OXIDATIVE TREATMENT OF ANTIBIOTICS IN PHARMACEUTICAL EFFLUENTS

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ABSTRACT

Antibiotics are intensively used both in veterinary and human medicine and up to 90 per cent they are excreted through urine and feces into municipal sewage after administration. They can also enter to the sewage treatment plants through the wastewater generated from the formulation process in pharmaceutical industry. Since antibiotic containing effluents are inert towards conventional biotreatment, antibiotics may reach to the aquatic environment and thus such effluents require an oxidative pretreatment.

Cephalosporine (I) and penicillin (II) group human antibiotics and quinolone group veterinary antibiotic in synthetically prepared pharmaceutical formulation effluents were treated by ozonation in order to improve their biodegradability. The effects of pH, initial antibiotic concentration, dose of hydrogen peroxide and applied ozone doses were investigated on treatment performance.

Elevating the reaction pH, inlet ozone concentration and hydrogen peroxide dose resulted in an enhancement on treatment efficiencies of all antibiotic formulation effluents.

While in the veterinary antibiotic effluent BOD_5/COD ratio was increased from 0.077 to 0.38 with an applied O_3 dose of 2960 mg/L h, at pH = 7, for human antibiotic I and human antibiotic II, BOD_5/COD ratio was increased from 0 to 0.1 and 0.27 respectively. In order to correlate biodegradability results with a structure of antibiotics, degradation rates of antibiotics were related with energies required for the degradation by H-abstraction.

Combination of ozone with UV, accelerated aromaticity removal from 63 per cent to 83 per cent, and oxygen uptake rate from 0.21 mg/L min to 0.28 mg/L min for veterinary antibiotic (COD_i = 900 mg/L), however, did not appear to be more effective than applying mere ozonation in terms of COD removal rates.

ÖZET

İnsan ve veteriner amaçlı kullanılan antibiyotiklerin yüzde 90'ı kullanıldıktan sonra dışkı ve idrar yollarıyla vücuttan atılarak evsel atıksulara karışmaktadır. Ayrıca ilaç endüstrisinde formülasyon prosesinden kaynaklanan atıksular ile evsel atıksu arıtma tesislerine girebilirler. Antibiyotikler konvansiyonel biyolojik arıtma sistemleriyle arıtılamadıklarından, alıcı su ortamlarına ulaşabilmekte ve bu nedenle bu tip atıksular kimyasal bir ön arıtma gerektirmektedirler.

Sentetik olarak hazırlanmış cephalosporin (I) ve penisilin (II) grup insan antibiyotikleri ve quinolone grup veteriner antibiyotiği içeren ilaç formülasyon atıksuları, biyolojik parçalanabilirliklerini artırmak amacıyla ozonlama prosesine tabi tutulmuştur. pH, antibiyotik konsantrasyonu, H_2O_2 dozunun ve uygulanan ozone dozunun arıtma verimine etkileri incelenmiştir.

Reaksiyon pH'nın ozon ve H_2O_2 dozunun yükseltilmesi bütün antibiyotik formülasyon atıksularının arıtma verimliliğini artırmıştır.

2960 mg/L saat ozon dozuyla pH 7 de ozonlama sonucu $BOİ_5$/KOİ oranı veteriner antibiyotiği için 0.077 den 0.38'e arttırılırken, bu oran insan antibiyotiği (I) ve insan antibiyotiği (II) için sırasıyla 0 dan 0.1 ve 0.27 ye kadar yükseltilmiştir. Antibiyotik yapıları ile biyolojik parçalanabilirlikleri arasında bağlantı kurabilmek amacıyla, antibiyotiklerin parçalanma hızları, H-çıkarılması ile parçalanmaları için gerekli enerji miktarları ile ilişiklendirilmiştir.

Ozonun UV ile kombinasyonu, veteriner antibiyotiğinin aromatiklik giderimini yüzde 63'ten yüzde 83'e, oksijen tüketim hızını da 0.21'den 0.28 mg/L dak'ya çıkarırken, KOİ giderim hızları üzerinde herhangi bir etkisi olmamıştır.

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1. INTRODUCTION

Pharmaceutical industry, which includes four different type of manufacturing processes, fermentation, chemical synthesis, extraction and formulating, (EPA, 1991), often generates high strength wastewater changing in character and quantity depending upon the used manufacturing processes and season (Nemerow, 1978). Besides, pharmaceutical wastewater is usually characterized by both a considerable fraction of organic substrates readily biodegradable by aerobic and anaerobic treatment as well as a fraction that is practically inert towards conventional bioremediation. The effluents originated from the formulation of antibiotics, contain almost only active substance, which are specially designed to control bacteria in humans and animals. Therefore a chemical pretreatment is necessary for pharmaceutical effluents, like antibiotic formulation, containing high concentrations of biorecalcitrant compounds.

On the other hand, during recent decades it was reported that antibiotics have been found in surface water (Stan *et al.*, 1994) and effluent of sewage treatment plant (Richardson and Bowron, 1985; Halling-Sørensen *et al.*, 1998; Kümmerer *et al.*, 2000). These results inferred that antibiotics cannot be eliminated during biological treatment and they are emitted into receiving water systems. Antibiotics are used both therapeutically for human and animals and as growth promoters in intensive farming. After administration up to 90 per cent of antibiotics may be excreted through urine and feces into sewage. Therefore significant amount of antibiotics may pass through target organisms and become spread in the terrestrial and aquatic environment. Consequently, the concentration of antibiotics in surface and ground water may rise to $\mu g\ l^{-1}$ and $ng\ l^{-1}$ range respectively (Halling-Sørensen *et al.*, 1998). In an environmental aspect, the most prominent effect of antibiotics is the exerting toxic effects to aquatic organisms that would upset the ecological balance (Migliore *et al*, 1997; Lansky and Halling-Sørensen, 1997). Moreover, presence of antibiotics in natural systems leads to the development of multi- resistant strains of bacteria. Hence, it is necessary to treat the effluents containing antibiotics and other biorecalcitrant pharmaceuticals adequately before discharging into receiving water systems.

During the last two decades, in order to augment the biodegradability and also increase the efficacy of subsequent treatment, advanced oxidation processes (AOPs) that are combinations of powerful oxidizing agents with UV or near-UV light have been applied to refractory organic pollutants and xenobiotics found in groundwater, surface water and industrial wastewater (Takahashi *et al.*, 1994; Scott and Ollis, 1995; Alvares *et al.*, 2001). However, few studies reported in the literature dealt with pharmaceuticals (Rey *et al.* 1999; Zwiener and Frimmel, 2000) and pharmaceutical effluents (Gulyas *et al.*, 1995; Höfl *et al.*, 1997).

Considering the above mentioned facts, the present investigation aimed to study the treatability of three different effluents originated from antibiotic formulation process by O_3, O_3/H_2O_2 and O_3/UV advanced oxidation processes for the improvement of biodegradability. The oxidative performance of ozonation was evaluated in terms of conventional wastewater measures such as COD and absorbance (UV_{254} and maximum absorption values of antibiotics) removal and biodegradability enhancement (BOD_5/COD and OUR).

2. THEORETICAL BACKGROUND

Many tons of drugs are manufactured per year and used in production of human and veterinary medicine. Drugs could be emitted to the sewage treatment plants and environment mainly from two different sources:

1. Manufacturing processes in pharmaceutical industry
2. Activities and actions of individuals and hospitals;
   a. Excretion of drugs and the metabolites of the parent drug by humans and animals after administration
   b. Discharge of outdated and surplus drugs

2.1. Manufacturing Processes in Pharmaceutical Industry

The primary goal of the pharmaceutical industry is to produce substances that have therapeutic value for humans and animals. It manufactures bulk substance pharmaceutical intermediates and active ingredients, which are further processed into finished products. Pharmaceutical products manufactured by the industry can be divided into four groups according to the Standard Industrial Classification (SIC) (EPA, 1998):

1. Medicinal chemicals and botanical products
2. Pharmaceutical preparations
3. Diagnostic substances
4. Biological products

Since the production processes are not considered in this classification, it is hard to correlate between raw materials, processes and wastewater characteristics. The activities of the pharmaceutical industry can be classified into three main categories:

1. Research and development
2. Primary manufacturing to produce the bulk drugs
    a. Chemical Synthesis
    b. Fermentation
    c. Biological and natural extraction
3. Secondary manufacturing (mixing, compounding or formulating)

2.1.1.Research and Development

New drug research and development are complex, costly, and time-consuming endeavors. Research and development in the pharmaceutical industry is a diverse activity that relies on the cooperative efforts of specially trained personnel to screen, isolate, and develop new drug substances, pharmaceutical applications, and products. Distinct areas of research include chemical research, biological research, and pharmaceutical research. Due to the great diversity of research and development efforts in the industry, the types of raw materials utilized and wastes generated vary greatly. Examples of common wastes resulting from pharmacological research and development include halogenated solvents, non-halogenated solvents, organic chemicals, natural products, biomass, radionuclides, oxidizers, acids, bases, and myriad of reagents. In general, a major portion of the raw materials that are used to conduct research and development would potentially end up as waste. These quantities, while significant, are small in comparison to those from manufacturing operations.

2.1.2. Fermentation

Most antibiotics and steroids are produced by the large-scale fermentation process, which involves three basic steps, inoculum and seed preparation, fermentation, and product recovery. Steam is the major sterilizing medium for most equipment in the fermentation process. However, detergents and disinfectants, to the extent that they are used, can contribute to waste loads. An example of a commonly used chemical disinfectant is phenol, a priority pollutant. Air pollution control equipment sometimes installed to clean fermentation waste off-gas is another wastewater source. The air and gas vented from the fermenters usually contain odoriferous substances like oxides of nitrogen and sulfur, and

carbon dioxide. Treatment is necessary to deodorize the gas before release to the atmosphere. Some plants use incineration while others use liquid scrubbers. The blowdown from scrubbers may contain absorbed chemicals, soluble organic compounds, and insoluble organic oils and waxes.

Product recovery is another wastewater generation source in fermentation process, which can be done by three different methods; 1. Solvent extraction, 2. Precipitation, and 3. Ion exchange and adsorption. Fermentation wastes are very amenable to biological treatment.

Process wastewater from fermentation plants is characterized by high BOD_5, COD and TSS concentrations, relatively large flows and a pH range of approximately 4.0 to 8.0.

2.1.3. Biological and Natural Extraction

Many materials used as pharmaceuticals are derived from such natural sources as the roots and leaves of plants, animal glands, and parasitic fungi. Priority pollutants, including methylene chloride, toluene, chloroform, 1,2 – dichloroethane, and phenol, were identified as being used in the manufacturing of extractive pharmaceuticals. The cation of lead and zinc are known to be used as precipitating agents. Phenol was identified as a disinfectant chemical. The other priority pollutants found were used as processing solvents such as ethanol, methanol, n- amyl acetate, iso-propanol, and acetone. The principal sources of wastewater from biological/natural extraction operations are

1. Spent raw materials (waste plasma fractions, spent media broth, plant residues)
2. Floor and equipment wash water
3. Chemical wastes (spent solvents)
4. Cleanup of spills

Wastewater from extraction plants is generally characterized by low BOD_5, COD, and TSS concentrations, small flows, and pH values of approximately 6.0 to 8.0.

2.1.4. Chemical Synthesis

Most of the active ingredients marketed and sold as drugs are manufactured by chemical synthesis. Chemical synthesis is the process of manufacturing pharmaceuticals using organic and inorganic chemical reactions. Since most of these compounds are produced in batch operations, the conventional batch reaction vessel is the major piece of equipment used on the process line.

A variety of priority pollutants are used as reaction and purification solvents during chemical synthesis. Priority pollutants during the chemical synthesis process include benzene, chlorobenzene, chloroform, chloromethane, 1-2 dichloroethane, methylene chloride, phenol, toluene, and cyanide. Primary sources of wastewater from chemical synthesis operation are

1. Process wastes such as spent solvents, filtrates, and concentrates,
2. Floor and equipment wash water,
3. Pump seal water,
4. Wet scrubber wastewater,
5. Spills.

The pollutants in chemical synthesis wastewater vary with respect to toxicity and biodegradability. The production steps may generate acids, bases, cyanides, metals, and other pollutants, while the waste process solutions and vessel wash water may contain residual organic solvents. Therefore, chemical synthesis wastewater is incompatible with biological treatment systems because it is too concentrated or too toxic for the biomass in the treatment system. Thus it may be necessary to chemically pretreat some chemical synthesis wastewater prior to biological treatment.

2.1.5. Mixing, Compounding, or Formulating

Pharmaceutically active ingredients are generally produced by batch processes in bulk form and must be converted to dosage form consumer use. Common dosage forms for

the consumer market are tablets, capsules, liquids, and ointments. In addition, active ingredients can also be incorporated into patches and time-release capsules.

Tablets are formed in a tablet press machine by blending the active ingredient, filler and binder. The filler such as starch, sugar is required to dilute the active medicinal ingredient to the proper concentration, and a binder such as corn syrup or starch is necessary to bind the tablet particles together. A lubricant may be added for proper tablet machine operation. The dust generated during the mixing and tableting operation is collected and usually recycled directly to the same batch, while broken tablets generally are collected and recycled to the granulation operation in a subsequent lot. Some tablets are coated by tumbling with a coating material and then dried. After the tablets have been coated and dried, they are sent to the packaging unit where they are bottled. Tablet coating operations can be significant source of air emissions of solvents if solvent based coatings are used, and can contribute solvents to the plant wastewater if certain types of air pollution control equipment (wet scrubbers or activated carbon) are used to capture solvent vapors from tablet coating operations. Wastewater from the wet scrubber is likely to be sewered as is the condensate from the steam used to regenerate the activated carbon.

The first step in capsule production is to form a hard gelatin shell. The shells are produced by machines that dip rows of rounded metal dowels into a molten gelatin solution, and then strip the capsules from the dowels after the capsules have cooled and solidified. The active ingredient and filler are mixed before being poured by machine into the empty gelatin capsules. The filled capsules are bottled and packaged. As in tablet production, some dust is generated, which is recycled to the production line. Liquid preparations are formulated for injection or oral use. In both cases, the liquid active ingredient is first weighed and then dissolved in water. Injectable solutions are bulk sterilized by heat or filtration and then poured into sterilized bottles. Oral liquid preparations can be bottled directly without the sterilization steps. Wastewater is generated by general cleanup operations, spills, and breakage.

Ointments are produced by blending an active ingredient with an ointment base such as polyethylene glycol. The blended product is then poured into tubes by machine and

packaged. Wastewater generated from these operations is all from the equipment cleaning operations.

The primary objective of mixing, compounding, or formulating operations is to convert the manufactured products into a final, usable form. The necessary production steps typically have small wastewater flows because very few of the unit operations generate wastewater. The primary use of water is in the actual formulating process, where it is used for cooling and for equipment and floor washing. Wastewater sources from mixing, compounding, or formulating operations are

1. Floor and equipment wash water
2. Wet scrubbers
3. Spills

The washouts from mixing tanks may be used to prepare the master batches of the pharmaceutical compounds and may contain inorganic salts, sugar and syrup. Other sources of contaminated wastewater are dust and fume scrubbers, either in building ventilation systems or on specific equipment. In general, this wastewater is readily treatable by biological treatment systems. However, in the case of antibiotic formulation process, because of the low biodegradability of active substances, generated wastewater could not be treated biologically without a pre treatment.

An analysis of the pollutant information in the pharmaceutical manufacturing database shows that wastewater from mixing, compounding, or formulating plants normally has low BOD_5, COD, and TSS concentrations, relatively small flows, and pH values of 6.0 to 8.0.

2.2. Activities and Actions of Individuals

While the point source emission of pharmaceuticals from manufacturing waste streams have been monitored and subject to controls, the environmental impact of

pharmaceutical substances originating from the activities and actions of individuals and hospitals is more difficult to assess.

2.2.1. Exposure Routes and Fate of Pharmaceutical Drugs

Drugs are designed to have particular characteristics. In order to be more effective on their therapeutic work, most of them are designed to be lipophilic and persistent. Lipophilicity gives them the ability to pass through cell membranes and act inside cells, and persistence provides to retain their chemical structure long enough to do their work. After administration, drugs are often absorbed by organisms (human or animal) and are subject to metabolic reactions such as oxidation, reduction, hydrolysis, conjugation, and hydroxylation (Hirsch *et al.*, 1999). According to the constitution of patient or the time of administration, the degree of metabolites could be changed and about 50 – 90 per cent of drug is excreted in unchanged form or metabolites might be reconverted to their parent compound after leaving the patient (Kümmerer *et al.*, 2000; Wollenberger *et al.*, 2000; Hirsch *et al.*, 1999). Researchers report that some of the metabolites are more lipophilic, persistent and toxic than the original drug from which they are derived (Halling-Sorensen *et al.*, 1998). The fate of veterinary and human drugs and their metabolites after urine or fecal excretion are quite different each other (Ternes, 1998).

The drugs used by humans and its metabolites will be discharged to the sewer systems together with the urine or faeces and enter the sewage treatment plant (Figure 2.1, a-d). Outdated or surplus pharmaceutical drugs could also be disposed of down household or hospital drains (Figure 2.1, b-c). Removal efficiency in a sewage treatment plant is a joint function of structure, physical and chemical characteristics of the drugs, biodegradation, chemical degradation characteristics and the treatment technology employed (Rogers, 1996; Daughton, 2001). The fate of drugs and the metabolites of the parent drug in the sewage treatment plants may be divided into three groups (Halling-Sorensen *et al.*, 1998);

1. The drug or its metabolites might be readily biodegradable and mineralised by microorganisms to carbon dioxide and water.

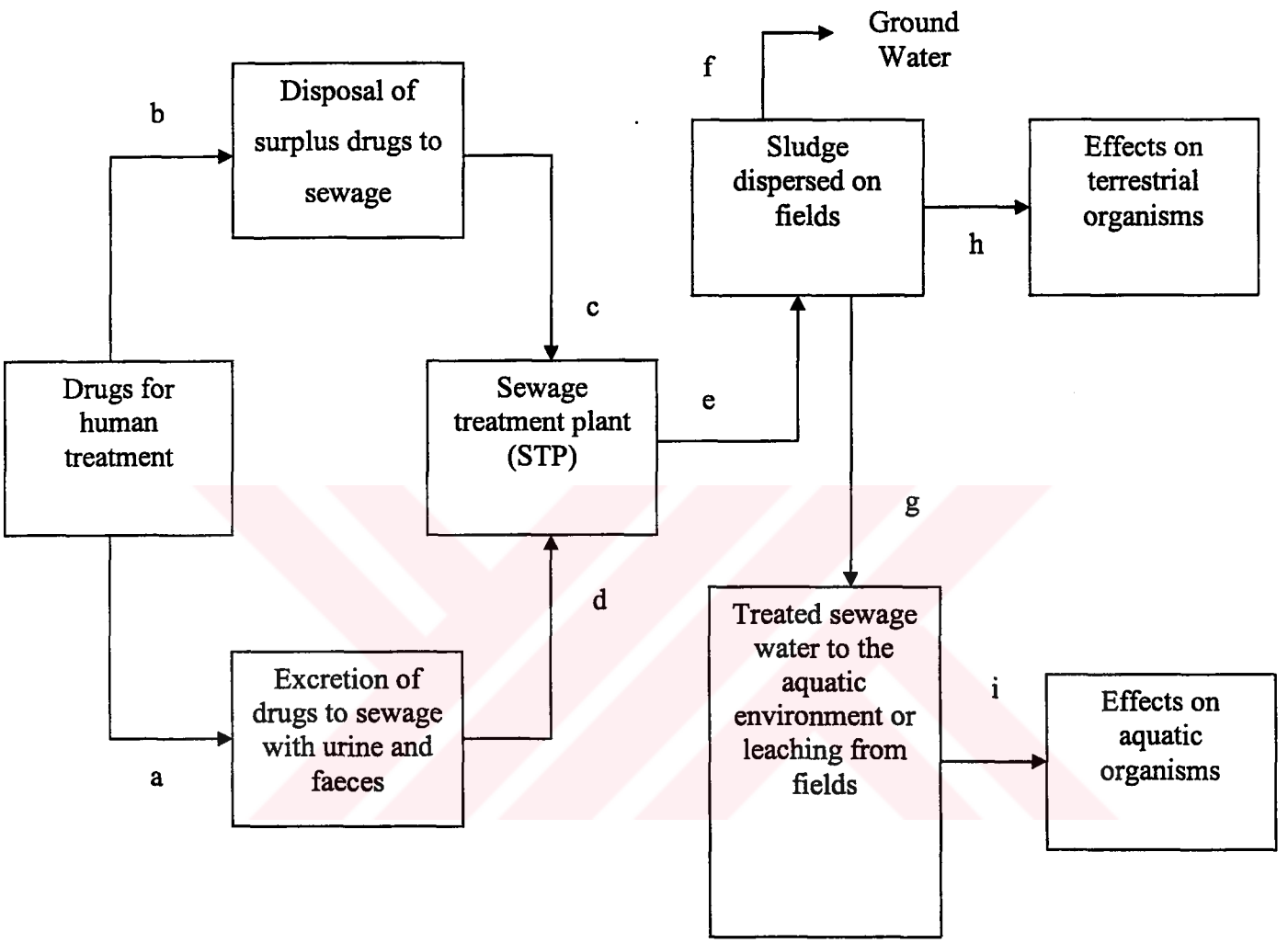


Figure 2.1.Exposure routes and fates of pharmaceuticals used for human treatment.

2. The drug or its metabolites could be more or less persistent in the sewage treatment plant. Depending on the sorption capacity onto solid surfaces, a part of substance will be retained in the sludge and can cause the contamination of soil and ground water (Figure 2.1, e-f, e-h).

3. Lastly, drug or its metabolites might be persistent, polar and non-binding to solids. Recent studies elucidated that elimination of this kind of drugs in municipal sewage treatment plants was often incomplete and elimination rates have been determined as ranging between 60 and 90 per cent for a variety of polar drugs (Ternes, 1998). Therefore, pharmaceutical drugs that are refractory to degradation and transformation do indeed have the potential to reach the aquatic environment (Figure 2.1, i) and tend to persist in the environment.

In addition to human drugs, veterinary drugs could also be emitted to the environment from different sources (Figure 2.2) (Halling – Sorensen, 1998):

1. Most of the drugs used for veterinary purposes (therapeutic treatment and growth parameter) will end up in manure, which is conserved in tank systems before emitted into the soil. Leaching through the soil profile cause to contamination of aquatic environment (Figure 2.2, c-d-i). Also drugs used in fish farming are collected in local water treatment plants and the sludge from these treatment plants is used as soil conditioner leading drugs end on agricultural soil (Figure 2.2, a-e-m).

2. Another possibility for environmental pollution is direct discharge of parent drug used in fish farming, to the receiving water, since large portion of applied drug is not eaten by the fish (Figure 2.2, k)

3. Lastly, if drugs are applied to grazing animals, drug or drug metabolites will be urinated or defecated directly on the field and undergo the same fate as the drugs dispersed with manure (Figure 2.2, b-g).

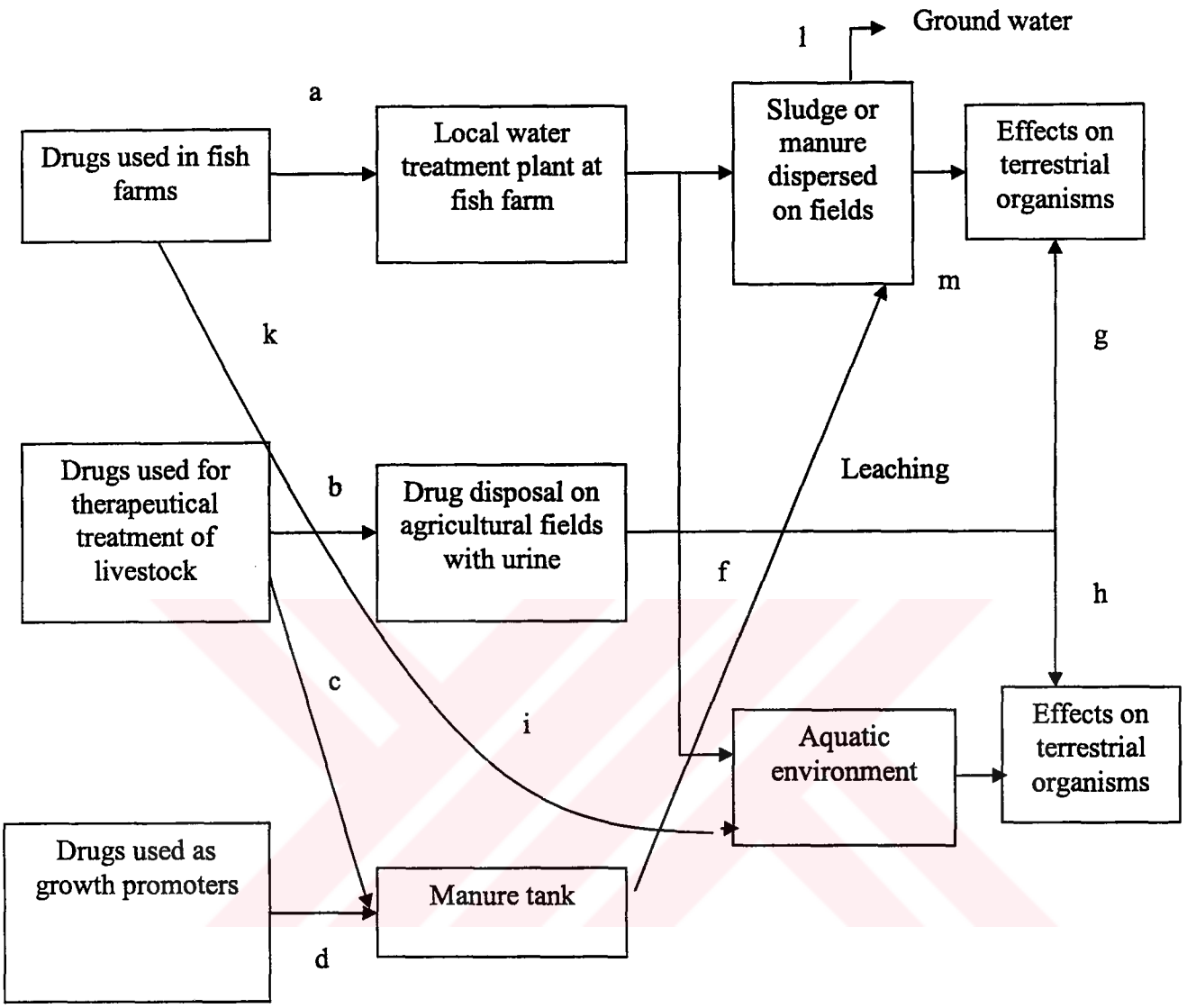


Figure 2.2. Exposure routes and fates of pharmaceuticals used for veterinary treatment.

2.2.2. Types of Pharmaceutical Substances in the Environment:

Since the 1980's, pharmaceutical drugs and diagnostic agents, like lipid lowering agents, cytostatic drugs, antibiotics, and X-ray contrast media have been reported to be present in effluent of sewage treatment plant (STP), surface water and ground water in the μg/L and ng/L range (Table 2.1).

Table 2.1. Occurrence of different drug classes in STP effluents, surface water and ground water

	Concentration (μg/L)			
Drug Class	**STP Effluent**	**Surface Water**	**Ground Water**	**Reference**
Cytostatic Drugs	-	0.02	-	Kümmerer *et al.* (2000)
Analgesics Agents				
Ibuprofen	3.45	0.53	-	Ternes (1998)
Aspirin	1	-	-	Richardson and Bawron (1985)
Acetylsalycylic acid	1.5	0.34	-	Ternes (1998)
Lipid Lowering Agents				
Clofibric acid	-	0.04	-	Richardson and Bawron (1985)
		0.009	-	Buser *et al.* (1998)
		-	0.165 (TW)*	Heberer *et al.* (1997)
Diagnostic Agents	-	0.15	0.07 (DW)**	Hirsch *et al.* (2000)
Antibiotics				
Erythromycin	-	1.7	-	Hirsch *et al.* (1999)
		1	-	Watts *et al.* (1983)
Sulphamethoxazole	-	-	0.47	Hirsch *et al.* (1999)
Tetracycline	-	1	-	Watts *et al.* (1983)
Penicilloyl groups	-	0.025	0.010	Richardson and Bawron (1985)

* Tap water
** Drinking water

a. *Cytostatic Drugs:* Under the aspect of potential effluence into the environment cytostatics are an important group of drugs in terms of their risk potential for humans and the environment. They have carcinogenic, mutagenic or embryotoxic properties. The active substances are expected to pass unchanged through sewage treatment plants and thus reach surface water (Kümmerer *et al.*, 1997).

b. *Analgesics Agents*: There are several types of analgesic agents that were detected in surface water such as, ibuprofen, diclofenac, and acetylsalicylic acid. Ibuprofen was found in the concentration of 3.45 µg/L in the effluent of sewage treatment plant, while 0.53 µg/L in water samples from different rivers. Another analgesics drug, acetylsalyclic acid was also detected in sewage treatment plant effluents and river samples in the concentration of 1.5 and 0.34 µg/L respectively (Ternes, 1998).

c. *Lipid lowering Agents*: One of the lipid lowering agents, clofibric acid is used by many people in large quantities to reduce cholesterol levels in the blood. Clofibric acids were found in river water at about 40 ng/L (Richardson and Bawron, 1985), in various Swiss lakes in the range of 1-9 ng/L and even at the North Sea up to 7-8 ng/L (Buser *et al.*, 1998). In Germany and Italy measurable quantities of clofibric acid was also detected in river water (Heberer *et al.*, 1997). Authors also found that tap water in Berlin contains clofibric acid at concentrations between 10 and 165 ng/L. Although these concentrations are very low, they are significant in that they are persistent and bioaccumulative in the environment. Regular therapeutic use is the source of clofibric acid, which is carried by sewage effluent into the aquatic system (Daughton and Ternes, 1999).

d. *Diagnostic Agents*: Diagnostic agents like X-ray contrast media might contribute to the adsorbable organic halogen (AOX) compounds in the environment. These agents were found in surface water at about 0.15 µg/L and in drinking water up to 0.07 µg/L (Hirsch *et al.*, 2000)

e. *Antibiotics:* Antibiotics are an important group of pharmaceutical substances in today's medicine. In addition to the human infections, they are also used in veterinary medicine.

2.2.3. Antibiotics

2.2.3.1. Occurrence of Antibiotics in the Environment. Since the sewage treatment plants are designed to treat wastewaters primarily via degradative action of microorganisms, biodegradability of the drug substances is an important characteristic in terms of removal of these substances from sewage water. Because of the acute toxicity to various microbial species, poor removals for antibiotics are expected in sewage treatment plants (Daughton, 2001).

Richardson and Bawron, (1985) examined a number of antibiotics such as ampicillin, erythromycin, sulphamethoxazole, tetracycline and penicilloyl groups for their biodegradability during sewage treatment. The study indicated that these antibiotics are nonbiodegradable and had the potential to survive sewage treatment. This property poses a persistence of these compounds in the environment and potential for bioaccumulation (Wollenberger *et al.*, 2000). Recent studies concerning antibiotics in the aquatic environment have also clearly shown that elimination in municipal sewage treatment plant is often incomplete. Hirsch *et al*, (1999) investigated that the concentration of the erythromycin in surface water was 1.7 μg/L. Sulphamethoxazole also found in ground water in the concentration of 0.47 μg/L. Additionally, Watts *et al.*, (1983) reported the presence of several antibiotics (erythromycin, sulphamethoxazole, tetracycline) in river water samples. However, in contradiction to the findings obtained by Watts *et al.*, (1983), Hirsch *et al.*, (1999) could not find any detectable amount of tetracycline and penicillin in 14 surface water samples. Besides biodegradability, sorption and chemical degradation are also important factors in terms of removal at sewage treatment plants as mentioned. The absence of tetracycline in surface water can be explained by the complexing properties of tetracycline, which can easily bind to divalent cations causing this antibiotic found in soils and sediments as stable complexes (Robelle and Spliid, 2000). The reason for the absence of penicillin in surface water samples is the presence of chemically unstable β–lactam rings which are readily susceptible to hydrolysis (Morrison and Boyd, 1987).

2.2.3.2 Effects of Antibiotics on the Environment. Among all other pharmaceutical drugs and substances, the literature on antibiotics is much more developed due to its serious irreversible effects on aquatic and terrestrial environment. Effects of antibiotics on

organisms, e.g. bacteria, algae, *Daphnia magna*, have been found not only in high concentrations, but also in low concentrations in chronic tests.

Antibiotic agents have the potential for forming resistance and bacterial toxicity with the potential to disturb environmental bacterial processes, and alter microbial species diversity (Daughton, 2001). Therefore, increasing production and consumption of antibiotics resulted in changing in the genetic pool of microorganisms in nature and increased resistance towards certain antibiotics (Jacobsen and Guildal, 2000; Rabolle and Spliid, 2000; Iwane *et al.*, 2001) that is a long term and high extent irreversible effect.

Additionally, the toxic effect data (EC_{50}) of human and veterinary antibiotics in the mg/L range on various aquatic species found in the literature, indicating the toxicity of antibiotics towards algal species have shown growth inhibiting effects (Migliore *et al.*, 1997; Lansky and Halling Sorensen, 1997; Harras, 1985; Macri *et al.*, 1988; Holten Lutzhoft, 1999).

Aside with algae, *Daphnia magna* was also used as a test organism for the evaluation of the toxicity of antibiotics on aquatic environment. Wollenberger *et al.*, (2000) worked with several veterinary antibiotics and found that, oxalinic acid and tiamulin have an acute toxic effect on *Daphnia magna* (EC_{50} 4.6 and 40 mg/L respectively) while oxytetracycline, sulphadiazine and tetracycline leads to reproductive toxicity in the range of 5 to 50 mg/L. Additionally, acute toxicity of furazolidone which is largely used in medicated fish feed, on *Daphnia magna* have been investigated by Macri *et al.*, (1988). The authors also found that, *Artemia salina* is less sensitive to these antibiotics than *Daphnia magna*. Migliore *et al.*, (1997) showed the toxicity of several agricultural antibiotics to *Artemia salina*.

Based on the studies related to the toxicity of antibiotics on aquatic organisms, it can be said that effects of antibiotics on bacteria and micro algae are generally found two to three orders of magnitude below the toxic values for higher trophic levels.

2.3. Treatment Technologies of Pharmaceutical Wastewater

Since there is a continuous development of new drugs and production methods causing the wastewater to change with time, it is necessary to find a broad, flexible and stable treatment scheme for pharmaceutical industry wastewater. Pharmaceutical wastewater is considered as high strength wastewater most of the time because of its high organic content and presence of toxic materials.

In recent years, pH adjustment/neutralization, equalization, advanced biological treatment, polishing pond treatment, multi media filtration, steam stripping, granular activated carbon, incineration and oxidation have been used for the treatment of pharmaceutical wastewater (EPA, 1998). These methods can be applied in various combinations according to the strength and characteristics of the wastewater.

2.3.1.Aerobic and Anaerobic Treatment of Pharmaceutical Wastewater

At the pharmaceutical industries, which are chemical synthesis and fermentation based, generally conventional biological processes are used for the treatment of wastewater. However, if biological methods are applied, the system should be monitored when new products are synthesized in order to control the biodegradability of new wastes (Rosen *et al.*, 1998).

Aerobic treatment systems have been employed for the treatment of pharmaceutical wastewater including activated sludge systems (Andersen, 1980; Bernard and Gray, 2000; Gohary *et al.*, 1995), with or without the addition of granular activated carbon (Rosen *et al.*, 1998; Osantowski *et al.*, 1985).

A treatment system including comminuter, basket screen, equalization basin and biological tower preceding the activated sludge system, disinfection and filtration was developed by Andersen (1980), for the treatment of pharmaceutical wastewater, which was high in organic strength (BOD_5 = 750-2000 mg/L, COD = 1000-2800 mg/L) and low in suspended solids. Results of the study showed that the two stage biological system

provided a high degree of treatment efficiency for this kind of wastewater (99 per cent BOD_5 and 91 per cent COD removal).

Gohary *et al.* (1995) carried out the biological treatment of wastewater from pharmaceutical and chemical company, which was very acidic and contains high concentrations of organic compounds and total solids, using both batch and continuous flow system. The effectiveness of three different biological treatment processes that were activated sludge process, fixed film reactor, and fixed film reactor followed by activated sludge processes were compared. The results obtained indicated that biological treatment using extended aeration activated sludge or biological filters followed by activated sludge process significantly removed the organic contaminants in wastewater (approximately 95 per cent of COD and BOD reduction). It was found that average BOD_5 and COD concentrations in the treated effluent complied with the regulatory standard.

Rosen *et al.* (1998) found that the treatment of biologically pretreated chemical synthesis based pharmaceutical wastewater with activated carbon provided high removal of COD and toxicity. He also studied with the multi stage bio-film process with fungal and bacterial treatment. It was found that this technique resulted in a better removal of toxicity than the modified activated sludge process, while the removal of organic material was approximately the same in both processes.

Anaerobic systems utilized for pharmaceutical wastewater treatment include membrane reactors (Livingston, 1994), continuously stirred tank reactors (Terzis, 1994), upflow filters (Seif *et al.*, 1992), and fluidized bed reactors (Stronach *et al.*, 1987).

Yeole *et al.* (1996) evaluated a dual biological treatment system consisting of anaerobic digestion followed by an activated sludge process that was developed for the treatment of wastewater arising in natural product extraction process. The wastewater had a pH of 5.8, COD and BOD_5 concentrations of 21000 mg/L and 14200 mg/L, respectively, and a suspended solids concentration of 20000 mg/L. The overall anaerobic-aerobic treatment process resulted in 98 per cent reduction in COD and 99 per cent reduction in BOD_5.

2.3.2. Chemical Treatment of Pharmaceutical Wastewater

Some kind of wastewaters include difficult substrates for biological treatment due to their varying content of a wide range of organic chemicals, both natural and xenobiotic, which may not be readily metabolized by the microbial associations present in the bioreactors (Henry *et al.*, 1996). For example, during the formulation process of pharmaceutical substances, highly toxic liquid wastes containing recalcitrant organic compounds, which cannot be treated by the conventional treatment systems are generated. At times, incineration was the only possible treatment processes for this kind of wastewater (Nemerow, 1978; Rey *et al.*, 1999), however recently chemical methods have been considered as an alternative for the treatment. There are few studies with chemical methods reported in the literature dealt with pharmaceuticals (Rey *et al.*, 1999; Zwiener and Frimmel, 2000) and pharmaceutical effluents (Gulyas *et al.*, 1995; Höfl *et al.*, 1997; Kadbasli *et al.*, 1999). However, no studies were found in the literature related to oxidative treatment of antibiotics.

Rey *et al.* (1999) studied the ozonation of the cytostatic drugs containing 5-fluoroacil, methotrexate, azathioprine, and cytarabine which are highly toxic and even carcinogenic and found ozonation as a suitable method for the inactivation of cytostatics in both acid and neutral media.

Oxidation experiments with the aim to degrade the lipid lowering agent, clofibric acid and the analgesic agents, ibuprofen and diclofenac were carried out in bench scale using ozone (O_3) and ozone/hydrogen peroxide (H_2O_2) by Zwiener and Frimmel (2000). The combined application of ozone and hydrogen peroxide leading to OH radical formation improved the degradation efficiency of all investigated compounds. The application of increased oxidant concentration resulted in a better degradation of all compounds to more than 90 per cent at a concentration of 3.7 mg/L ozone and 1.4 mg/L hydrogen peroxide and more than 98 per cent at a concentration of 5 mg/L ozone and 1.8 mg/L hydrogen peroxide.

Musterman and Boero (1992) treated the high strength non-biodegradable pharmaceutical wastewater by wet air oxidation (WAO), prior to discharge to the activated

sludge process. COD reduction by 45 per cent to 85 per cent and increase in biodegradation was obtained by WAO for waste streams from chlorobenzene and toluene recovery bottoms.

Gulyas *et al.* (1995) studied the O_3 and O_3/H_2O_2 advanced oxidation processes for removal of preservative 1,1,1-trichloro-2-methyl-2-propanol (TCMP). However, the oxidation of the wastewater failed to be effective in TCMP degradation because of competitive ozonation of other organic solutes in the wastewater. Therefore, it was concluded that for the pharmaceutical wastewaters containing TCMP, biological treatment prior to ozonation stage is necessary to remove biodegradable organic solutes, which will compete with refractory organics for oxidants.

Another study with oxidative treatment of pharmaceutical wastewater was done by Höfl *et al.* (1997) by using several advanced oxidation processes. Using two samples of pharmaceutical wastewater, the efficiency of three advanced oxidation processes namely O_3/UV, H_2O_2/UV and Fenton were compared for the removal of COD and AOX. While Fenton treatment was found most appropriate method for the wastewaters with high amounts of COD (>5000 mg/L), photochemical processes were found suitable for the degradation of AOX.

Kabdasli *et al.* (1999) studied with three chemical synthesis wastewaters, which were completely different in character in terms of their biodegradability. Chemical oxidation with H_2O_2 (35 per cent) and NaOCl (50 per cent) were used as a pre treatment for omeprazole and mephenoxalone wastewaters to increase their biodegradability. Combination of chemical oxidation and biological treatment provided up to 80 per cent COD removal at reasonable F/M ratios for omeprazole wastewater, while chemical pretreatment did not enhance the biodegradability of mephenoxalone wastewater.

2.4. Advanced Oxidation Processes

Chemical oxidation processes are typically applied to wastewater containing non-biodegradable organic compounds, which are toxic or inhibitory to microbial growth. In

order to inactivate inhibitory compounds and improve the biodegradability of recalcitrant organics, their structure is altered by means of oxidizing agents, such as ozone, hydrogen peroxide, permanganate, chlorine dioxide, chlorine or hypo chloride. Since the chemical oxidation is not economically feasible, its combination with cheaper biological processes is preferred instead of carrying chemical oxidation to the fullest extent of reaction (Eckenfelder, 2000). Chemical oxidation is generally used as a pretreatment in order to provide partial oxidation of wastes highly resistant to conventional biological treatment. In addition to oxidizing agents, chemical oxidation systems may utilize the synergy derived from a combination of oxidants and UV lights in advanced oxidation processes (Glaze and Kang, 1989). Advanced oxidation processes have been defined as treatment processes that involve the generation of highly reactive radical intermediates, particularly the hydroxyl radical (Glaze and Kang, 1989; Legrini *et al.*, 1993). Hydroxyl radical generation could be achieved by both photochemical and non-photochemical homogeneous AOPs or heterogeneous AOPs.

1. Non-photochemical Homogenous AOPs
   * Ozone / OH^-
   * Ozone / Hydrogen peroxide
   * The Fenton's Reaction (Fe^{2+} / H_2O_2)

2. Photochemical Homogenous AOPs
   * Ozone / UV-C
   * Hydrogen peroxide /UV-C
   * The photo-Fenton's Reaction (Fe^{2+} /H_2O_2/UV-C)

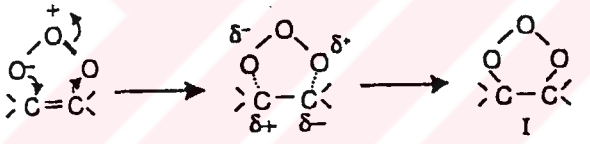
3. Photochemical Heterogeneous AOPs
   * Photocatalytic oxidation (e.g. TiO_2/UV-A)

2.5. Ozonation Process

In water, ozone may react with dissolved organic substances directly or it may decompose leading to more reactive species such as hydroxyl radicals, which determines

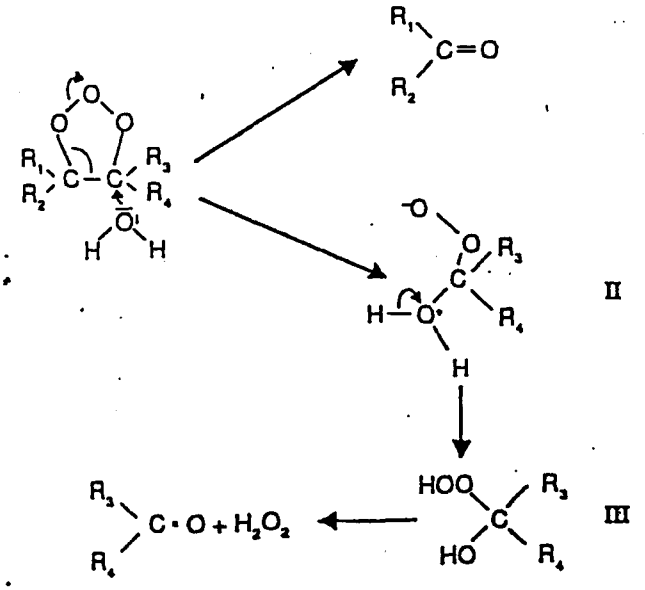
the subsequent reactions. The ozone reaction mechanism is uniquely dependent on the rate of mass transfer into an aqueous system. According to pH of reaction medium, reactivity exists in different ways. At high pH, the major reaction mechanism with solutes entails hydroxyl radical, which has little selectivity for oxidation of organic compounds. At neutral pH values both direct ozone and hydroxyl radical reactions are important while, at low pH, direct oxidation of solutes by ozone is the controlling reaction (Eckenfelder, 2000). Molecular ozone reactions are extremely selective and limited to unsaturated aromatic and aliphatic compounds as well as to specific functional groups (atoms carrying a negative charge like N, S, P and O). Direct attack of molecular ozone occurs by electrophilic, nucleophilic or dipolar cyclo addition (Langlais *et al.*, 1991).

Cyclo addition: As a result of its dipolar structure, the ozone molecule may lead to 1-3 dipolar cyclo addition on unsaturated bonds, with the formation of primary ozonide (I) corresponding to the following reaction:



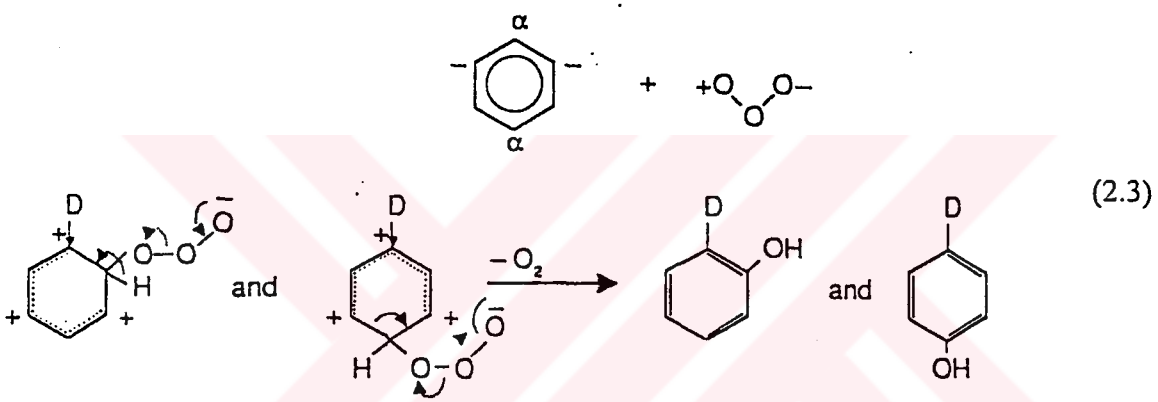
(2.1)

In a protonic solvent such as water, this primary ozonide decomposes into a carbonyl compound and a zwitterion (II) that quickly leads to a hydroxy-hydroperoxide (III) stage that, in turn, decomposes into a carbonyl compound and hydrogen peroxide.



(2.2)

Electrophilic reaction: The electrophilic reaction is restricted to molecular sites with a strong electronic density and, in particular, certain aromatic compounds. Aromatics substituted with electron donar groups like OH and NH_2, show high electronic densities on carbons located in the ortho and para positions, and so are highly reactive with ozone at these positions. On the contrary, the aromatics substituted with electron withdrawing groups like COOH and NO_2, are weakly ozone reactive. In this case, the initial attack of the ozone molecule takes place mainly on the least deactivated meta position. The result of this reactivity is that the aromatic compounds bearing the electron donor groups such as phenol and aniline, react quickly with ozone.



(2.3)

This initial attack of the ozone molecule leads to the formation of ortho and para hydroxylated by products. These hydroxylated compounds are highly susceptible to further ozonation

Nucleophilic reaction: The nucleophilic reaction is found locally on moleculer sites showing an electronic deficit and, on carbons carrying electron withdrawing groups.

Depending upon the nature of the organic compound, two types of initial attack of generated hydroxyl radical are (Langlais *et al.*, 1991);

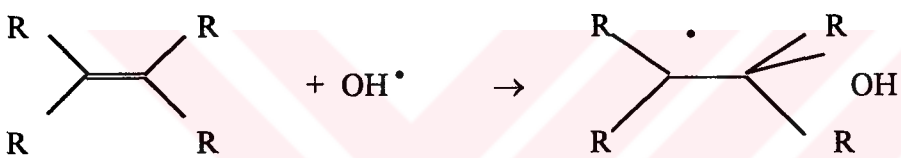
Hydrogen Abstraction: As in the case of alcohols or alkenes, the hydroxyl radical abstracts a hydrogen atom to form water. Organic radicals are generated yielding peroxyl radicals by addition of oxygen molecule.

$$HO^{\bullet} + RH \rightarrow R^{\bullet} + H_2O \quad (2.4)$$

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet} \quad (2.5)$$

These intermediates initiate thermal reactions of oxidative degradation, leading finally to CO_2, H_2O, and inorganic salts.

Radical Addition: The hydroxyl radical adds itself to the contaminant, as in the case for olefins or aromatic compounds. It adds to double bonds in unsaturated and aromatic molecules. The hydroxyl radical can attack halogenated organics resulting with replacement of the halogen with hydroxyl radical.



(2.6)

The reactivity and rate constant of organic compounds with hydroxyl radical, which is formed by decomposition of ozone is higher than the direct attack of ozone molecule itself (Langlais *et al.*, 1991). The reaction rates of various organic compounds with ozone and hydroxyl radical are presented in Table 2.2.

Table 2.2. Reaction rate constants of organics with ozone and hydroxyl radical (CCOT, 1996)

Organic compound	k_{O3} (L mole^{-1} s^{-1})	$k_{OH^{\bullet}}$ (L mole^{-1} s^{-1})
Chlorinated Alkenes	10^{-1} to 10^{3}	10^{9} to 10^{11}
Phenols	10^{3}	10^{9} to 10^{10}
N-containing Organics	10 to 10^{2}	10^{8} to 10^{10}
Aromatics	1 to 10^{2}	10^{8} to 10^{10}
Ketones	1	10^{9} to 10^{10}
Alcohols	10^{-2} to 1	10^{8} to 10^{9}
Alkanes	10^{-2}	10^{6} to 10^{9}

2.6. Reaction Mechanisms for the Formation of Hydroxyl Radical

Ozone at high pH and combination of ozone with hydrogen peroxide and/or UV light are the main processes for the decomposition of ozone in order to generate more reactive hydroxyl radicals.

2.6.1. O_3/OH^-

Decomposition of ozone may be initiated by hydroxyl ion at alkaline solutions. This kind of decomposition is pH dependent and is first order both in ozone and in hydroxyl ion concentration (Staehelin and Hoigne, 1982).

The initiation and propagation steps for the hydroxyl ion initiated decomposition of ozone are the followings (Glaze *et al.*, 1987).

Initiation steps

$O_3 + OH^- \rightarrow O_2^- + HO_2^\bullet$ $\quad k_{2.7} = 70\ M^{-1}\ s^{-1}$ (2.7)

$HO_2^\bullet \leftrightarrow H^+ + O_2^-$ $\quad pK = 4.8$ (2.8)

Propagation steps:

$^\bullet O_2^- + O_3 \rightarrow {}^\bullet O_3^- + O_2$ $\quad k_{2.9} = 1.6 \times 10^9\ M^{-1}\ s^{-1}$ (2.9)

$^\bullet O_3^- + H^+ \rightarrow HO_3^\bullet$ $\quad k_{2.10} = 5.2 \times 10^{10}\ M^{-1}\ s^{-1}$ (2.10)

$HO_3^\bullet \rightarrow OH^\bullet + O_2$ $\quad k_{2.11} = 1.1 \times 10^5\ M^{-1}\ s^{-1}$ (2.11)

The overall reaction for the hydroxyl ion initiated decomposition of ozone is:

$2\ O_3 + OH^- \rightarrow OH^\bullet + 3O_2$ (2.12)

These reactions can be followed by hydroxyl radicals interacting with further ozone as in the following reaction;

$OH^\bullet + O_3 \rightarrow HO_2^\bullet + O_2$ $\quad k_{2.13} = 3.0 \times 10^9\ M^{-1}\ s^{-1}$ (2.13)

However, the reactive hydroxyl radical might be scavenged by carbonate, bicarbonate, phosphate or organic solutes present in the medium leading a decrease in the treatment efficiency (Staehelin and Hoigne, 1985).

$$OH^{\bullet} + HPO_4^{-2} \rightarrow OH^- + {}^{\bullet}HPO_4^- \qquad k_{2.14} = 5 \times 10^6\ M^{-1}\ s^{-1} \qquad (2.14)$$

$$OH^{\bullet} + HCO_3^- \rightarrow OH^- + {}^{\bullet}HCO_3 \qquad k_{2.15} = 1.5 \times 10^7\ M^{-1}\ s^{-1} \qquad (2.15)$$

$$OH^{\bullet} + CO_3^{-2} \rightarrow OH^- + {}^{\bullet}CO_3^- \qquad k_{2.16} = 4.2 \times 10^8\ M^{-1}\ s^{-1} \qquad (2.16)$$

However, it is also known that when ${}^{\bullet}HPO_4^-$ reacts with organic compound to form superoxide radical ($O_2^{\bullet-}$) leading to further decomposition of ozone.

$$^{\bullet}HPO_4^- + RCH_2OH \rightarrow RCHOH \xrightarrow{O_2} O_2^{\bullet-} \qquad (2.17)$$

Since it provides the formation of $O_2^{\bullet-}$ radical, which leads to further decomposition of ozone, phosphate also act as promoter of the free radical reaction (Staehlin and Hoigne, 1982).

2.6.2. O_3/H_2O_2

Hydrogen peroxide is a weak acid and when combined with water, it partially dissociates into hydroperoxide ion. The conjugate base of hydrogen peroxide (HO_2^-) can initiate the decomposition of ozone to form hydroxyl radicals (Glaze *et al.*, 1987). The reaction between H_2O_2 and O_3 produces hydroxyl radical, however this reaction is very slow (Staehlin and Hoigne, 1982). The decomposition rate of ozone by hydrogen peroxide increases with increasing pH (Langlais *et al.*, 1991), but at very high pH values, the reaction of ozone with hydroxide ion predominates and the peroxide has little benefit (CCOT, 1996). Therefore, the pH of the medium should be maintained at between 7 and 8.

The initiation and propagation reactions for the hydroperoxide anion initiated decomposition of ozone are

Initiation steps:

$H_2O_2 + H_2O \rightarrow HO_2^- + H_3O^+$ pK = 11.6 (2.18)

$O_3 + HO_2^- \rightarrow O_3^- + HO_2^\bullet$ $k_{2.19} = 2.8 \times 10^6\ M^{-1}\ s^{-1}$ (2.19)

$HO_2^\bullet \leftrightarrow H^+ + O_2^-$ pK = 4.8 (2.20)

Propagation steps:

$^\bullet O_2^- + O_3 \rightarrow {}^\bullet O_3^- + O_2$ $k_{2.21} = 1.6 \times 10^9\ M^{-1}\ s^{-1}$ (2.21)

$^\bullet O_3^- + H^+ \rightarrow HO_3^\bullet$ $k_{2.22} = 5.2 \times 10^{10}\ M^{-1}\ s^{-1}$ (2.22)

$HO_3^\bullet \rightarrow OH^\bullet + O_2$ $k_{2.23} = 1.1 \times 10^5\ M^{-1}\ s^{-1}$ (2.23)

The overall reaction for the hydrogen peroxide initiated decomposition of ozone

$2\ O_3 + H_2O_2 \rightarrow 2\ OH^\bullet + 3O_2$ (2.24)

However, hydrogen peroxide might act as a hydroxyl radical scavenger as well as an initiator when the concentration is so high (Glaze *et al.*, 1987).

$H_2O_2 + OH^\bullet \rightarrow O_2^- + H_2O + H^+$ $k_{2.25} = 2.7 \times 10^7\ M^{-1}\ s^{-1}$ (2.25)

$HO_2^- + OH^\bullet \rightarrow OH^- + HO_2^\bullet$ $k_{2.26} = 7.5 \times 10^9\ M^{-1}\ s^{-1}$ (2.26)

2.6.3. O_3/UV

Ozone / UV treatment is more effective for destruction of recalcitrant organic compounds than mere ozone treatment (Peyton and Glaze, 1988). In aqueous solution, ozone absorbs UV radiation maximum at 253.7 nm and during this process, the hydrogen peroxide accumulated.

$O_3 + H_2O + hv \rightarrow O_2 + H_2O_2$ (2.27)

At neutral and basic pH, hydrogen peroxide accumulation is smaller than that of acidic pH. Peroxide is produced at a rate of ozone mass transfer into solution. This hydrogen peroxide along with ozone participates in secondary reactions to produce hydroxyl radical.

1. $H_2O_2 + hv \rightarrow 2\,OH^{\bullet}$ or (2.28)

2. $HO_2^- + O_3 \rightarrow HO_2 + O_3^- \rightarrow OH^{\bullet} + 2O_2$ (2.29)

Although O_3/UV and O_3/H_2O_2 processes seems to follow the same mechanism for the generation of hydroxyl radical, in the former hydrogen peroxide is formed in situ, rather than adding it from an external source. Ozone/UV process has the advantage for dissolved substances that undergo direct UV photolysis contributing to the decomposition rate of substance, k_0 as given by the following formula (Glaze *et al*, 1987):

$$k_0 = k_p + k_{ox} D^n \quad (2.30)$$

where

k_0 = Decomposition rate of substance (1/s),

k_p = Direct photolysis constant (1/s),

k_{ox} = Constant for OH radical reaction with substrate (L/mole s),

D = Ozone dose (mole/L),

n = Kinetic coefficient.

When a dissolved substance absorbs light strongly in the UV region, large fluxes of UV radiation will accelerate the destruction of it where the k_p is so large that using ozone has no significant effect. On the other hand, when the substance is not photolyzed directly with high efficiency, the use of UV radiation to generate hydrogen peroxide makes little sense. In such cases, it is preferable to add hydrogen peroxide from an external source in order to increase destruction rate of substance.

Photon flux, concentration of O_3, pH, and concentration of radical scavengers are important factors affecting treatment efficiency of O_3/UV process and optimum conditions for photolytic ozonation can also vary with substrate type and concentration (Peyton and Glaze, 1988).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Synthetic Ceftriaxone Sodium (Human Antibiotic I) Formulation Wastewater

For the preparation of synthetic formulation wastewater, a cephalosporine group human antibiotic was used. Finished product of antibiotic contains only the active substance, ceftriaxone sodium, which is white to yellowish orange powder. The molecular structure of ceftriaxone sodium is represented in Figure 3.1. and some properties of it are given in Table 3.1.

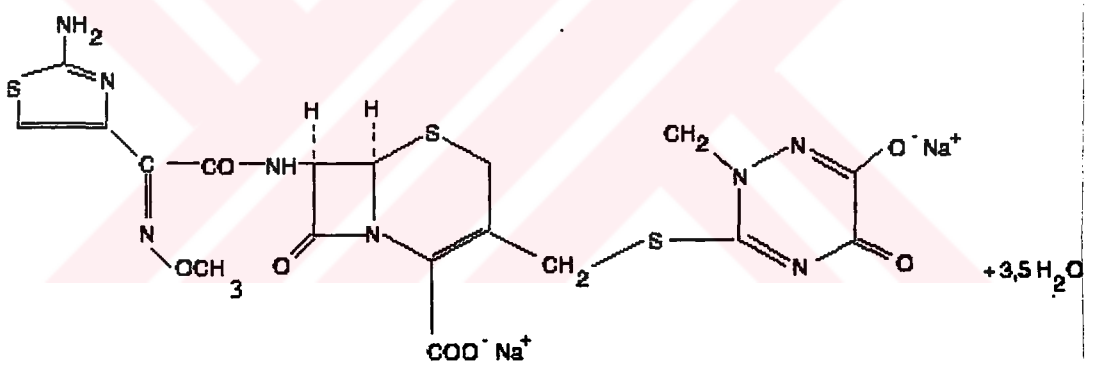


Figure 3.1. The molecular structure of ceftriaxone sodium.

Table 3.1. Characteristics of ceftriaxone sodium

Trade Name	Rocephin®
Antibiotic Group	Cephalosporine
Supplier	Roche
Solubility (g/L)	470*
Molecular Weight (g/mole)	661.59
COD (mg/ g)	993
BOD_5 (mg/mg)	Non-biodegradable
λ_{max} (nm)	241

*Data from safety sheet of ceftriaxone sodium

The absorption spectra of synthetic ceftriaxone sodium formulation wastewater at its natural pH which is approximately 6.7 in deionized water is presented in Figure 3.2.

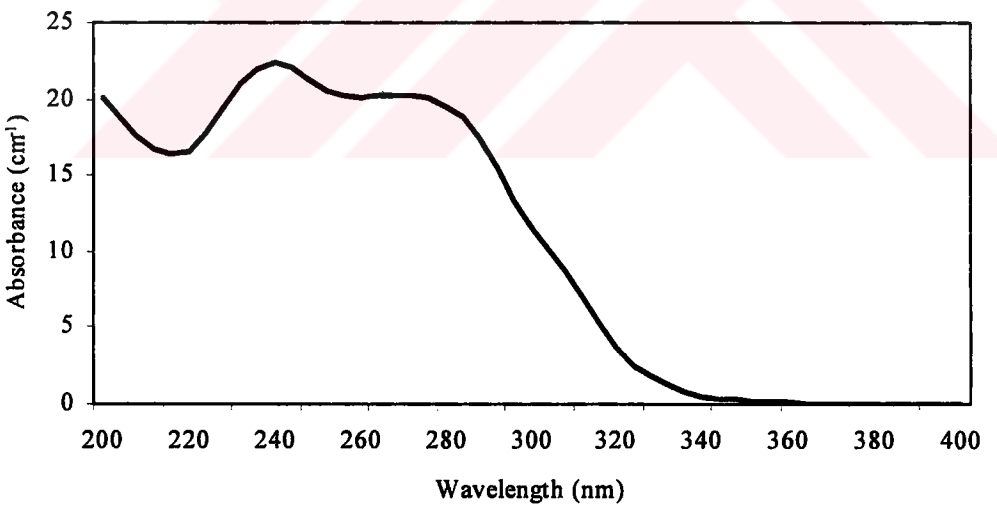


Figure 3.2. Absorption spectra of ceftriaxone sodium with the initial COD of 450 mg/L at pH 6.7.

3.1.2. Synthetic Penicillin VK (Human Antibiotic II) Formulation Wastewater

Second human antibiotic used for the preparation of synthetic wastewater was penicillin group and similar to human antibiotic I, its finished product contains only the active substances, penicillin VK which is white powder. The molecular structure and some properties of penicillin VK are presented in Figure 3.3 and Table 3.2 respectively.

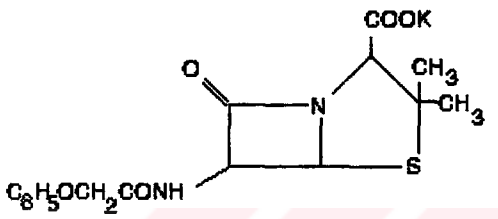


Figure 3.3. The molecular structure of penicillin VK.

Table 3.2. Characteristics of penicillin VK

Trade Name	Penicillin®
Antibiotic Group	Penicillin
Supplier	Hoechst Marion Roussel
Solubility (g/L)	≥100*
Molecular Weight (g/mole)	388
COD (mg/ g)	1426
BOD_5 (mg/mg)	Non - biodegradable
λ_{max} (nm)	267.5

*Data from safety sheet of penicillin VK

The absorption spectra of synthetic penicillin VK formulation wastewater at its natural pH that is 5.7 in deionized water is presented in Figure 3.4.

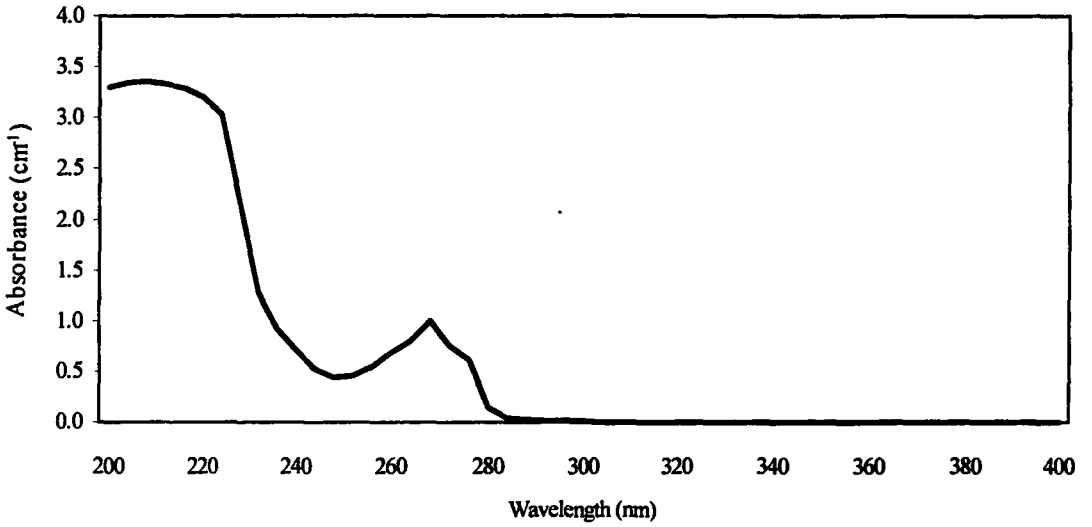


Figure 3.4. Absorption spectra of penicillin VK at pH 5.7 with the initial COD of 450 mg/L.

3.1.3. Synthetic Enrofloxacin (Veterinary Antibiotic) Formulation Wastewater

Lastly, a quinolone group veterinary antibiotic was used for the preparation of synthetic formulation wastewater. In contrast to human antibiotics, it is in liquid form containing 10 per cent active substance (enrofloxacin). Molecular structure and some characteristics of enrofloxacin is presented in Figure 3.5 and Table 3.3 respectively.

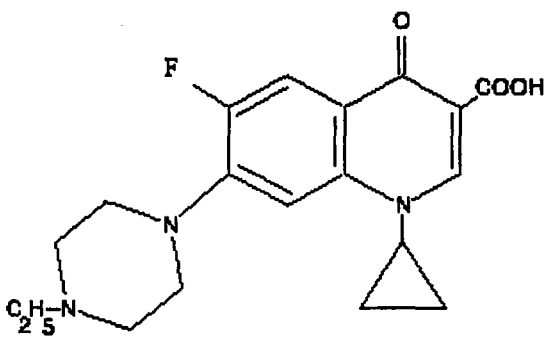


Figure 3.5.The molecular structure of enrofloxacin.

Table 3.3. Characteristics of enrofloxacin

Trade Name	Baytril®
Antibiotic Group	Quinolone
Supplier	Bayer
Solubility (g/L)	Soluble pH≥11
Molecular Weight (g/mole)	359.4
COD (mg/ g)	2250
BOD_5 (mg/mg)	Non biodegradable
λ_{max} (nm)	280-334.5

Formulation mixture of enrofloxacin has 199 mg COD/mL formulation mixture and 15.5 mg BOD/mL formulation mixture. Similar to the active substance, the mixture gave the maximum absorption at 334.5 nm and 280 nm wavelengths. The absorption spectra of formulation mixture of enrofloxacin is given in Figure 3.6.

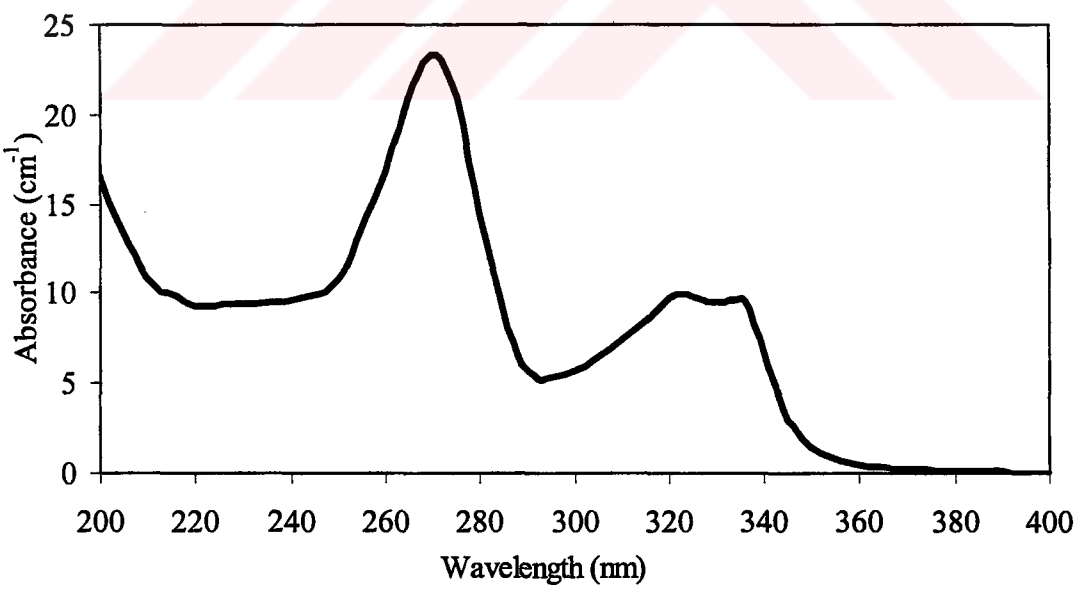


Figure 3.6. Absorption spectra of enrofloxacin formulation mixture with the initial COD of 450 mg/L at pH 7.5.

3.1.4. Oxidants

H_2O_2 stock solution (30 per cent w/w) was supplied from Merck and kept at 4°C.

3.1.5. Buffer solutions

Ozonation of the antibiotics was conducted on synthetic solutions at three different pH values. The solutions were buffered with phosphate buffer having the same ionic strength, 0.2 μ (Christian, 1994). All buffer solutions were prepared with deionized water and for the preparation of 1750 mL synthetic effluent, 1250 mL phosphate buffer was used. The chemicals used for buffer were obtained from Merck in analytical grade (Table 3.4).

Table 3.4. The composition of phosphate buffer solutions

pH	KH_2PO_4 (g/L)	Na_2HPO_4 (g/L)	H_3PO_4 (mL/L)	Na_3PO_4 (g/L)
3	27.2	-	1.35	-
7	4.64	7.84	-	-
11	-	8.16	-	1.7365

3.2. Methods

3.2.1. Chemical Reactors

3.2.1.1. Ozone Reactor. The semi-batch ozone reactor was a 1.5 L volume glass bubble column (78 cm height and 6 cm diameter). The reactor had ports for ozone inlet, outlet and liquid sample removal. Ozone was produced from oxygen in a laboratory scale ozone generator unit (Fisher OZ 500) at a flow rate of 100 L/h. The oxygen–ozone mixture was fed to the reactor through a porous plate located at the bottom of the column. All tubings from the ozone generator to the reactor were made of Neoprene and fittings from Teflon. The reactor was equipped with a recirculating system of the liquid phase (Figure 3.7). During each experiment aliquots of sample solutions were withdrawn periodically from sampling port.

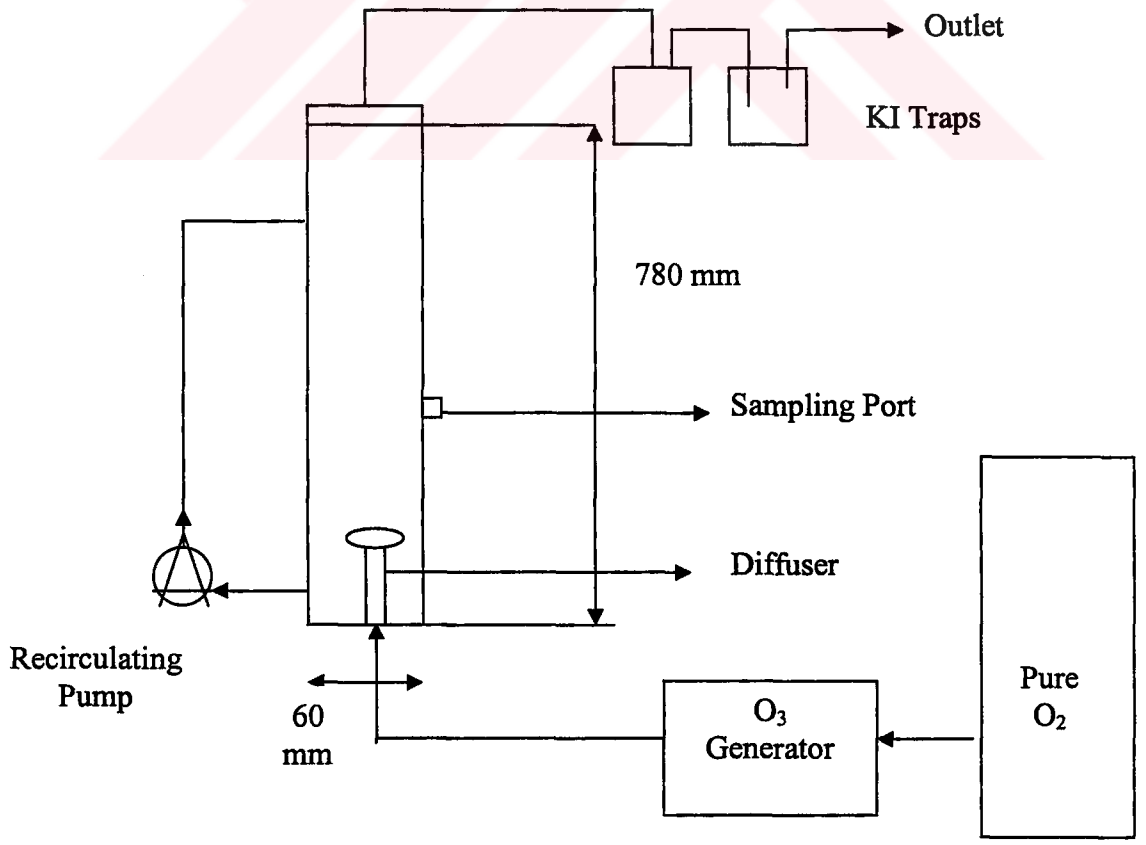


Figure 3.7. Schematic presentation of ozone reactor.

3.2.1.2. Photochemical Reactor. O_3 / UV-C oxidation of synthetic antibiotic formulation wastewaters were conducted in a 2 L capacity photoreactor (97 cm height and 7 cm diameter) at recirculating batch mode. The UV-C reactor was equipped with 20 W low-pressure mercury lamp (Ultramax, 48 cm height and 2 cm diameter) that was placed into a quartz envelope (94 cm height and 3 cm diameter) and mainly emitted at 253.7 nm. The incident radiation flux of the UV-C lamp and effective path length in the photochemical reactor were determined by hydrogen peroxide actinometry (Nicole, 1990). Mixing of the reaction solution was provided by Cole Parmer recirculating pump (Figure 3.8.).

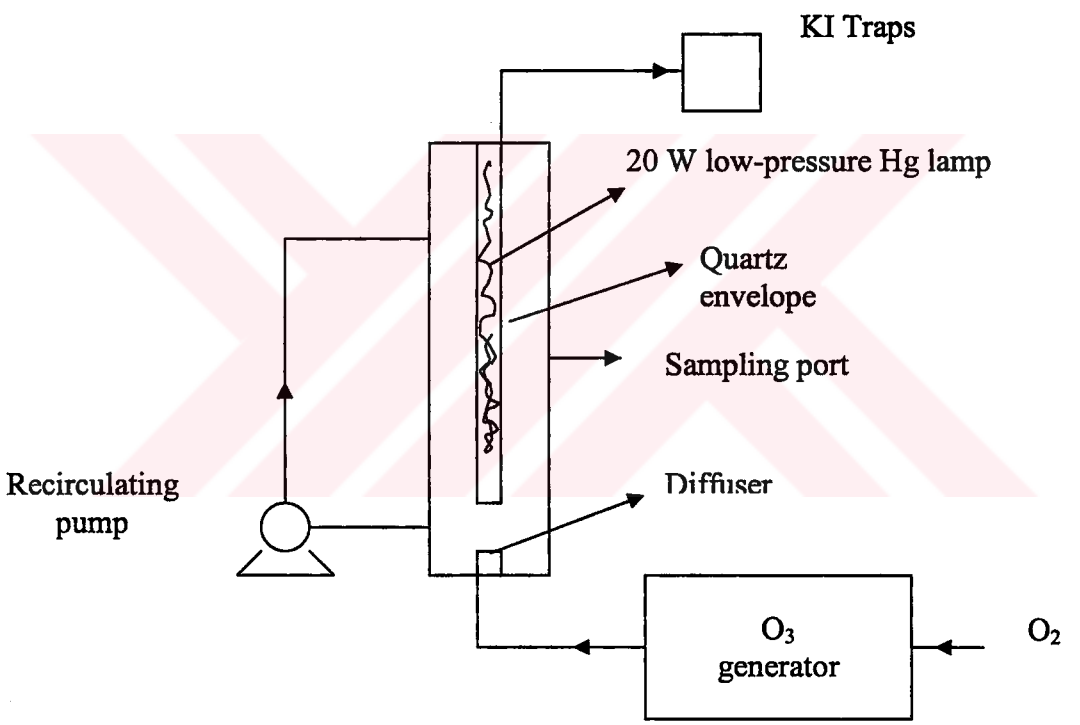


Figure 3.8. Schematic presentation of photochemical reactor.

3.2.2. Analytical Parameters

3.2.2.1. Spectrophotometric Measurements. The light absorbances at the 254 nm wavelength that is the maximum absorbance wavelength of the organic compounds (Beltran et al., 2001) and at the characteristic wavelength of synthetic formulation wastewater were recorded. For spectrophotometric measurements, Schimadzu UV-160 model spectrophotometer was used.

3.2.2.2. COD Measurements. COD measurements were conducted in accordance with Standard Methods by the closed reflux colorimetric method (APHA/AWWA/WPCF, 1989).

3.2.2.3. BOD_5 Measurements. BOD_5 measurements were carried out by monometric method (Velp). Domestic sewage was used as the microbial inoculum at a volume 10 per cent of the sample volume.

3.2.2.4 Residual Hydrogen Peroxide Determination. Molibdate catayzed iodometric titration was used for the determination of residual hydrogen peroxide concentration (Klassen *et.al.*, 1994).

3.2.2.5 Destruction of Residual Hydrogen Peroxide with the Enzyme Catalase. In order to prevent further reaction with samples and positive interference with the COD measurements any excessive H_2O_2 used as an oxidant was destroyed with enzyme catalase from bovine liver (176000 A.U., Sigma), immediately after sampling. 1 A.U. of the catalase destroys 1μmoles of H_2O_2 per minute at pH=7 at 25°C.

3.2.2.6. Respirometric Measurements. The Oxygen Uptake Rate (OUR_0) of the raw and ozonated synthetic veterinary antibiotic formulation wastewater treated in photochemical and ozone reactor was measured by WTW Oxi 3000 model respirometer. For this purpose, 50 mL of acclimatized and pre-settled mixed culture obtained from aeration tank of a local wastewater treatment plant were mixed with 350 mL raw and ozonated wastewater and aerated with diffuser. Acclimatization of biomass used for OUR measurements was

conducted during several weeks by synthetic domestic wastewater The feed base was diluted to obtain a COD feed rate of 250 mg/L O_2 /day. The composition of synthetic domestic wastewater is given at Table 3.5.

Table 3.5. Composition of the synthetic domestic effluent solution (Aktas, 1999)

Chemical Component	Concentration (g/L)
CH_3COONa	6.0
Glucose	5.6
Peptone	2.0
$(NH_4)_2SO_4$	5.0
$MgSO_4$	1.0
KH_2PO_4	2.0
K_2HPO_4	2.0
$CaCl_2$	0.45
$FeCl_3.6H_2O$	0.2
$NaHCO_3$	3.0

3.2.3 Ozone Analysis

3.2.3.1. Determination of Dissolved Ozone. Dissolved ozone concentrations were determined by the indigo colorimetric method in accordance with Standard Methods (APHA/AWWA/WPCF, 1989). Absorbance values of solutions were measured at 600 nm and concentration of the dissolved ozone was calculated according to the following formula;

$$\text{Ozone concentration } mg\ O_3/L = \frac{100 \times \Delta A}{f \times b \times V} \qquad (3.1)$$

where

ΔA = difference in absorbance between sample and blank,

b = path length of cell (cm),

V = volume of sample (mL),

f = the slope of the calibration curve at 600 nm (0.42 ± 0.001 cm^{-1} per mg O_3/L).

3.2.3.2. Determination of Gas Phase Ozone Concentration. The concentration of ozone in the input and off gas was determined by the iodometric method (IOA, 1987). Ozone was absorbed in 110 mL KI solution and then solution was acidified by 10 mL H_2SO_4 (25 per cent) before titration with sodium thiosulfate. The concentration of gas phase ozone was calculated by the following formula:

$$O_3(mg/\min) = \frac{V_{S_2O_3^{2-}}(mL) \times N_{S_2O_3^{2-}}(eqg/L) \times 24\ g/eqg}{t(\min)} \qquad (3.2)$$

3.2.3.3. Determination of Ozone Mass Transfer Coefficient (k_La). The rate limiting step in the ozonation of wastewater is the mass transfer of ozone from the gas phase to the wastewater. Ozone mass transfer coefficient mainly depends upon the system (e.g. air flow rate) and composition of water (Wu and Wang, 2001). The volumetric mass transfer coefficient of ozone at ozone and photochemical reactors were determined by the ozonation of organic free water at pH = 2. Dissolved ozone concentrations as a function of ozonation time at ozone and photochemical reactors are shown in Figure 3.9 and Figure 3.10 respectively.

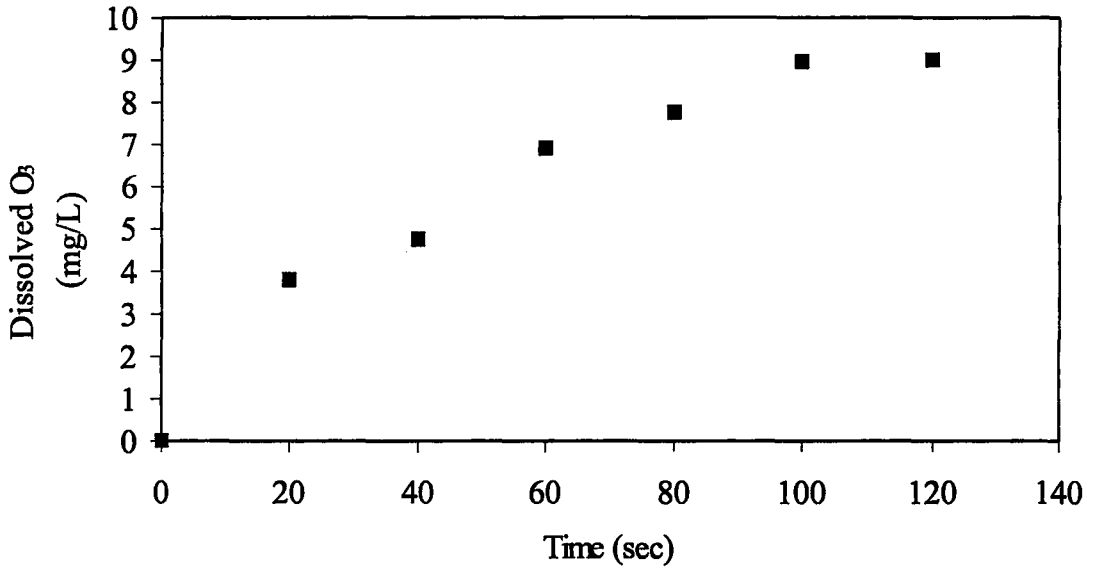


Figure 3.9. Dissolved ozone concentrations as a function of ozonation time for organic free water in ozone reactor.

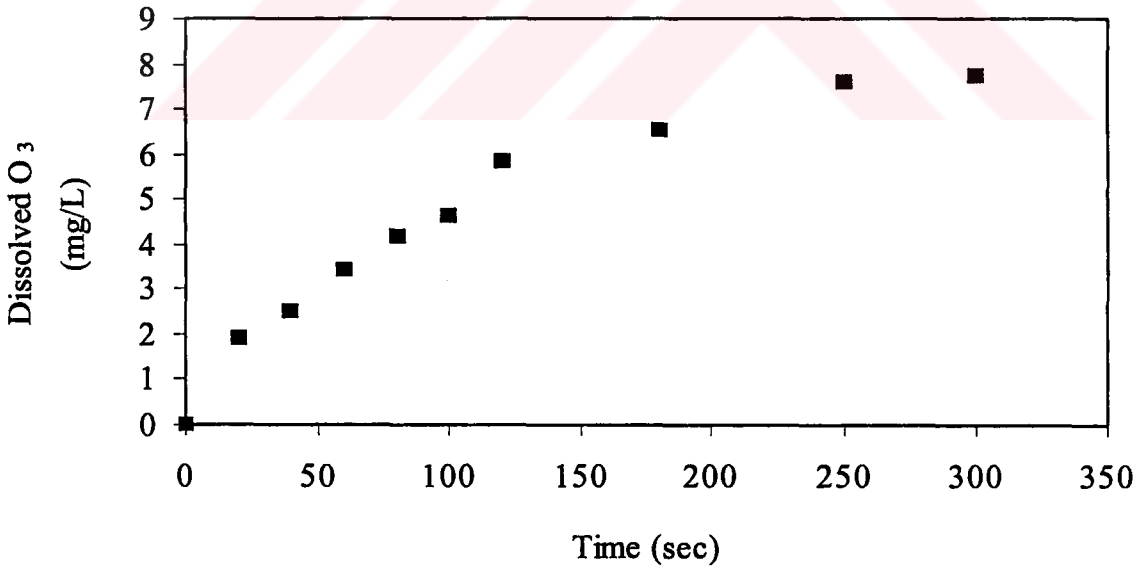


Figure 3.10. Dissolved ozone concentrations as a function of ozonation time for organic free water in photochemical reactor.

Volumetric mass transfer coefficients for ozone and photochemical reactors were calculated as 0.0293 sec^{-1} and 0.0104 sec^{-1} respectively, by the following formula ignoring the self decomposition of ozone;

$$\ln\frac{(C_s - C_t)}{C_s} = -k_L a \times t \qquad (3.3)$$

where

C_s = saturation concentration of ozone at the gas liquid interphase,

C_t = dissolved ozone concentration at time t,

$k_L a$ = volumetric mass transfer coefficient,

t = time.

3.2.4. H_2O_2 Actinometry

H_2O_2 actinometry was performed according to the experimental procedure described by Nicole, (1990). This procedure was used for the determination of incident photonic fluxes at 253.7 nm emitted by low-pressure mercury vapour lamps and for the determination of effective path length for photochemical reactor. Decomposition of hydrogen peroxide to H_2O and O_2 by the absorption of incident radiation at 253.7 nm is given in the following equations:

$$H_2O_2 \rightarrow 2OH^{\bullet} \qquad (3.4)$$

$$OH^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \qquad (3.5)$$

$$HO_2^{\bullet} + H_2O_2 \rightarrow OH^{\bullet} + H_2O + O_2 \qquad (3.6)$$

$$OH^{\bullet} + HO_2 \rightarrow H_2O + O_2 \qquad (3.7)$$

Initial concentrations of H_2O_2 were chosen as 1 mM and 100 mM to measure the decomposition of H_2O_2 during the H_2O_2/UV-C reaction. Residual H_2O_2 was determined by the molybdate catalyzed iodometric method (Klassen *et al.*, 1994). Residual H_2O_2 concentration was found according to the following equation:

$$C_{H_2O_2}(mg/L) \times V_{H_2O_2}(mL) = N_{S_2O_3^{2-}}(eqg/L) \times V_{S_2O_3^{2-}}(mL) \times 17000(mg/eqg) \qquad (3.8)$$

UV – C Photolysis of 100 mM H_2O_2

H_2O_2 concentrations in 25 mL samples were determined by the iodometric method and 0.2 M $Na_2S_2O_3$ was used as a titrant. Figure 3.11 indicated that the decomposition of high concentration hydrogen peroxide by UV-C lamp follows zero order kinetics.

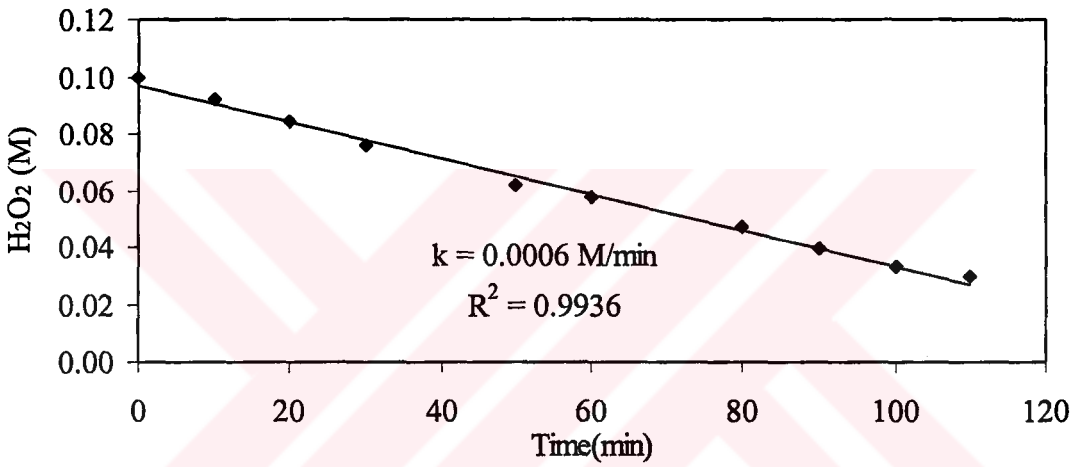


Figure 3.11. Decomposition of 100mM H_2O_2 by UV-C photolysis.

According to data obtained, the zeroth order decomposition rate constant of 100 mM H_2O_2 was found as 0.0006 M/min (1.0×10^{-5} M/s).

For high concentration of H_2O_2 the decomposition rate of it can be described by the following equation:

$$\frac{d[H_2O_2]}{dt} = I_0 \times \Phi \qquad (3.9)$$

where

Φ_{254} = quantum yield for photolysis of H_2O_2 at 254 nm, 1.04 mole H_2O_2 / mole photons (Klassen *et al.*, 1994).

$I_0 = (1.0 \times 10^{-5}\ M/s)/(1.04\ mole H_2O_2/mole\ photons) = 9.6 \times 10^{-6}$ mole photons/Ls

$= 9.6 \times 10^{-6}$ Einstein / Ls

The energy of one mole of photon is:

$E_{photon} = (h \times c/\lambda) \times 6.022 \times 10^{23}$ photons/mole photon

$= (6.63 \times 10^{-34}\ Js \times 3 \times 10^{8}\ m/s)/(254 \times 10^{-9}\ m) \times 6.022 \times 10^{23}$ photons/mole photon

$= 4.7 \times 10^{5}$ J/mole photon $= 4.7 \times 10^{5}$ J/Einstein

The incident photonic flux for the photochemical reactor is:

$I_0 = 9.6 \times 10^{-6}$ Einstein / Ls $\times 4.7 \times 10^{5}$ J/Einstein $= 4.51$ J/Ls $= 4.51$ W/L

UV – C Photolysis of 1mM H_2O_2

For the determination of 1 mM H_2O_2 decomposition, 5mM $Na_2S_2O_3$ was used as a titrant. Figure 3.12 indicates that the decomposition of 1mM hydrogen peroxide by UV-C photolysis follows first order kinetics.

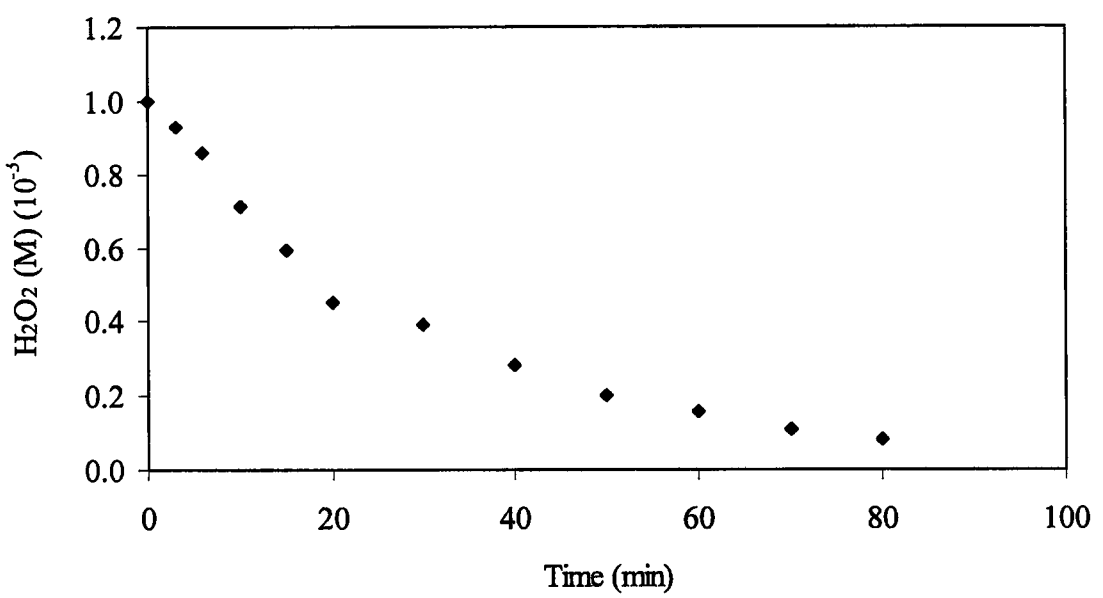


Figure 3.12. Decomposition of 1mM H_2O_2 by UV-C photolysis.

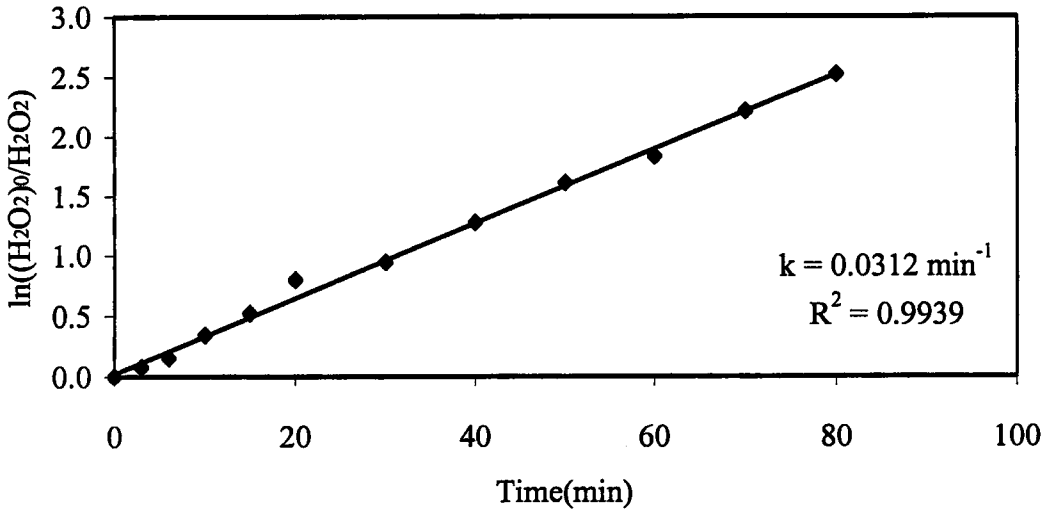


Figure 3.13. Kinetic plot for 1mM H_2O_2 decompostion by UV-C photolysis in photochemical reactor.

The equation for the first order decomposition of H_2O_2 at low concentrations is (Nicole, 1990):

$$\frac{-d\ln[H_2O_2]}{dt} = I_0 \times \Phi_{254} \times \epsilon_{H_2O_2} \times d \qquad (3.10)$$

where

ε_{H2O2} = molar extinction coefficient at 254 nm (19.6 L/mole H_2O_2 x cm),

d = effective path length (cm),

Φ_{254} = quantum yield for photolysis of H_2O_2 at 254 nm (1.04 mole H_2O_2 / mole photons).

$-d \ln[H_2O_2] / dt = k_{H2O2} = 0.0312\ min^{-1} = 5.2 \times 10^{-4}\ s^{-1}$

$d = 2.66$ cm

3.2.5. Kinetic Evaluation

The efficiency of ozonation process for the treatment of synthetic effluents was mainly assessed in terms of COD abatement. Theoretically, ozonation experiments depend on both ozone and investigated chemical concentration (Bellamy *et al.*, 1991).

$$\frac{-d[C]}{dt} = k \times [C][O_3] \qquad (3.11)$$

where

k = second order reaction rate constant (min^{-1}),

$[O_3]$ = concentration of ozone (mol/L),

[C] = concentration of substance (mol/L).

By implying that ozone concentration in the reacting medium remains constant, COD abatement can be assumed to follow pseudo-first order kinetics:

$$\ln\frac{[COD]}{[COD_0]} = -k_1 \times t \qquad (3.12)$$

where

k_1 = pseudo-first order COD removal rate constant (min^{-1}),

[COD] = COD value at time t (mg/L),

$[COD_0]$ = initial COD value,

t = time (min).

In all experiments pseudo- first order rate constants were calculated within the 30 minutes. The correlation coefficient indicated a good fit of the observed data to the pseudo first order model (correlation coefficient, $R^2 > 0.9$) at that reaction period. Discrepancies from first order kinetics were observed at longer reaction times.

3.2.6. Determination of Bond Dissociation Energies

The bond dissociation energies of antibiotics to form radicals through H abstraction by hydroxyl radical were determined by using the computer program PC Spartan Pro. First, the molecules were constructed and conformational space was searched through rotation of every flexible bond by 120^0 using molecular mechanics method. The most stable conformer was then extracted. Heat of formation of the most stable conformer was calculated using semi-empirical PM3 method. The optimized geometry was used to generate radicals through H-abstraction at every different site of the structure. Single point heats of formation values of radical structures were then calculated by PM3 method.

4. RESULTS AND DISCUSSION

4.1. Application of O_3, O_3/H_2O_2 Processes to Synthetic Antibiotic Formulation Wastewater in the Ozone Reactor

In this study, O_3 and O_3/H_2O_2 processes were applied to pharmaceutical formulation effluents that were synthetically prepared from two human antibiotics and a veterinary antibiotic. Since the actual COD value of antibiotic formulation effluents varies 450-1500 mg/L, the initial COD values of synthetically prepared formulation effluents were also chosen in this range. The effects of pH, initial antibiotic concentration, inlet ozone dose as well as dose of hydrogen peroxide on ozonation kinetics were investigated. Chemical oxygen demand (COD), biochemical oxygen demand (BOD), oxygen uptake rate (OUR), aromatic content (UV_{254}), and the maximum absorption values of antibiotics were the parameters followed in order to evaluate the performance of ozonation process.

4.1.1. The Effect of pH

In general, ozone reacts with organic compounds found in water and wastewater via two different pathways namely direct molecular and indirect radical chain type reaction depending upon pH and composition of water. Direct oxidation predominated under acid conditions is more selective, while radical oxidation that is dominate at basic pH is less selective (Langlais *et al.*, 1991).

To elucidate the effect of pH on the oxidation of antibiotic compounds, three different synthetic antibiotic formulation effluents having COD value of 450 mg/L were subjected to ozonation with an applied ozone dose ($(O_3)_i$) of 2960 mg/L h in buffered solutions at pH 3, 7 and 11. Figure 4.1 displays the time dependent changes in normalized UV_{254} and COD at varying pH values.

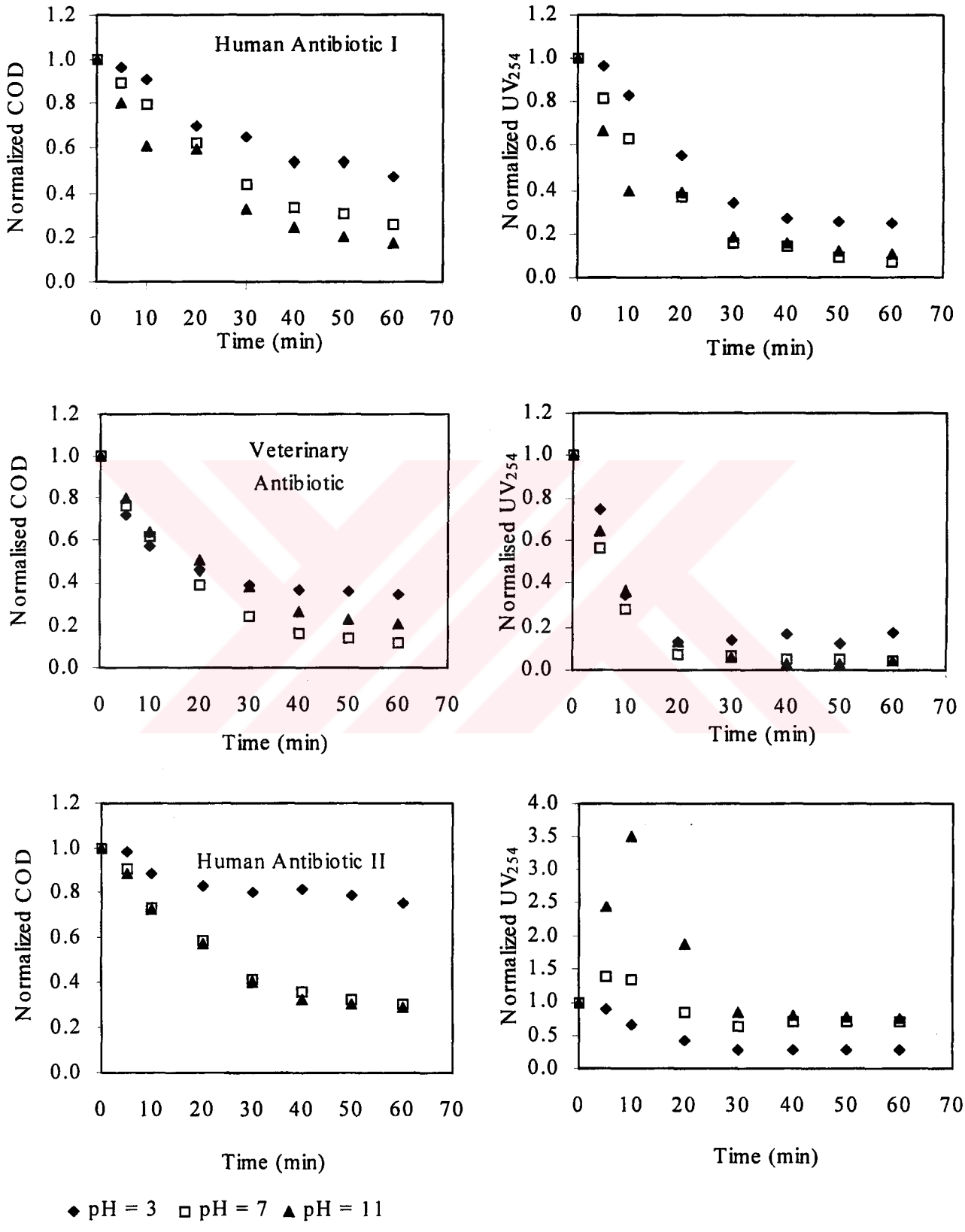


Figure 4.1. Variations in normalized COD and UV_{254} values of synthetic human antibiotic I, veterinary antibiotic, and human antibiotic II formulation effluents as a function of treatment time for different pH values (COD_i = 450 mg/L).

With the increment of the solution pH from 3 to neutral and alkaline values, COD and UV_{254} abatements were enhanced for three effluents as expected. Since the oxidation potential of hydroxyl radicals is much higher than that of ozone molecule, direct oxidation is slower than radical oxidation and furthermore causes incomplete oxidation of aromatic compounds. However, the results indicated that COD and UV_{254} abatement of veterinary antibiotic, which showed a little dependence on pH, was also achieved by direct ozonation. Similar results were also reported in the literature for the ozonation of cyanide (Rice, 1977) and methyl orange (Chen, 2000).

Aside with COD and UV_{254}, removal of maximum absorption values of synthetic antibiotic effluents was also followed in order to evaluate the performance of ozonation process. While veterinary antibiotic gave the maximum absorption at 334.5 and 280 nm (Figure 4.2), for human antibiotic II this value was determined as 267.5 nm (Figure 4.3).

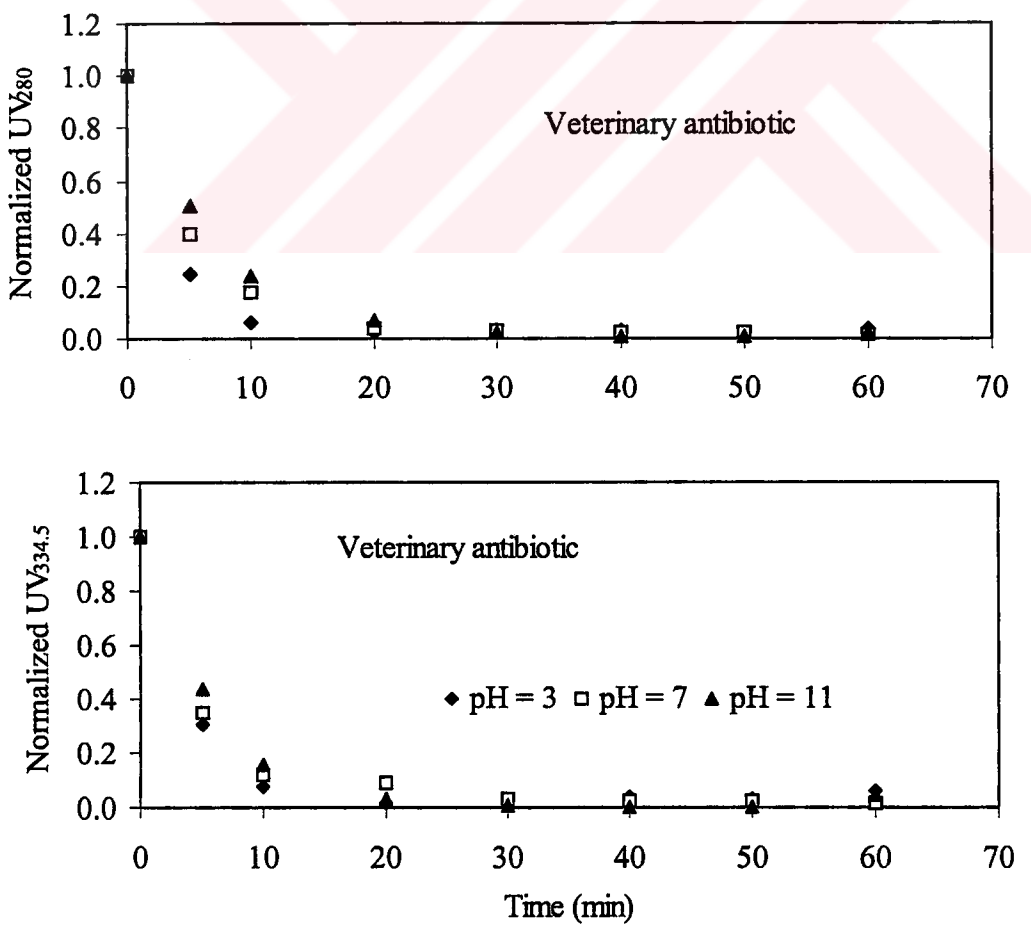


Figure 4.2. Removal kinetics for maximum absorption of veterinary antibiotic (COD_i = 450 mg/L) for different pH values.

As can be seen from the Figure 4.2, independent of pH, removal of the absorption values at 280 and 334.5 nm for veterinary antibiotics are very rapid and complete removal was achieved within the 20 minutes of ozonation process.

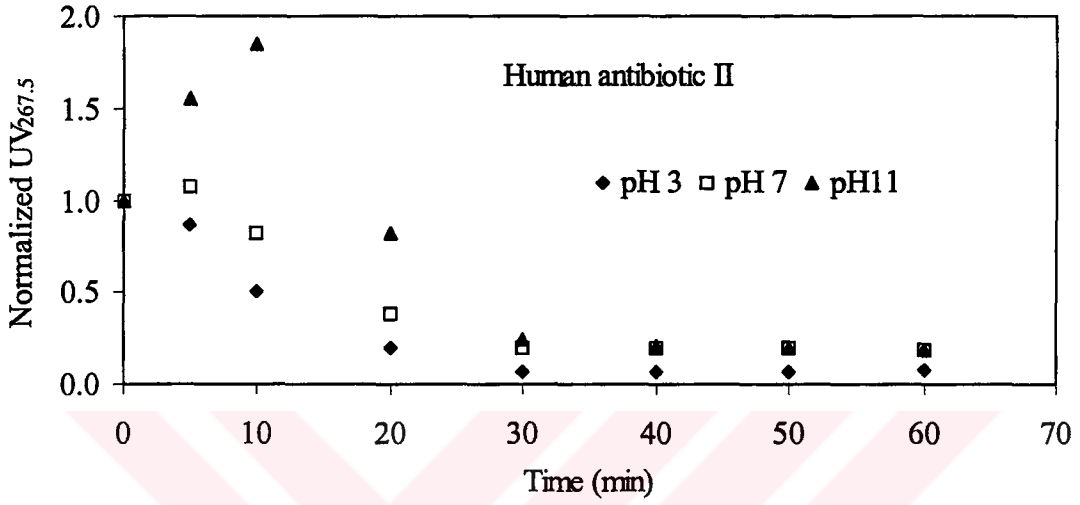


Figure 4.3. Removal kinetics for maximum absorption of human antibiotic II (COD_i = 450 mg/L) for different pH values.

Figure 4.3 indicates that the removal of absorption of human antibiotic II at 267.5 nm as a function of ozonation time was relatively higher than that of aromatic content of it, even at alkaline pH where formation of intermediates with higher absorption values were obtained.

Pseudo first order rate constants for COD reduction and overall COD, UV_{254} and maximum absorption removal percentages for one hour ozonation of the antibiotic effluents as a function of pH are summarized at Figure 4.4.

Pseudo first order rate constants for COD reduction were calculated within the 30 minutes of ozonation, since the correlation coefficient indicated a good fit of the observed data to the pseudo first order model at that reaction period ($R^2 > 0.9$). Due to the variations in the absorbance values of human antibiotic II at 254 nm within the first 10 minutes, variations in absorbance values were represented as removal percentage in one hour.

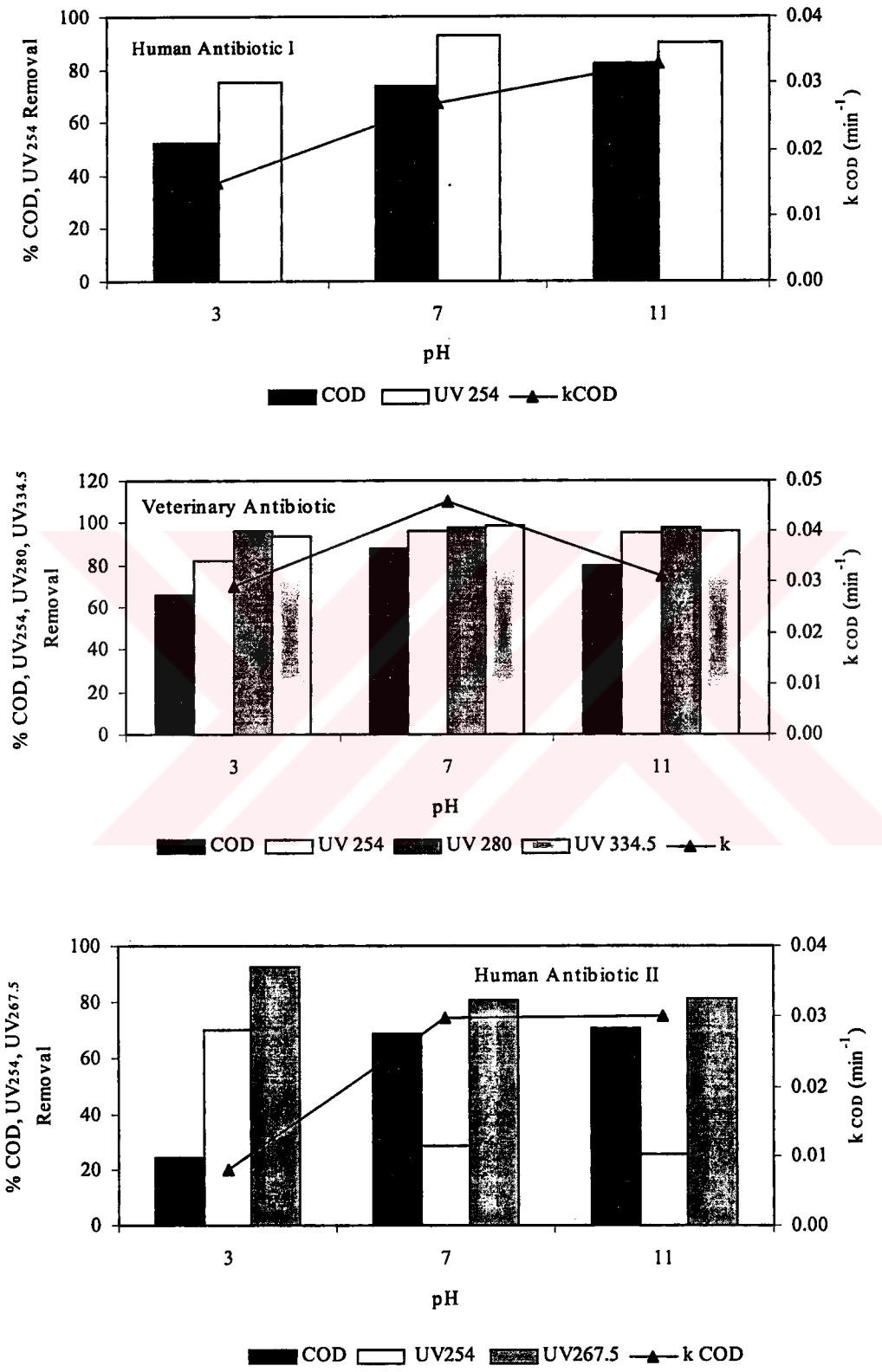


Figure 4.4. Effect of pH on COD removal rate constant, COD and absorbance removal percentages of synthetic antibiotic effluents (COD_i = 450 mg/L, $(O_3)_i$ = 2960 mg/L h).

Although the effect of pH on ozonation of each selected antibiotic exhibited different trend, removal of COD was low at pH 3 for all three of antibiotics. While the highest COD removal rate was obtained at pH 7 for veterinary antibiotic, that of human antibiotic I and human antibiotic II was more pronounced at pH 11. Raising the pH from 3 to 11 for ozonation of human antibiotic II results in more than 3 fold increase in pseudo first order COD removal rates (increased from 0.0079 min^{-1} to 0.03 min^{-1}). While increase in the solution pH provided a slight enhancement for UV_{254} removal of human antibiotic I, had no effect on that of veterinary antibiotic. However, removal of aromaticity for human antibiotic II exhibited a completely different trend due to the significant increases in UV_{254} absorbance values at pH 7 and 11 within the first 10 minutes of ozonation (Figure 4.1). This may indicate the formation of intermediates possessing higher UV absorbance values as a result of the different reaction mechanism followed at these pH values.

In order to indicate importance of pH control during ozonation process, separate ozonation experiments ($(O_3)_i$ = 2960 mg/Lh) were carried out by using unbuffered solution of human antibiotic I and variations of pH as well as COD were detected (Figure 4.5).

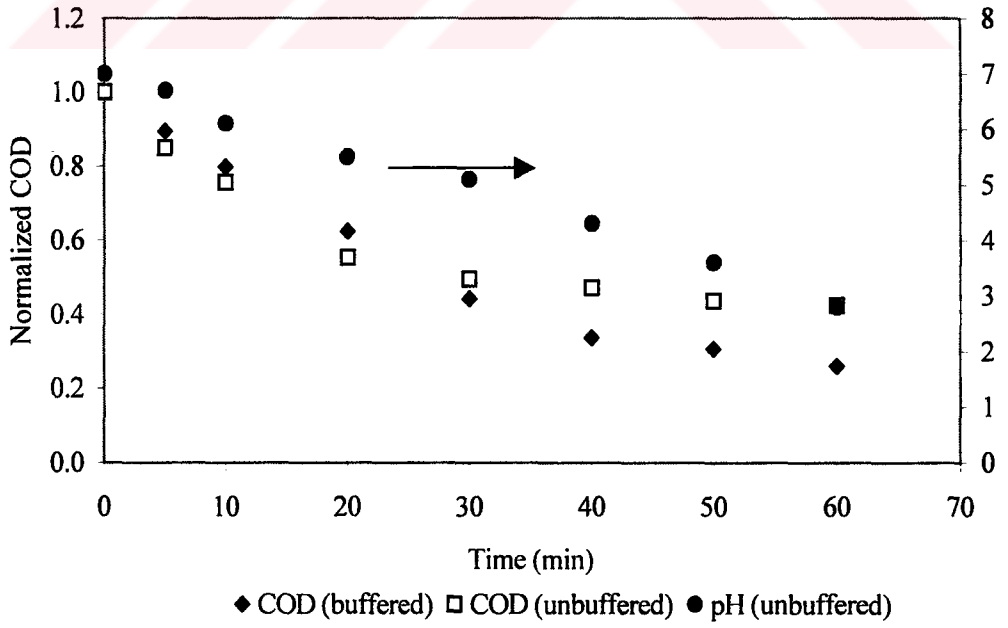


Figure 4.5. COD abatement kinetics of buffered and unbuffered human antibiotic I formulation effluents and variations in pH value of unbuffered solution (COD_i = 450 mg/L).

As can be seen from Figure 4.5, steadily pH drop, which indicated the formation of acidic reaction intermediates, was observed during the ozonation reaction. Low pH is known to suppress the formation of hydroxyl radicals from ozone and ozone is reacting directly by an electrophilic attack that leads to incomplete COD removal. Reaction products formed at acidic pH were resistant to oxidation by ozone. However in case of ozonation at pH 7 both $OH^{\bullet}$ radicals and ozone are the oxidizing agent hence, significant portion of COD was removed by the progress of ozonation. In addition, this result may suggest that the generated intermediates and the acids become increasingly important scavengers of hydroxyl radicals (Beltran, 2001). Even at high pH, ozonation reaction rates slowed down by the progress of ozonation and the reaction rate constant was about half of the initial stages as ozonation (Figure 4.1).

4.1.2. The Effect of Initial Concentration

Since effluents from formulation process in pharmaceutical industry might show a fluctuation in its quality, it is important to examine the effect of initial COD of wastewater on the treatment efficiency of ozonation process. For this purpose, synthetic wastewater samples having the COD values 250 mg/L–1400 mg/L were ozonated at pH = 7 in phosphate buffered solutions with an applied ozone dose of 2960 mg/L h and removal kinetics in terms of COD and UV $_{254}$ were investigated (Figure 4.6). COD and absorbance values of three different antibiotics at different concentrations are presented in Table 4.1. Preparation of veterinary antibiotic effluent with the initial COD of 1400 mg/L could not be accomplished due to its low solubility at pH = 7.

Table 4.1. COD and absorbance values of antibiotics at different concentrations

Human Antibiotic I				
Concentration (mg/L)	COD (mg/L)	UV_{254} (1/cm)		
238	250	10.25		
450	450	19.425		
926	900	41.4		
1389	1400	60.4		
Veterinary Antibiotic				
Concentration (mL/L)	COD (mg/L)	UV_{254} (1/cm)	UV_{280} (1/cm)	$UV_{334.5}$ (1/cm)
1.25	250	8.43	10.5	4.92
2.2	450	11.025	14.45	8.675
4.5	900	24.78	31.78	19.74
Human Antibiotic II				
Concentration (mg/L)	COD (mg/L)	UV_{254} (1/cm)	$UV_{267.5}$ (1/cm)	
177	250	0.266	0.504	
317	450	0.456	0.883	
630	900	0.96	1.8	
982	1400	1.53	2.84	

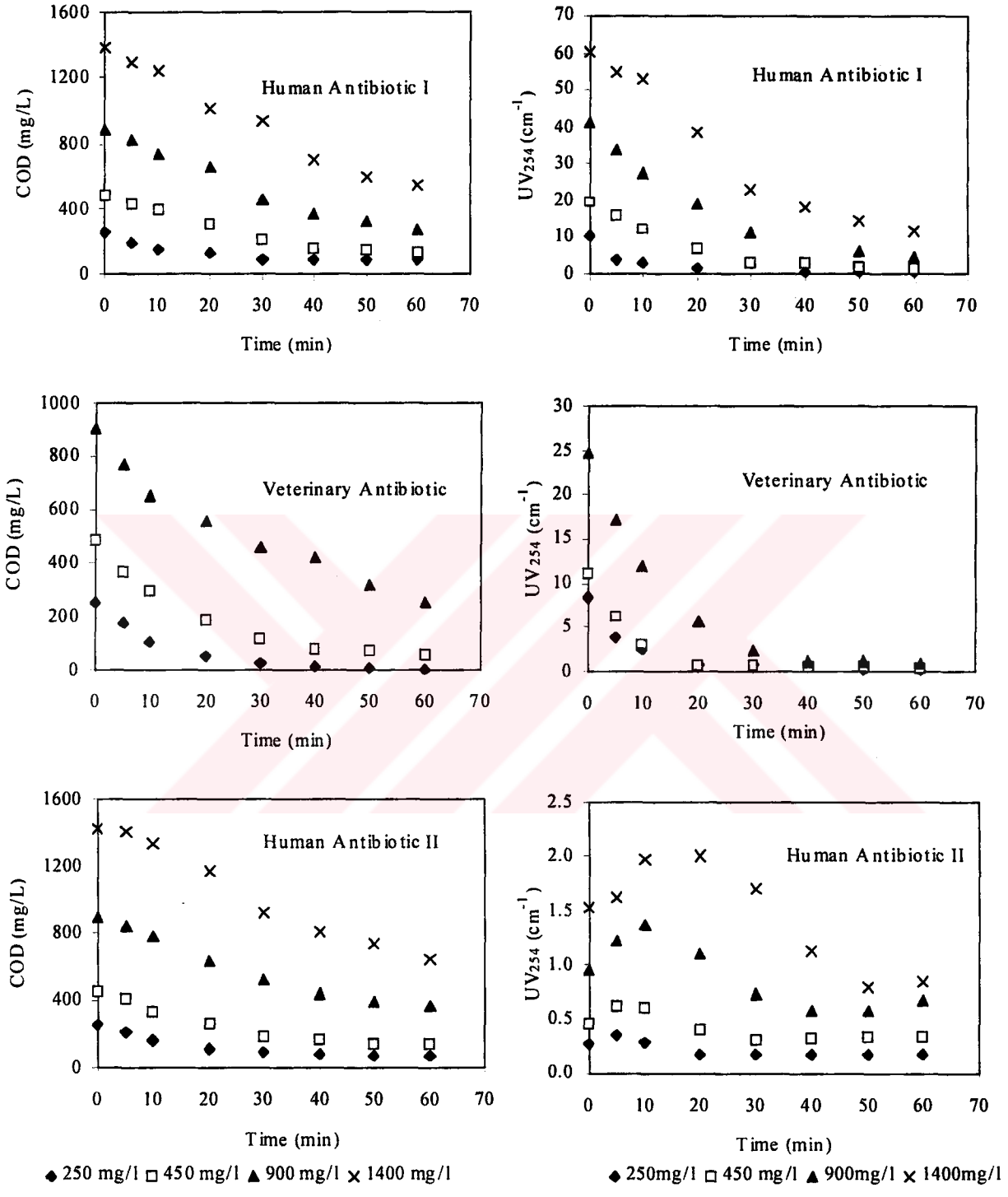


Figure 4.6. COD and UV_{254} abatement kinetics of synthetic human antibiotic I, veterinary antibiotic and human antibiotic II for different initial COD values (pH = 7, $(O_3)_i$= 2960 mg/L h).

It is obvious that in terms of COD removal rate, the treatment efficiency of synthetic wastewater samples decreased considerably with an increase in the initial COD value. Increasing initial COD concentration in the range of 250 mg/L – 1400 mg/L also had a significant effect upon UV_{254} absorption as well as COD removals.

It is also clearly seen from the Figure 4.6 that, UV_{254} removal of veterinary antibiotic was very rapid and completed by one hour ozonation, while residual absorption at 254 nm remained in effluents of human antibiotic I and human antibiotic II at high initial COD concentrations. This result can be explained by the fact that, contrary to human antibiotics, veterinary antibiotic contains only 10 per cent active substance (enrofloxacin), in its formulation leading a rapid degradation of aromaticity.

Figure 4.7 shows the absorption removal kinetics of veterinary antibiotic at 280 nm and 334.5 nm for one hour ozonation (pH = 7, $(O_3)_i$ = 2960 mg/L h) for different initial COD concentrations.

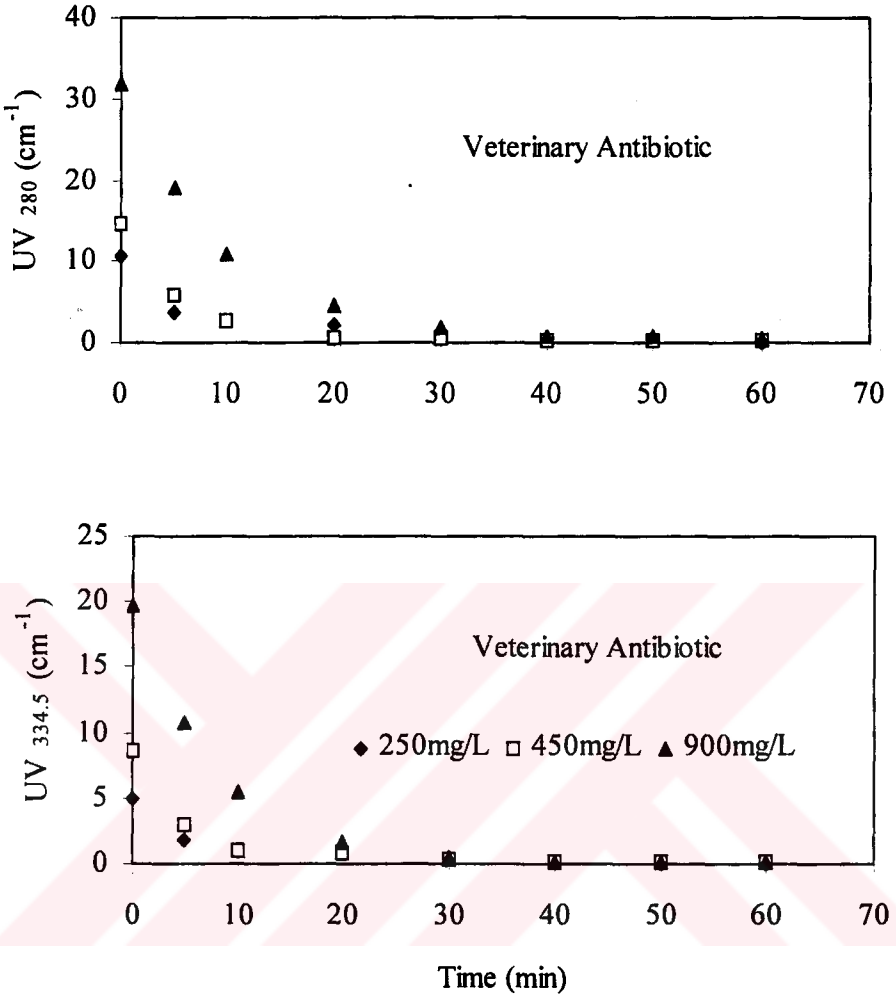


Figure 4.7. Abatement kinetics for absorption of veterinary antibiotic at 280 nm and 334.5 nm, for different initial COD concentrations as a function of ozonation time.

Removals of absorption values at 280 nm and 334.5 nm exhibited same trend with UV_{254} values as indicated in Figure 4.7. Complete removal of these absorption values were also accomplished by one hour ozonation at pH=7 indicating the degradation of chromophore groups on veterinary antibiotics.

Figure 4.8 represents the abatement kinetics for absorption values of human antibiotic II at 267.5 nm for one hour ozonation at pH = 7, for different initial COD concentrations.

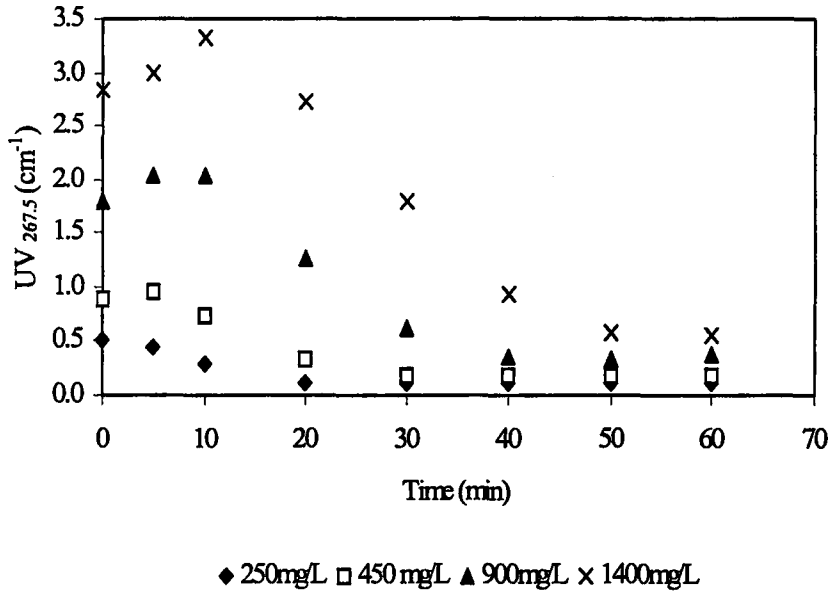


Figure 4.8. Abatement kinetics for absorption of human antibiotic II at 267.5 nm for different initial COD concentrations as a function of ozonation time.

Formation of intermediates possessing higher absorption at 267.5 nm within the 10 minutes of ozonation was more pronounced at higher initial COD values as expected.

Each set of data obtained from Figure 4.6 was regressed to the pseudo first order rate equation in terms of COD removal kinetics. Calculated pseudo first order COD reduction rate constants of synthetic formulation wastewater samples subjected to ozonation ($(O_3)_i$ = 2960 mg/L h) at pH = 7 are illustrated in Figure 4.9 as a function of initial COD concentration.

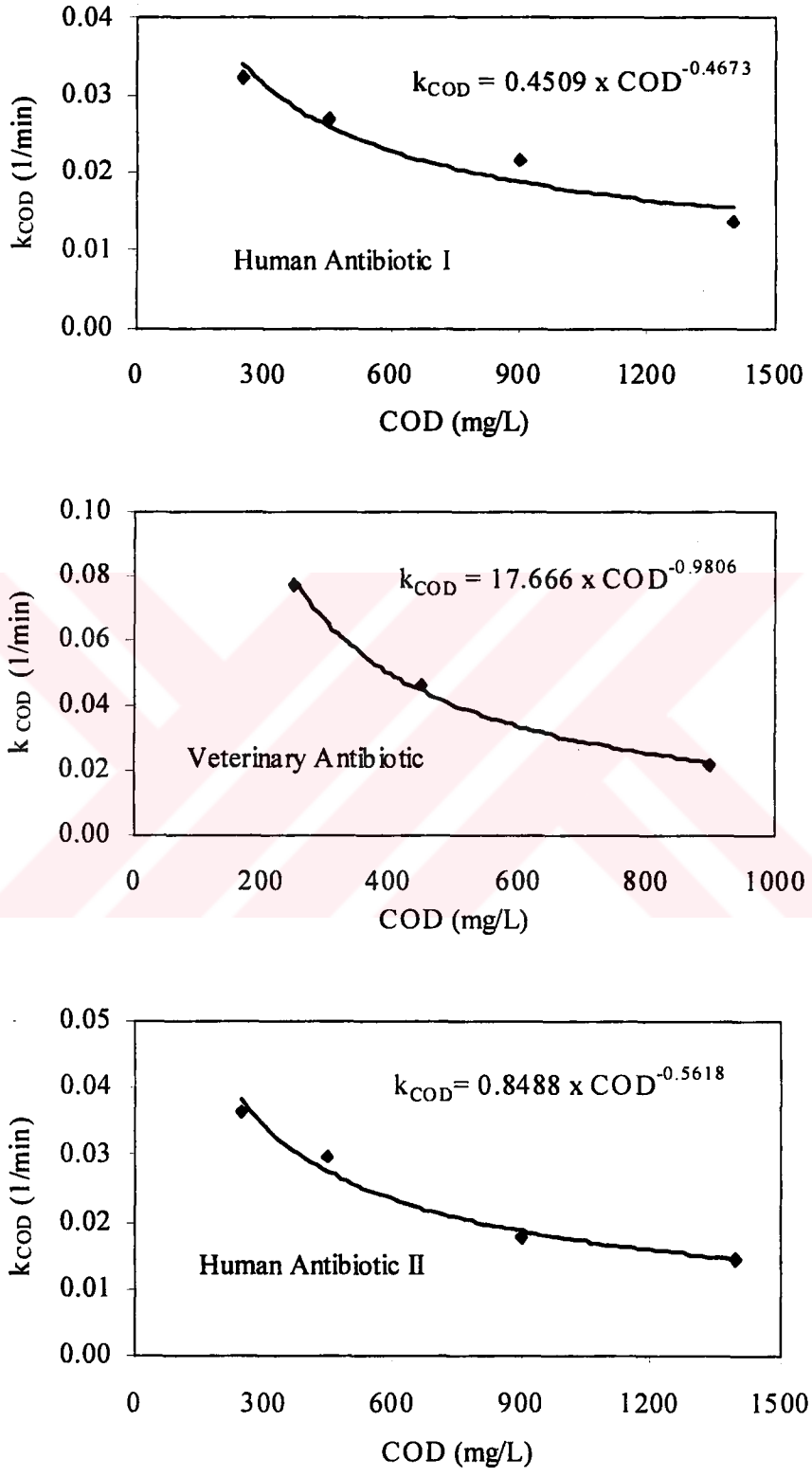


Figure 4.9. Relationship between pseudo first order COD removal rate constant and initial COD concentration for the ozonation of synthetic antibiotic formulation effluents.

For all antibiotic formulation effluents simple kinetic relationship was established between first order rate constants and initial COD concentration (250 mg/L – 1400 mg/L) obtained for ozonation at pH = 7 according to the Figure 4.9.

Human Antibiotic I ;

$$k_{COD} = 0.4509 \times COD^{-0.4673} \quad (4.1)$$

Veterinary Antibiotic ;

$$k_{COD} = 17.666 \times COD^{-0.9806} \quad (4.2)$$

Human Antibiotic II

$$k_{COD} = 0.8488 \times COD^{-0.5618} \quad (4.3)$$

From the equations, it is obvious that COD removal rate of veterinary antibiotic shows the highest dependence on initial COD concentration.

4.1.3. The Effect of Hydrogen Peroxide Dose

Combining ozone with hydrogen peroxide to enhance oxidizing ability has been extensively researched recently and is considered to be a promising alternative for refractory organics removal from aqueous solutions (Glaze *et al*, 1987; Masten and Davis, 1993). It was shown that the conjugate base of H_2O_2 at milimolar concentrations could initiate the decomposition of ozone much more rapidly into hydroxyl radicals than with the hydroxide ion (Staehelin and Hoigné, 1982).

With the above mentioned facts in mind, ozonation of synthetic antibiotic formulation wastewater having initial COD value of 450 mg/L was performed in the presence of 10, 20, 50, and 75 mM H_2O_2, at pH = 7 with an applied ozone dose of 2960 mg/L h. A lower limit for the effectiveness of the H_2O_2/O_3 AOP is in a pH range of 5 to 7 based on results of Staehelin and Hoigne (1982), therefore H_2O_2/O_3 process was applied to synthetic wastewater samples at pH 7.

Additionally, synthetic human antibiotic II formulation effluent was treated with mere hydrogen peroxide at same concentrations for one hour. Since the hydrogen peroxide alone is not a strong oxygen transfer agent for COD and UV_{254} absorbance, only 10 per cent and 2 per cent removals were obtained with a 75 mM H_2O_2 at the end of one hour experiment (Data not shown).

Results obtained from the O_3/H_2O_2 experiments in the presence of 10, 20, 50, and 75 mM H_2O_2 are represented in Figure 4.10 in terms of COD and UV_{254} abatement kinetics.

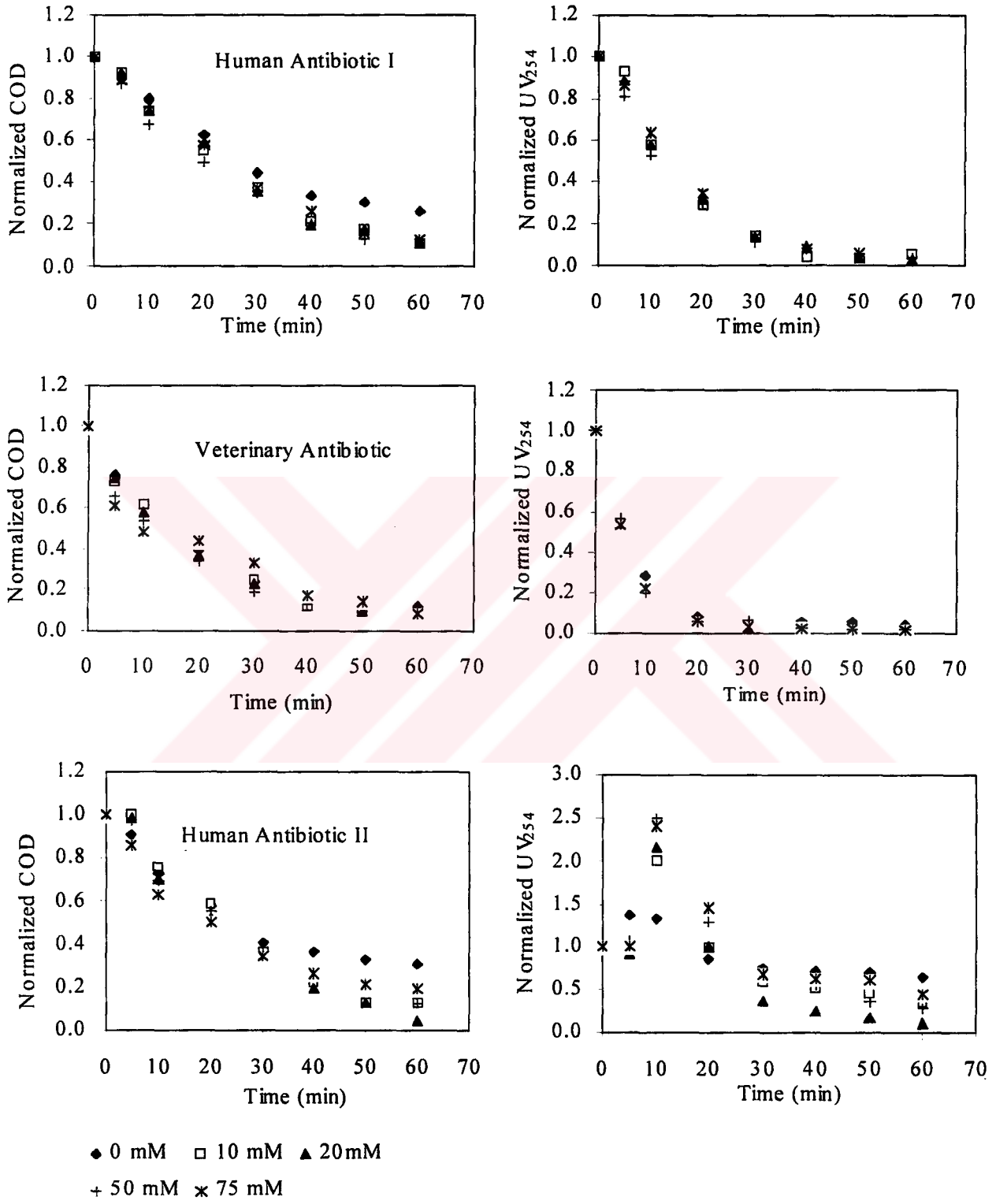


Figure 4.10. COD and UV_{254} abatement kinetics for O_3/H_2O_2 experiment with synthetic antibiotic formulation effluents for different H_2O_2 concentrations (pH = 7, COD_i = 450 mg/L, $(O_3)_i$ = 2960 mg/L h).

Almost same results were obtained for COD and aromaticity removals in the presence and absence of H_2O_2, especially in the case of human antibiotic I and veterinary antibiotic. These similarities may be due to the high reactivity of compounds to ozonation and the presence of high ozone concentration in reaction medium.

Degradation kinetics of chromophore groups of veterinary antibiotic and human antibiotic II are presented in Figure 4.11 and Figure 4.12 respectively.

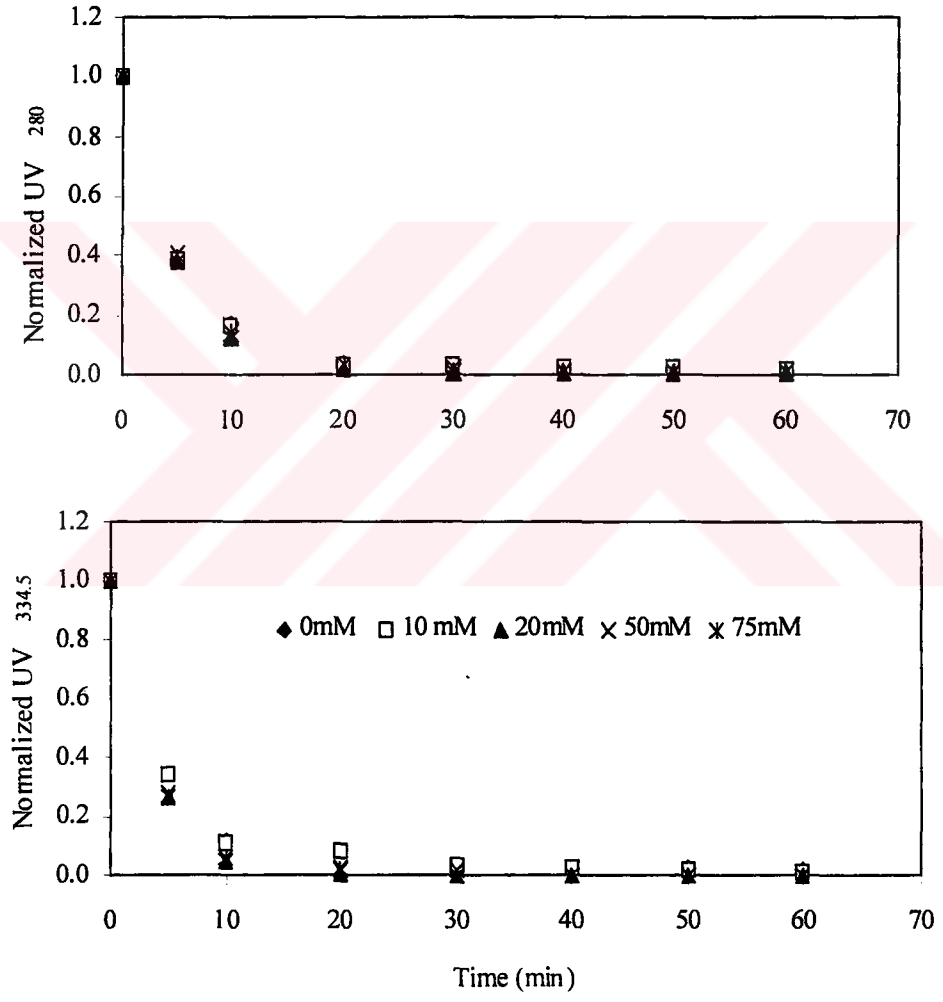


Figure 4.11. Normalized UV $_{280}$ and UV $_{334.5}$ values for the O_3/H_2O_2 process of veterinary antibiotic at pH = 7.

Similar to UV_{254} removal, all absorption values at maximum wavelengths of veterinary antibiotic were completely removed by one hour O_3/H_2O_2 experiment regardless of the initial H_2O_2 concentration.

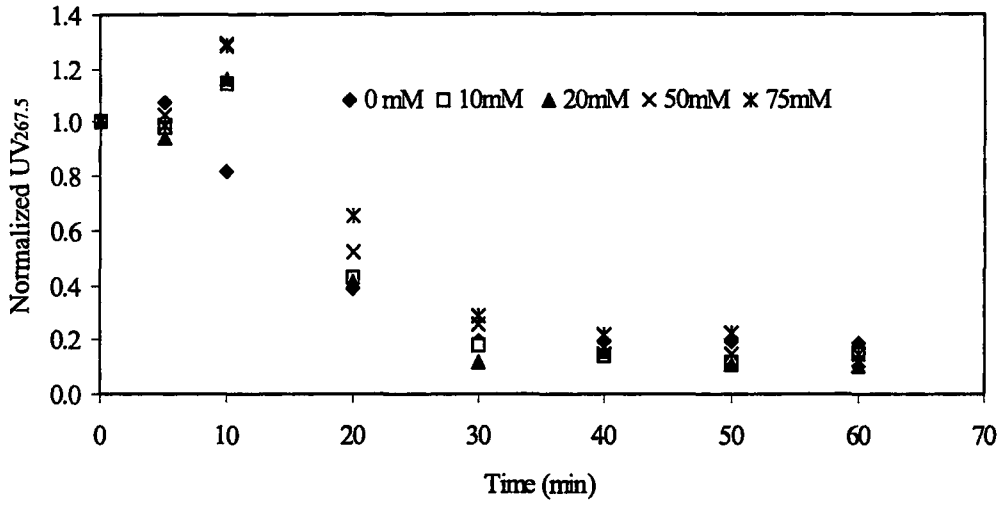


Figure 4.12. Normalized UV $_{267.5}$values for the O_3/H_2O_2 process of human antibiotic II at pH = 7.

In case of human antibiotic II the effect hydrogen peroxide dose was less pronounced for the degradation of absorption at 267.5 nm (Figure 4.12), compared to COD and UV_{254} removals. Although there was no significant change in the overall $UV_{267.5}$ removals, the addition of hydrogen peroxide at different doses caused a change in the reaction pathway. In other words, as the hydrogen peroxide dose increased, the absorbance values of intermediate products became higher due to the variations in the reaction mechanism (Figure 4.12).

Figure 4.13 represents the pseudo first order COD removal rate constants and overall COD and absorption removal percentages for ozonation of synthetic antibiotic formulation effluents for five different hydrogen peroxide doses.

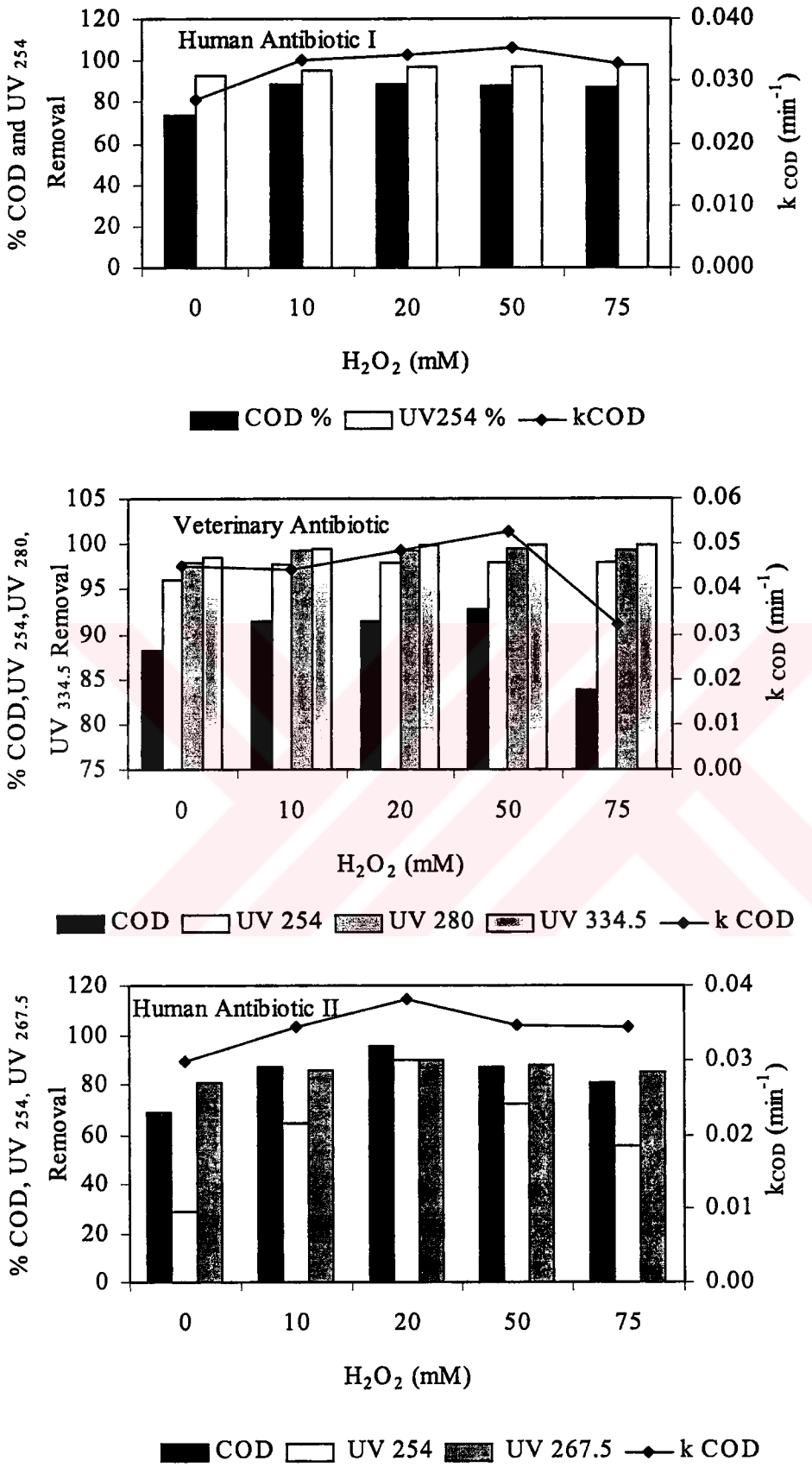


Figure 4.13. k_{COD} values and overall COD and absorption removal percentages for ozonation of synthetic antibiotic formulation effluents for different H_2O_2 concentrations at pH = 7.

The addition of optimum dose of hydrogen peroxide (50 mM) did not have a significant contribution on pseudo first order COD removal rate constants, overall COD and UV_{254} removals for human antibiotic I and veterinary antibiotic. However, a further increase of the introduced H_2O_2 dose to 75 mM resulted in a significant reduction in the obtained k_{COD} (0.032 min^{-1}) and overall COD removal (83 per cent). This event is attributable to the scavenging effect of hydrogen peroxide overdosing as explained in Section 2.5.2.

Although there was no significant enhancement in the total COD and UV_{254} reduction with the addition of hydrogen peroxide in the case of human antibiotic I and veterinary antibiotic, for human antibiotic II it was obviously observed that almost all COD and UV_{254} absorbance were removed in the presence of 20 mM hydrogen peroxide. This observation could be explained by the fact that, the structure of human antibiotic II does not contain much unsaturated bonds and functional groups that ozone can readily attack directly compared with human antibiotic I and veterinary antibiotic. Therefore, hydroxyl radical formation rate during the ozonation process affected the human antibiotic II most prominently in terms of treatment efficiency. The remarkable difference between direct ozonation at pH 3 and hydroxyl radical reaction at alkaline pH was also observed in the case human antibiotic II (Figure 4.1).

Highest pseudo first order rate constants for removal of COD were achieved with a H_2O_2/O_3 ratio of 0.57 (w/w) for human antibiotic I and veterinary antibiotic, and 0.23 for human antibiotic II. Further increase in the H_2O_2/O_3 ratio had no contribution on reaction rate values. Similarly, Laplanche *et al.*, (1995), and Glaze and Kang, (1989) showed that the ozone/hydrogen peroxide treatment efficiency in terms of pseudo first order removal rate constants of organic compounds did not increase significantly with a ratio value of O_3/H_2O_2 greater than 0.4. Bellamy *et al.*, (1991), determined the optimum H_2O_2 / O_3 ratio as 0.5, at which maximum volatile organic carbon oxidation rate constants were obtained.

Figure 4.14 shows the plots of hydrogen peroxide residuals in solution for various hydrogen peroxide doses (10-75 mM) as a function of ozonation time for the O_3/H_2O_2 treatment of human antibiotic II. As can be seen from the figure, hydrogen peroxide was consumed completely within the 40 minutes of ozonation in case of 10 mM H_2O_2 dose while in the presence of 20 mM H_2O_2, where the optimum treatment efficiency was obtained for human antibiotic II, utilization of hydrogen peroxide maintained until the end of one hour ozonation. High levels of residual peroxide for 50 and 75 mM doses acted as a hydroxyl radical scavenger leading to a slight decrease in treatment efficiency when compared with 20 mM dose.

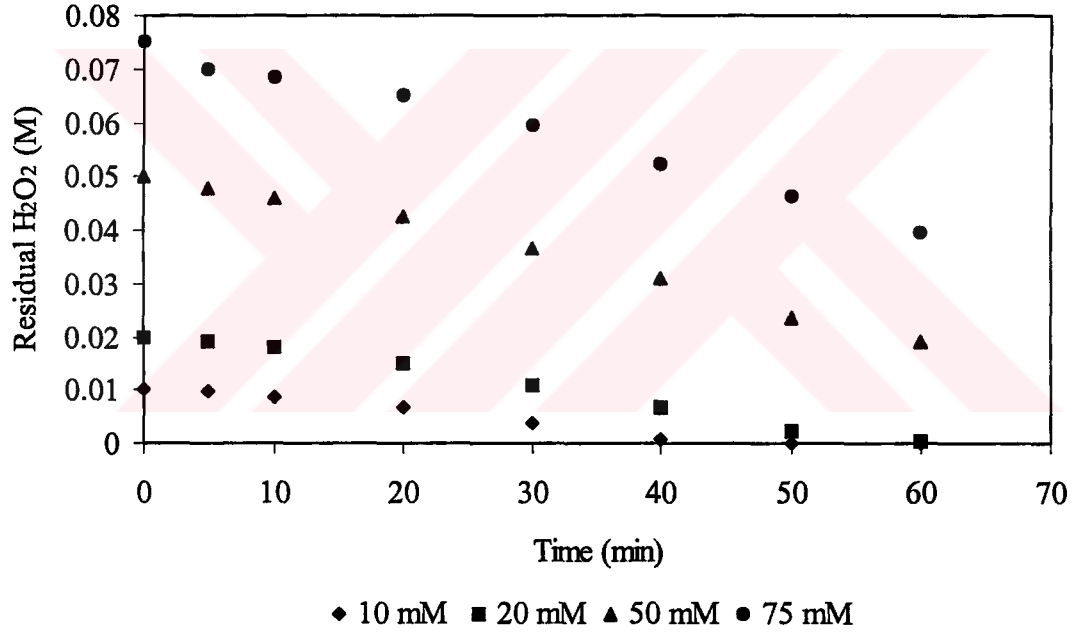


Figure 4.14. Residual peroxide concentration for various peroxide doses (10-75 mM) as a function of ozonation time for the O_3/H_2O_2 treatment of human antibiotic II at pH = 7.

4.1.3.1. Effect of pH on O_3/H_2O_2 Treatment: To explore the effect of pH on the H_2O_2/O_3 process, ozonation was performed for human antibiotic I in the presence of 50 mM H_2O_2 at pH values of 3, 7, and 11 and abatement kinetics of COD and UV $_{254}$ values are presented in Figure 4.15.

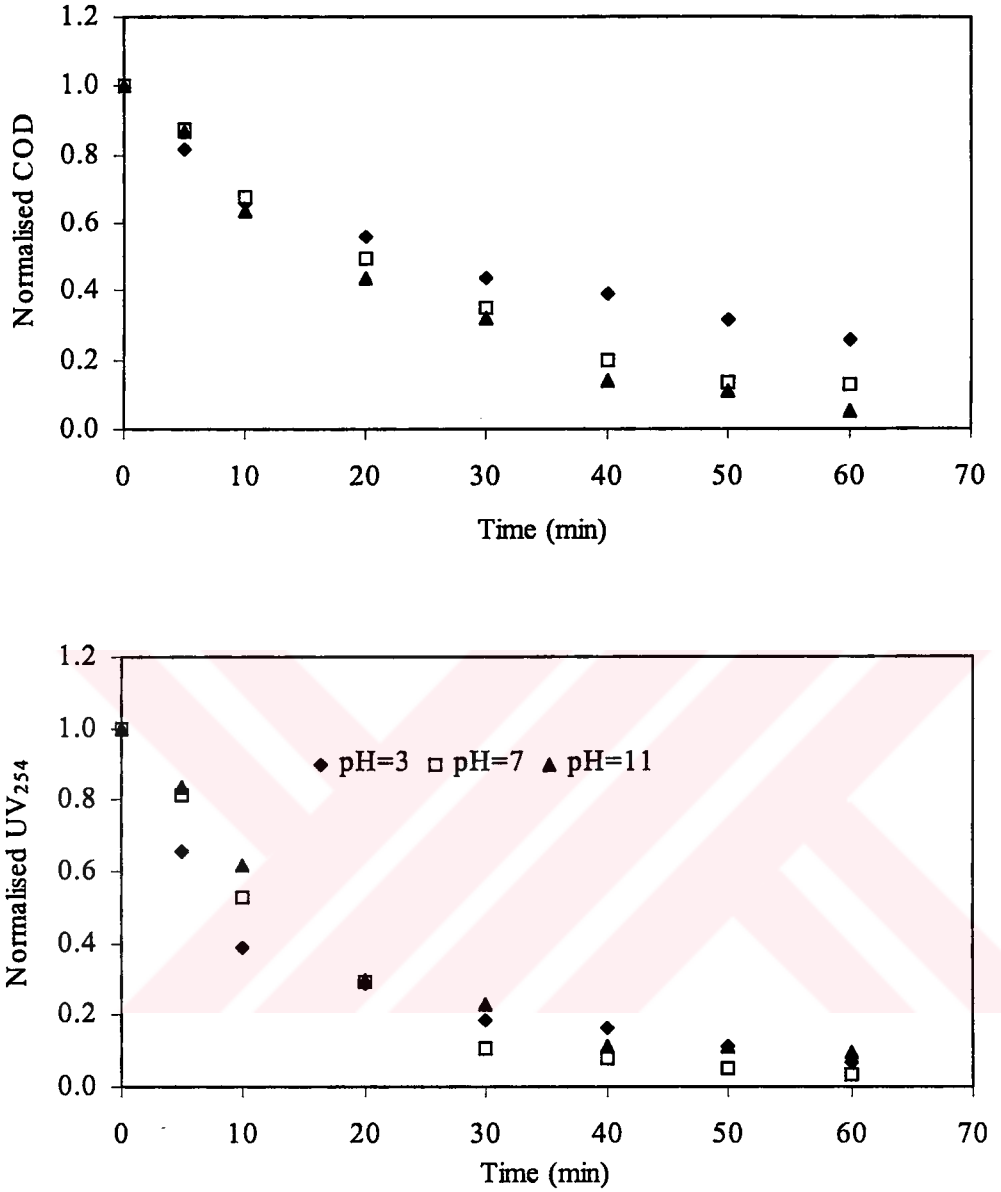


Figure 4.15. COD and UV $_{254}$ abatement kinetics for the O_3/H_2O_2 treatment of human antibiotic I for different pH values (COD_i = 450 mg/L, $(O_3)_i$ = 2960 mg/L h).

Figure 4.16 represents the pseudo first order COD removal rate constants and overall COD and UV_{254} removal percentages for the O_3/H_2O_2 treatment of human antibiotic I having the initial COD of 450 mg/L for different pH values (H_2O_2 = 50 mM, $(O_3)_i$ = 2960 mg/L h)

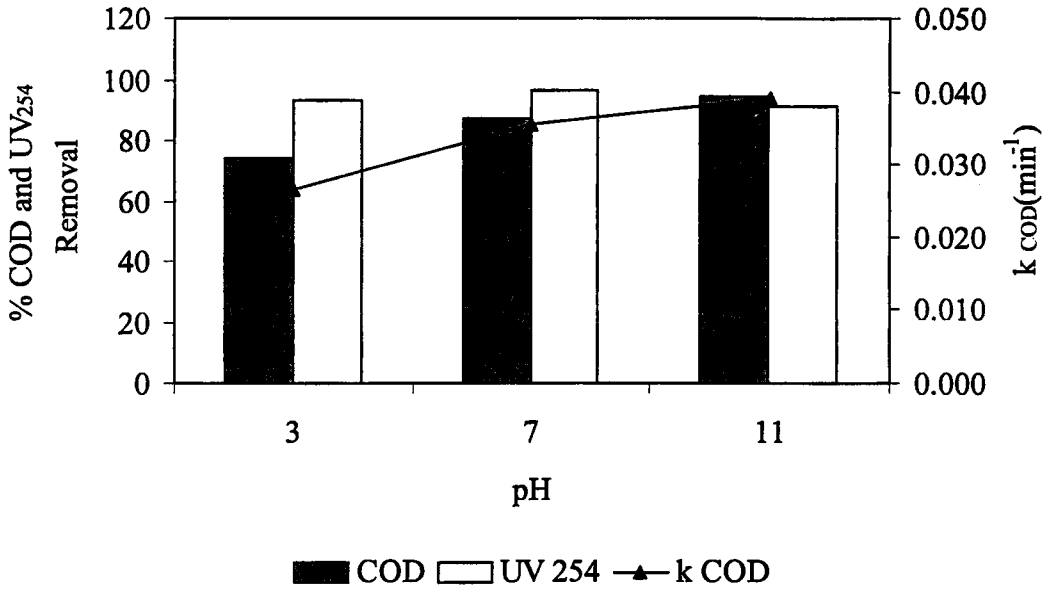


Figure 4.16. Pseudo first order COD reduction rate constants and overall COD and UV $_{254}$ removal for the O_3/H_2O_2 (50 mM) treatment of human antibiotic I for different pH values (COD_i = 450 mg/L).

At low pH values decomposition of hydrogen peroxide to hydroperoxide anion is not favored (Eq 2.18). The reaction of ozone with hydroperoxide anion to generate hydroxyl radical (Eq. 2.19) is faster than that of H_2O_2 having the k value less than 10^{-2} $M^{-1}s^{-1}$ (Staehelin and Hoigne, 1982). Therefore, elevating the pH from 3 to 11 resulted in an improvement of k_{COD} value (increased from 0.027 to 0.039 min^{-1}) and overall percent COD removals (about 20 per cent). However, when compared with mere ozonation at pH 3, addition of 50 mM hydrogen peroxide leaded to an important enhancement in k_{COD} (increased from 0.015 to 0.027 min^{-1}), overall COD and UV_{254} removals (22 per cent and 20 per cent improvement respectively).

4.1.4. Effect of Inlet Ozone Concentration

Ozone is partially water insoluble (0.082 atm m^3/mole at 298^{0}K) and for effective treatment must dissolve adequately in wastewater. Ozone dose has been reported as either applied or absorbed (i.e. consumed) doses. The absorbed dose value is more informative because it indicates the amount of dissolved ozone available for oxidation, and so aids optimization for the treatment efficiency (Alvares *et al.*, 2001).

In order to elucidate the effect of ozone dose on treatment efficiency, ozonation experiments were conducted for veterinary antibiotic formulation wastewater with the initial COD value of 900 mg/L, at four different inlet ozone doses (960 mg/h – 4440 mg/h).

Subtracting the outlet ozone concentration from the inlet concentration conventionally derives the absorbed ozone dose. Table 4.2 summarizes absorbed ozone concentrations and overall percent ozone consumption for one hour ozonation of veterinary antibiotic at four different inlet ozone doses.

Table 4.2. Total cumulative ozone absorption and overall percent ozone consumption for the ozonation of veterinary antibiotic for different inlet ozone doses (pH=7)

Inlet Ozone (mg/h)	Applied Ozone (mg/Lh)	Outlet Ozone (mg/h)	Absorbed Ozone (mg/h)	Consumed Ozone Concentration (mg/Lh)	% Ozone Consumption
960	640	181	779	519	81
2448	1632	840	1608	1072	66
3888	2592	1459	2429	1619	62
4440	2960	1800	2640	1760	59

Figure 4.17 gives the reduction kinetics of COD and UV $_{254}$ for one hour ozonation of synthetic veterinary antibiotic formulation wastewater at pH = 7, for different ozone doses, as well as the concentration of consumed ozone concentration during the experiment.

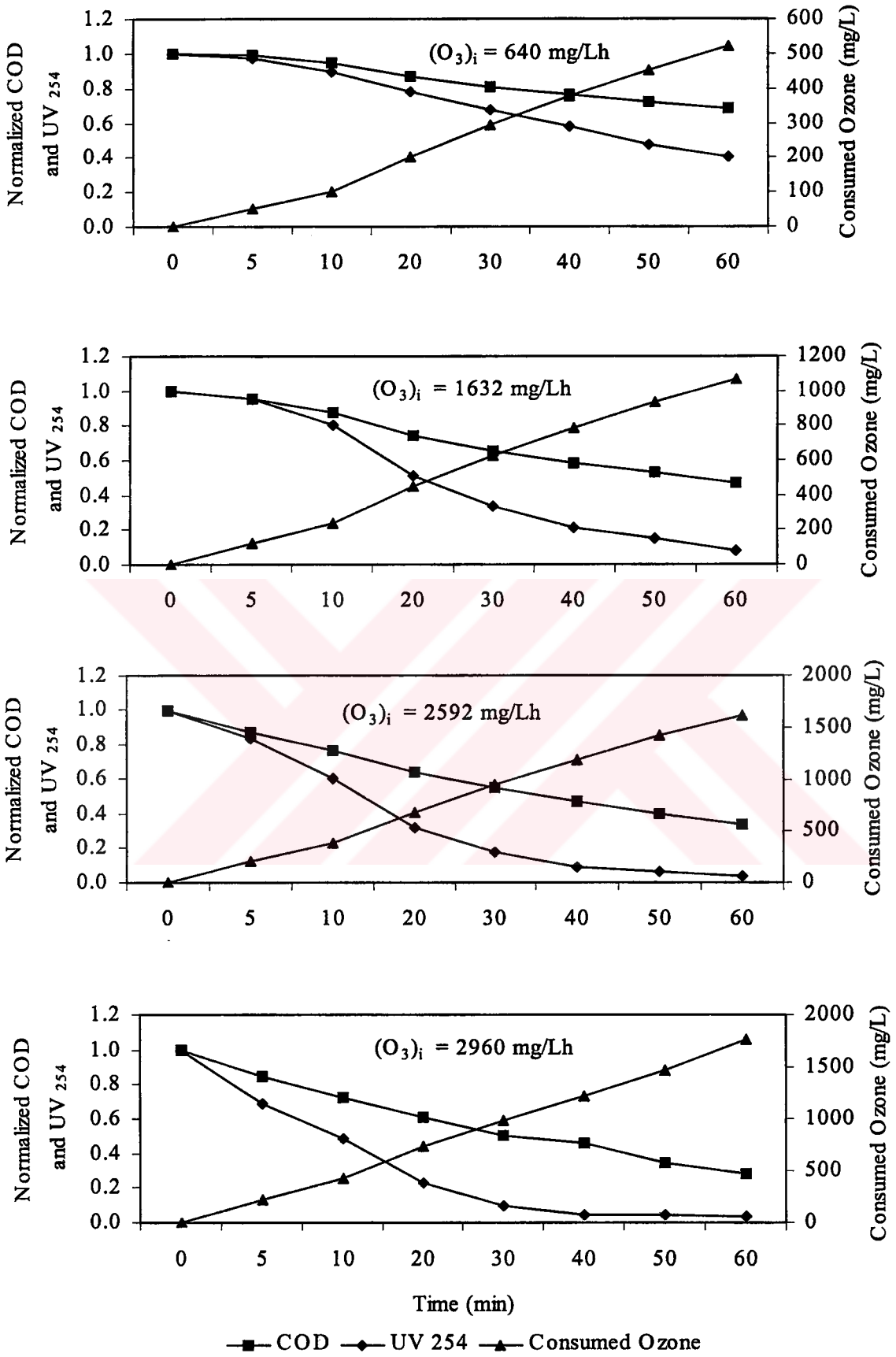


Figure 4.17. Normalized COD, UV $_{254}$ values and consumed ozone concentrations of veterinary antibiotic (COD_i = 900mg/L) for different applied ozone doses (pH= 7).

The effect of applied ozone dose on overall COD and UV_{254} removal percentages and pseudo first order COD removal rate constants of veterinary antibiotic formulation effluent are summarized in the Figure 4.18.

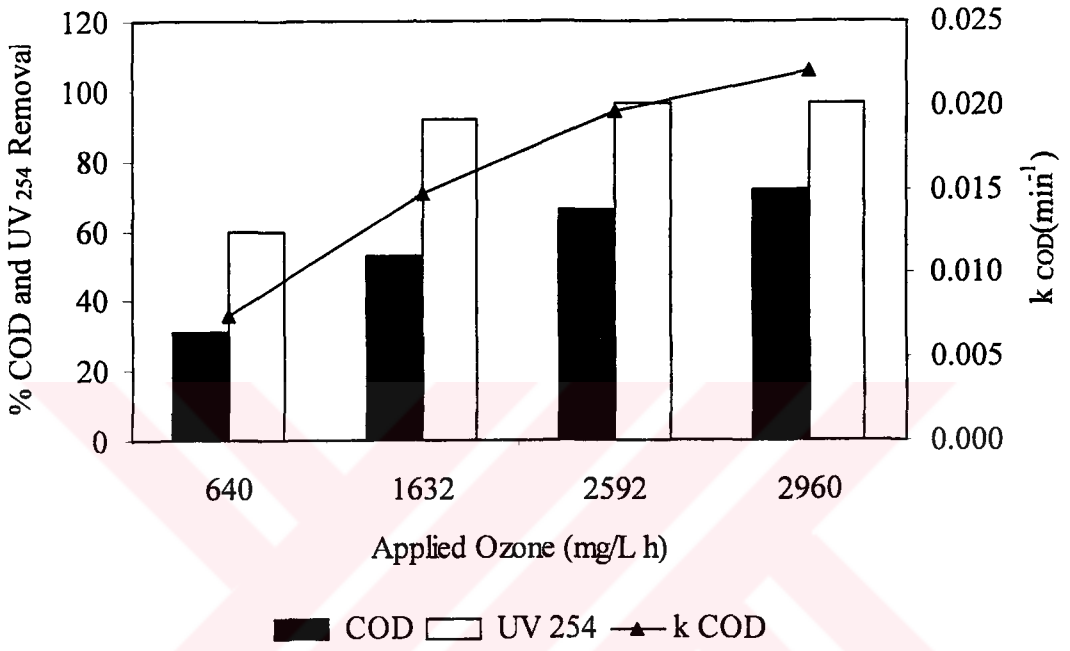


Figure 4.18. Overall COD and UV_{254} removals and pseudo first order COD reduction rates for one hour ozone treatment of veterinary antibiotic with the initial COD value of 900 mg/L at pH = 7, for different applied ozone doses.

Pseudo first order COD reduction rates were increased from 0.0074 to 0.0196 min^{-1} by raising the applied ozone dose to 2592 mg/L h. However, this enhancement slowed down with a further increase in the applied ozone. This is probably due to the fact that within a given retention time, there appeared to be a maximum amount of ozone that the synthetic veterinary antibiotic formulation wastewater was able to absorb, beyond which, further increase in applied ozone concentration only the resulted in more unabsorbed ozone in the outlet gas existing the reactor. Similar results were obtained by Lin and Liu (1994), who conducted the ozonation process for the treatment of textile wastewater.

These findings can also be explained in terms of fractional ozone utilization which is defined as the ratio of the COD removal (mg/L) to the ozone consumption (Lin and Liu, 1994).

Fractional Ozone Utilization = COD Removal / Ozone Consumption (4.4)

Equation 4.4 gives the amount of COD removed per unit of ozone utilized. Figure 4.19 demonstrates this quantity as a function of the applied ozone doses.

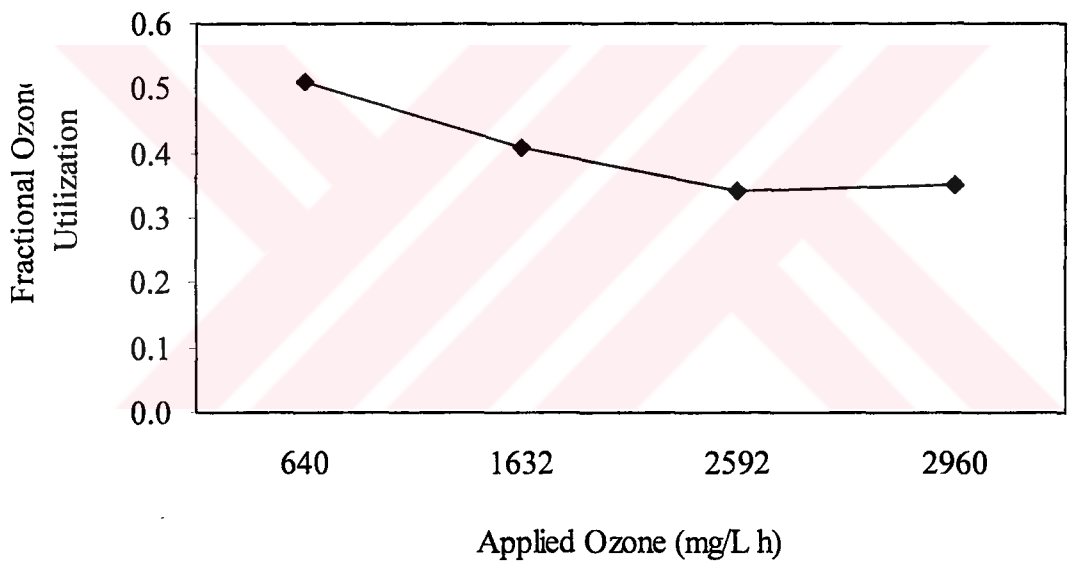


Figure 4.19. Fractional ozone utilization for different applied ozone doses.

As can be seen from the figure, the ozone utilization was more efficient at lower ozone doses, however, since the effectiveness of ozone oxidation is significantly reduced at low applied ozone doses, longer retention times might be required for optimal treatment efficiency.

4.2. Biodegradability Enhancement in the Ozone Reactor

Regarding ozonation as a pretreatment process for conventional treatment methods, it is important to examine its influence to properties of organic substances like biodegradability. In pretreatment studies, mostly BOD_5 and OUR measurements are performed in order to asses the biodegradability of wastewaters (Alvares, 2001).

Biodegradability ratio (BOD_5/COD): Represents the amount of oxygen consumed by biological oxidation compared to the oxygen required for complete mineralization. An increase in the ratio after pretreatment is indicative of improved biodegradability due to an increase in the proportion of the COD amenable to biological mineralization.

Microbial oxygen uptake rate (OUR): Oxygen Uptake Rate is an alternative means of measuring the biodegradability of ozonation by products since the biomass respiration rate is correlated with substrate removal.

The present part of the study focused on the possibility to improve the biodegradability of the synthetic antibiotic formulation wastewaters by applying the ozonation process at different conditions.

4.2.1. Biodegradability Measurements by BOD_5/COD Ratio

The biodegradability of industrial recalcitrant substrates has been shown to improve upon ozonation, so that the BOD_5/COD ratios increased from 0 between 0.15 and 0.5 under optimum ozone conditions (Alvares, 2001; Scott *et al.*, 1995). In order to assess the effect of ozonation on the biodegradability of the synthetic antibiotic formulation wastewaters, BOD_5 measurements were conducted and biodegradability of effluents were represented as BOD_5/COD ratio. Initially, the BOD_5/COD ratio for all synthetic wastewater was noticeably low and changes in this ratio as a function of ozonation time are presented in Figure 4.20.

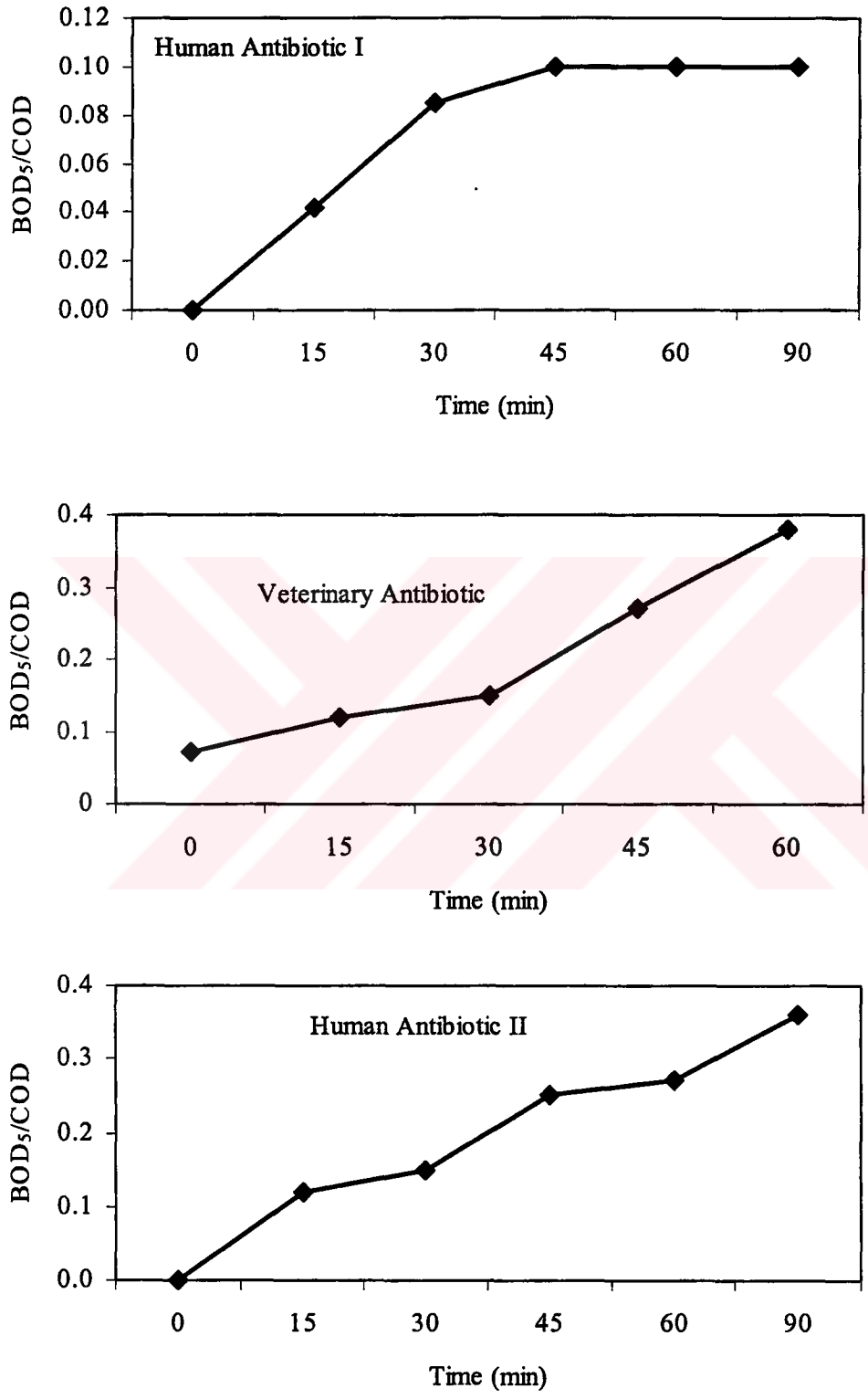


Figure 4.20. Variations in BOD_5/COD ratio of human antibiotic I (COD_i=1400mg/L), veterinary antibiotic (COD_i=900 mg/L) and human antibiotic II (COD_i=1400mg/L) formulation effluents as a function of treatment time (pH=7, $(O_3)_i$ = 2960 mg/L h).

While for the veterinary antibiotic effluent BOD_5/COD ratio was increased from 0.077 to 0.38 after one hour ozonation , in case of human antibiotic I and human antibiotic II BOD_5/COD ratio was increased from 0 to 0.1 and 0.27 respectively.

It has been previously suggested that increasing the ozone contact time first produces more biodegradable intermediates, and that upon extension of the ozonation period biodegradability might remains constant (Balcioglu Akmehmet and Arslan 2001), decrease (Takahashi *et al.*, 1994; Joachimsen and Jekel, 1997; Imai *et al.*, 1998) or in some cases even further increase (Gilbert, 1987; Benitez *et al.*, 2001) depending upon the specific pollutant type in question. In this study, while the biodegradability of human antibiotic I remained almost constant, that of human antibiotic II and veterinary antibiotic indicated further increase with an increasing contact time.

4.2.1.1. Effect of Ozone Dose. In the literature, generally biodegradability parameters such as BOD/COD ratio, examined as a function of ozone dosage have revealed the optimum ozone dose for maximum biodegradability improvement (Sevimli *et al.*, 2000; Stockinger *et al.*, 1995; Liakou *et al.*, 1997). Lower ozone doses could not be efficient since did not promote sufficient partial oxidation, while higher doses above the optimum may caused a decline in biodegradability due to the complete mineralization of biodegradable ozonation by products.

With the above mentioned facts in mind, in order to asses the effect of applied ozone dose on the biodegradability of veterinary antibiotic effluent, BOD_5 values for the ozonation of it (COD_i = 900 mg/L) were determined for different inlet ozone doses as a function of ozonation time. Changes in the biodegradability of veterinary antibiotic as a function of ozonation time for the applied ozone doses of 640, 1632, 2592 and 2960 mg/L h, are represented in Figure 4.21 in terms of BOD_5/COD ratio.

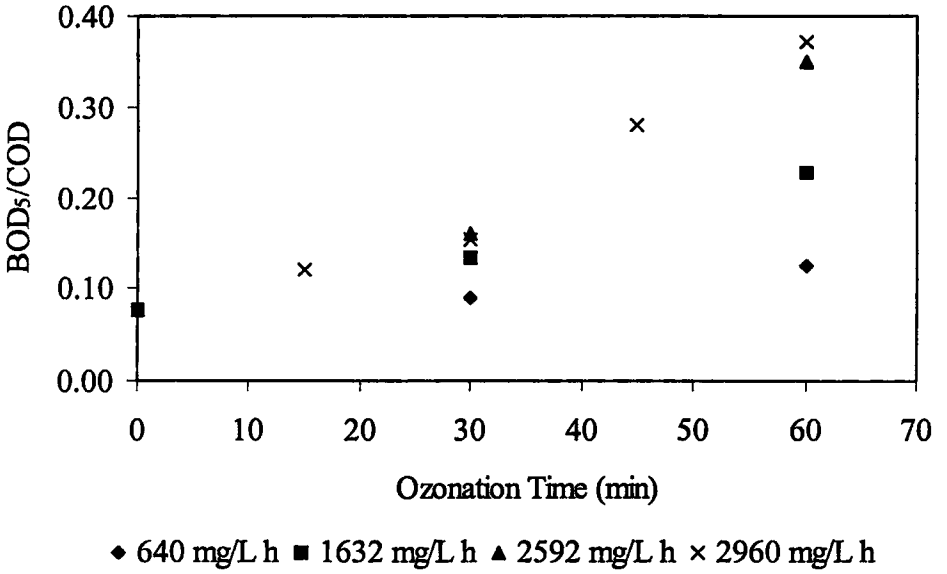


Figure 4.21. Changes in the biodegradability of veterinary antibiotic as a function of ozonation time for different inlet ozone doses (COD_i = 900 mg/L, pH = 7).

An increase in the BOD_5 values accompanied by a corresponding decrease in the COD values resulted in an enhancement in the BOD_5/COD ratio indicating the conversion of the structure of veterinary antibiotic to more biodegradable compounds. According to the Figure 4.21 biodegradability was improved with the increasing ozonation time for each applied ozone doses. At the applied ozone dose of 2960 mg/L h, an increase in the BOD_5/COD ratio was observed from 0.077 to 0.15 and 0.38 within the 30 and 60 minutes of ozonation respectively. However, no remarkable enhancement was obtained with the lowest inlet ozone dose in one hour period.

Figure 4.22 implies the biodegradability of veterinary antibiotic as a function of consumed ozone concentration.

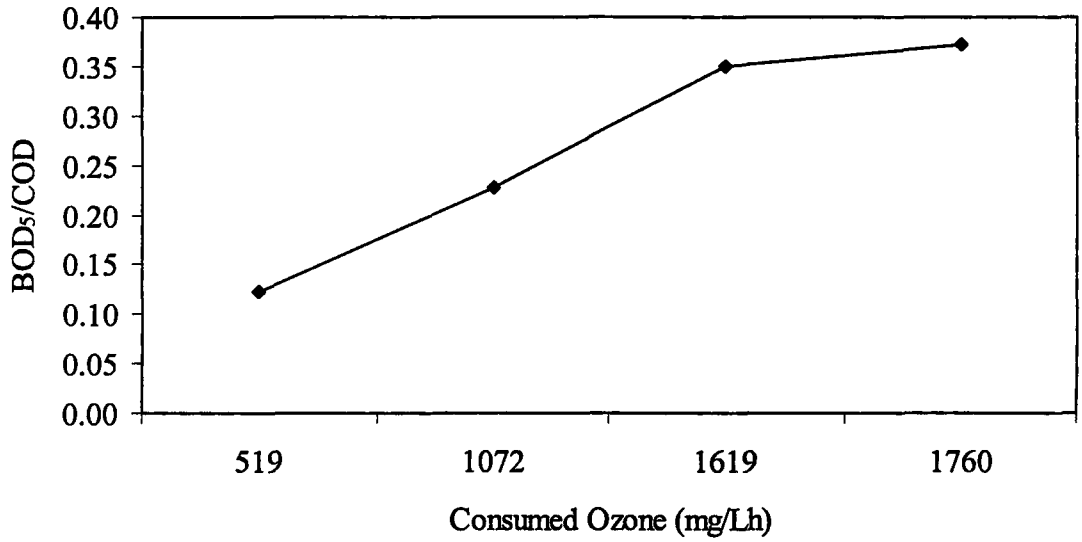


Figure 4.22. BOD_5/COD ratios for one hour ozonation of veterinary antibiotic with the initial COD of 900 mg/L as a function of consumed ozone concentration (pH = 7).

In the case where the concentration of consumed ozone is the lowest, since the partial oxidation of veterinary antibiotic effluent could not be promoted sufficiently, no significant enhancement on biodegradability was obtained. As the consumed ozone dose was increased to 1619 mg/L h ($(O_3)_i$ = 2592 mg/L h), three fold improvement on the BOD_5/COD ratio was established and remained almost constant thereafter. Similar results were obtained by Medley and Stover (1983) and Beltran *et al.* (1999) who assessed the effects of ozone on biodegradability of 2,4 -dinitrophenol and domestic wastewater respectively.

4.2.1.2. Effect of pH. Aside with applied ozone concentration, pH is another factor affecting the biodegradability improvement by ozonation process. In order to evaluate the effect of pH on biodegradability of synthetic veterinary antibiotic formulation wastewater, ozonation was performed at the pH values of 3, 7 and 11, and BOD_5 values were determined as a function of ozonation time. Figure 4.23 shows the variation of BOD_5/COD ratio for different pH values as a function of ozonation time.

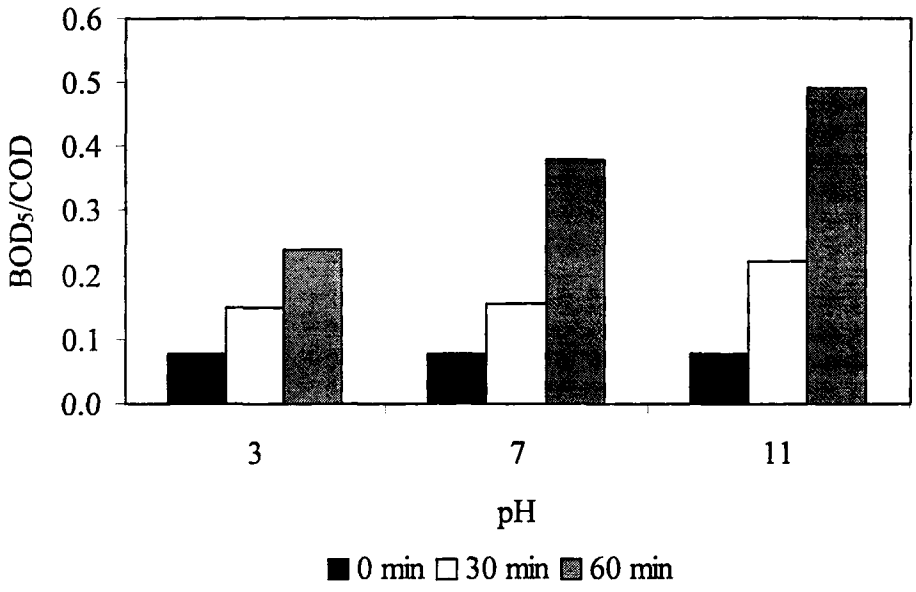


Figure 4.23. Changes in the biodegradability of veterinary antibiotic (COD_i = 900 mg/L) by the ozonation at pH values of 3, 7, and 11, ($(O_3)i$ = 2960 mg/L h).

Although oxidative degradation of veterinary antibiotic was found highest at pH 7 in terms of COD and UV absorbance removals (Figure 4.4), by increasing the pH value BOD5/COD ratio was increased (Figure 4.23).

4.2.2. Biodegradability Measurements by OUR Value

Oxygen Uptake Rate (OUR) can also be used to evaluate the biodegradability of treated wastewater samples. For this purpose, the OUR_0 measurements of raw and ozonated synthetic veterinary antibiotic formulation wastewater (COD_i = 900 mg/L, $(O_3)_i$ = 2592 mg/L h) were performed. By taking into consideration the volume and COD value of actual wastewater originated from pharmaceutical industry, raw and ozonated synthetic antibiotic formulation effluents were diluted with the synthetic domestic wastewater at a ratio of 1:2 (v/v). Raw and treated wastewater samples ozonated at certain time intervals were aerated in mixed culture just before oxygen uptake measurements. Figure 4.24 shows OUR_0 values achieved for the samples contained synthetic domestic and veterinary antibiotic formulation effluent, as a function of ozonation time.

achieved for the samples contained synthetic domestic and veterinary antibiotic formulation effluent, as a function of ozonation time.

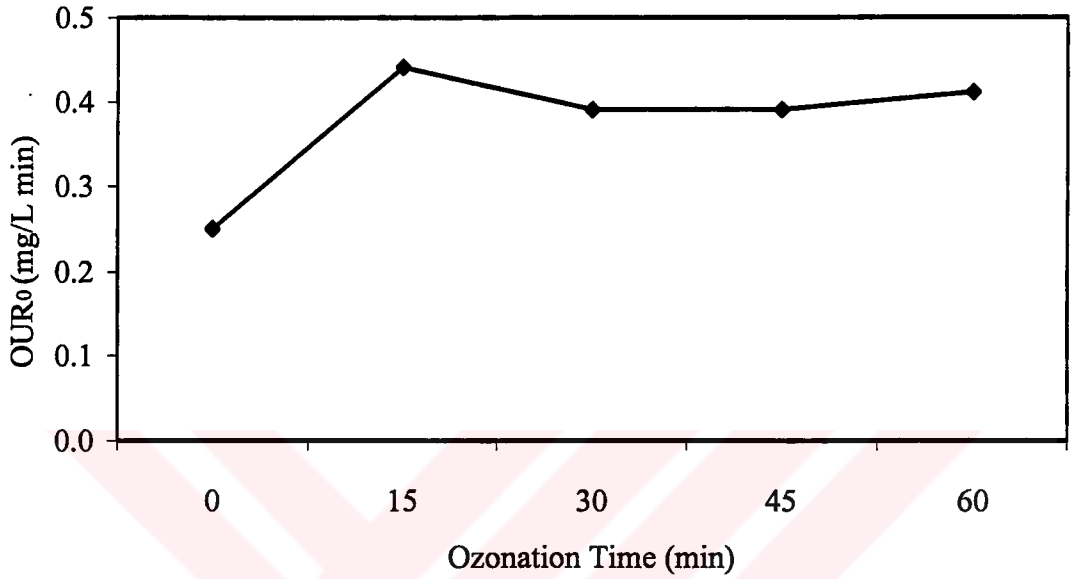


Figure 4.24. OUR_0 values of ozonated veterinary antibiotic formulation wastewater samples diluted with synthetic domestic wastewater at a ratio of 1:2.

It can be concluded from the figure that, ozonated wastewater samples provided a much higher oxygen uptake rate (0.44 mg/L min for 15 min ozonated wastewater) indicating that the ozonation products were more biodegradable and less toxic than the untreated synthetic veterinary antibiotic formulation effluent which has considerably low OUR (0.25 mg/L min).

In addition a reference experiment was executed with synthetic domestic wastewater that enabled the comparison of the OURs obtained for ozonated veterinary antibiotic formulation effluents. The OUR obtained for the synthetic domestic wastewater (1.4 mg/L min) was three times higher than that of the synthetic veterinary antibiotic formulation effluent subjected to 15 min ozonation.

4.3. Determination of Required Bond Dissociation Energies for Radical Formation by H-Abstraction

It is well known that one of the possible attacks of hydroxyl radical is H-abstraction for the ozonation of organic substances at alkaline pH values (Langlais *et al.*, 1991). In order to explain results obtained from biodegradability studies, a quantum mechanical computational method was used. H abstraction by hydroxyl radical for both human and veterinary antibiotics was modeled. By using this computational method, heat of formation values of possible radical structures generated through H abstraction by hydroxyl radical was determined and required bond dissociation energies were calculated by the following formula:

$$\Delta E = (\Delta H_{R\bullet} + \Delta H_{H\bullet}) - \Delta H_{RH} \qquad (4.5.)$$

where;

ΔH_H = heat of formation value of hydrogen radical (52 kcal / mole)

ΔE = bond dissociation energy

$\Delta H_{R\bullet}$ = heat of formation value of generated radical

ΔH_{RH} = heat of formation value of antibiotics

Figure 4.25 represents the molecular structures of three antibiotics labeled with possible H abstraction sites and Table 4.3 summarizes the heat of formation values of possible radicals generated by H abstraction and calculated bond dissociation energies.

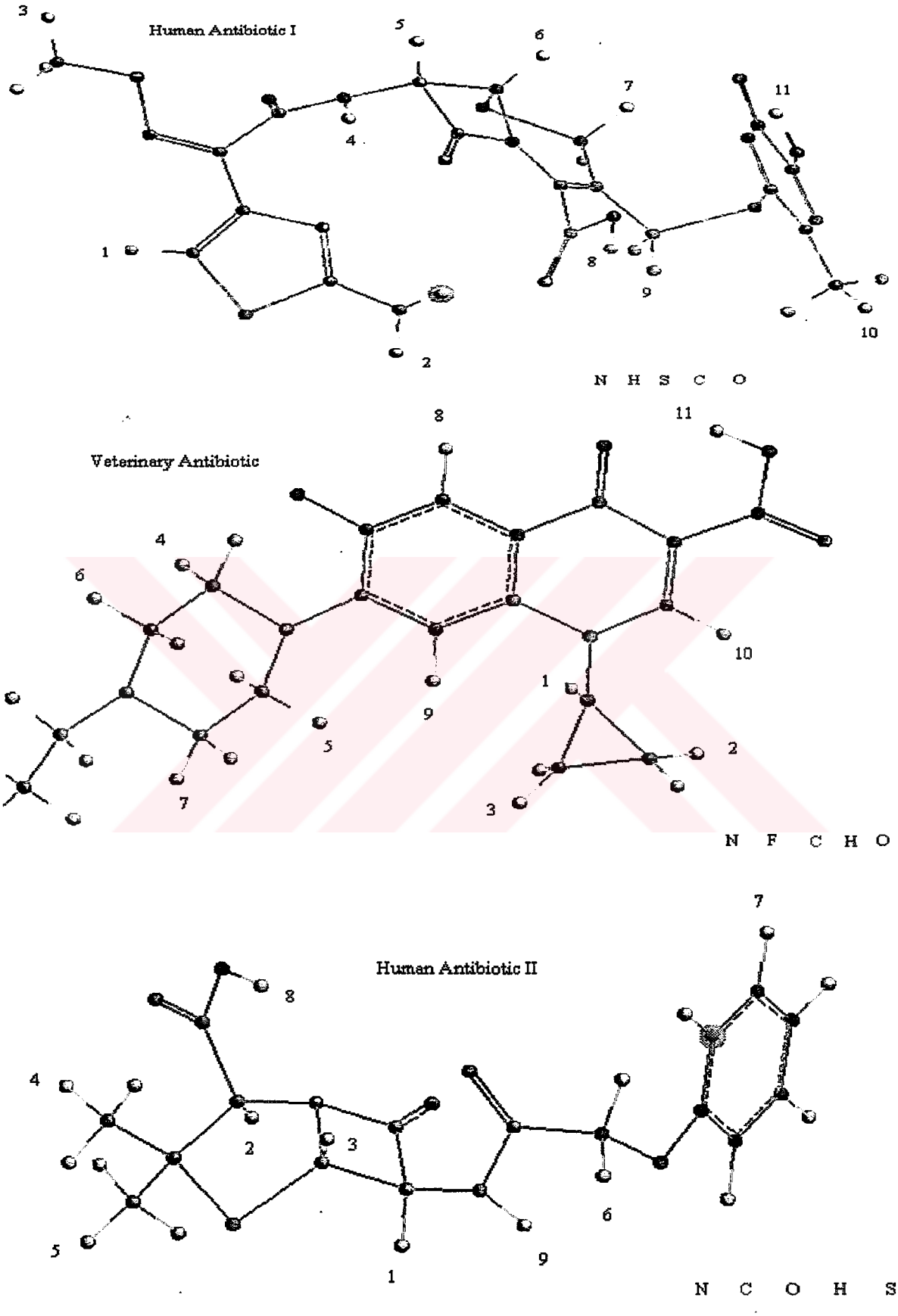


Figure 4.25. The molecular structures of three antibiotics labeled with possible H abstraction sites.

Table 4.3. Heat of formation values of possible radicals generated by the reaction of hydroxyl radical with antibiotics through H abstraction and calculated bond dissociation energies

Human Antibiotic I (Δ H = -67.368 kcal/mole)		
Number of H atom	$\Delta H_{R\cdot}$ (kcal/mole)	Δ E (kcal/mole)
1	56.528	175.368
2	21.163	140.531
3	19.512	138.88
4	21.987	141.265
5	21.867	141.235
6	-1.299	118.069
7	22.043	141.403
8	57.033	176.401
9	16.486	135.854
10	10.511	129.871
11	17.874	137.242
Veterinary Antibiotic (Δ H = -108.133 kcal/mole)		
Number of H atom	$\Delta H_{R\cdot}$ (kcal/mole)	Δ E (kcal/mole)
1	-56.212	103.921
2	-48.033	112.21
3	-47.694	112.439
4	-73.784	86.349
5	-73.092	87.041
6	-71.958	88.175
7	-73.128	87.005
8	-39.292	120.841
9	-45.055	115.078
10	-42.470	117.663
11	-69.276	90.857

Table 4.3 continued

Human Antibiotic II		
Number of H atom	$\Delta H_{R\cdot}$ (kcal/mole)	ΔE (kcal/mole)
1	-86.575	105.425
2	-95.355	96.645
3	-84.311	107.689
4	-80.130	111.870
5	-78.637	113.363
6	-88.506	103.494
7	-66.213	125.787
8	-61.157	130.843

The calculated bond dissociation energies seemed to be in agreement with the results obtained from biodegradability studies. The lowest bond dissociation energy (86.349 kcal/mole) for degradation through the most stable radical formation was found for veterinary antibiotic by which the highest COD removal and biodegradability enhancement was obtained through ozonation. Biodegradability enhancement could not be achieved for human antibiotic I indicating that the oxidative degradation of its structure to the intermediates with smaller molecular size is not as favored as veterinary antibiotic. The highest bond dissociation energy required for human antibiotic I (118.069 kcal/mole) confirm this result. Biodegradability measurements and calculated bond dissociation energy for the radical formation of human antibiotic II reveals that its degradation to small and low molecular weight organic compounds was also accomplished as much as veterinary antibiotic.

4.4. Application of Ozone and Ozone / UV Processes to Synthetic Veterinary Antibiotic Formulation Wastewater in the Photochemical Reactor

Photolytic ozonation (O_3 / UV) has been shown by several investigators to be more effective for the destruction of some organic compounds found in wastewater than ozonation alone (Peyton *et al.*, 1982; Ku *et al.*, 2000). Especially, in the case where a organic substance absorbs strongly in the UV region, large fluxes of UV irradiation will accelerate the destruction of the substance (Glaze et al., 1987).

As stated at Table 4.2 the absorption values of veterinary antibiotic in the UV region is considerably high. In the present study, O_3 and O_3 / UV processes were performed on synthetic veterinary antibiotic formulation wastewater in the photochemical reactor with an applied ozone dose of 2592 mg/L h, at pH = 7. Figure 4.26 and Figure 4.27 illustrate the abatement kinetics of COD and UV_{254}, and concentrations of consumed ozone for the O_3 and O_3 / UV treatment of veterinary antibiotic at pH = 7, respectively.

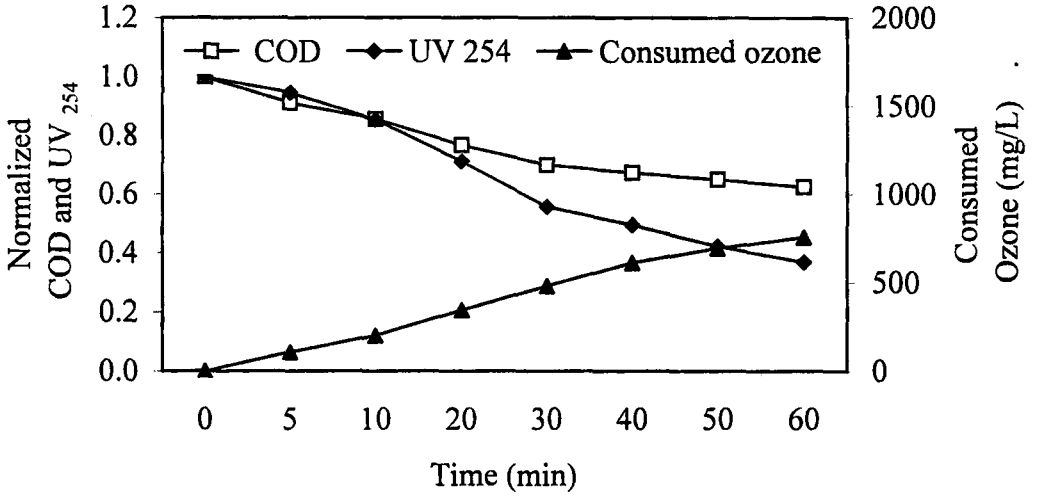


Figure 4.26. Normalized COD and UV_{254}, consumed ozone concentrations for the ozonation ($(O_3)_i$ = 2592 mg/L h) of veterinary antibiotic (COD_i=900 mg/L), at pH = 7.

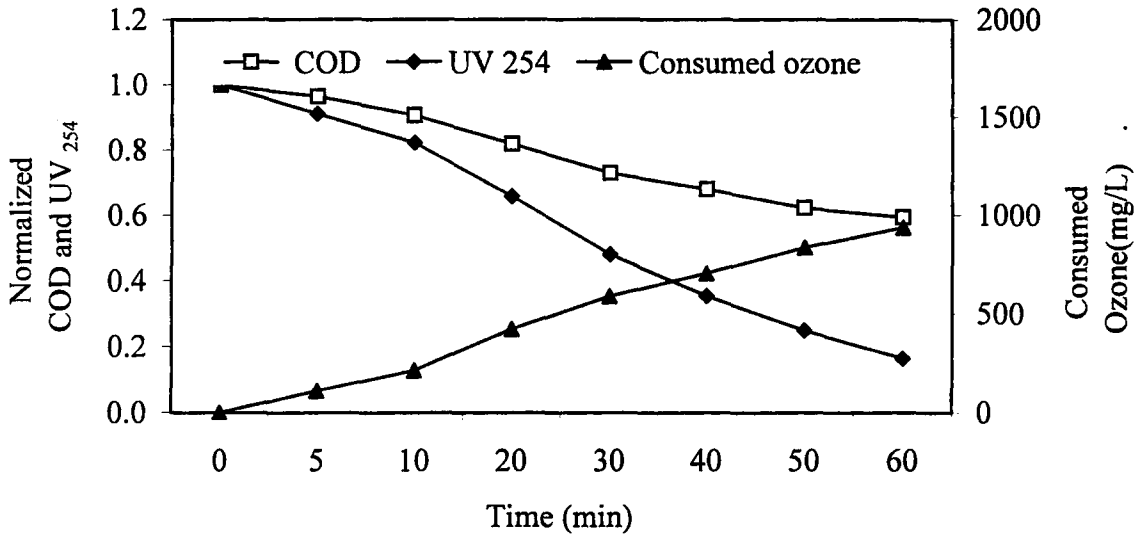


Figure 4.27. Normalized COD and UV_{254}, consumed ozone concentrations for the photolytic ozonation ($(O_3)_i$ = 2592 mg/L h) of veterinary antibiotic (COD_i=900 mg/L), at pH = 7.

Even though photolytic ozonation did not provide remarkable enhancement for overall COD removal percentages and pseudo first order COD reduction rate constants (Table 4.4), abatement of UV_{254} and $UV_{334.5}$ values were greatly improved. Considerably high absorption values of veterinary antibiotic in the UV region might be an explanation for this observation. Pseudo first order COD removal constants and overall COD, UV_{254}, and $UV_{334.5}$ removals for O_3 and O_3 /UV treatments of synthetic veterinary antibiotic formulation wastewater (COD_i = 900 mg/L, $(O_3)_i$ = 2592 mg/L h) in the photochemical reactor are given at Table 4.4. Additionally for the comparison of treatment efficiencies of ozone and photochemical reactor, results obtained from ozonation of veterinary antibiotic in the ozone reactor (COD_i = 900 mg/L, $(O_3)_i$ = 2592 mg/L h) are also presented at Table 4.4.

Table 4.4. Treatment efficiency of veterinary antibiotic formulation wastewater (COD_i=900 mg/L) by O_3 and O_3/UV processes in ozone and photochemical reactor

Parameter	O_3 Treatment (Ozone Reactor)	O_3 Treatment (Photochemical Reactor)	O_3/UV Treatment (Photochemical Reactor)
k_{COD} (min^{-1})	0.0197	0.01	0.01
% COD Removal	66	37.5	40.7
% UV_{254} Removal	96.3	63	83.6
% $UV_{334.5}$ Removal	99.6	82.6	95
Consumed Ozone Concentration (mg/L h)	1619	755	935

Since the ozone mass transfer coefficients of ozone and photochemical reactor were different considerably, significant differences in consumed ozone concentrations were observed in two different reactors. Due to the fact that the rate-limiting step in the ozonation of wastewater is the mass transfer of ozone from the gas phase to the wastewater and ozone absorption is accelerated through increasing ozone mass transfer coefficient. Hence, higher treatment efficiency was obtained in ozone reactor (Table 4.4).

Although combination of UV light and ozone did not contribute the overall COD removal and pseudo first order rate constants, it provided a significant increase in the aromaticity removal of veterinary antibiotic.

From these findings, it can be concluded that degradation of veterinary antibiotic into intermediates with smaller molecular size was accomplished by photolytic ozonation rather than mere ozonation, indicating the formation of more biodegradable products. In order to support this result, oxygen uptake rates of wastewater samples subjected to ozonation and photolytic ozonation were measured as a function of treatment time separately. Figure 4.28 shows the OUR_0 values of synthetic veterinary antibiotic

formulation wastewater samples subjected to ozonation and photolytic ozonation at certain time intervals in the photochemical reactor.

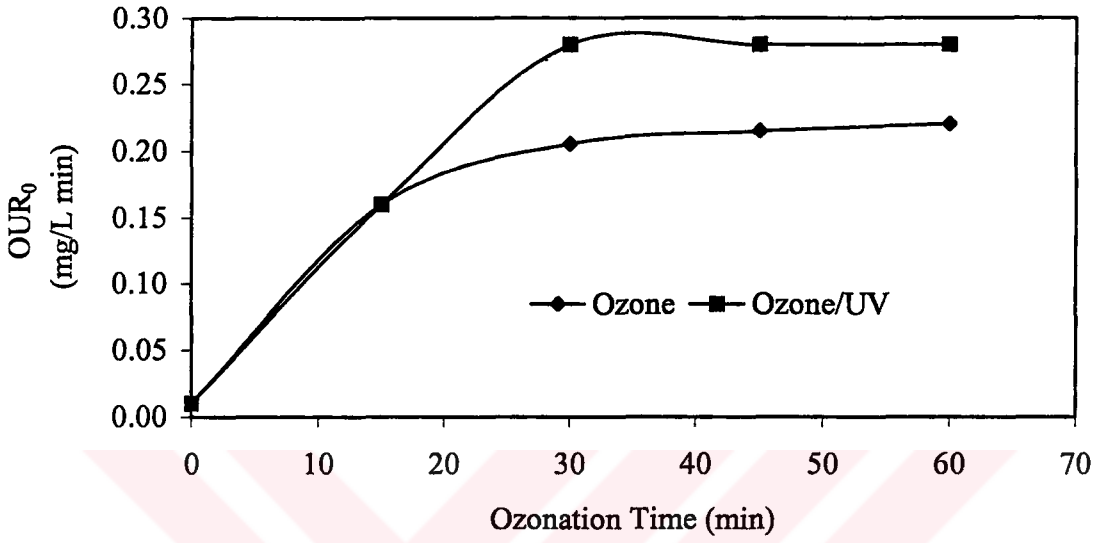


Figure 4.28. OUR_0 of synthetic veterinary antibiotic formulation wastewater samples (COD_i = 900 mg/L), subjected to ozonation and photolytic ozonation at certain time intervals in the photochemical reactor ($(O_3)_i$ = 2592 mg/L h, pH = 7).

As can be seen from Figure 4.28, wastewater subjected to photolytic ozonation provided a much higher oxygen uptake rate showing that the products were more biodegradable than that of wastewater samples subjected to mere ozonation confirming the above findings.

5. CONCLUSION

The present study was undertaken to ascertain the effect of O_3, O_3/H_2O_2 and O_3/UV processes on three different synthetic antibiotic formulation wastewater.

The effect of pH on ozone treatment was found to be highest in the case of human antibiotic II. Elevating the ozonation pH from 3 to 11 increased the pseudo first order COD rate constants of human antibiotic II from 0.0079 min^{-1} to 0.03 min^{-1} and enhanced total COD removal 45 per cent with an applied ozone dose of 2960 mg/L h. The positive effect of pH was less pronounced for the treatment of human antibiotic I and veterinary antibiotic. Results for the ozonation of human antibiotic I in buffered and non-buffered solutions revealed that, pH control was essential to obtain efficient COD and UV_{254} removals and COD removal decreased from 74 per cent in buffered solution to 58 per cent in non-buffered solution due to the formation of acidic products.

Increasing the initial COD concentration in the range of 250 mg/L – 1400 mg/L inhibited the COD and UV_{254} abatements as well as the pseudo first order COD rate constants for both antibiotic effluents. The kinetic relationship between the initial COD values of antibiotics and COD abatement rate constants obtained for ozonation at pH = 7, at ozone dose of 2960 mg/Lh, was found as an inverse power function for the concentrations between 250 mg/L and 1400 mg/L.

For the O_3/H_2O_2 treatment of human antibiotic I and veterinary antibiotic (initial COD = 450 mg/L) at different hydrogen peroxide doses and pH = 7, introduction of an optimum dose of H_2O_2 (50mM) accelerated the pseudo first order COD rate constants from 0.027 min^{-1} to 0.035 min^{-1}, and from 0.046 to 0.0526 min^{-1} respectively, whereas no significant enhancement was obtained in overall COD and UV_{254} removals. However, beyond the optimum dose, the oxidation rate of veterinary antibiotic decreased from 0.0526 min^{-1} to 0.032 min^{-1} due to the scavenging effect of hydrogen peroxide at elevated doses. On the other hand, ozonation of human antibiotic II in the presence of 20 mM H_2O_2

provided almost 100 per cent COD removal and 30 per cent enhancement in UV_{254} removal with an applied ozone dose of 2960 mg/L.

From these findings it can be concluded that, molecular ozone was found to be more selective towards the human antibiotic II due to the lack of unsaturated bonds in its structure. Oxidative degradation of human antibiotic was improved with the corresponding increase in the formation rate of hydroxyl radical. On the other hand, degradation rate of veterinary antibiotic was determined as highest in both treatment process and was not depend upon the reaction conditions compared with human antibiotics.

Veterinary antibiotic formulation wastewater having the initial COD value of 900 mg/L was subjected to ozonation at pH = 7, at four different inlet ozone doses ranging between 960 mg/h – 4440 mg/h. Results from the experiment revealed that ozone utilization decreased from 81 per cent to 59 per cent, as the inlet ozone concentration increased. However, elevating ozone dose provided about 40 per cent improvement for both COD and UV_{254} removals and increased the pseudo first order COD rate constants from 0.0074 min^{-1} to 0.022 min^{-1}. Therefore, although utilization of ozone was found to be better at low inlet ozone doses, longer retention times required for optimal treatment efficiency. Hence, it would be worthwhile to perform ozonation process at low ozone doses for an extended time period.

Studies on enhancement of biodegradability indicated that, while the increase in the ratio for human antibiotic I remained constant after one hour ozonation at the value 0.1, further increase with a contact time was observed in the ratio for human antibiotic II and veterinary antibiotic which reached to 0.27 and 0.38 respectively. The reason for the low effectiveness of ozonation on biodegradability of human antibiotic I could be explained by the complex molecular structure of it. Parallel to this finding, the required energy for the degradation of human antibiotic I through H-abstraction by hydroxyl radical was found to be highest (118.069 kcal/mole), while that of veterinary antibiotic and human antibiotic II were 86.349 kcal/mole and 96.345 kcal/mole respectively.

Biodegradability enhancement studies of veterinary antibiotic formulation wastewater carried out at four different inlet ozone doses revealed that the increase of

ozone absorption was parallel to the enhancement of BOD_5/COD ratio. Up to inlet ozone dose of 3888 mg/h, an effective improvement was observed in this ratio (increased from 0.12 to 0.35), however, beyond this concentration, no further enhancement on biodegradability was obtained. Additionally, elevating the reaction pH from 3 to 7 and 11 resulted in a remarkable increase in the BOD_5/COD ratio (0.24, 0.38 and 0.49 respectively) of veterinary antibiotic subjected to ozonation with an inlet ozone dose of 4440 mg/h.

The relative enhancement of the OUR_0 of veterinary antibiotic formulation effluent diluted with synthetic domestic wastewater in a ratio of 1:2, could be improved by a factor two after 15 minute ozonation at neutral pH and inlet ozone dose of 3888 mg/h.

Results obtained from the O_3 and O_3/UV treatments of veterinary antibiotic formulation effluent at photochemical reactor obviously elucidated the contribution of UV light on overall aromaticity removal was more pronounced than that of COD abatement. UV_{254} removal was increased from 63 per cent to 83.6 per cent, whereas only 3 per cent enhancement was obtained for overall COD removal.

Compared to the effectiveness of photochemical and ozone reactors for the ozonation of synthetic veterinary antibiotic effluent (initial COD = 900 mg/L) at an inlet ozone dose of 3888 mg/h, increasing the absorbed ozone concentration (for ozone reactor =1619 mg/L h, for photochemical reactor = 755 mg/L h) as a consequence of the enhanced mass transfer coefficient, resulted in a corresponding 30 per cent increase in the overall COD and UV_{254} removals.

The OUR_0 values of synthetic veterinary antibiotic formulation wastewaters subjected to one hour ozone and ozone/UV processes (inlet ozone dose = 3888 mg/h) at pH = 7, were determined as 0.21 mg/L min and 0.28 mg/L min respectively, indicating the contribution of ozone UV combination on biodegradability enhancement.

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