 2, 5, 10, 15, 25, 50 and 100 mg/L, respectively.

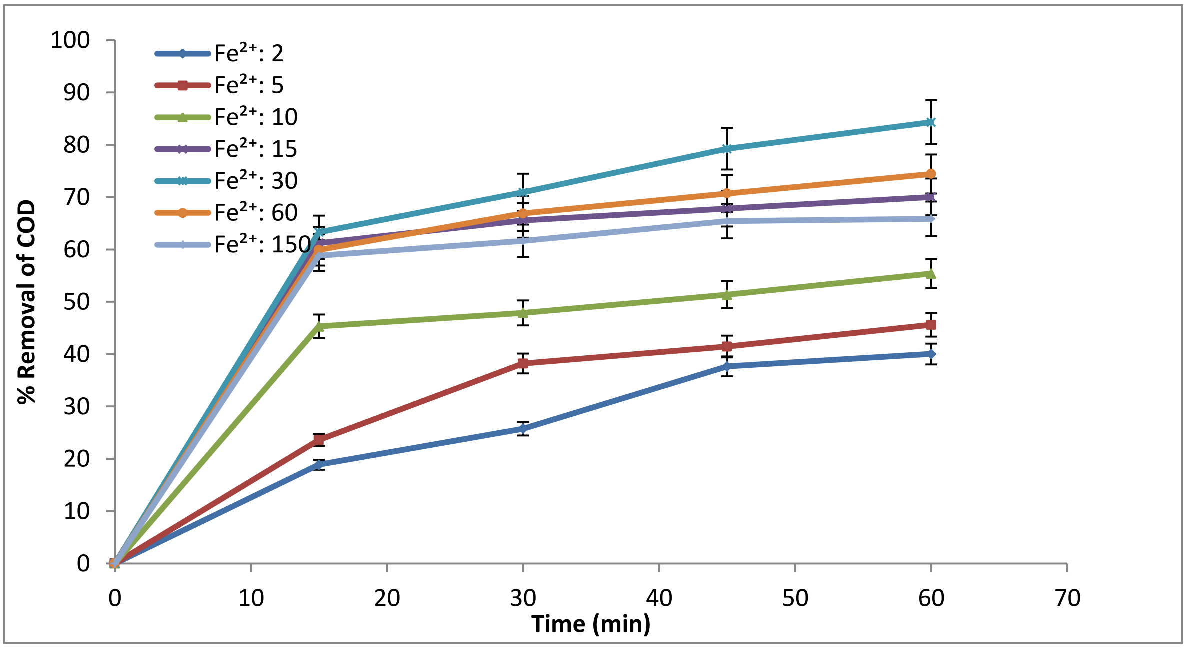

Figure 3.25. Effect of Fe(II) on removal of COD using Photo-Fenton reaction from solution which contain amoxicillin

(conditions: Amx 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) 2-150 mg/L)

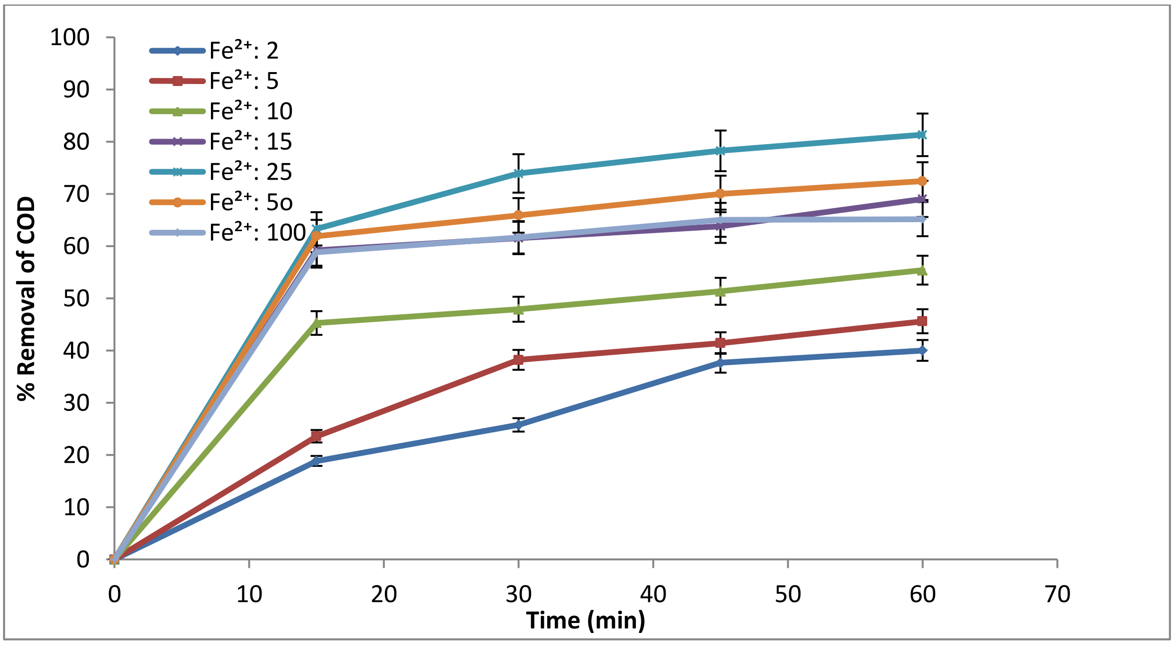

Figure 3.26. Effect of Fe(II) on removal of COD using Photo-Fenton reaction from solution which contain ampicillin

(conditions: Amp 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) 2-100 mg/L)

The results indicate that for different Fe(II) doses, COD degradation increased with increasing Fe(II) concentration up to 30 mg/L and 25 mg/L for amoxicillin and ampicillin respectively and then decrease with more increases in iron ions doses. Apparently, high Fe(II) doses caused scavenging effect on hydroxyl ions. As results show the COD degradation percent decrease with the increase of Fe(II) concentrations, this may be due to direct reaction of $OH^{\cdot}$ radical with metal ions in high doses of Fe(II) [75] as shown in reaction:

$Fe^{2+} + HO^{\cdot} \rightarrow Fe^{3+} + HO^{-}$ $K = (2.5\text{-}5) \times 10^{8}\ L\ mol^{-1}\ S^{-1}$

It was known that the Fe^{2+} had a catalytic decomposition effect on H_2O_2. When Fe^{2+} concentration increased, the catalytic effect also accordingly increased. When the concentration of Fe^{2+} was higher, a great amount of Fe^{3+} from the process of H_2O_2 decomposition by Fe^{2+} was easy to exit in the form of $Fe(OH)^{2+}$ in acidic medium. This may be due to strong absorption of $Fe(OH)^{2+}$ for UV light from 290 to 400nm, the strength of UV light would decrease [84]. Therefore, the decrease in the COD degradation of antibiotic would occur.

The results agree well with Elmolla and Chaudhuri (2009) and Kavitha et al., findings (2005) [62] [73].

3.3.3.2. Effect of Fe(II) on Removal of Antibiotic

Figure 3.27 and Figure 3.28 show variations of percent amoxicillin and ampicillin removal with different Fe(II) concentrations, respectively. Amoxicillin degradation percent was 58.85, 69.73, 76.14, 87.31, 92.98, 88.6 and 79.3 at Fe (II) concentrations of 2, 5, 10, 15, 30, 60 and 150 mg/L, respectively (Figure 3.27). Also, ampicillin degradation was 57.85, 70.13, 75.24, 86.31, 92.88, 88.62 and 78.3 at Fe(II) concentrations of 2, 5, 10, 15, 25, 50 and 100 mg/L, respectively (Figure 3.28).

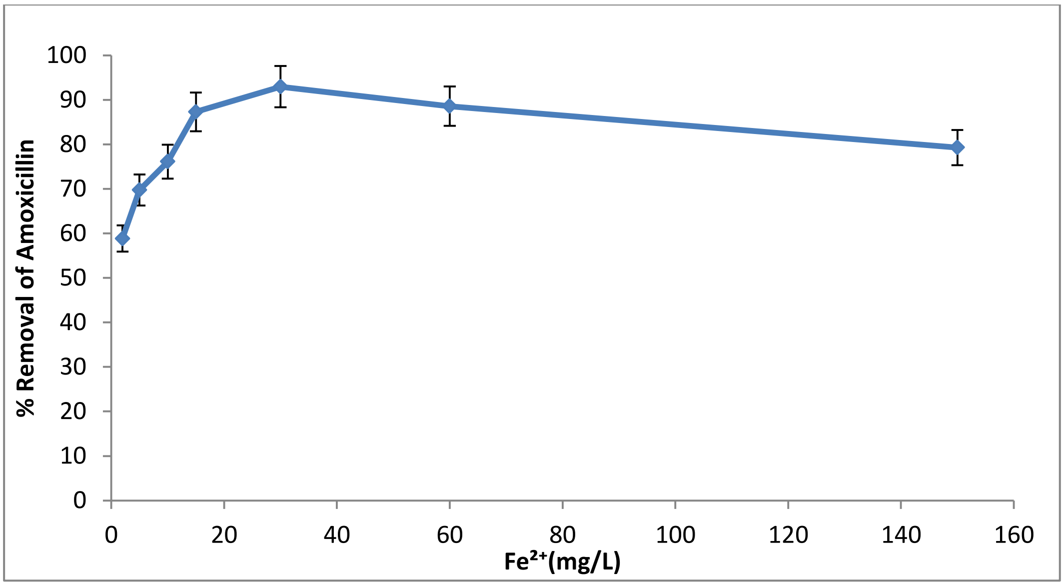

Figure 3.27. Effect of Fe(II) on removal of amoxicillin using Photo-Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) 2-150 mg/L)

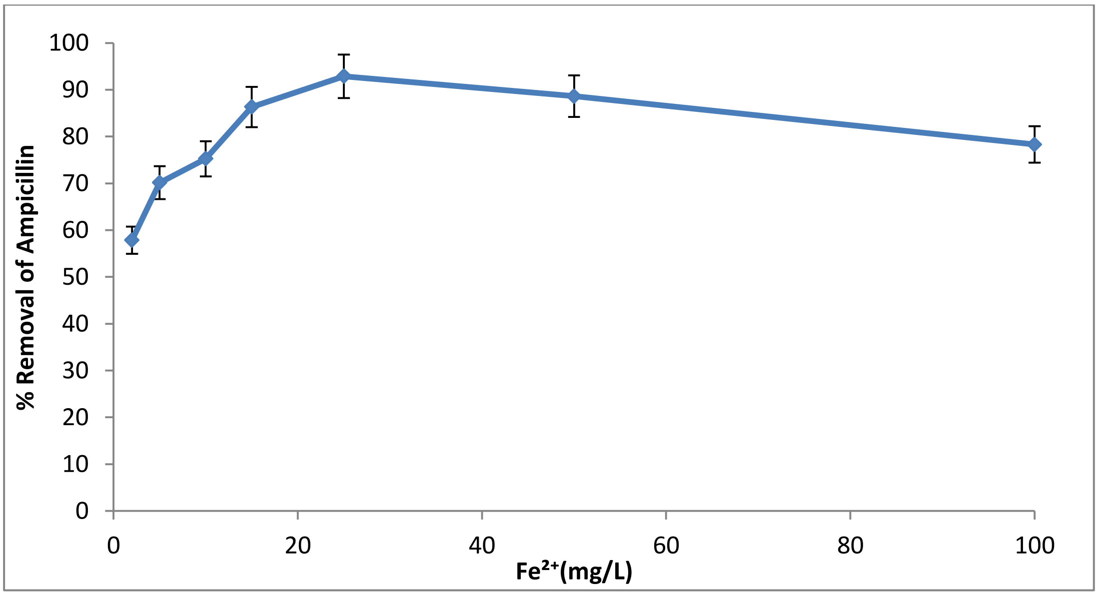

Figure 3.28. Effect of Fe(II) on removal of ampicillin using Photo-Fenton reaction at equilibrium time

(conditions: Amp 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe(II) 2-100 mg/L)

Effect of iron ions concentrations on percent antibiotics removal are depicted in Figure 3.27 and Figure 3.28. As the results indicate percent antibiotic removal increased with the Fe(II) doses up to 30 mg/l and 25 mg/L antibiotic concentrations for amoxicillin and ampicillin, respectively. “Further increases in Fe(II) dose to 50 or 100 mg/L resulted in decreases in antibiotic removal probably due to hydroxyl ion scavenging effect of high Fe(II) doses” [76].

3.3.4. Effect of Hydrogen Peroxide Concentration

To examine the effect of hydrogen peroxide on COD and antibiotic degradations, initial H_2O_2 concentration was varied and the other operating conditions were constant.

3.3.4.1. Effect of H_2O_2 Concentration on Removal of COD

Variation of percent COD removals with H_2O_2 doses are depicted in Figure 3.29 and Figure 3.30 for amoxicillin and ampicillin, respectively. COD degradation percent for amoxicillin after 60 min irradiation was 21.02, 42.11, 57.37, 62.43, 73.44 and 67.14 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.29). As results show in Figure 3.30, COD degradation percent for ampicillin was 16.72, 38.11, 50.37, 58.43, 70.46 and 62.04 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively.

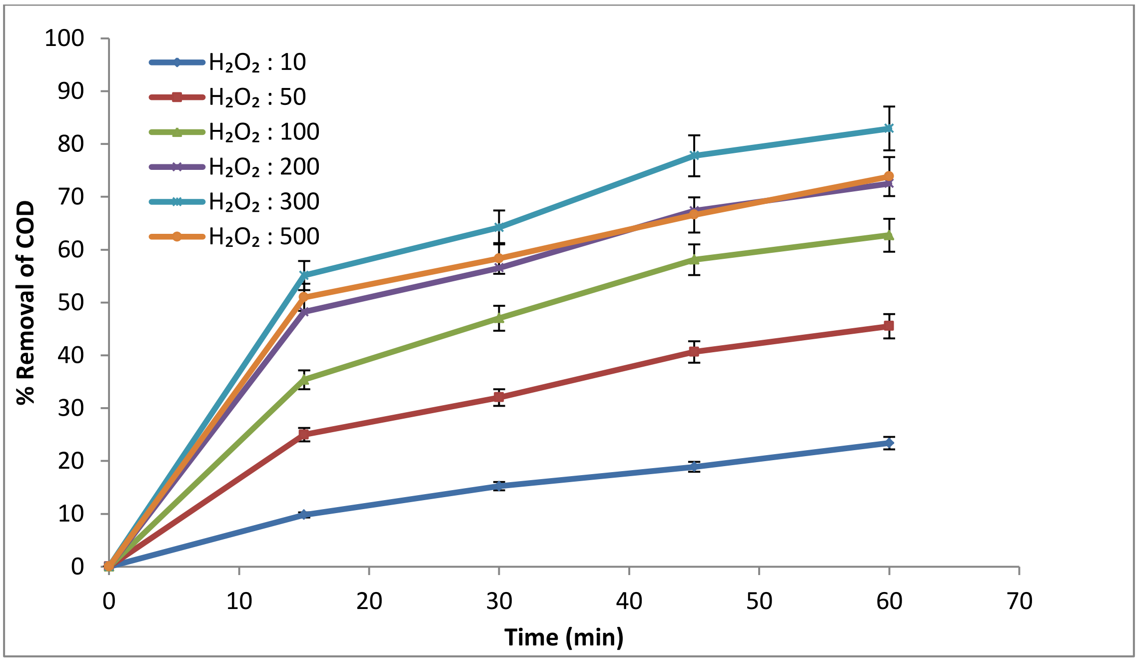

Figure 3.29. Effect of H_2O_2 on removal of COD using Photo-Fenton reaction from solution which contain amoxicillin

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

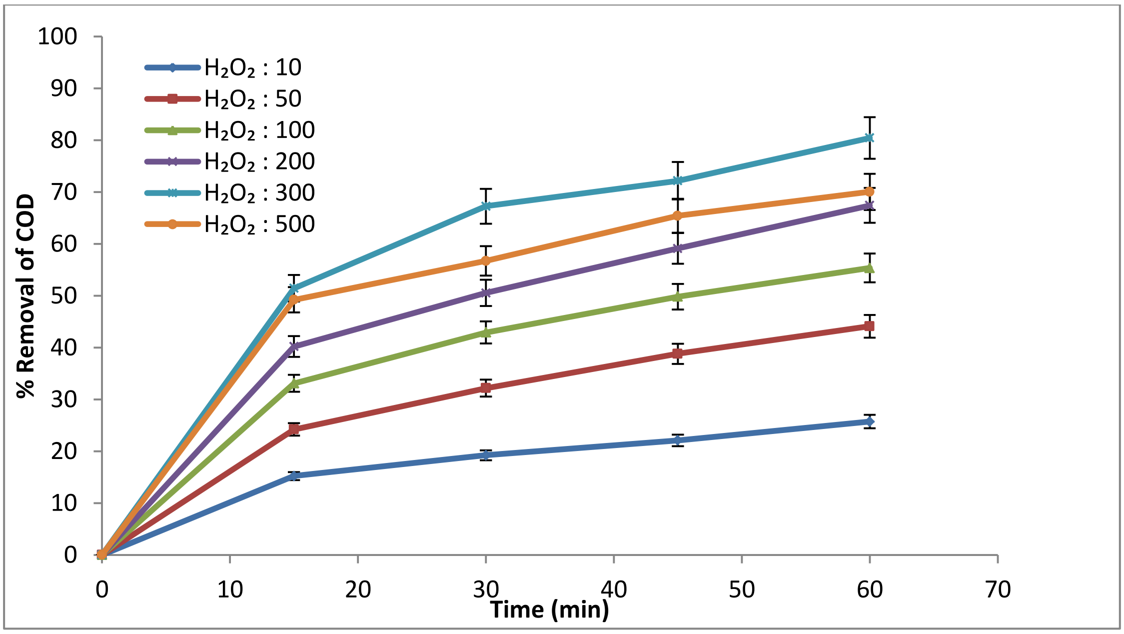

Figure 3.30. Effect of H_2O_2 on removal of COD using Photo-Fenton reaction from solution which contain ampicillin

(conditions: Amp 100 mg/L; Fe(II) 25 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

Hydroxyl radicals are the major parameter that influences oxidation efficiency. Results indicate that percent COD degradation increased with increasing peroxide dose for both amoxicillin and ampicillin. Further increasing in hydrogen peroxide concentration did not improve the removal. “This may due to auto-decomposition of H_2O_2 to oxygen and water and scavenging of $OH^{\cdot}$ by H_2O_2” [77] according to these reactions:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

$H_2O_2 + OH^{\cdot} \rightarrow H_2O + HO^{\cdot}_2$ K= (1.7-4.5) ×10^7 L mol^{-1} S^{-1}

“Moreover, the excess H_2O_2 reacts with ferric ions to form hydroperoxyl radical” [73] as in this reaction:

$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO^{\cdot}_2 + H^+$ K= (1-2) ×10^{-2} L mol^{-1} S^{-1}

The same results were also reported by Sun et al. (2007) and Lucas and Peres (2006) [54] [78]. Therefore, the optimum concentration of H_2O_2 should be added to antibiotic solution to achieve the best removal.

3.3.4.2. Effect of H_2O_2 Concentration on Removal of Antibiotic

Figure 3.31 and Figure 3.32 show variations of percent amoxicillin and ampicillin removal with different H_2O_2 concentrations, respectively. Amoxicillin degradation percent was 37.39, 65.94, 82.17, 87.43, 93.14 and 90.65 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.31). Also, ampicillin degradation was 35.19, 62.94, 78.07, 84.43, 94.14 and 89.65 at H_2O_2 concentrations of 10, 50, 100, 200, 300 and 500 mg/L, respectively (Figure 3.32).

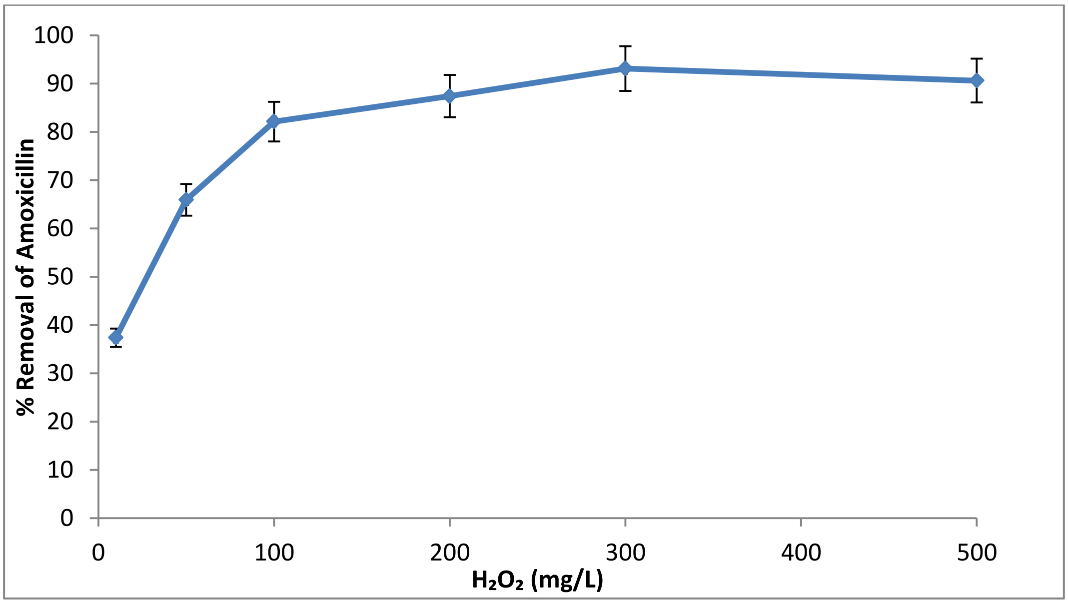

Figure 3.31. Effect of H_2O_2 concentration on removal of amoxicillin using photo-Fenton reaction at equilibrium time

(conditions: Amx 100 mg/L; Fe(II) 30 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

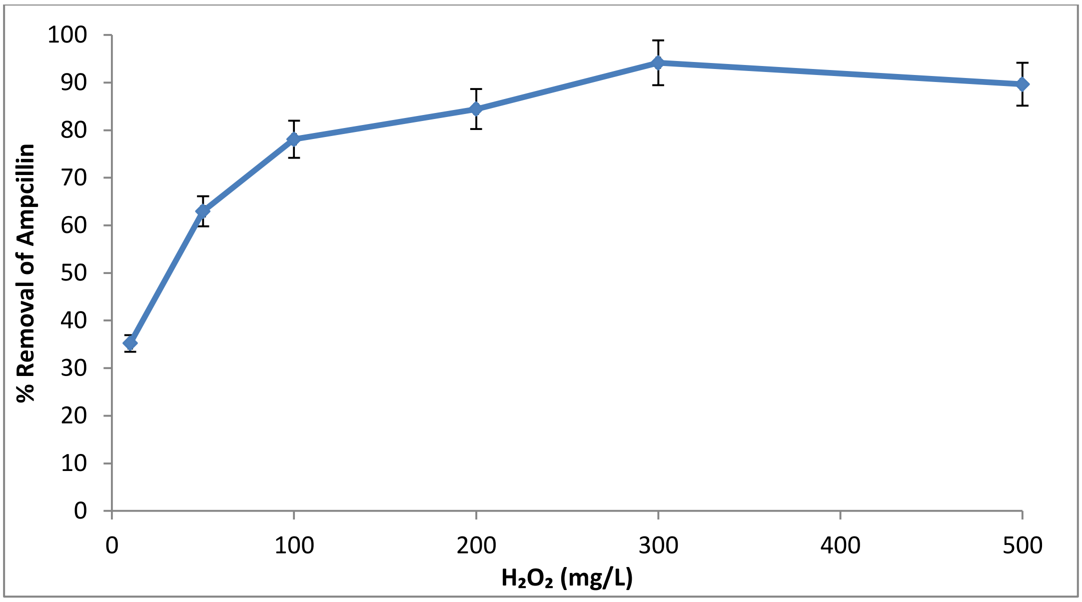

Figure 3.32. Effect of H_2O_2 concentration on removal of ampicillin using photo-Fenton reaction at equilibrium time

(conditions: Amp 100 mg/L; Fe(II) 25 mg/L; pH 3; temperature 25 ˚C; H_2O_2 10-500 mg/L)

As shown in Figure 3.31 and Figure 3.32, percent antibiotics removal increased with increasing H_2O_2 concentration. The H_2O_2 concentration, play a major role in degradation of antibiotics because it is the source of hydroxyl radicals. The maximum removals were occurred at high concentration of H_2O_2 for both amoxicillin and ampicillin antibiotics. Further increase in amount of H_2O_2 cause to decrease degradation of antibiotic due to auto-decomposition of H_2O_2 to oxygen and water and scavenging of $OH^{\cdot}$ by H_2O_2 [77]. The results were in good agreement with reported results at literature [62] [56].

3.3.5. Effect of Initial Antibiotics Concentration

The effect of antibiotic concentration on the removal efficiency, were investigated at different concentrations of amoxicillin and ampicillin.

3.3.5.1. Effect of Initial Antibiotic Concentration on Removal of COD

Figure 3.33 and Figure 3.34 show variations of percent COD removal with different concentrations of amoxicillin and ampicillin. COD degradation percent for amoxicillin after 60 min irradiation was 64.76, 69.97, 84.75, 81.09, 59.22 and 44.43 at amoxicillin concentrations of 10, 25, 50, 100, 200 and 500 mg/L, respectively (Figure 3.33). Similarly, as results show in Figure 3.34, COD degradation percent for ampicillin was 60.8, 67.27, 78.71, 74.37, 54.03 and 44.17 at ampicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively.

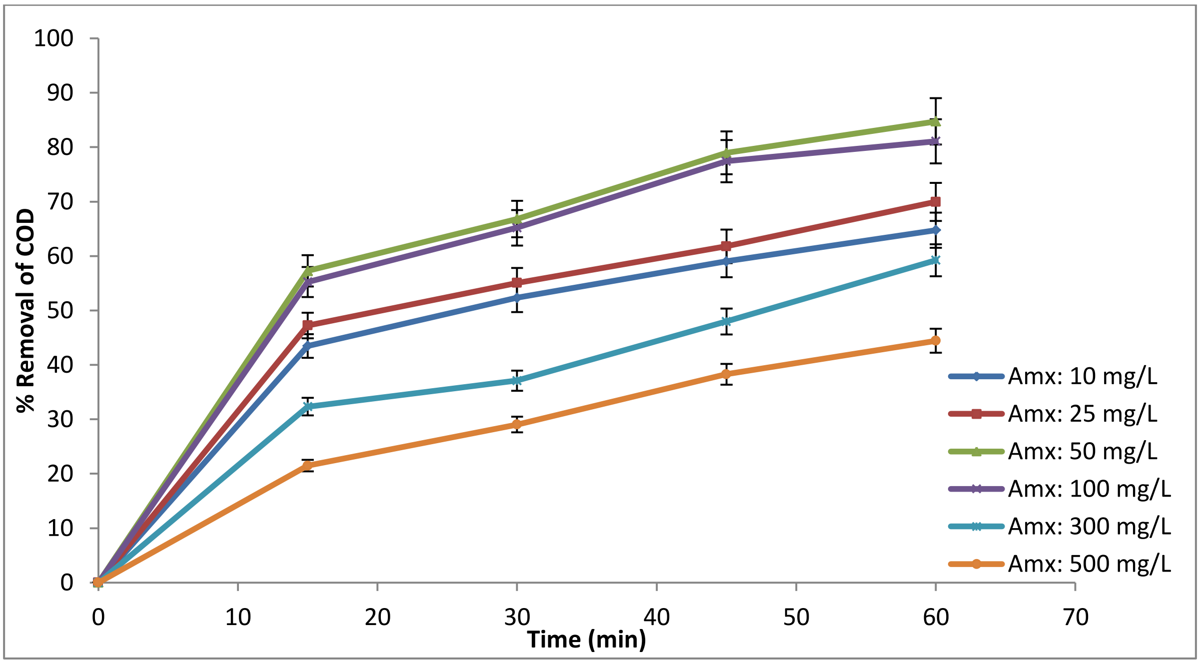

Figure 3.33. Effect of initial antibiotic concentration on removal of COD using Photo-Fenton reaction from solution which contain amoxicillin

(conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amx concentration 10-500 mg/L)

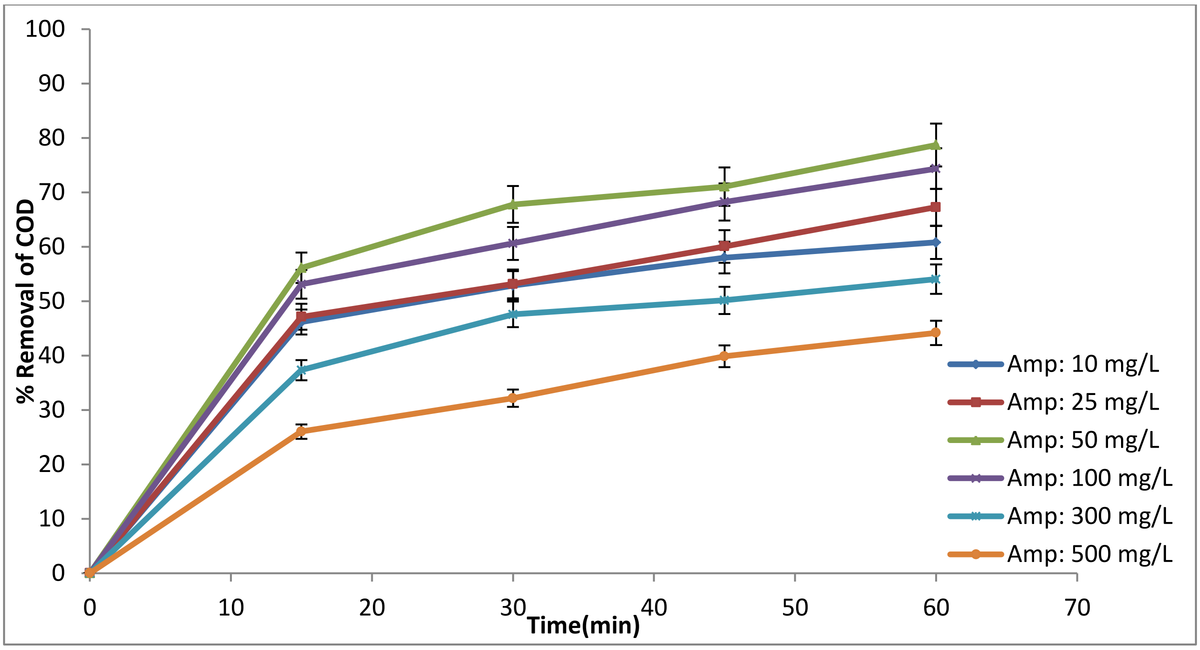

Figure 3.34. Effect of initial antibiotic concentration on removal of COD using Photo-Fenton reaction from solution which contain ampicillin

(conditions: pH 3; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amp concentration 10-500 mg/L)

Effect of initial antibiotics concentrations on COD removal are presented in Figure 3.33 and Figure 3.34. The results indicate that COD degradation increase with antibiotic concentration up to 50 mg/L for both amoxicillin and ampicillin, further increase in antibiotic concentration above 50 mg/L resulted in decrease in COD degradation. The reason of this may be due to limitations by other reactants of H_2O_2 and iron ions [76]. High antibiotic concentrations required high peroxide dose [76].

3.3.5.2. Effect of Initial Antibiotic Concentration on Removal of Antibiotic

Figure 3.35 and Figure 3.36 show variations of percent amoxicillin and ampicillin removal with different antibiotic concentrations, respectively. Amoxicillin degradation percent was 85.54, 87.79, 94.32, 90.86, 82.21 and 79.68 at amoxicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively (Figure 3.35). Whereas, for ampicillin degradation was 81.34, 88.89, 93.83, 90.07 81.11 and 75.68 at ampicillin concentrations of 10, 25, 50, 100, 300 and 500 mg/L, respectively (Figure 3.36).

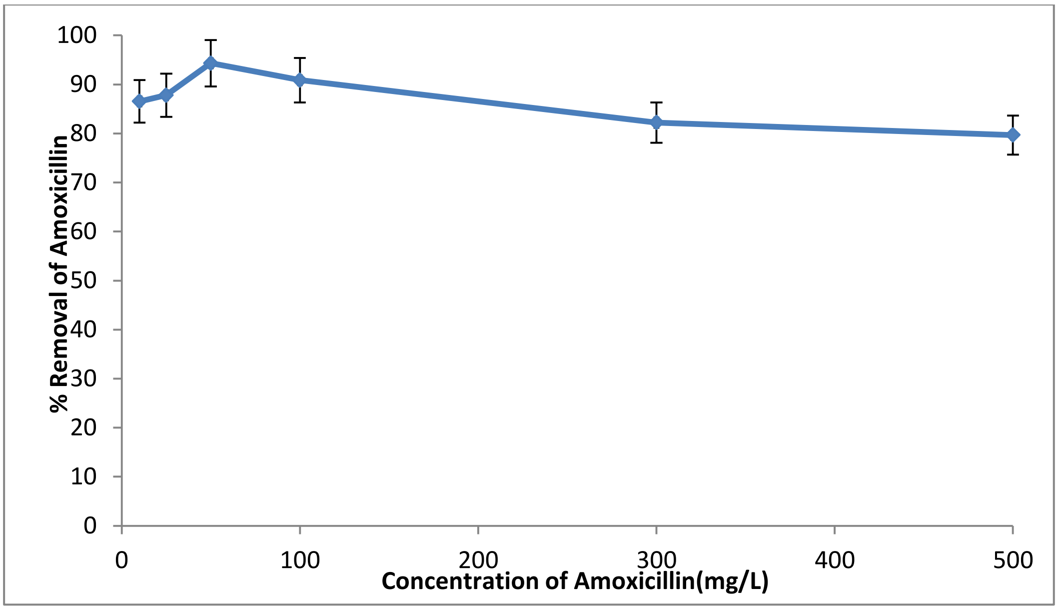

Figure 3.35. Effect of initial antibiotic concentration on removal of amoxicillin using Photo-Fenton reaction at equilibrium time

(conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amx concentration 10-500 mg/L)

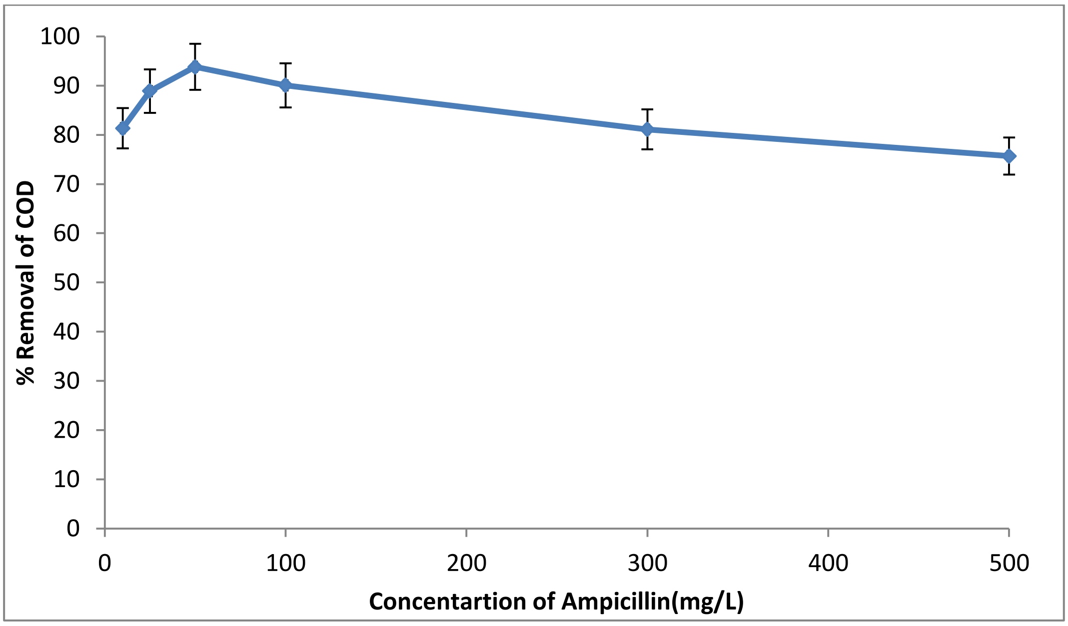

Figure 3.36. Effect of initial antibiotic concentration on removal of ampicillin using Photo-Fenton reaction at equilibrium time

(conditions: pH 3; Fe(II) 25 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; amoxicillin concentration 10-500 mg/L)

Variation of percent amoxicillin and ampicillin removal at different antibiotic doses, are depicted in Figure 3.35 and Figure 3.36. Antibiotic removal also increased with antibiotic concentration up to 50 mg/L for both amoxicillin and ampicillin. Further increases in antibiotic concentration above 50 mg/L resulted in decreases in percent antibiotic removal due to limitations by other reactants of peroxide and Fe(II) [76]. When the initial antibiotic concentrations increase, the hydroxyl radical concentrations remain constant for all antibiotic molecules and hence the removal rate decreases [78] [79].

3.3.6. Effect of Temperature

To determine the effect of temperature on COD and antibiotic degradations, temperature was varied in range of 25-60 ˚C and the other operating conditions were constant.

3.3.6.1. Effect of Temperature on Removal of COD

Variation of percent COD removals with temperature are depicted in Figure 3.37 and Figure 3.38 for amoxicillin and ampicillin, respectively. COD degradation percent for amoxicillin after 60 min reaction was 73.41, 73.95, 74.83, 75.81 and 75.82 at temperature of 25, 30, 40, 50 and 60 ˚C, respectively (Figure 3.37). As results show in Figure 3.38, COD degradation percent for ampicillin was 73.7, 74.11, 74.14, 73.91 and 74.67 at temperature of 25, 30, 40, 50 and 60 ˚C, respectively.

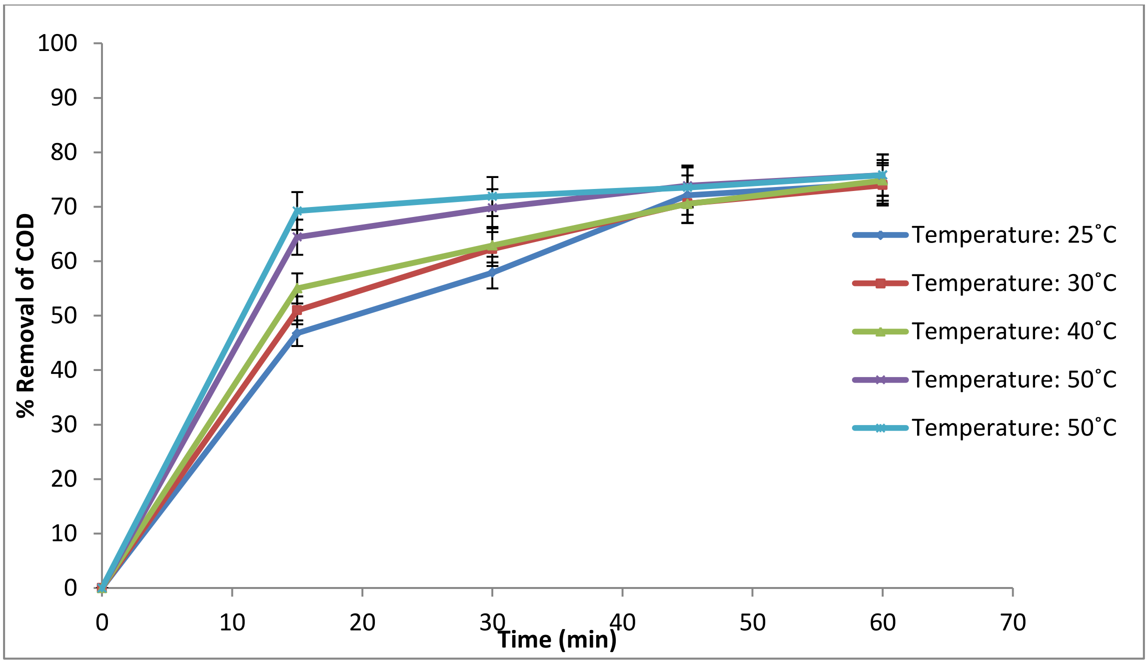

Figure 3.37. Effect of temperature on removal of COD using Photo-Fenton reaction from solution which contain amoxicillin

(conditions: Amx 50 mg/L; Fe(II) 30 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25-60 ˚C)

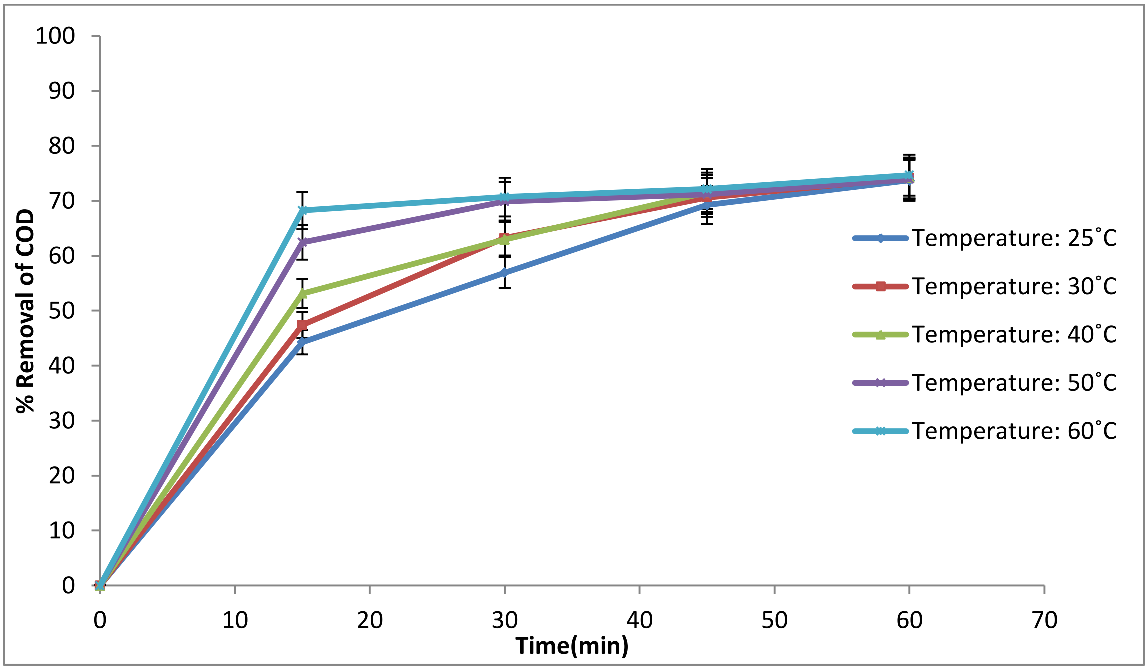

Figure 3.38. Effect of temperature on removal of COD using Photo-Fenton reaction from solution which contain ampicillin

(conditions: Amp 50 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25-60 ˚C)

As results indicated, no significant differences were observed in COD removal for tested temperatures. The reported results show that the temperature of wastewater nearly does not affect the efficiency of COD degradation in Fenton reaction [80]. The results are in line with the findings Tekin. et al., [81].

3.3.6.2. Effect of Temperature on Removal of Antibiotic

Figure 3.39 and Figure 3.40 indicate variations of percent amoxicillin and ampicillin removal at different temperatures, respectively. Amoxicillin degradation percent was 93.14, 92.91, 92.05, 93.15 and 92.83 at temperature 25, 30, 40, 50 and 60˚C respectively (Figure 3.39). Also, ampicillin degradation was 92.14, 90.51, 91.78, 92.65 and 92.83 at temperature 25, 30, 40, 50 and 60˚C respectively (Figure 3.40).

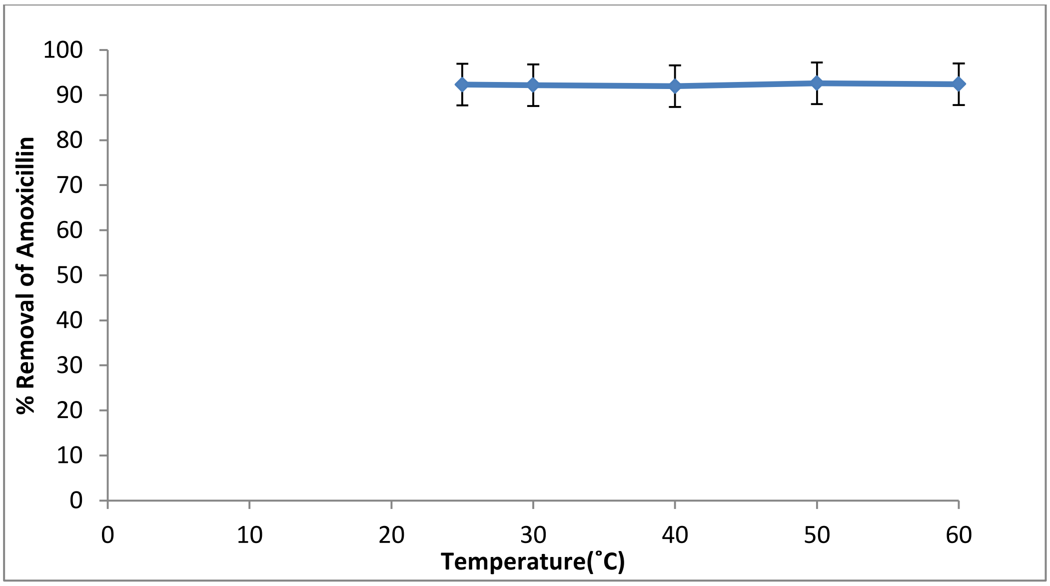

Figure 3.39. Effect of temperature on removal of amoxicillin using Photo-Fenton at equilibrium time

(conditions: Amx 50 mg/L; Fe(II) 30 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25-60 ˚C)

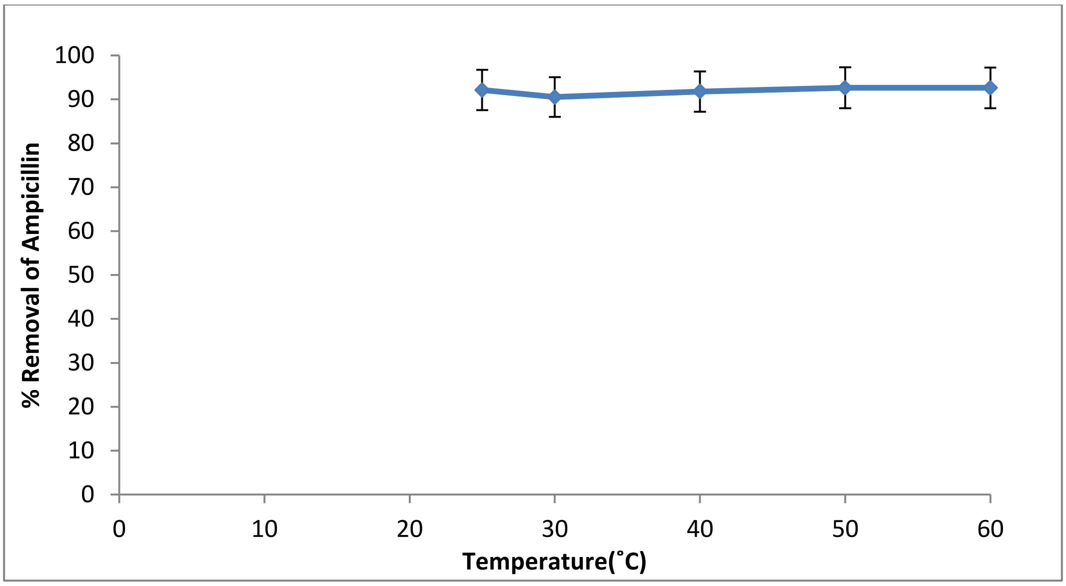

Figure 3.40. Effect of temperature on removal of ampicillin using Photo-Fenton at equilibrium time

(conditions: Amp 50 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25-60 ˚C)

As results indicated, no significant differences were observed in antibiotic removal for tested temperatures for both amoxicillin and ampicillin. The reported results show that the temperature of wastewater nearly does not affect the efficiency of degradation in Fenton reaction [80]. The finding results are in good agreement with findings Tekin et al., [81].

3.4. STATISTICAL ANALYSIS FOR PHOTO-FENTON OXIDATION

Table 3-3- One-way ANOVA for COD removal of Amoxicillin by Photo-Fenton oxidation

Factor	Average		Standard Deviation	F ratio	P value
pH	2	75.4833	0.786469	**911.28**	**0.009**
	3	83.38	1.35303		
	4	71.9833	0.645781		
	5	65.2467	0.840555		
	6	54.29	0.75		
	7	41.0933	0.732006		
Fe^{2+} (mg/L)	2	40.56	0.592706	**480.76**	**0.0000**
	5	44.83	0.713583		
	10	55.2267	0.945639		
	15	56.1733	0.816844		
	30	70.2033	0.846896		
	60	64.4933	1.26895		
	150	40.56	0.846896		
H_2O_2 (mg/L)	10	22.95	2.44624	**590.16**	**0.0089**
	50	44.1733	1.42732		
	100	60.7167	1.78889		
	200	70.3967	1.05249		
	300	82.5333	1.155		
	500	72.7333	0.971665		
Antibiotic (mg/L)	10	64.1	1.75565	**404.07**	**0.000**
	25	70.5067	0.65041		
	50	84.5067	1.06604		
	100	79.8867	1.48291		
	300	59.6633	0.683106		
	500	43.92	1.52056		
Temperature(˚C)	25	72.58	1.61577	**3.25**	**0.0597**
	30	73.15	0.943186		
	40	74.5167	0.74144		
	50	75.1633	0.750755		
	60	74.7167	1.00684		

Table 3-1 shows the Analysis of Variance (ANOVA) test results for removal of COD by Photo-Fenton oxidation for amoxicillin. ANOVA analysis performed on the results presented that, COD removal was significantly affected by pH, Fe(II) concentration, H_2O_2 concentration and initial antibiotic concentration, because the P-value is less than 0.05 at the 95% confidence level. The results are in agreement with Elmolla et.al; [56] and Ay et.al; [76]. A statistical analysis (one-way ANOVA) for temperature results indicated that, COD removal was not significantly affected by temperature because the P-value is greater than 0.05 at the 95% confidence level.

Table 3-4- One-way ANOVA for COD removal of Ampicillin by Photo-Fenton oxidation

Factor	Average		Standard Deviation	F ratio	P value
pH	2	61.61	1.48647	**148.02**	**0.0000**
	3	72.5333	2.06969		
	4	67.02	1.68057		
	5	58.1967	1.16749		
	6	52.3933	1.13059		
	7	41.9233	1.54765		
Fe^{2+} (mg/L)	2	40.1533	1.11015	**579.41**	**0.0000**
	5	44.38	1.2105		
	10	54.3933	0.870766		
	15	69.1433	1.01894		
	25	81.1033	0.894893		
	50	71.3033	1.13509		
	100	64.0933	1.22871		
H_2O_2 (mg/L)	10	24.6967	1.30286	**738.35**	**0.0000**
	50	45.2433	1.10151		
	100	56.6633	1.50151		
	200	66.26	1.14582		
	300	79.9567	1.45198		
	500	70.2233	1.00759		
Antibiotic (mg/L)	10	64.0733	1.71631	**369.28**	**0.0000**
	25	68.4967	1.38944		
	50	83.2833	1.34098		
	100	80.28	0.736682		
	300	58.2933	0.940284		
	500	42.9567	1.62731		
Temperature(˚ C)	25	70.3967	5.0028	**1.27**	**0.3458**
	30	72.6467	1.21698		
	40	73.0467	1.55217		
	50	74.2367	1.51837		
	60	74.5833	1.09144		

Table 3-1 shows the Analysis of Variance (ANOVA) test results for removal of COD by Photo-Fenton oxidation for ampicillin. ANOVA analysis performed on the results presented that, COD removal was significantly affected by pH, Fe(II) concentration, H_2O_2 concentration and initial antibiotic concentration, because the P-value is less than 0.05 at the 95% confidence level. The results are in agreement with Elmolla et.al; [56]. A statistical analysis (one-way ANOVA) for temperature results indicated that, COD removal was not significantly affected by temperature because the P-value is greater than 0.05 at the 95% confidence level.

3.5. INDIVIDUAL CONTRIBUTION OF CONTROL PARAMETERS ON DEGRADATION OF ANTIBIOTICS

To evaluate the efficiency of each condition on the antibiotic removal, experiments were designed under these conditions: (1) antibiotic + Fe(II), (2) antibiotic + H_2O_2, (3) antibiotic + UV, (4) antibiotic + UV+ H_2O_2, (5) antibiotic + UV+ Fe(II), (6) antibiotic+ Fe(II)+ H_2O_2 (Fenton process), (7) antibiotic+ UV+ Fe(II)+ H_2O_2 (Photo-Fenton process). Figure 3.41 and Figure 3.42 show the results of COD degradation for both amoxicillin and ampicillin, respectively.

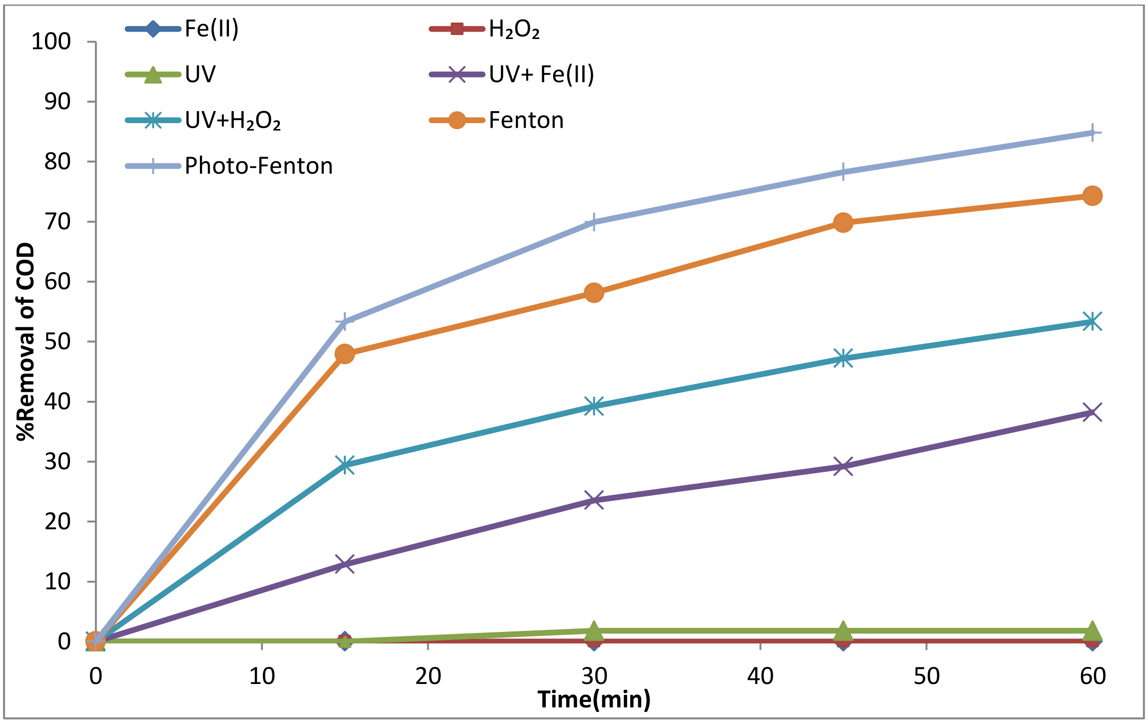

Figure 3.41. COD degradation of amoxicillin under different conditions (conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Amoxicillin 100 mg/L)

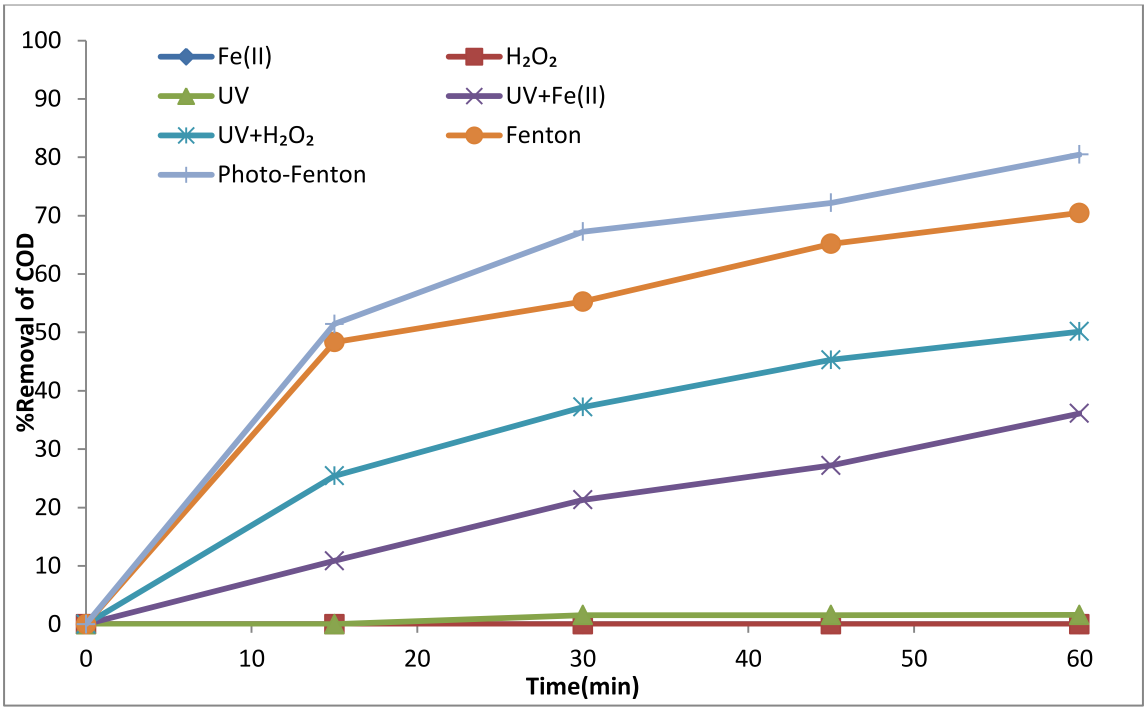

Figure 3.42. COD degradation of ampicillin under different conditions (conditions: pH 3; Fe(II) 30 mg/L; H_2O_2 300 mg/L; temperature 25 ˚C; Ampicillin 100 mg/L)

As results show the efficiency of COD removal for photo-Fenton process is more than other processes. The relative efficiencies of these processes are in the following order: UV+ Fe(II)+ H_2O_2 (Photo-Fenton) > Fe(II)+ H_2O_2 (Fenton process) > UV+ H_2O_2 > UV+ Fe(II) > UV > H_2O_2 and Fe(II).

3.6. ANTIBIOTIC ANALYSIS USING HPLC

Some of samples were chosen to antibiotic analysis by HPLC. For this purpose, Agilent 1100 Series High Performance Liquid Chromotography (HPLC) was used.

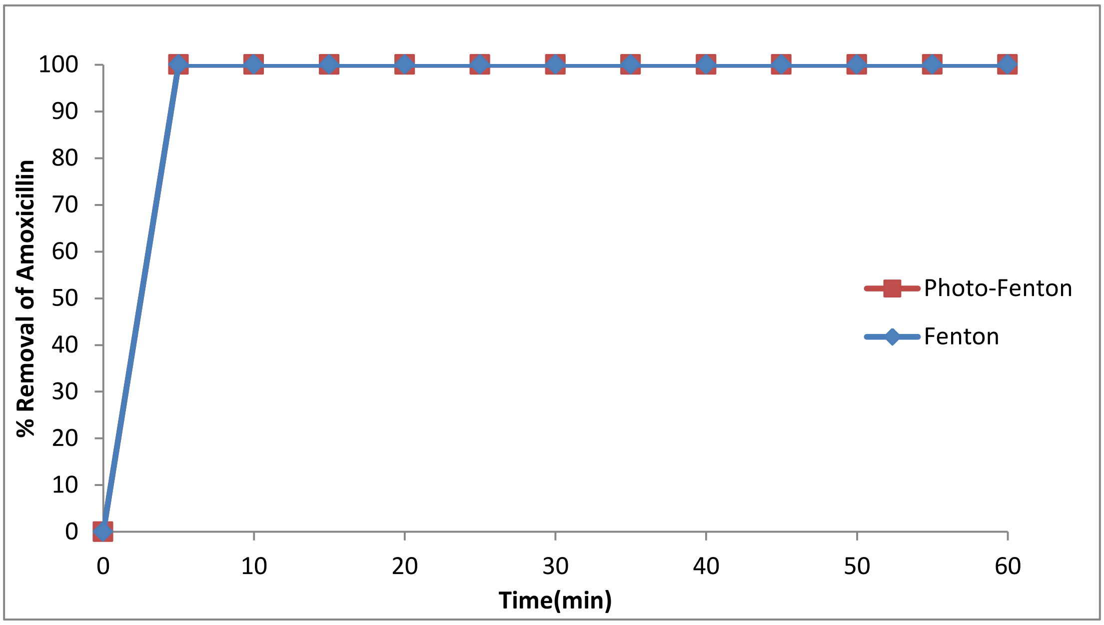

Figure 3.43. Variation of Amoxicillin removal with time in Fenton and Photo-Fenton oxidation under optimum conditions based on HPLC

(conditions: Amx 50 mg/L; Fe(II) 30 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C)

Under optimum conditions, amoxicillin analysis was determined by High Performance Liquid Chromotography (HPLC). As results show complete degradation of amoxicillin was occurred in 5min of reaction for both Fenton and Photo-Fenton oxidation (Figure 3.41).

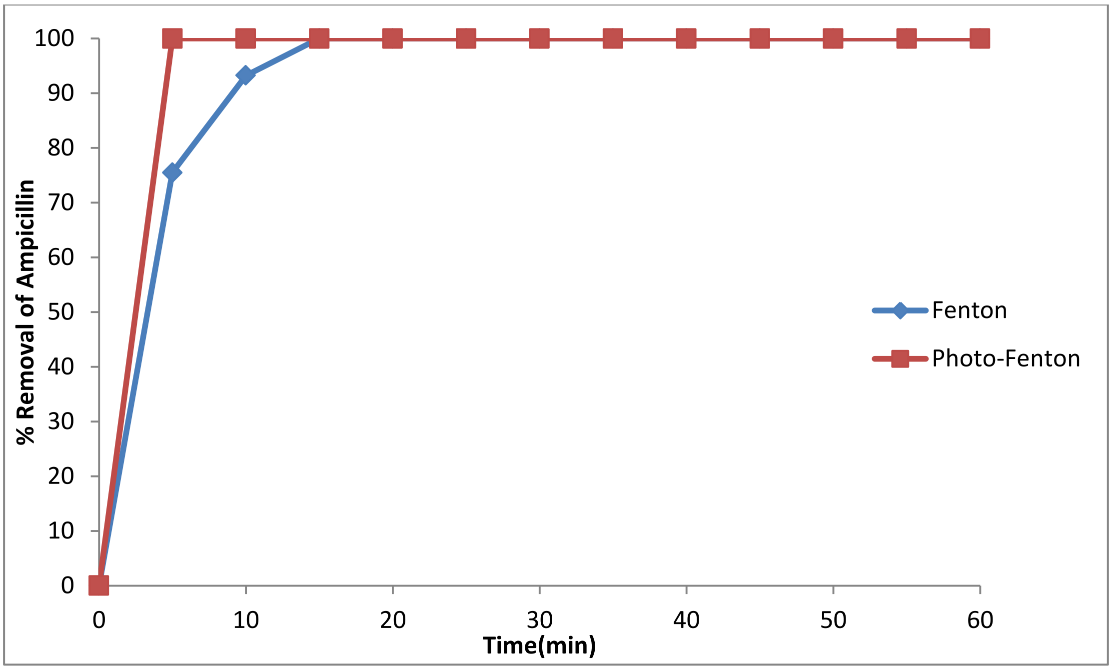

Figure 3.44. Variation of Ampicillin removal with time in Fenton and Photo-Fenton oxidation under optimum conditions based on HPLC

(conditions: Amp 50 mg/L; Fe(II) 25 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C)

Under optimum conditions, ampicillin analysis was determined by High Performance Liquid Chromotography (HPLC). As seen in Figure 3.42 complete degradation of ampicilin was occurred in 15min and 5min of reaction for Fenton and Photo-Fenton oxidation, respectively.

To compare the results of antibiotics removal that determined with UV/VIS, some of the samples were chosen to antibiotic removal analysis by HPLC. As Figure 3.43 indicates, the results that determined by UV/VIS are in high correlation with antibiotic analysis by HPLC.

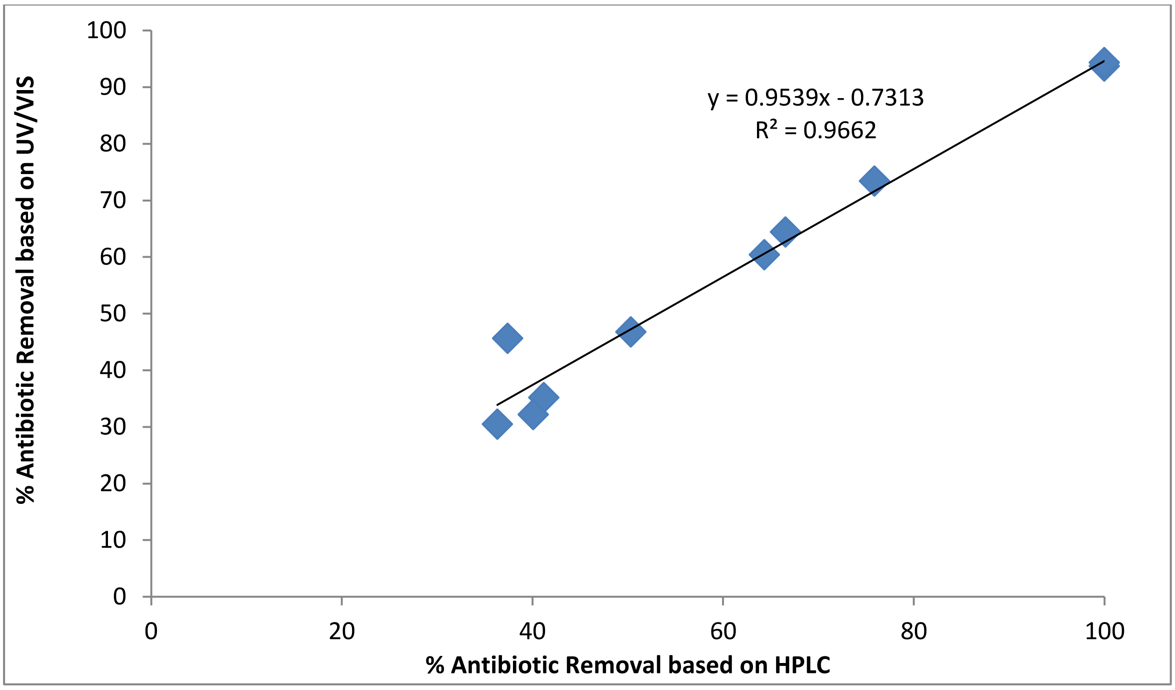

Figure 3.45. The correlation between HPLC and UV/VIS in determining %Antibiotic removal

Table 3-5- Some important results of the literature studies as compared to this study

Reference	***Target Drug***	***Treatment Methods***	***Summary of Results***
Elmolla and Chaudhuri (2009)	Amoxicillin (500mg/L)	Photo-Fenton	The maximum biodegradability ratio (>0.40) was achieved at H_2O_2 /COD molar ratio=2, H_2O_2/Fe^{2+}molar ratio=50 and pH=3-3.5, after 30-45 min of reaction. Under these conditions, complete degradation was achieved in 1 min (TOC removal=71%)
Rozas et al.(2010)	Ampicillin (20mg/L)	Fenton Photo - Fenton	Under the optimized conditions (pH=3.7, 87mM Fe^{2+}, 373mM H_2O_2 for Fenton and pH=3.5, 87mM Fe^{2+}, 454mM H_2O_2 for photo-Fenton) a complete degradation was reached. –It was achieved a higher mineralization (50%TOC removal) with photo-Fenton than Fenton (20%TOC removal). –The degradation products not present in antibacterial activity
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.
Elmollaand Chaudhuri (2009)	Ampicillin(105mg/L) Cloxacillin(103mg/L) Amoxicillin(104mg/L)	Fenton	Under the optimal conditions (H_2O_2/Fe^{2+} molar ratio=10, pH=3) it was achieved the complete degradation of the antibioticsin2min. –The biodegradability was improved from 0 to 0.37 in 10 min and COD and DOC degradation were 81.4% and 54.3%, respectively in 60min.

This Study	Amoxicillin(50 mg/L) Ampicillin (50 mg/L)	Fenton and Photo-Fenton	Complete antibiotic removal and nearly 84% COD removals were achieved in our studies using Fenton and photo-Fenton oxidation within 60 min.

3.7. AMOXICILLIN AND AMPICILLIN DEGRADATION KINETICS

The model of kinetic that used for this study was zero order, first order and second order. The results were shown in Table 3-6. The results were fitted to pseudo-first order kinetic.

The model of kinetic that use for oxidation of amoxicillin and ampicillin antibiotics by Fenton and photo-Fenton can be described as pseudo-first order rate equation as follow [85]:

$$\ln\left(\frac{COD_o}{CODt}\right) = K \times t$$

Where, COD_o and COD_t are the amount of COD at beginning of reaction and time t, respectively. The K is the observed rate constant (min $^{-1}$).

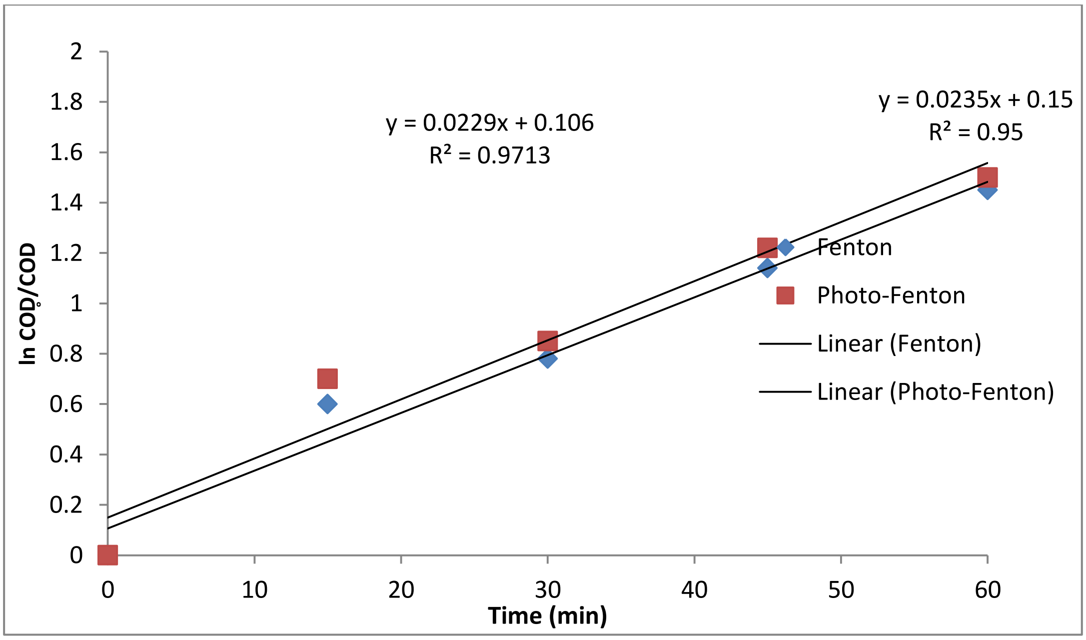

Figure 3.46. Pseudo-first order kinetic model plot of Fenton and Photo-Fenton oxidation for amoxicillin removal

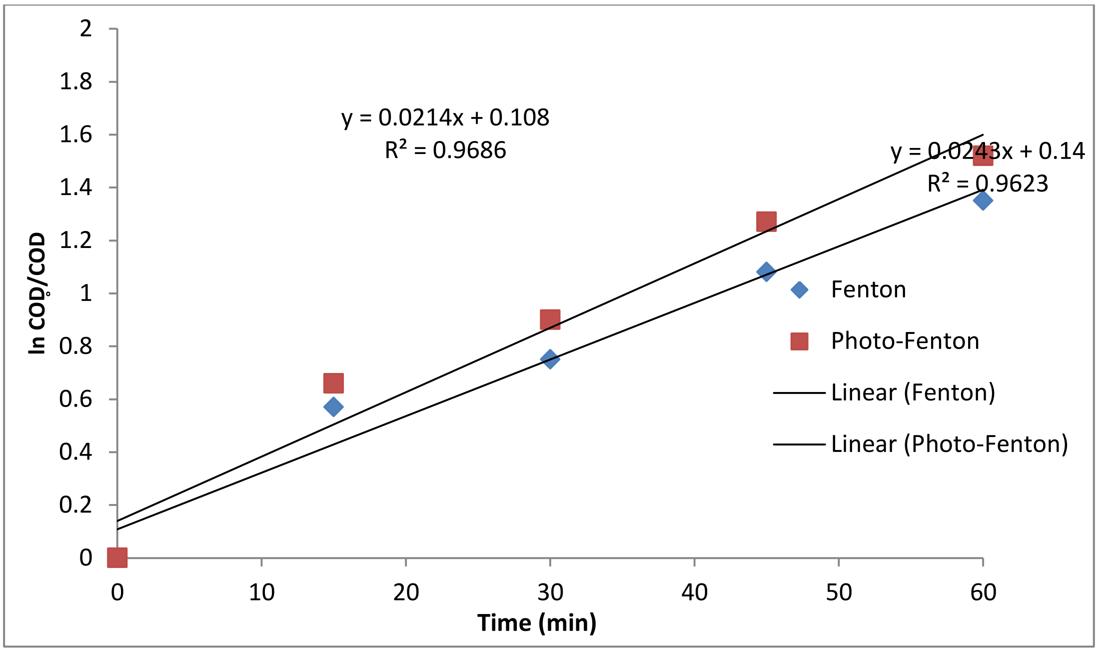

Figure 3.47. Pseudo-first order kinetic model plot of Fenton and Photo-Fenton oxidation for ampicillin removal

Figure 3.41 and Figure 3.42 indicate the value of pseudo-first order kinetic constant for amoxicillin and ampicillin under optimum conditions. Rate constant K, was obtained by fitting the experimental data to a straight line. The results for both antibiotics are shown in Table 3.9. As results indicate photo-Fenton process had the higher k to compare with Fenton process. This may be due to photochemical regeneration of Fe^{2+} ions by photo reduction of Fe^{3+} ions in photo-Fenton process and hence increase hydroxyl radical production rate [86].

Table 3-6 Kinetic rate constants for antibiotics under their best conditions

Process	**K_0 Zero order(M min $^{-1}$)**	**R^2 Zero order**	**K (min $^{-1}$) First order**	**R^2 first order**	**K (M^{-1}min $^{-1}$) Second order**	**R^2 Second order**	**Antibiotic**
Fenton	0.02	0.79	0.022	0.97	0.001	0.9	Amoxicillin
Photo-Fenton	0.021	0.8	0.023	0.95	0.011	0.91	Amoxicillin
Fenton	0.019	0.81	0.021	0.96	0.002	0.87	Ampicillin
Photo-Fenton	0.02	0.83	0.024	0.96	0.013	0.88	Ampicillin

3.8. REMOVAL OF ANTIBIOTICS BY USING Fe_2O_3 MAGNETITE PARTICLES IN FENTON AND PHOTO-FENTON OXIDATION

We used Fe_2O_3 magnetite particles as catalyst in Fenton and photo-Fenton oxidations. The experiments were carried out under optimum conditions that were found for amoxicillin and ampicillin by Fenton and Photo-Fenton oxidations.

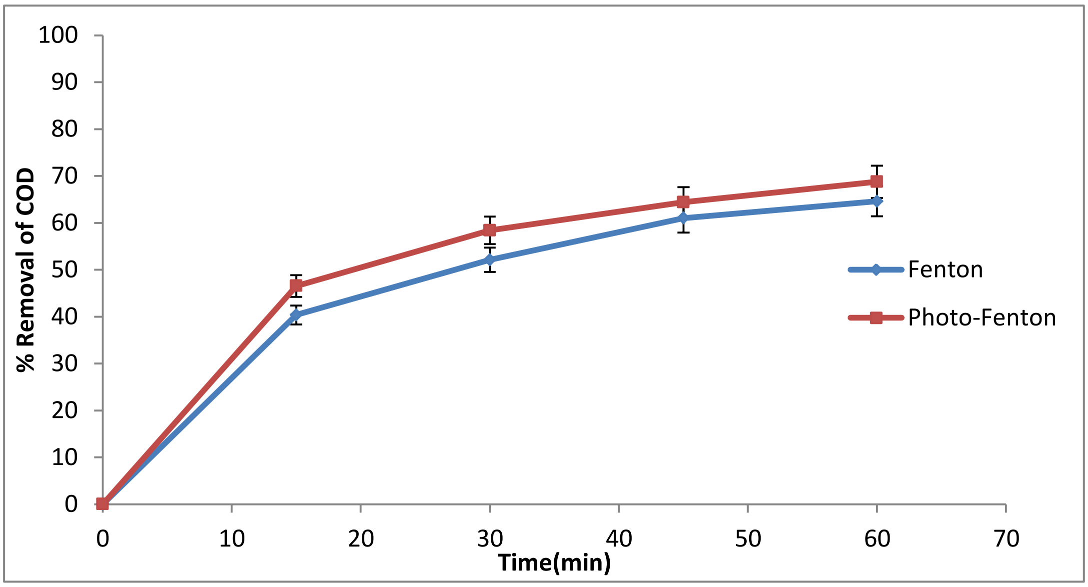

Figure 3.48. Effect of Fe_2O_3 particles on removal of COD using Fenton and Photo-Fenton reaction from solution which contain amoxicillin

(conditions: Amx 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe_2O_3 30 mg/L

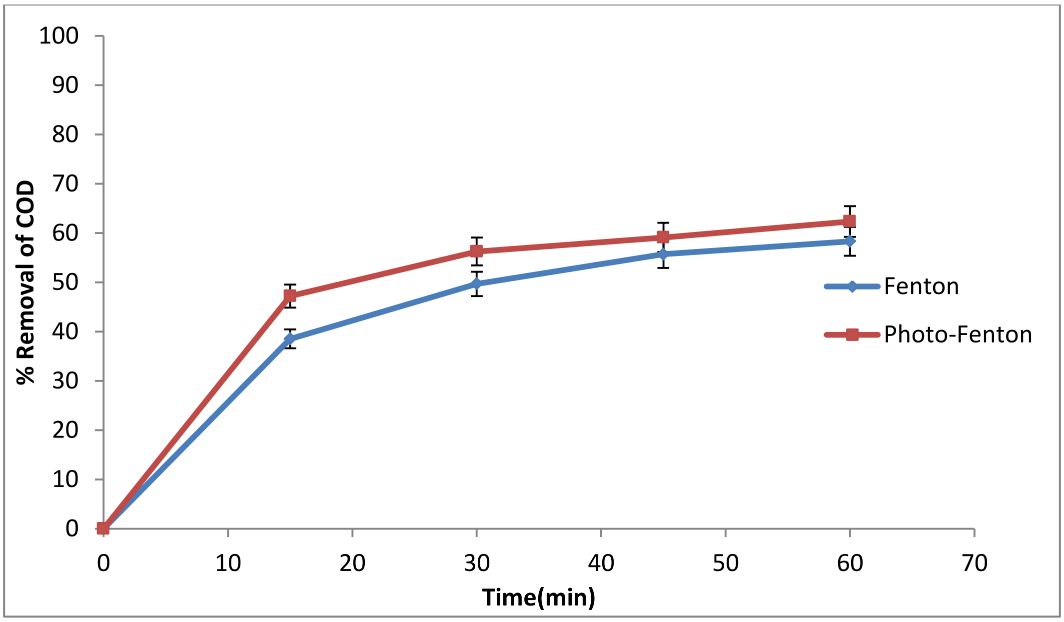

Figure 3.49. Effect of Fe_2O_3 particles on removal of COD using Fenton and Photo-Fenton reaction from solution which contain ampicillin

(conditions: Amp 100 mg/L; H_2O_2 300 mg/L; pH 3; temperature 25 ˚C; Fe_2O_3 25 mg/L)

Figure 3.48 and Figure 3.49 show the percent COD removal for amoxicillin and ampicillin, respectively. These experiments carried out under optimum conditions by Fenton and Photo-Fenton oxidation with using Fe_2O_3 particles as catalyst. As results show the percentage of COD removal decreased when we use Fe_2O_3 particles as catalyst in Fenton and Photo-Fenton for both antibiotics under optimum conditions.

4. CONCLUSION

In this study, the degradation of amoxicillin and ampicillin (penicillin group) antibiotics by Fenton and Photo-Fenton oxidation were investigated. It has been found that Fenton and Photo-Fenton process is effective in treatment of aqueous amoxicillin and ampicillin solutions. Antibiotics and COD measurements were carried out to determine the most effective conditions. The optimum condition at the concentration of 50 mg/L amoxicillin was found as pH 3, Fe^{2+} 30 mg/L, H_2O_2 300 mg/L. Under these conditions the removal of COD and amoxicillin was determined as 76.8% and 100% for Fenton reaction and 84.81% and 100% for photo-Fenton reaction, respectively. The optimum condition at the concentration of 50 mg/L ampicillin was found as pH 3, Fe^{2+} 25 mg/L, H_2O_2 300 mg/L. Under these conditions the removal of COD and ampicillin was determined as 72.67% and 100% for Fenton reaction and 78.71% and 100% for photo-Fenton reaction, respectively.

Under these optimized conditions complete removal of amoxicillin achieve within 5 minutes for both Fenton and photo-Fenton process. In addition, under best conditions for ampicillin, complete removal of ampicillin occurs in 15 and 5 min of reaction for Fenton and photo-Fenton reaction, respectively.

Both methods are proven to be effective for amoxicillin and ampicillin degradation. However photo-Fenton process is most effective for COD degradation and photo-Fenton process indicated higher rate constant than Fenton process.

Fenton and Photo-Fenton can be used for pretreatment of amoxicillin and ampicillin antibiotics wastewater for biological treatment. We can achieve complete removal of amoxicillin and ampicillin by Fenton and Photo-Fenton methods. So, removal of antibiotics was not a problem, but some intermediates were produced during Fenton and Photo-Fenton reaction which could not be removed by methods that investigated in this study. We can say that mineralization of amoxicillin and ampicillin was not complete by Fenton and Photo-Fenton oxidation. But as results show Photo-Fenton method improved COD removal compared with Fenton method.

Recommended studies in this field for future research can be summarized as follows:

- A combined oxidation process can be used; such as photo-Fenton by peroxone (peroxide-ozone) treatment,
- To improve the effect of photo-Fenton method, different light intensities or different wavelenghts of UV light can be investigated,
- As an alternative, modified Fenton or electro-Fenton methods can be investigated.

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Row	Amx (mg/L)	Fe (mg/L)	H_2O_2 (mg/L)	pH	Temperature (˚C)	%COD Removal after 60 min	%Amx Removal after 60 min	%Amx Removal(HPLC)	Method
1	100	30	300	2	25	65.12	86.65		Fenton
2	100	30	300	3	25	74.28	92.23		Fenton
3	100	30	300	4	25	62.01	86.12		Fenton
4	100	30	300	5	25	57.32	72.42		Fenton
5	100	30	300	6	25	49.73	69.31		Fenton
6	100	30	300	7	25	38.94	60.41	64.32 (30 min)	Fenton
7	100	2	300	3	25	37.87	55.87		Fenton
8	100	5	300	3	25	43.39	61.39		Fenton
9	100	10	300	3	25	52.12	72.13		Fenton
10	100	15	300	3	25	65.31	87.31		Fenton
11	100	30	300	3	25	75.28	92.18		Fenton
12	100	60	300	3	25	70.3	86.6		Fenton
13	100	150	300	3	25	62.6	77.3		Fenton
14	100	30	10	3	25	21.02	32.19	40.08 (60 min)	Fenton
15	100	30	50	3	25	42.11	62.54		Fenton
16	100	30	100	3	25	57.37	78.37		Fenton
17	100	30	200	3	25	62.43	83.54		Fenton
18	100	30	300	3	25	73.44	92.84		Fenton
19	100	30	500	3	25	67.14	88.14		Fenton
20	10	30	300	3	25	60.4	85.54		Fenton
21	25	30	300	3	25	66.97	88.79		Fenton
22	50	30	300	3	25	76.81	94.32	100 (15 min)	Fenton
23	100	30	300	3	25	73.87	91.86		Fenton
24	300	30	300	3	25	53.21	81.21		Fenton
25	500	30	300	3	25	40.67	77.68		Fenton
26	50	30	300	3	25	73.61	92.34		Fenton
27	50	30	300	3	30	73.95	92.21		Fenton

28	50	30	300	3	40	74.93	91.98		Fenton
29	50	30	300	3	50	75.81	92.65		Fenton
30	50	30	300	3	60	75.83	92.43		Fenton
31	100	30	300	2	25	73.37	90.25		Photo-Fenton
32	100	30	300	3	25	84.81	93.27		Photo-Fenton
33	100	30	300	4	25	70.61	89.32		Photo-Fenton
34	100	30	300	5	25	65.4	76.42		Photo-Fenton
35	100	30	300	6	25	54.29	72.31		Photo-Fenton
36	100	30	300	7	25	41.21	65.42		Photo-Fenton
37	100	2	300	3	25	40.03	58.85		Photo-Fenton
38	100	5	300	3	25	45.61	69.73		Photo-Fenton
39	100	10	300	3	25	55.39	76.14		Photo-Fenton
40	100	15	300	3	25	70.04	87.31		Photo-Fenton
41	100	30	300	3	25	84.32	92.98		Photo-Fenton
42	100	60	300	3	25	74.43	88.6		Photo-Fenton
43	100	150	300	3	25	65.88	79.3		Photo-Fenton
44	100	30	10	3	25	23.4	37.39	45.61(60 min)	Photo-Fenton

45	100	30	50	3	25	45.51	65.94		Photo-Fenton
46	100	30	100	3	25	62.73	82.17		Photo-Fenton
47	100	30	200	3	25	71.53	87.43		Photo-Fenton
48	100	30	300	3	25	82.94	93.14		Photo-Fenton
49	100	30	500	3	25	73.84	90.65		Photo-Fenton
50	10	30	300	3	25	60.8	86.54		Photo-Fenton
51	25	30	300	3	25	67.27	87.79		Photo-Fenton
52	50	30	300	3	25	78.71	94.32	100 (15 min)	Photo-Fenton
53	100	30	300	3	25	74.37	90.86		Photo-Fenton
54	300	30	300	3	25	54.03	82.21		Photo-Fenton
55	500	30	300	3	25	44.17	79.68		Photo-Fenton
56	50	30	300	3	25	74.31	93.14		Photo-Fenton
57	50	30	300	3	30	73.95	92.91		Photo-Fenton
57	50	30	300	3	40	74.83	92.05		Photo-Fenton
59	50	30	300	3	50	75.81	93.15		Photo-Fenton
60	50	30	300	3	60	75.83	92.83		Photo-

Row	Amp(mg/L)	Fe (mg/L)	H_2O_2 (mg/L)	pH	Temperature (˚C)	%COD Removal 60 min	%Amp Removal after 60 min	%Amp Removal(HPLC)	Method
1	100	25	300	2	25	65.54	85.45		Fenton
2	100	25	300	3	25	68.22	91.87		Fenton
3	100	25	300	4	25	62.81	80.32		Fenton
4	100	25	300	5	25	58.91	73.42		Fenton
5	100	25	300	6	25	52.11	70.31		Fenton
6	100	25	300	7	25	47.21	64.42	66.54 (30 min)	Fenton
7	100	2	300	3	25	32.77	56.77		Fenton
8	100	5	300	3	25	40.49	60.69		Fenton
9	100	10	300	3	25	48.22	75.13		Fenton
10	100	15	300	3	25	60.61	86.41		Fenton
11	100	25	300	3	25	71.28	92.88		Fenton
12	100	50	300	3	25	64.13	87.5		Fenton
13	100	100	300	3	25	56.06	74.39		Fenton
14	100	25	10	3	25	16.72	30.49	36.32 (60 min)	Fenton
15	100	25	50	3	25	38.11	59.64		Fenton
16	100	25	100	3	25	50.37	74.17		Fenton
17	100	25	200	3	25	58.43	80.64		Fenton
18	100	25	300	3	25	70.46	90.79		Fenton
19	100	25	500	3	25	62.04	86.04		Fenton
20	10	25	300	3	25	58.8	84.34		Fenton
21	25	25	300	3	25	64.27	88.19		Fenton
22	50	25	300	3	25	73.81	93.7	100 (15 min)	Fenton
23	100	25	300	3	25	71.37	91.06		Fenton
24	300	25	300	3	25	51.03	80.11		Fenton
25	500	25	300	3	25	40.17	74.68		Fenton
26	50	25	300	3	25	73.61	92.44		Fenton

27	50	25	300	3	30	73.95	92.51		Fenton
28	50	25	300	3	40	74.04	91.98		Fenton
29	50	25	300	3	50	73.81	92.25		Fenton
30	50	25	300	3	60	74.63	92.83		Fenton
31	100	25	300	2	25	63.17	89.25		Photo-Fenton
32	100	25	300	3	25	74.18	94.17		Photo-Fenton
33	100	25	300	4	25	68.16	90.32		Photo-Fenton
34	100	25	300	5	25	59.82	72.42		Photo-Fenton
35	100	25	300	6	25	53.11	70.31		Photo-Fenton
36	100	25	300	7	25	43.32	65.42		Photo-Fenton
37	100	2	300	3	25	40.03	57.85		Photo-Fenton
38	100	5	300	3	25	45.61	70.13		Photo-Fenton
39	100	10	300	3	25	55.39	75.24		Photo-Fenton
40	100	15	300	3	25	69.04	86.31		Photo-Fenton
41	100	25	300	3	25	81.32	92.88		Photo-Fenton
42	100	50	300	3	25	72.43	88.62		Photo-Fenton
43	100	100	300	3	25	65.18	78.3		Photo-Fenton
44	100	25	10	3	25	25.72	35.19	41.2 (60 min)	Photo-

									Fenton
45	100	25	50	3	25	44.11	62.94		Photo-Fenton
46	100	25	100	3	25	55.37	78.07		Photo-Fenton
47	100	25	200	3	25	67.43	84.43		Photo-Fenton
48	100	25	300	3	25	80.46	94.14		Photo-Fenton
49	100	25	500	3	25	70.04	89.65		Photo-Fenton
50	10	25	300	3	25	60.8	81.34		Photo-Fenton
51	25	25	300	3	25	67.27	88.89		Photo-Fenton
52	50	25	300	3	25	78.71	93.82	100 (15 min)	Photo-Fenton
53	100	25	300	3	25	74.37	90.06		Photo-Fenton
54	300	25	300	3	25	54.03	81.11		Photo-Fenton
55	500	25	300	3	25	44.17	75.68		Photo-Fenton
56	50	25	300	3	25	74.31	92.14		Photo-Fenton
57	50	25	300	3	30	73.95	90.51		Photo-Fenton
57	50	25	300	3	40	74.83	91.78		Photo-Fenton
59	50	25	300	3	50	75.81	92.65		Photo-Fenton

60	50	25	300	3	60	75.83	92.63		Photo-Fenton

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- Public Relations and Marketing Assistance – Alp Sigorta – Ankara – Turkey (Sep. 2012 - Dec. 2012).
- Sales Manager – Cheap Tour – Ankara – Turkey (Sep. 2011- Aug. 2012)
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Areas of Experience

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Projects and Budgets

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Publications

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Oral and Poster Presentations

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