

EFFECTS OF WATER COMPONENTS ON THE PHOTOCATALYTIC AND OZONE
OXIDATION OF OXYTETRACYCLINE ANTIBIOTIC

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by

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation
C	Concentration
C ₀	Initial Concentration
C _e	Equilibrium Adsorbed Concentration
CTC	Chlortetracycline
HPLC	High Pressure Liquid Chromatography
K	Equilibrium Constant
k'	Pseudo First Order Model Rate Constant
K _{ow}	Octanol Water Partition Coefficient
OTC	Oxytetracycline
pK _a	Acid Dissociation constant
r	Correlation Coefficient
r ₀	Initial Degradation Rate
STP	Sewage Treatment Plant
t	Reaction Time
TC	Tetracycline
TOC	Total Organic Carbon
UV	Ultraviolet
ZPC	Zero Point Charge

1. INTRODUCTION

Antibiotics are an important group of pharmaceuticals in today's medicine. They are used for the treatment of human and animal infections and they are used as growth promoter in animal feeding operations. Human and veterinary antibiotics are continually being released into the environment mainly as a result of manufacturing processes, disposal of unused or expired products, and excreta. Veterinary drugs may enter into the environment more directly than does human drugs (Christensen, 1998). The existence of antibiotics in the environment and their possible effects on living organisms are giving rise to growing concern (Heberer, 2002).

Depending upon their physical and chemical properties, many of antibiotic substances or their bioactive metabolites end up in soils and sediments. Surface runoff and leaching cause the transport of the antibiotics from soil to surface and groundwater. In addition, effluent of sewage treatment plant can constitute a source for antibiotic pollution in the surface water (Halling-Sorensen, 1998; Kümmerer et al., 2000). Bacterial resistance is a significant problem related with the presence of antibiotics in the environment (Ternes et al., 1999). These compounds have also an important exerting toxic effect to aquatic organisms even in the $\mu\text{g L}^{-1}$ – mg L^{-1} concentration range that change the ecological balance negatively (Halling-Sorensen, 1998).

Conventional treatment processes are unable to eliminate pharmaceuticals in water and wastewater, thus it is necessary to investigate advanced treatment technologies for antibiotic pollution control. Different advanced treatment technologies have been recently evaluated for this purpose, including chemical oxidation using ozone and ozone/hydrogen peroxide (Zwiener and Frimmel, 2000; Ternes et al., 2002; Balcioglu and Otker, 2003; Alaton et al., 2004), membrane filtration such as nanofiltration and reverse osmosis (Hartig et al., 2001; Heberer et al., 2002; Nghiem et al., 2004), and activated carbon adsorption (Hartig et al., 2001; Ternes et al., 2002). Among these ozone oxidation and heterogenous photocatalysis can be a promising process for degradation of pharmaceuticals (Fan et al., 2002; Balcioglu and Otker, 2003; Ikehata et al., 2006; Abellan et al., 2007). However, in these studies the effects of water components on the oxidation of antibiotics were not investigated.

The present investigation was aimed to study the treatment of water synthetically contaminated with a tetracycline group antibiotic by TiO_2 mediated photocatalytic oxidation and ozone oxidation. The effects of initial antibiotic concentration, pH, and H_2O_2 concentration on the performance of photocatalytic degradation were investigated. In case of ozone oxidation experiments, the effects of pH and applied ozone dose on the degradation of antibiotic were evaluated. The antibiotic treatment performances of both photocatalytic and ozone oxidation were also investigated in the presence of Ca^{2+} , HCO_3^- , NO_3^- , PO_4^{3-} , SO_4^{2-} , Cl^- ions, and humic acid to observe the effect of water components on the degradation of antibiotic.

2. THEORETICAL BACKGROUND

2.1. Antibiotics

2.1.1. Definition of Antibiotics

An antibiotic is a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi or protozoa. The term originally referred to any agent with biological activity against living organisms. However, "antibiotic" now refers to substances with anti-bacterial, anti-fungal, or anti-parasitical activity. With advances in organic chemistry many antibiotics are now also obtained by chemical synthesis. On the other hand, antibiotics are suspicious environmental contaminants as they are biologically active, have lipophilic properties and a low biodegradability (Chrintensen, 1998).

2.1.2. Most Widely Used Antibiotics

After the discovery of penicillin by Alexander Fleming in 1928, numerous kinds of antibiotics took place, thus today, antibiotics are widely used to treat diseases in human and animals and to improve growth efficiency of animals (Sarmah et al., 2006). The most frequently used antibiotics in Turkey are penicillin (45 %), cephalosporines (20 %), macrolides (17.5 %), quinolones (17 %), and tetracyclines (4 %) (Karabay and Hosoglu, 2008). In USA, this order is reported as ionophores/arsenicals (47.5 %), tetracyclines (15.8 %), penicillins (4.3 %), and sulfonamides (2.3 %). Tetracyclines are the most widely used antibacterial compounds in the UK, followed by sulfonamides, β -lactams, macrolides, aminoglycosides, fluoroquinolones, and others (Ungemach et al, 2006). In Finland, the most common drug was tetracyclines (28 %), in Austria macrolides (26 %) and in Denmark and Sweden narrow-spectrum penicillins (40 % and 36 %, respectively) (Cars et al., 2001).

On the other hand, the use of antibiotics as growth promoters has been banned in European countries in 2003 (EC, 2003). In Turkey, the use of antibiotics as a growth promoter in the feed and feeding to the animals has been also restricted by a regulation in 2006. After the banning of antibiotics as growth promoter, an actual decline in antibiotics used in agricultural practices has been recorded (Kemper, 2008). Before the banning, the amounts of

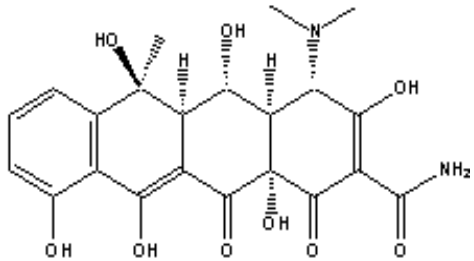
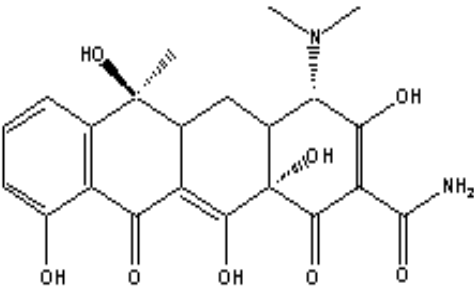
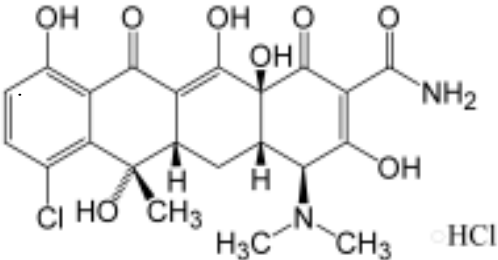
antibiotics used in one year were 13,288 tons in 1999 in the EU and Switzerland, of which 29 % were used in veterinary medicine, 6 % as growth promoters, and 65 % were used in human medicine (Kemper, 2008).

Antibiotic groups can be classified as tetracyclines, macrolides, sulfonamides, and quinolones..

2.1.2.1.Tetracyclines: The tetracyclines (TCs) are broad-spectrum antibacterials widely used in human and animal medicine. They are active against a range of organisms such as *Mycoplasma* and *Chlamydia*, as well as a number of gram-positive and gram-negative bacteria. Tetracycline, oxytetracycline (OTC), and chlortetracyclines (CTC) are widely used in animal feeds to maintain health and improve growth efficiency in many countries.

These chemicals are characterized by a partially conjugated four-ring structure with a carboxamide functional group (Fedeniuk, 1998). The molecule of tetracycline has several ionizable functional groups of a rather unusual type, and the charge of the molecule depends on the solution pH. There are three distinct acidic functional groups for tetracycline: tricarbonyl methane (pK_a 3.57), dimethyl ammonium cation (pK_a 9.44), and the phenolic diketone (pK_a 7.49). An examination of their pK_a (Table 2.1) values suggests that TC, OTC, and CTC have similar pH dependent speciation, which is also consistent with their structural relationship. The ionization of OTC in water at different pH values is presented in Figure 2.1.

Table 2.1. Some properties of tetracycline antibiotics.

Compound	Structure	Molecular Weight (g mol ⁻¹)	pK _a	Reference
OTC		460.4	3.57 7.49 9.44	(Figueoroa and Mackay, 2005)
TC		444.4	3.57 7.49 9.88	(Figueoroa et al. 2004)
CTC		478.9	3.3 7.7 9.7	(Figueoroa et al. 2004)

TCs are relatively stable in acidic media, but not in alkaline conditions, and form salts in both media (Halling-Sørensen et al., 2002). They have been found to form complexes with chelating agents such as divalent metal ions and β -diketones, and strongly bind to proteins and silanol groups (Oka et al., 2000). In general, these compounds are sparingly soluble in water. However, solubility of the corresponding hydrochlorides is reported to be much greater (Thiele-Bruhn, 2003).

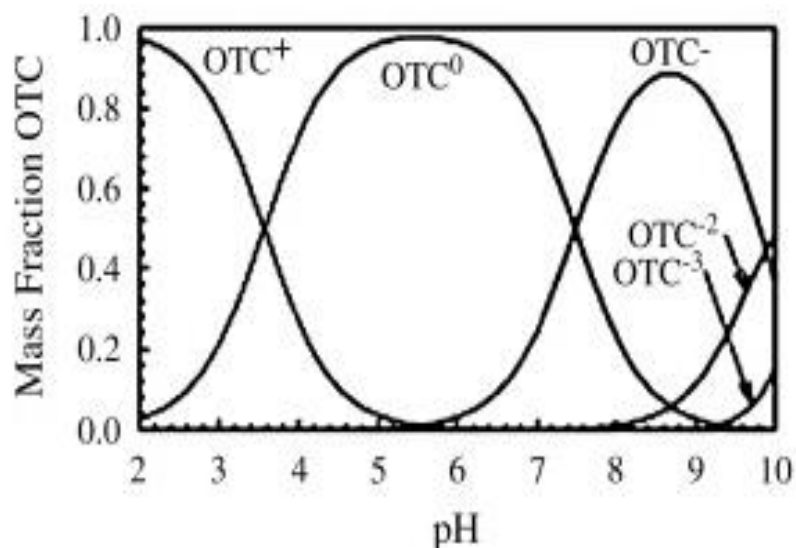
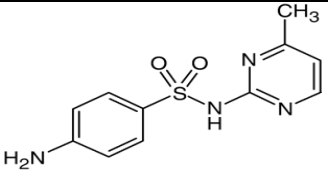
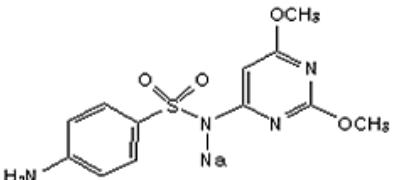
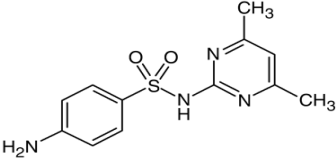
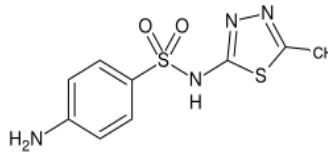
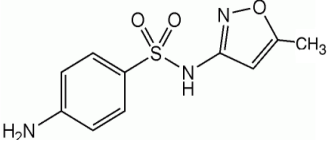
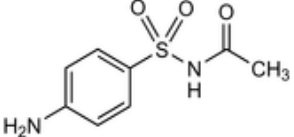
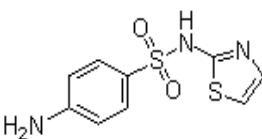
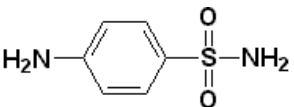


Figure 2.1. OTC speciation at environmentally relevant pH values (Tavares and McGuffin, 1994).

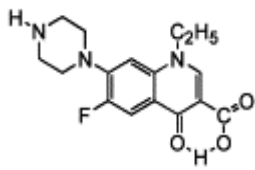
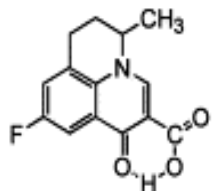
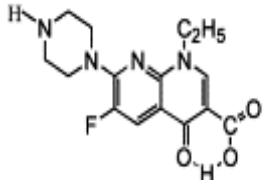
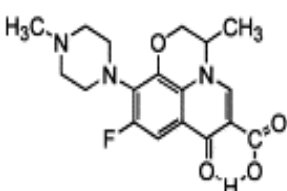
2.1.2.2. Macrolides: Tylosin falls within the macrolide group of antibiotics, and is a broad-spectrum antibiotic with a good antibacterial activity against most pathogenic organism such as gram-positive bacterium, some gram-negative bacterium, vibrio, spirochete, coccidian etc. (Sarmah et al., 2006). Tylosin consists of a mixture of the macrolides: Tylosin A, Tylosin B (desmycosin), Tylosin C (macrocin), and Tylosin D (relomycin), all of which contribute to the potency of the antibiotic. Tylosin is unstable in acidic and alkaline media, and relatively stable under neutral pH conditions (pH 7). The solubility of most of the macrolide group of antibiotics is high and has been found to increase with an increase in solvent polarity (Cherlet et al., 2002). The main properties and chemical structure of tylosin are presented in Table 2.2.

Table. 2.3. Properties of sulfonamides (Lindsey et al., 2001).

Compound	Structure	Molecular weight (g mol ⁻¹)	pK _a
Sulfamerazine		264.30	7.0
Sulfadimethoxine		310.33	5.9
Sulfamethazine		278.33	7.59
Sulfamethizole		270.33	5.50
Sulfamethoxazole		253.28	5.6
Sulfacetamide		214.2	5.4
Sulfathiazole		255.31	7.2
Sulfanilamide		172,2	10.4

2.1.2.4. Quinolones: They are used extensively for treatment of a broad range of clinical infections of human including those of the genitourinary, gastrointestinal, and respiratory tracts and of animals for preventing diseases. They are active against gram-positive and in some cases anaerobic bacteria. Quinolones rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA. The quinolones exhibit concentration-dependent bacterial killing. The effects of most combinations of antibiotics with quinolones are indifferent or additive., However, ciprofloxacin and rifampicin appear to be antagonistic against *Staphylococcus aureus*. In these drugs, there are two types of ring structures, a naphthyridine nucleus, with a nitrogens at position 1 and 8, and a nucleus with only one nitrogen in position 1, referred to as a quinoline nucleus. All compounds, both quinolones and naphthyridones, contain the keto oxygen at C-4 and carboxylic acid side chain at C-3, both of which have now been found to be essential to activity. Moreover, ofloxacin, norfloxacin, enoxacin etc. have a piperazinyl group at 7-carbon atom. Because these drugs contain carboxyl group, or carboxyl and amine groups, the acid–base behaviour will be influenced by the physicochemical properties of solvent (Park et al., 2000). Table 2.4. represents the chemical structures and properties of some quinolones.

Table 2.4. Properties of quinolones (Park et al., 2002)

Compound	Structure	Molecular weight (g mol ⁻¹)	pK _a
Norfloxacin		319.34	7.26
Flumequine		261.25	6.51
Enoxacin		320.32	7.53
Ofloxacin		361.37	7.18

2.2.Sources of Antibiotic Contamination and Pathways of Antibiotics in the Environment

Antibiotics may be disseminated into the environment from both human and agricultural sources, including excretion, flushing of old and out-of-date prescriptions, medical waste, discharge from wastewater treatment facilities, leakage from septic systems, and agricultural waste storage structures (Figure 2.2.).

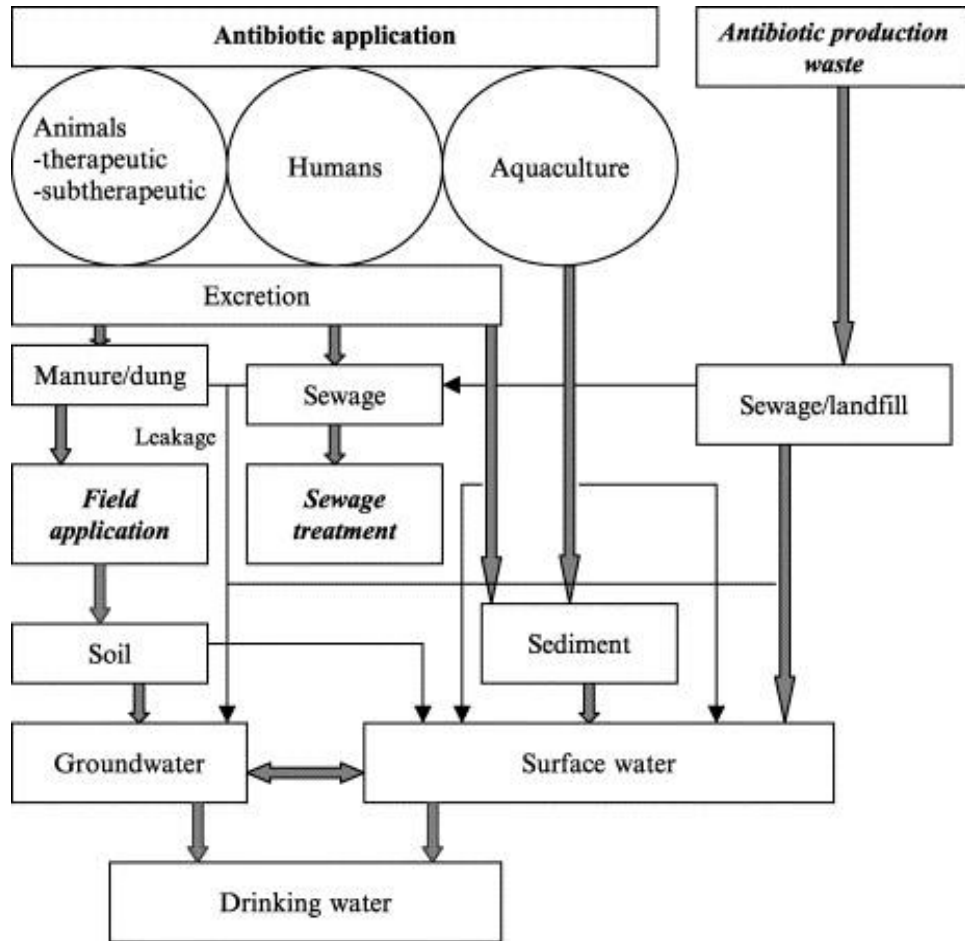


Figure 2.2. Anticipated exposure pathways for antibiotics in the environment (Kumar et al., 2005).

Sewage and wastewater from hospitals and veterinary clinics are also huge contributors to antibiotics pollution (Rhodes et al. 2000). Since many of the treatment plant are not designed and operated to eliminate very low concentrations of the antibiotics some of the antibiotics including tetracycline, sulfamethoxazole, and tylosin have been detected in sewage treatment plant effluents (Karthikeyan et al., 2006; Sarmah et al., 2006; Hirsch 1999). During sewage treatment, as many organic compounds, antibiotics sorbed onto sludge or residues remained in treated effluent. In this way, antibiotics can enter directly into surface water, sorbed in sediments or leach into groundwater depending on the physicochemical properties of antibiotics. In addition, unused or expired antibiotics can be end up in the household waste and if the waste not incinerated, antibiotics can reach to landfill (Eckel et al., 1993; Holm et al. 1995). Holm et al., (1995) analyzed the ground water down gradient of a landfill formerly used for the disposal of waste from pharmaceutical production. They found a large variety of sulfonamide concentrations ranging up to about 5 mg L^{-1} .

In animal husbandry, antibiotics are used to in large quantities to increase production and improve animal health care. Depending on the type of antibiotic, and age and species of the animal, a high amount of antibiotic can be excreted in an unmetabolized form (Sarmah et al., 2006). As most of the antibiotics are water-soluble, as much as 90% of one dose can be excreted in urine and up to 75% in animal feces (Halling-Sørensen, 2001). Sheep excrete nearly 21% of an oral dose of fertilization, and young bulls excrete about 17–75% of chlortetracycline as the parent compound (Sarmah et al, 2006). Animal manure containing excreted antibiotics can be used as a fertilizer hence; they may enter into the environment (Boxall et al., 2004). Therefore, leaching to the groundwater from soil and reach surface waters through surface runoff can come into existence (Hirsch et al., 1999).

Some of the largest sources of antibiotics in the waterways are animal farms, crop production, and fish farms (Wiggins et al., 1999). Antibiotics are also introduced into surface waters when fisheries use medicated foods or treat for disease outbreaks. The presence of residual feed additives may also taken up by fish crustaceans and exert toxic effects (Capone et al., 1996).

2.3. Occurrence of Antibiotics in Environmental Matrices

Over the last two decades concerns have been raised about occurrence of antibiotics in the environment and numerous studies has been documented comparatively. A number of these compounds have been detected in surface water, groundwater, manure, and soil (Halling-Sørensen et al., 1998; Ternes, 1998; Hirsch et al., 1999; Stumpf et al., 1999; Heberer et al., 2002; Kolpin et al., 2002; Sarmah et al., 2006).

Although they are found at trace levels, the continuous introduction of antibiotic into the environment is characterized as a “pseudo-persistence”, which may result in toxic effects (Bautitz and Nogueira, 2007). Since studies have shown these compounds are transported into surface water and ground water from urban and agricultural sources, researchers have begun to investigate their adverse effects (Cole et al., 2000; Loftin et al., 2005).

The occurrence of antibiotics in surface and groundwater, manure, and agricultural soil are summarized below.

2.3.1. Surface Water

The first reported case of surface water contamination by antibiotics was in England more than two decades ago, when Watts et al. (1982) detected at least one compound from the macrolide, sulfonamide, and tetracycline group of antibiotics in river water at concentrations of $1 \mu\text{g L}^{-1}$. Following this, a variety of other antibiotics was also detected in surface water in concentrations up to $1 \mu\text{g L}^{-1}$ (Ternes, 1998; Hirsch et al., 1999). For example, a German group detected residues of chloramphenicol in one small river in southern Germany at concentration of $0.06 \mu\text{g L}^{-1}$ (Hirsch et al., 1999). Tylosin has also been found in the river waters of Italy (Zuccato et al., 2000). In addition, Alder et al. (2001) detected sulfamethazine and other groups of antibiotics used in veterinary medicine in Swiss surface waters. Erythromycin was also detected in surface water in the U.S. and maximum reported concentration was 1.7 mg L^{-1} .

2.3.2. Groundwater

The occurrence of veterinary antibiotics in groundwater is the result of agricultural usage of antibiotics and it has been reported by some researchers. (Hirsch et al., 1999; Hamscher et al., 2000; Lindsey et al., 2001; Sarmah et al., 2006). Residues of sulfonamide antibiotics were detected in four samples collected from an agricultural area, with two samples showing sulfamethazine at concentrations of 0.08 and $0.16 \mu\text{g L}^{-1}$ (Lindsey et al., 2001). Multiple classes of antimicrobial compounds (tetracycline, macrolide, β -lactam, and sulfonamide) were also detected in groundwater samples collected in nearby swine farms in the US (Campagnolo et al., 2002).

2.3.3. Dung, Manure and Agricultural Soils

The intracorporal administration of antibiotics inevitably leads to residual concentrations in excrements (Thiele-Bruhn, 2003). It is, therefore, not surprising to find residues of antibiotics either as metabolite or parent compound in dung, manure, and subsequently, in agricultural fields (Hamscher et al., 2002). For instance, Hamscher et al. (2002) reported the presence of 4.0 and 0.1 mg kg^{-1} of TC and CTC in liquid manure, while in the soil samples fertilized with liquid manure, the concentrations of these compounds varied from an average $86.2 \mu\text{g kg}^{-1}$ in the top soil (0–10 cm) to as high as $171.7 \mu\text{g kg}^{-1}$ in the 20–30-cm layer. The

reason why higher concentrations found in deeper layers has been explained by the additional release of bound residues in the form of 4-epi-tetracycline (4-epi TC), a metabolite of TC, and 4-epi TC is transferred from the liquid manure into the soil (Hamscher et al., 2002). In addition, Hamscher et al. (2000) reported chlortetracycline, tetracycline, and oxytetracycline concentrations of 0.1–0.3 $\mu\text{g L}^{-1}$ in soil water samples collected from agricultural land in Germany.

2.4. Fate of Antibiotics

In the environment, main processes affecting the fate of the antibiotics in the environment can be classified as sorption, leaching, and degradation (Sarmah et al., 2006). Factors affecting these processes are temperature, water flow, and distance between cage and sediment, bacterial activity, chemical composition, and depth of the sediment (Samuelson, 1989).

Sorption of antibiotics in soil and sediment is affected by several factors such as organic carbon, clay content, particle size, and surface area. Some studies can explain sorption behavior of antibiotics. It was observed that sorption of quinoline group antibiotics mainly depends on the particle size of the soil (Nowara et al., 1997). In addition, Sarmah et al. (2006) found that oxytetracycline strongly sorbed on soil. They reported that cation exchange capacity, and organic carbon and clay content are responsible for oxytetracycline sorption on soil. Tolls (2000) determined that surface interactions of tetracycline with clay minerals were the reason for the strong sorption on soils. Leaching through the soil profile also causes the contamination of aquatic environment. Therefore, the leachate can reach sub soil layer and groundwater aquifers (Heberer, 2002).

The degradation of antibiotics can explain the environmental fate of antibiotics. Their degradation varies according to their chemical structure, temperature of the field and humidity, rainfall and nature of soil. Numerous studies were performed to explain the degradation of antibiotics (Halling –Sorensen, 2001; Halling-Sørensen et al., 2002; Sarmah et al., 2006). Sarmah et al. (2006) reported that the degradation of tetracycline antibiotics could occur by photodegradation and hydrolysis. The fate analyses of tetracyclines showed that abiotic degradation products, and reversible epimers, may be

formed due to hydrolysis and/or photolysis such as epi-tetracyclines, anhydrotetracyclines, as well as iso-tetracycline for chlortetracycline alone (Halling-Sørensen et al., 2002).

2.5. Effects of Antibiotics on the Environment

Antibiotic agents have the potential for forming resistance and bacterial toxicity with the potential to disturb environmental bacterial processes, and alter microbial species diversity (Sarmah et al., 2006). Therefore, increasing production and consumption of antibiotics resulted in changing the genetic pool of microorganisms in nature and increased resistance towards certain antibiotics (Reyes, et al., 2006; Iwane et al., 2001) that is a long term and high extent irreversible effect.

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are “resistant” and continue to multiply in the presence of therapeutic levels of an antibiotic. This is a serious threat for public health as more and more infections can no longer be treated with the presently known antidotes. Concentrations below therapeutic levels may play a role in the selection of resistance and its genetic transfer in certain bacteria.

The resistant bacteria may be selected by antibiotic substances in hospital effluent, municipal sewage, aeration tanks, anaerobic digestion process of STPs or in soil. Furthermore, resistant bacteria are excreted or discharged into sewage, soil, and other environmental compartments. Resistant and even multi-resistant pathogenic bacteria have been detected in wastewater and STPs, as well as in other environmental compartments. (Guardabassi et al., 1998). Furthermore, in arid regions, wastewater containing resistant bacteria and antibiotics is used for irrigation, and sewage sludge serves as a fertilizer. This allows resistant bacteria to enter the food chain directly. Resistance can be transferred to other bacteria living in other environments such as ground water or drinking water. In general, knowledge of sub inhibitory concentrations and their effects against environmental bacteria is poor, especially with respect to resistance. There are a number of recent and older publications about the mechanisms of very low antibiotic concentrations on the expression of bacterial virulence factors. Studies using test systems indicate that various antibiotics remain active against different groups of bacteria present in wastewater (Kümmerer, 2001).

In addition, toxic effect data (EC_{50}) of human and veterinary antibiotics in the $mg L^{-1}$ 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; Landsky and Halling Sorensen, 1997; Holten Lutzhoft, 1999).

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 $mg L^{-1}$ respectively) while oxytetracycline tetracycline, and sulphadiazine lead to reproductive toxicity in the range of 5 to 50 $mg L^{-1}$. Additionally, Migliore et al, (1997) showed the toxicity of several agricultural antibiotics to *Artemia salina*. Effects against algae and daphnids have been reported at surprisingly low concentrations (5–100 $\mu g L^{-1}$) (Holten-Lützhøft et al., 1999). The effects of antibiotics on organisms e.g. bacteria, algae, *Daphnia magna*, have been found not only in high concentrations, but also low concentrations in chronic tests. Therefore, the presence of antibiotics as low concentrations results in a selective reproduction of resistant bacteria, which could transfer the resistance genes to other bacterial species.

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 tropic levels.

2.6. Treatment Technologies for Removal of Antibiotics

Various treatment technologies have been alternatively used for the treatment of the antibiotics. These technologies can be classified as, membrane filtration such as nanofiltration and reverse osmosis (Hartig et al., 2001; Heberer et al., 2002; Nghiem et al., 2004; Nghiem et al., 2005), activated carbon adsorption and silica (Hartig et al., 2001; Ternes et al., 2002; Turku et al 2007; Choi et al.,2008) and chemical oxidation using ozone and ozone/hydrogen peroxide (Zwiener and Frimmel, 2000; Ternes et al., 2003; Balcioglu and Otter, 2003; Alaton et al., 2005).

Membrane technology including microfiltration, ultrafiltration, nanofiltration, and reverse osmosis is an indispensable treatment alternative for utilities with complex water and

wastewater management needs. There are a few studies about treatment of antibiotics by using membrane technology. Some researchers found that a membrane system including reverse osmosis and ultrafiltration is highly effective for the treatment of the recovery of oxytetracycline from the wastewater (Zhang et al., 2006; Li et al., 2004). Heberer et al. (2002) exhibited that reverse osmosis is appropriate to remove a variety of different pharmaceuticals from highly contaminated surface waters. Koyuncu et al. (2008) obtained almost complete removal of tetracycline and oxytetracycline by using nano filtration membrane system.

Adsorption is indicative in most natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins and water purification. Some researchers found that adsorption of antibiotics on natural zeolite and silica was found to be highly pH dependent, exhibiting increases correspondent to decreases in pH (Chaubal et al., 1995; Otker and Balcioglu., 2005; Turku et al., 2007). It was observed that considerable amount of enrofloxacin was removed from the solution via adsorption at acidic conditions (Otker and Balcioglu., 2005). Choi et al.,(2008) proved that granular activated carbon filtration is relatively effective method for the removal of tetracycline (TC), doxycycline-hyclate (DXC), and chlortetracycline-HCl (CTC), which were difficult to remove by coagulation and the performance of GAC filtration greater than 90 %. They also proved that pore volume is the most important factor for an effective removal. In addition, Turku et al., (2007) found that the adsorbed amounts of tetracycline on silica increases rapidly when the solution pH is decreases from 6 to 4, since electrostatic attraction occurred between the negatively charged silica surface and the positively charged tetracycline molecules at acidic conditions.

2.7. Advanced Oxidation Processes

Treatment of water or wastewater including antibiotics may require a chemical process in which toxic or hardly degradable compounds would be decomposed into innocuous or biodegradable products. Chemical oxidation systems may utilize the synergy derived from a combination of oxidizing agents and UV lights in advanced oxidation processes, which are used for water and wastewater treatment. Advanced oxidation processes consist of the generation of highly reactive radicals mainly, hydroxyl radicals (Glaze and Kang, 1989). Hydroxyl radical generation processes are classified as below:

- **Non-photochemical Homogenous AOPs**

Ozone / H_2O_2

Ozone / OH^\cdot

Fenton (Fe^{2+} / H_2O_2)

Fenton-like reaction (Fe^{3+} / H_2O_2)

- **Photochemical Homogenous AOPs**

Photolytic ozonation (O_3 / UV-C)

Photocatalytic ozonation (O_3 / TiO_2 /UV)

Photolysis of Hydrogen Peroxide (H_2O_2 / UV-C)

Photo- Fenton reaction (Fe^{2+} / H_2O_2 / UV)

- **Heterogeneous AOPs**

Photocatalytic reaction (e.g TiO_2 /UV-A)

2.7.1. Photocatalytic Oxidation Process

Photocatalysis is the acceleration of a photoreaction in the presence of a catalyst, which absorbs light to produce oxidative species. The photocatalytic activity (PCA) depends on the ability of the catalyst to create electron–hole pairs, which generate free radicals able to undergo secondary reactions.

Photocatalytic degradation has been proved a promising method for the treatment of water and wastewater contaminated with organic and inorganic pollutants. Several catalysts are used as potential photocatalysts for this purpose. These include mainly TiO_2 , CdS, ZnS, α - Fe_2O_3 , FeOOH, ZnO, ZrO_2 , and SnO_2 . Among the semiconductors TiO_2 ($E_g = 3.2 \text{ eV}$) is one of the most popular and promising photocatalyst, because of its stability under harsh conditions, commercial availability, different allotropic forms with high photoactivity, possibility of coating as a thin film on solid support, ease of preparation in the laboratory, etc. Its absorption spectrum overlaps with the solar spectrum and hence opens up the possibility of using solar energy as the source of irradiation (Hoffmann et al., 1995). A schematic presentation of TiO_2 based photocatalysis is shown in the Figure 2.3.

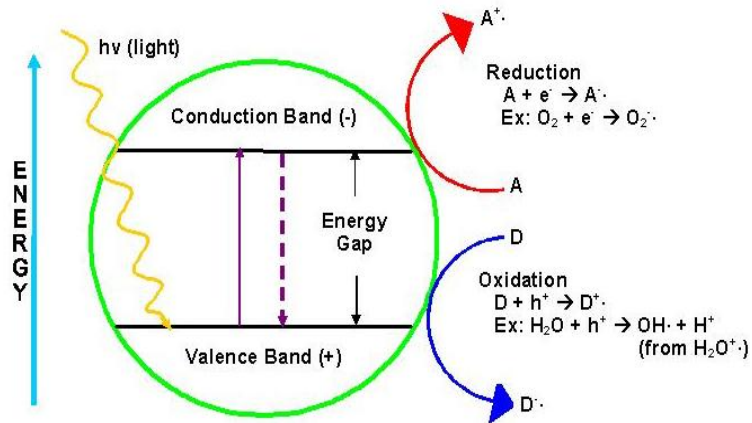


Figure 2.3. Photocatalysis mechanism of titanium,
(<http://dev.nsta.org/evwebs/1952/Operation.jpg>)

In aqueous solution, the photoinduced h^+ on TiO_2 photocatalyst may react with surface hydroxyl ions or surface bound water molecules to produce hydroxyl radicals (OH^{\bullet}), the primary oxidant in the photocatalytic system. Simultaneously, the photoinduced e^- could be trapped by oxygen to form superoxide radical anions ($O_2^{\bullet-}$). The degradation of organic substrates seems to be mediated by a series of reactions initiated by these primary oxidizing species, particularly OH^{\bullet} radicals. Due to the reactivity and nonselectivity of OH^{\bullet} radicals, UV/ TiO_2 process is more destructive for numerous organic substrates than traditional oxidation methods, especially for the removal of toxic and recalcitrant organic pollutants (Legrini et al., 1993; Lin et al., 1996).

The photocatalytic method offers many advantages over traditional wastewater treatment techniques such as activated carbon adsorption, chemical oxidation (Konstaninou and Albanis; 2003). These include;

- chemical additives are not required,
- inexpensive and commercially available light source and catalysts can be used,
- low-temperature operation allows the use of plastic piping and construction, reducing costs and minimizing acid corrosion problems (Hoffmann et al., 1995).

2.7.2. Ozone Oxidation Processes

Ozone may react with dissolved organic substances directly or it may decompose leading to more reactive species such as hydroxyl radicals that determines the subsequent

reactions. The ozonation reaction mainly based on the rate of mass transfer into an aqueous system. Due to its structure, molecular ozone can react as a dipole, an electrophilic or nucleophilic agent and can oxidise water impurities via direct, selective reactions or can undergo decomposition via a chain reaction mechanism resulting in the production of free hydroxyl radicals.

The decomposition of ozone follows a pseudo first-order kinetics law (Kasprzyk-Hordern et al., 2003).

$$-\left(\frac{d[\text{O}_3]}{dt}\right)_{\text{pH}} = k' [\text{O}_3] \quad (2.1.)$$

where k' is a pseudo first-order constant for a given pH value.

The initiation and propagation steps of hydroxyl ion initiated decomposition of ozone are followings (Glaze and Kang, 1989):

Initiation steps:



Propagation steps:



The overall reaction for the hydroxyl ion initiated decomposition of O_3



It was reported that, at increased pH levels, there is an increase in removal efficiency due to accelerated ozone decomposition to free radicals (Staehelin and Hoigne, 1982).

However, at low or neutral pH values these radical reactions are of minor importance regarding degradation reactions of micro pollutants (Sonntag et al., 1993).

2.7.3. Advanced Oxidation Processes for the Removal of Antibiotics

In recent years, photocatalytic degradation of antibiotics such as tetracycline and β -lactam group of antibiotics and ozone oxidation of sulfamethoxazole, oxytetracycline, ibuprofen, and diclofenac antibiotics were investigated in various studies (Zwiener and Frimmel, 1999; Fan et al., 2002; Balcioglu and Otker, 2003; Huber et al, 2003; Ternes et al. 2003; Di Paola et al., 2004; Vogna et al., 2004; Addamo et al., 2005; Li et al., 2008;). Water components can affect the performance of both photocatalytic and ozone oxidation of organic materials. However, in these studies the effects of these components on oxidation of antibiotics has not been yet investigated.

Effective degradation and partial mineralization of tetracycline by TiO_2 mediated photocatalysis was reported by Di Paola et al. (2004) and Addamo et al. (2005). Almost complete conversion of 50 mg L^{-1} tetracycline was achieved in two hour by the $\text{TiO}_2/h\nu$ treatment with 0.4 g L^{-1} of catalyst and about 90 % of TOC was removed in 6 h (Di Paola et al., 2004). However, Addamo et al., (2005) reported that direct photolysis was much less effective in tetracycline conversion and mineralization, while the formation of 4a, 12a-anhydro-4-oxo-4-dedimethylaminotetracycline was suggested as a photolytic by-product and no degradation by-product/intermediate was determined for the tetracycline decomposition catalyzed by TiO_2 .

Degradation of cefradine, a cephalosporin-type β -lactam antibiotic has been studied using an immobilized titanium dioxide photocatalyst and degradation byproduct was not determined (Fan et al., 2002). Addamo et al. (2005) achieved complete conversion of 50 mg L^{-1} lincomycin in 2 h by the $\text{TiO}_2/h\nu$ and the actual degradation intermediates and by-products were not determined with the exception of sulfate ion.

It was revealed that ozonation at natural pH values provided a promising technique for the treatment of synthetic antibiotic formulation wastewater (Balcioglu and Otker, 2003; Alaton et al., 2005). In another study, higher oxidant concentrations, and ozone and hydrogen

peroxide combination were recommended for the treatment of drinking water containing some pharmaceuticals (Zwiener and Frimmel, 1999; Huber et al., 2003).

It was found that both ozonation and $\text{H}_2\text{O}_2/\text{UV}$ systems were effective for the diclofenac antibiotic degradation under the appropriate experimental conditions in aqueous solutions (Vogna et al., 2004; Zwiener and Frimmel, 2000).

Ternes et al. (2003) demonstrated that as low as 5 mg L^{-1} of applied ozone could eliminate 0.62 mg L^{-1} sulfamethoxazole present in a biologically treated municipal wastewater below its detection limit. Similar results were also reported elsewhere (Huber et al., 2005). However, it was found that even though almost 90 % OTC decomposition was obtained after 5 min of ozonation, by-products of the partial ozonation of OTC were more toxic than the parent compound (Li et al., 2008).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Titanium Dioxide

Commercially available Degussa P25 was used as a catalyst in the photocatalytic experiments. The characteristics of the catalyst are represented in Table 3.1.

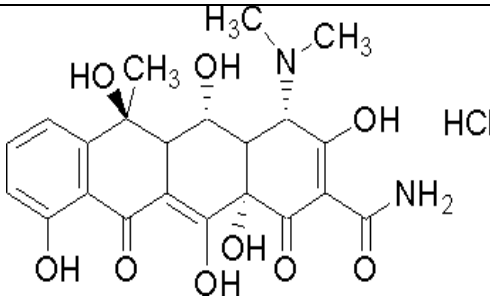
Table 3.1 Characteristics of P 25 titanium dioxide.

	Specific Surface Area (m ² g ⁻¹)	Crystallinity	Particle Size (nm)
Degussa P 25 TiO ₂	49.4	70 % anatase , 30 % rutile	~30

3.1.2. Oxytetracycline hydrochloride

Hydrochloride salt of oxytetracycline was used as a model tetracycline antibiotic to contaminate the water since this form is widely used in veterinary medicine and quite soluble in water. Oxytetracycline hydrochloride, (OTC. HCl, C₂₂H₂₄N₂O₉.HCl, 95 % purity) was purchased from Sigma Aldrich and it was used without any purification. Chemical properties of OTC are presented in Table 3.2.

Table 3.2. Chemical properties of OTC.

Structure		(http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/75966)
Mol. Weight (g mol ⁻¹)	496.89	
pK _{a1}	3.57	
pK _{a2}	7.49	(Figueoroa and Mackay, 2005)
pK _{a3}	9.44	
Log K _{ow}	1.22	(Boleas et al., 2005)
Solubility (g L ⁻¹)	6.9	(http://www.fda.gov/cvm/FOI/038-439_EA.pdf).

3.1.3. Other chemicals

Disodium hydrogen phosphate, potassium nitrate, sodium sulfate, and sodium hydrogen carbonate were used to investigate effects of phosphate, nitrate, sulfate, and bicarbonate anions on the degradation of OTC. Calcium chloride and humic acid were also used to explore the effects of them on the degradation of OTC. Hydrogen peroxide was used as an electron scavenger in the photocatalytic experiments. All chemicals used in the experiments are listed in table 3.3.

Table 3.3. Chemicals used in the experiments.

Name	Chemical Formula	Supplier
Humic Acid	Sodium Salt	Aldrich
Potassium Nitrate	KNO ₃	Mallinckrodt
Calcium Chloride	CaCl ₂ . 2 H ₂ O	Merck
Disodium Hydrogen Phosphate	Na ₂ HPO ₄ .12 H ₂ O	Merck
Sodium Hydrogen Carbonate	NaHCO ₃	Merck
Sodium Hydroxide	NaOH	Riedel Haen
Sodium Sulfate	Na ₂ SO ₄	Riedel Haen
Hydrogen Peroxide	H ₂ O ₂ (30 % w/w)	Riedel Haen

3.2. Methods

3.2.1. Photocatalytic Reactor

Photocatalytic experiments were performed in a 1 L-capacity annular borosilicate glass reactor in batch mode (Figure 3.1). Photoreactor was illuminated with a 20 W BLB lamp (General Electric F20T12.BLB) which was placed at the center of the reactor. The slurry in the reactor was stirred by both recirculation of the solution with a peristaltic pump (Master flex L/S Model 77250-62) and passing air through sintered glass discs placed at the bottom of the reactor. The flow rate of the pump was 153 mL min⁻¹. Teflon tubing was used for all connections in the experimental setup. Light intensity in the reactor was 8.4x10⁻⁷ Einstein s⁻¹.

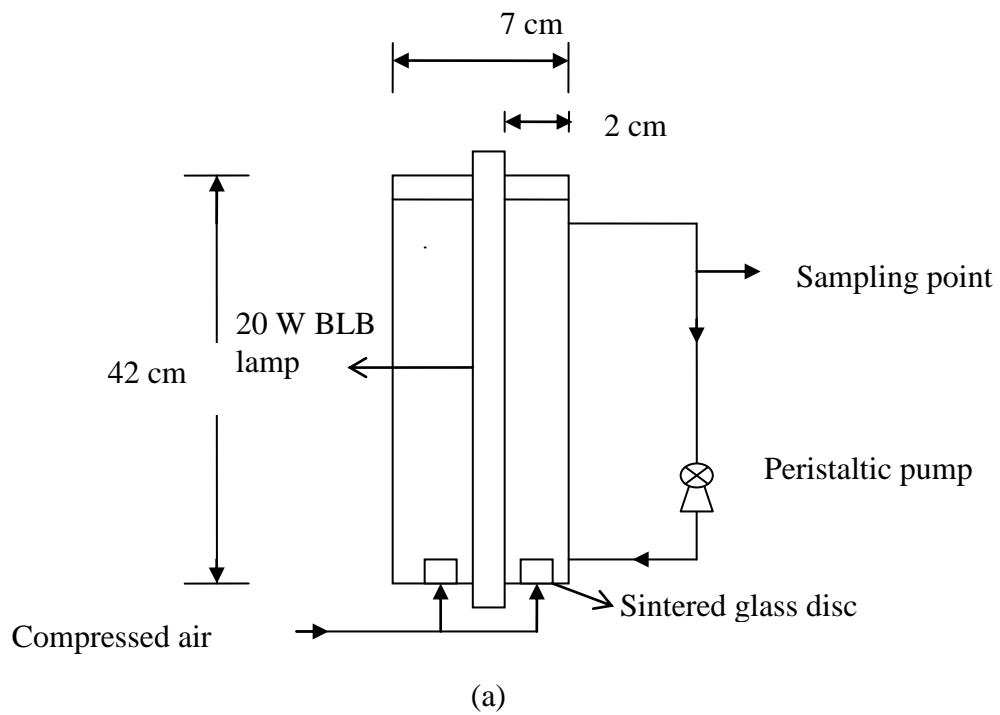


Figure 3.1. Schematic presentation (a) and image of photocatalytic reactor (b).

3.2.2. Ozonation Reactor

Ozonation experiments were performed in another borosilicate glass reactor (Figure 3.2). Ozonation of 1.5 L solution in ozone bubble column (78 cm height; 6 cm diameter) was carried out in semi-batch mode with a gas flow rate of $0.1 \text{ m}^3 \text{ h}^{-1}$. Ozone and oxygen gas mixture was continuously sparged through a sintered glass disc placed at the bottom of the reactor. Experiments were performed with different inlet gas phase ozone concentrations ranging from 9.1 to 26.8 g m^{-3} . The reactor has three ports for ozone and oxygen gas mixture inlet and outlet, and water sampling. Teflon tubing was used for all connections in the experimental setup. The solution in the ozone bubble column was circulated with a peristaltic pump (Cole Palmer) to obtain good mixing conditions. The flow rate of liquid with the pump was 133 mL min^{-1} . Ozone was produced from dry and pure oxygen in a laboratory scale corona discharge ozone generator (Fisher OZ 500). The effluent gas leaving the column from the top of it was passed through an ozone analyzer (Fisher Ozotron 23) to monitor ozone concentration in the off- gas.

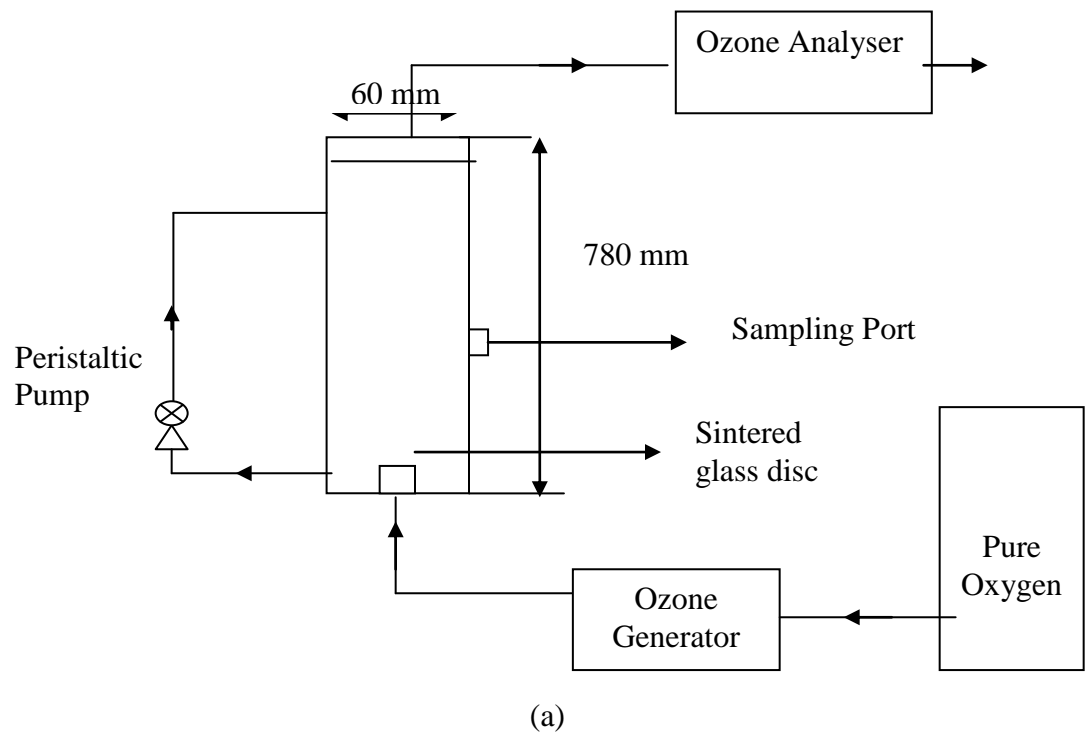


Figure 3.2. Schematic presentation (a) and image of ozone reactor (b).

3.3. Experimental Procedure

All solutions in this study were prepared with Milli-pore water (Gradient Milli-Q). OTC solutions, photocatalytic and ozonation reactors, and samples taken from reactors were all covered with aluminum foil for protection against the light.

In the photocatalytic experiments, after the preparation of OTC solution, TiO₂ was added to the solution and the prepared slurry was subsequently mixed in a sonicator (Bondelin Sonorex Super RK510) for 10 minutes. The suspension was left mixing by means of a magnetic stirrer (Micro Sitirrer Velf Scientific) in the dark for one hour to achieve the adsorption of OTC on TiO₂. The samples of 8 mL were taken into glass centrifuge tubes at certain time interval. Each sample was centrifuged (Nuve NF 1205) at 3000 rpm for 30 min and the supernatant in the tube was subsequently filtered through 0.45 µm membrane filter (Sartorius Minisart).

In ozone oxidation experiments, the OTC solution was prepared as 1.5 L and pH adjusted to 7 and 9 with sodium hydroxide and no adjustment was required for pH 4. pH measurement was done with a portable pH-meter (WTW pH 330). Then, the solution was filled into the ozone reactor and samples were taken from sampling port of the reactor at certain time intervals. Antibiotic concentration in the samples was determined by spectrophotometric (Schimadzu Model 1208) and high pressure liquid chromatographic (HPLC) (Agilent Technologies 1100 series) analysis. For the evaluation of aromaticity removal, UVA₂₅₄ measurements were also performed.

3.3.1. Analytical Methods

3.3.1.1. High performance liquid chromatographic analysis of OTC : HPLC system used for OTC analysis was equipped with a tertiary pump, a photodiode array and an auto sampler with an automated injection system. Gradient elution was carried out with acetonitrile (solvent A) and water containing 0.1% formic acid (solvent B) using analytical column Eclipse XDB C18 (5µ, 150 x 4 mm) equipped with ODS guard column. Elution started with A:B:10:90 and then changed to 40:60 from 0 to 5 min and then returning to initial composition from 5 to 8 minutes. OTC was detected at 360 nm with a retention time of 6.0 min. OTC quantification

was performed by external calibration curve (Appendix A). A typical chromatogram of OTC is presented in Figure 3.3.

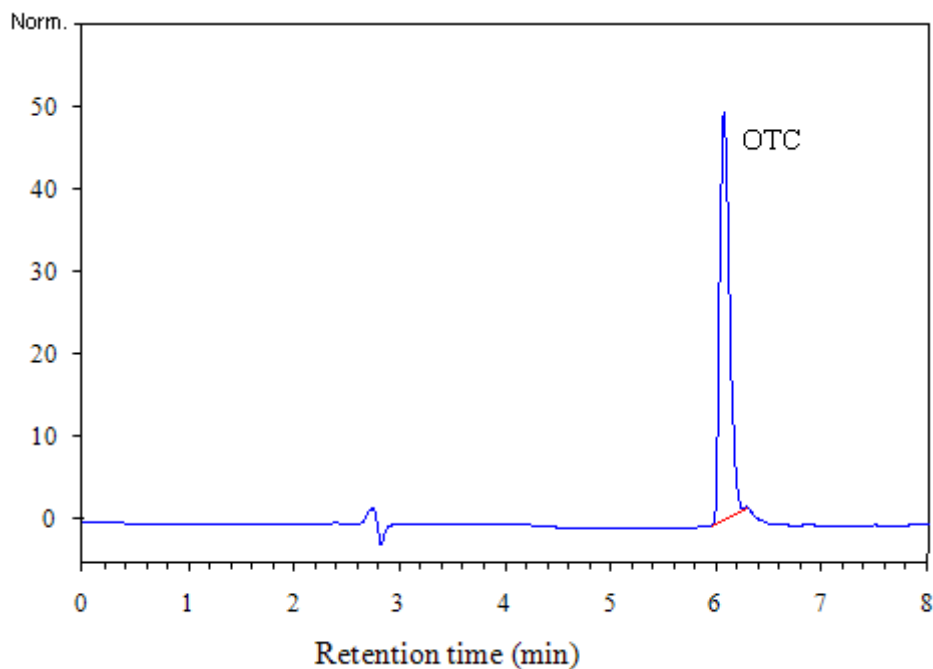


Figure 3.3. A typical chromatogram of the OTC.

3.3.1.2. Spectrophotometric analysis of OTC: The concentration of OTC was also quantified by UV/Vis spectrophotometer at a wavelength corresponding to the maximum absorbance of OTC and dilutions were undertaken when absorbance exceeds the value of 1.0. Ionization and complexation interactions of OTC led to a spectral shift and the maximum absorbance value of OTC exhibited variation depending upon the presence of Ca^{2+} and pH value of solution. The spectrum of 0.06 mM OTC solution at pH 6.5 is presented in Figure 3.4.

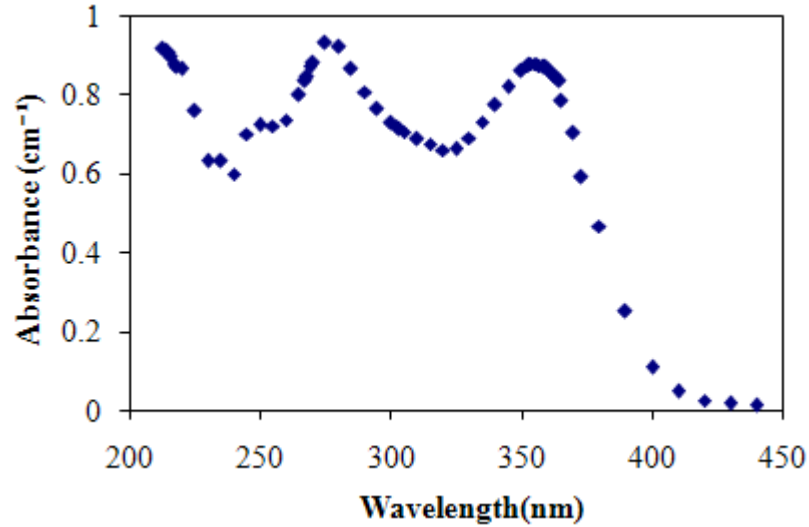


Figure 3.4. Absorption spectra of 0.06 mM OTC at pH 6.5.

A separate calibration curve was prepared at the maximum absorbance wavelength for each of the pH values (Appendix B). Initial and final antibiotic concentrations in aqueous solutions were found using these calibration curves. In accordance with the Lambert-Beer Law, a linear relationship was found between the absorbance values and the concentrations of OTC.

3.4. Kinetic Evaluations

In order to evaluate the photocatalytic and ozone oxidation rate of OTC, pseudo first-order kinetics (eq. 3.1) was applied to the obtained data.

$$\text{Degradation rate of OTC} = -\frac{dC}{dt} = k' C \quad (3.1)$$

where k' is the pseudo first order rate constant, t is the reaction time, and C is the concentration of OTC.

The integration of equation yields the following equation (3.2).

$$\ln\left(\frac{C_0}{C}\right) = k' t \quad (3.2)$$

where C_0 is the initial concentration of OTC. In case of photocatalytic oxidation, this value was expressed as C_e , which was the equilibrium concentration of OTC after dark adsorption in the photocatalytic experiments.

4. RESULTS AND DISCUSSION

4.1. Photocatalytic Oxidation of OTC

In this study, all photocatalytic oxidation experiments were conducted with 1 g L^{-1} TiO_2 . The effect of initial OTC concentration on the degradation rate of antibiotic was investigated in the concentration range between 0.04 mM and 0.5 mM at five different concentrations. In addition, the effect of pH was studied at three different pH values namely, pH 4, 7, and 9. In order to enhance the photocatalytic oxidation rate of OTC by scavenging the electrons hence increasing the radical concentration, H_2O_2 was added to the suspension at three different concentrations (25, 50, and 100 mg L^{-1}). Considering the presence of Ca^{2+} , HCO_3^- , NO_3^- , PO_4^{3-} , SO_4^{2-} , Cl^- ions, and humic acid in natural and polluted water systems, additional experiments were performed to investigate their effects on the photocatalytic degradation rate of OTC.

4.1.1. Control Experiments

A series of preliminary tests were carried out in the dark in order to determine the adsorption of OTC on TiO_2 and under illumination without using TiO_2 to determine the photolysis of OTC. Photocatalytic experiments were performed after the suspensions were mixed in dark for one-hour dark period to ensure that adsorption equilibrium was achieved. Figure 4.1 indicates the results of preliminary experiments.

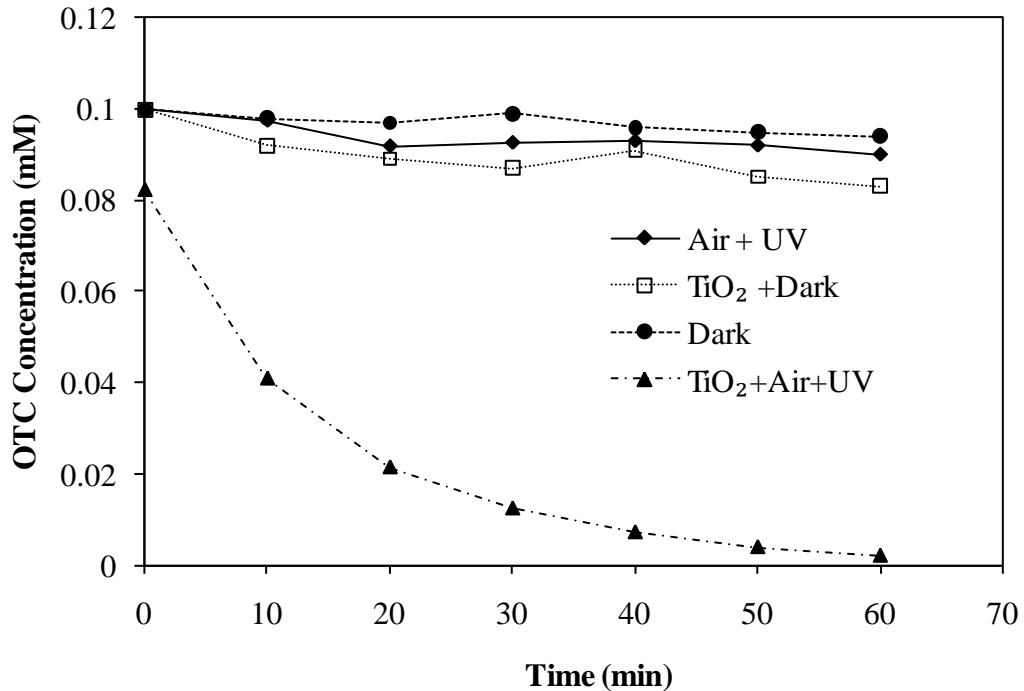


Figure 4.1. Degradation of OTC under different experimental conditions ($C_{\text{OTC}} = 0.1$ mM, pH= 7, $\text{TiO}_2 = 1 \text{ g L}^{-1}$).

The results of control experiments clearly illustrated that the degradation of OTC involves mainly TiO_2 surface mediated photocatalysis. Modest decrease in the concentration of OTC was observed for the dark control experiments in the presence of TiO_2 due to the adsorption, which was 17.7 % within 60 min. However, almost negligible adsorption of tetracycline onto TiO_2 was observed by another researcher (Addamo et al., 2005) indicating that tetracycline and oxytetracycline exhibited different adsorption properties onto TiO_2 .

Although it was known that tetracyclines are not resistant to light (Miskoski et al., 1998; Metz et al., 1996), in this study, the degradation of OTC by direct photolysis in the absence of TiO_2 was insignificant, and 10 % degradation of OTC was obtained within 60 min treatment period. In the absence of light and photocatalyst, 6 % of decrease in OTC concentration was observed due to the adsorption of OTC on the walls of reactor or hydrolysis. Consequently, 95 % of 0.1 mM OTC was degraded at the end of the treatment period.

4.1.2. Effect of Initial OTC Concentration

It was reported that the concentrations of tetracyclines and their breakdown products are found in groundwater ranging from $0.05 \mu\text{g L}^{-1}$ to 32 mg L^{-1} (Krapac et al., 2005; Mackie et

al., 2006; Hirsch et al. 1999). In this study, the photocatalytic oxidation of OTC was examined at five different initial antibiotic concentrations ($C_0 = 248 - 19.84 \text{ mg L}^{-1}$) for 60 min at pH 7. Although, this selected concentration range is much higher than those determined in the natural aquatic environment (Hirsch et al., 1999), in order to assess the effect of antibiotic concentration on the photocatalytic degradation rate more clearly high concentration range was selected. Since the preliminary experiments indicated that the equilibrium was achieved after one-hour adsorption period, suspensions were stirred in dark for one hour before illumination. The concentration of OTC in the first sample taken from photocatalytic reactor (C_e) was also used to evaluate dark adsorption results. Figure 4.2 indicates the effects of five different initial concentrations of OTC from 0.5 mM to 0.04 mM on dark adsorption and photocatalytic oxidation of OTC.

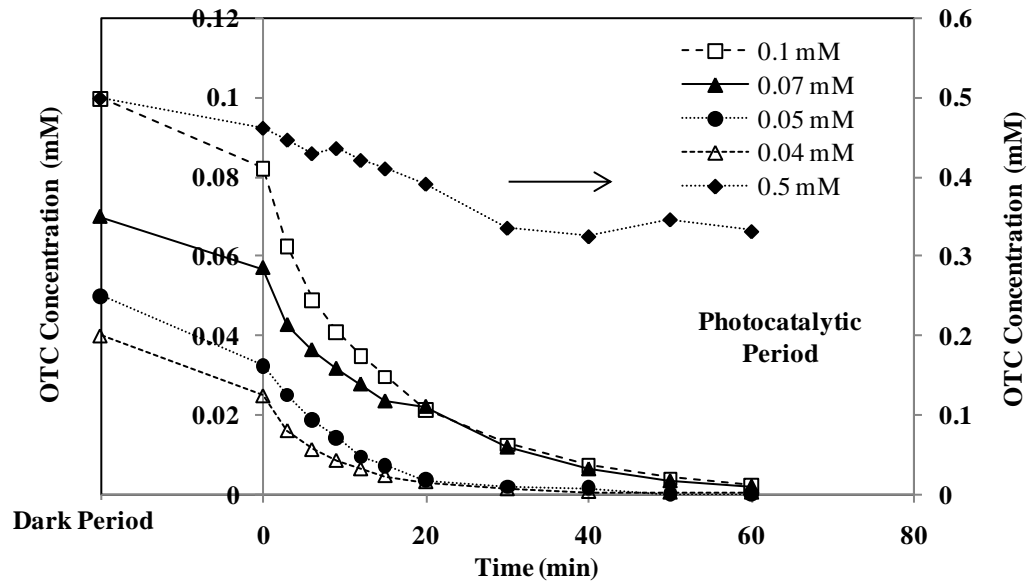


Figure 4.2. Effect of initial OTC concentration on dark adsorption and photocatalytic oxidation of OTC (pH = 7, $\text{TiO}_2 = 1 \text{ g L}^{-1}$).

As can be seen from Figure 4.2, the amount of adsorbed OTC percentage on the photocatalyst decreased with an increase in the initial concentration of antibiotic. By increasing the initial OTC concentration from 0.04 mM to 0.5 mM, the adsorbed amount of OTC was decreased from 37.9 % to 7.5 %. The higher adsorption of OTC led to the higher photocatalytic degradation rate of OTC. While 35 % of 0.5 mM OTC was degraded within 60 min of the photocatalytic oxidation, about 90 % degradation was obtained within 10 min by decreasing the initial OTC concentration to 0.04 mM. The obtained results indicated that photocatalytic oxidation could be especially promising at low antibiotic concentrations.

The Langmuir–Hinshelwood (L-H) model is usually used to describe the effect of initial concentration of pollutant on the photocatalytic degradation rate for its simplicity and the ability to fit well with the experimental results in heterogeneous photocatalysis (Sauer et al., 2002; Lathasree et al., 2004; Beltran et al., 2005). The effect of initial OTC concentration in the photocatalytic process was analysed using the linear form of the L-H model given by eq. 4.1. The intermediate products formed during photocatalytic degradation of OTC could undergo photocatalytic oxidation as well as parent compound. Simultaneous release of H⁺ influences the pH of the reaction. Therefore, initial reaction rate was used to evaluate the effect of OTC concentration in order to minimize the effects of pH variations and competitive of reaction products.

$$\frac{1}{r_0} = \frac{1}{k} + \frac{1}{kKC_e} \quad (4.1)$$

r_0 is the initial degradation rate of OTC, k is the limiting rate constant of reaction at maximum coverage, K represents the equilibrium constant for the adsorption of OTC on the semiconductor, C_e is the equilibrium OTC concentration after the adsorption on TiO₂ in the dark.

The applicability of L-H equation for the degradation of OTC has been confirmed by the linear plot of r_0 against C_e as shown in Figure 4.3. The r_0 values were independently obtained by a linear fit of the C_e versus time in the initial period of treatment.

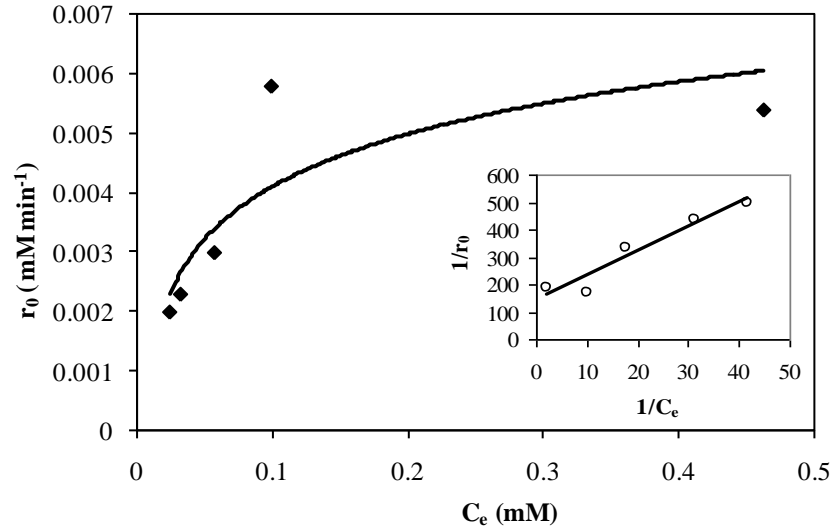


Figure 4.3. Dependence of the initial reaction rate, r_0 , on the concentration of OTC (pH= 7, $\text{TiO}_2 = 1 \text{ g L}^{-1}$). Inset: linear transform of r_0^{-1} vs. C_e^{-1} according to eq. 4.1.

The k and K values calculated according to eq. 4.1 from the slope of straight line and from the intercept with the $1/r_0$ axis were $6.99 \times 10^{-6} \text{ M min}^{-1}$ ($3.47 \text{ mg L}^{-1} \text{ min}^{-1}$) and $1.613 \times 10^4 \text{ M}^{-1}$ ($0.032 \text{ mg}^{-1} \text{ L}$), respectively. Similar values for two L-H parameters were reported for photocatalytic degradation of chloramphenicol ($k = 4.15 \times 10^{-6} \text{ M min}^{-1}$ and $K = 1.76 \times 10^4 \text{ M}^{-1}$) (Chatzitakis et al., 2008), sulfamethoxazole ($k = 7.54 \times 10^{-6} \text{ M min}^{-1}$ and $4.21 \times 10^4 \text{ M}^{-1}$), and sulfamethazine ($3.27 \times 10^{-6} \text{ M min}^{-1}$ and $K = 1.7 \times 10^4 \text{ M}^{-1}$) (Hu et al., 2007).

4.1.3. Effect of pH

The surface charge of TiO_2 and ionization or speciation of a pollutant can be profoundly affected by the solution pH. Therefore, the pH of the solution can play a key role in the adsorption and photocatalytic oxidation of pollutants.

To elucidate the effect of pH on the photocatalytic degradation of 0.1 mM OTC, experiments were performed at three different pH values (4, 7, and 9) and obtained results are presented in Figure 4.4. While pH values of OTC suspensions were adjusted to pH 7 and 9 by the addition of dilute NaOH, for the pH value of 4 any chemical was not added since the natural pH of OTC solution was 4. After the adjustment of pH to the desired values, the suspensions were mixed in dark for 60 min to ensure that the adsorption equilibrium for OTC was achieved. The first sample taken from the photocatalytic reactor before turning on the lamp was also used to evaluate dark adsorption results with varying pH values.

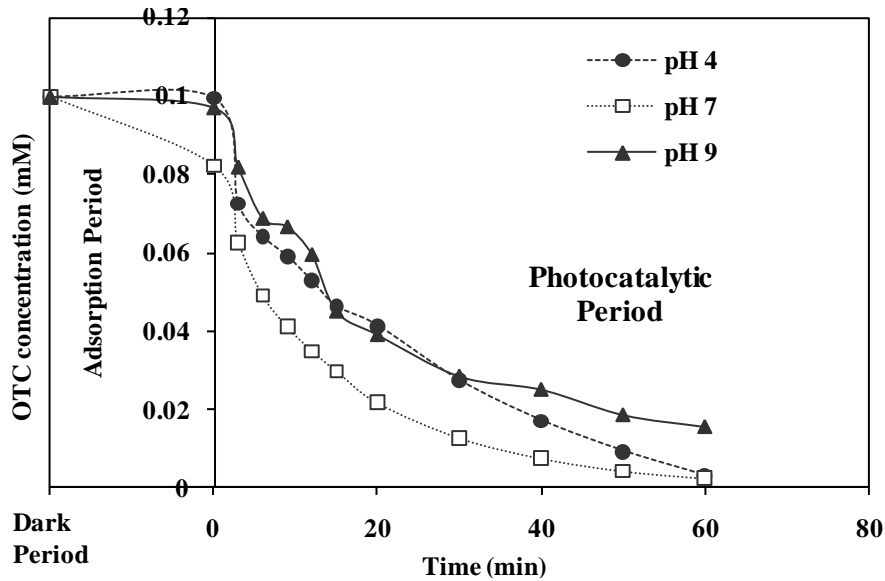
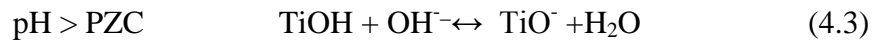
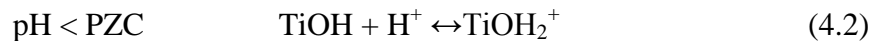


Figure 4.4. Effect of pH on the photocatalytic degradation of 0.1 mM OTC.

The results showed that after one-hour equilibration period, 0.3 %, 17.8 %, and 2.9 % of 0.1 mM OTC were adsorbed on TiO_2 at pH 4, 7, and 9, respectively (Table 4.1). This result can be explained by the charge of TiO_2 surface and OTC molecule. The zero point charge (ZPC) of TiO_2 is about 6.3 (O'Shea and Cardona, 1995). Under acidic conditions, the positive charge of the TiO_2 surface increases (eq. 4.2) but above pH 6.3, the negative charge at the surface of TiO_2 increases with the increase in the pH (eq.4.3).



On the other hand, pK_a values of OTC are $\text{pK}_1 = 3.57$, $\text{pK}_2 = 7.49$, and $\text{pK}_3 = 9.44$ (Figuroa and Mackay, 2005). Therefore, OTC is found in zwitterionic form at the investigated pH values. However, negative charge density of OTC is higher at pH 9 where electrostatic repulsion between OTC and negatively charged TiO_2 surface led to a significant decrease in the adsorption. Hydroxide ion may also compete with OTC for surface adsorption at pH 9. Both TiO_2 and OTC have mainly positive charges at pH 4. Thus electrostatic repulsion resulted in lower adsorption of OTC (Figure 4.2) on TiO_2 surface at both pH 4 and pH 9. Molinari et al. (2006) observed the adsorption of seven pharmaceuticals on TiO_2 , related with the acid–base properties of the pharmaceuticals at different pH values.

As shown in Figure 4.4 as well as the adsorption, the degradation percentage of OTC changed by varying the pH values from 4 to 9. The adsorption of organic pollutants on TiO_2 surface depending upon the pH of solution was previously investigated in various studies (Gaya and Abdullah, 2008; Mrowetz and Selli, 2006; Wang and Ku, 2007) in order to explain the importance of adsorption on the photocatalytic oxidation. From these studies, it can be inferred that photocatalytic degradation rate depends upon the photolysis of pollutant and adsorption of pollutant has negative impact on the photocatalytic degradation if the photodegradation of pollutant occurs in liquid phase (Hu et al., 2003). On the contrary, adsorption of pollutant enhances efficiency of pollutant, which does not undergo photolysis in the solution (Hu et al., 2001). In accordance to these findings, in this study, the lowest residual OTC concentration was achieved at pH 7 at which the highest adsorption was achieved. The higher adsorption at pH 7 can provide the higher probability of the reaction between reactive radicals produced on the surface of illuminated TiO_2 and adsorbed OTC.

In most of the studies, it was found that the photocatalytic degradation of pollutants at low concentration obeys the pseudo first-order kinetics (Di Paola et al., 2004; Yurdakal et al., 2007). In this study, the pseudo first-order rate constants were compared to evaluate the effect of pH on photocatalytic degradation rate of OTC. Figure 4.5- a, b, and c indicates the pseudo first-order kinetic plot of OTC at three different pH values. The figures also represent the variation of suspension pH during the photocatalytic degradation process.

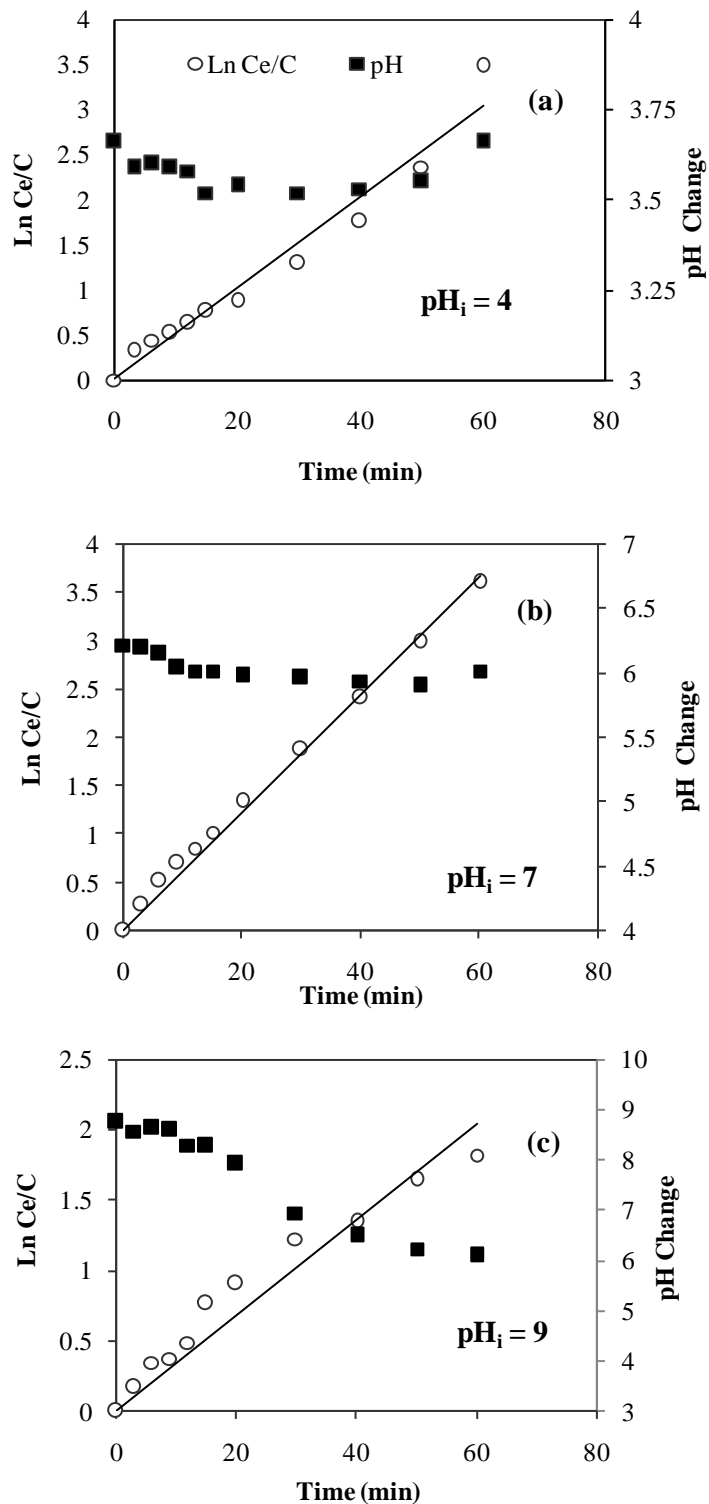


Figure 4.5. Kinetic plot of OTC degradation at three different pH values and variation of pH during the photocatalytic reaction.

The pH of OTC solution decreased during the photocatalytic oxidation (Figure 4.5). When the initial pH of the solution was 4 it decreased to 3.7 by a slight abatement at the end of the treatment period. On the other hand, by increasing the initial pH of solution, variation in

the pH value of solution by photocatalytic oxidation was more remarkable. The pH values of reaction solutions decreased from 7, 9 to 6, and 6.1, respectively. The reason of the decrease in pH value of reaction solution can be explained by the formation of acidic reaction products and/or production of H^+ ions as well as the adsorption of OH^- ions on the TiO_2 surface.

The degradation rates of OTC were calculated within the 60 min of photocatalytic process and the high correlation coefficients at each pH indicated a good fit of the observed data to pseudo first-order kinetics. The pseudo first-order degradation rate constants, correlation coefficients, and dark adsorption percentages of OTC at different pH values are summarized in Table 4.1.

Table 4.1. Effect of pH on the pseudo first order rate constant and dark adsorption rate of OTC.

Initial pH	Dark adsorption (%)	k (min^{-1})	r^{2*}
4	0.30	0.05	0.96
7	17.70	0.06	0.99
9	2.90	0.04	0.95

*Correlation coefficient for k value

As can be seen from Table 4.1, while the fastest degradation rate of OTC was observed at pH 7, the lowest degradation rate was obtained at pH 9. The highest adsorption percentage was obtained at pH 7 with 17.70 %, around the ZPC of TiO_2

As well as the adsorption of OTC on TiO_2 surface, the pH of the solution effects the formation of hydroxyl radicals by the reaction between hydroxide ions and photo-produced holes on TiO_2 surface. The positive holes are considered as the major oxidation species at low pH whereas hydroxyl radicals are considered as the predominant species at neutral or high pH levels (Tang and Huang, 1995; Tunesi and Anderson, 1991). It would be expected that production of OH^\bullet radicals is higher due to presence of more available hydroxide ions on TiO_2 surface, thus the efficiency of the process should be logically enhanced at high pH (Shourong et al., 1997; Galindo et al., 2000; Sharma et al., 1995 and Sakthivel et al., 2003). However, the results of this study indicated that the adsorption of OTC has a remarkable effect on the

photocatalytic degradation rate. As can be seen from Table 4.1 and Figure 4.5, the photocatalytic degradation rate of OTC has a maximum value at pH 7 with 0.06 min^{-1} rate constant. Similarly, some researchers obtained the highest photodegradation rate of pollutants at neutral pH and a decrease in degradation rate in alkaline media was observed (Chiou, et al., 2007; Palaminos, et al., 2008). On the other hand, numerous studies showed that alkaline medium favored photodegradation of different organic compounds (Doong et al., 2001; Florencio et al., 2004; Malygina et al., 2005). Moreover, it was found that pH has no remarkable effect for the photocatalytic degradation of sulfamethoxazole (Abellan et al., 2007; Hu et al., 2007).

4.1.4. Effect of Hydrogen Peroxide on the Photocatalytic Degradation of OTC

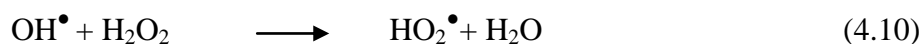
The efficiency of the photocatalytic process is related with the separation of photo-electron and photo-hole pair. In the absence of electron acceptor and donor, the recombination of electron hole pair results in a decrease in the efficiency of photocatalytic process. In conventional photocatalytic process, oxygen acts as electron trap and slow electron transfer occurs from TiO_2 to O_2 . Consequently, hydroxyl radicals are generated by the reactions through eq. 4.4- 4.7.



Various electron acceptors can be added to the photocatalytic oxidation process in order to obtain efficient electron hole pair separation (Hoffman et al., 1995). It is known that addition of H_2O_2 increases the efficiency of photocatalytic reaction due to inhibition of electron hole recombination (eq. 4.8 and 4.9).



On the other hand, an excess of H_2O_2 can act as a hole or hydroxyl radical scavenger (Legrini et al., 1993).



With the above mentioned facts in mind, photocatalytic degradation of OTC was performed in the presence of 50 and 100 mg L^{-1} H_2O_2 at pH 7. The effect of H_2O_2 addition on the photocatalytic reaction is presented in Figure 4.6.

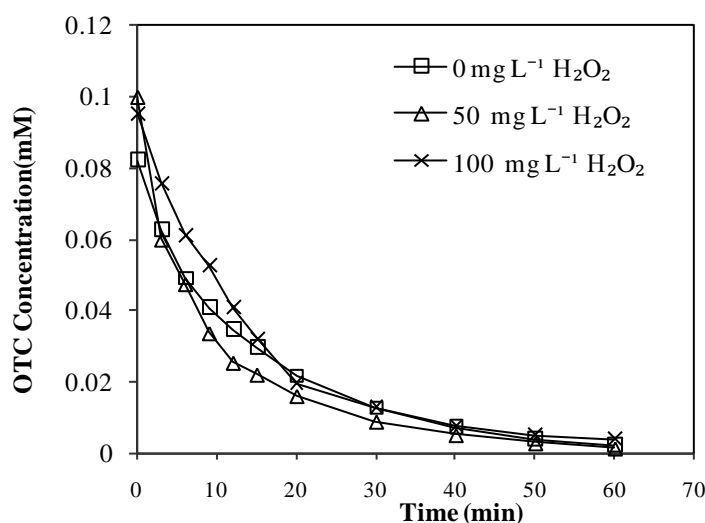


Figure 4.6. Effect of H_2O_2 addition on the photocatalytic degradation of OTC (pH= 7, $C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$).

The pseudo first-order degradation rate constants, correlation coefficients, and dark adsorption percentages of OTC in the presence of different H_2O_2 concentrations are given in Table 4.2.

Table 4.2 Effect of H_2O_2 concentration on the pseudo first-order photocatalytic degradation rate constant.

H_2O_2 concentration (mg L^{-1})	k (min^{-1})	r^2
0	0.07	0.99
50	0.08	0.99
100	0.06	0.96

The results showed that the addition of 50 mgL^{-1} hydrogen peroxide to the solution enhanced the oxidation process slightly. When the concentration was increased to 100 mg L^{-1} the photocatalytic degradation rate of OTC decreased. Similar results were obtained for the photodegradation of chlorfenapyr pesticide (Cao et al., 2005). However, Palaminos et al., (2008) observed that hydrogen peroxide did not affect the photocatalytic degradation of antibiotic flumequine. Most of the studies showed that addition of hydrogen peroxide accelerated the degradation of many organics, presumably due to enhanced production of hydroxyl radicals through the photodecomposition of hydrogen peroxide (Wolfrum and Ollis, 1994; Sioi et al., 2006; Chiou, et al., 2007).

In the presence of hydrogen peroxide, almost no adsorption of OTC was observed on TiO_2 surface without light. Hydrogen peroxide is more likely adsorbed on the catalyst surface (Gao et al., 2003), so the degradation of hydrogen peroxide on positive holes could hinder the formation of OH^\bullet radicals. Furthermore, due to the low amounts of OTC adsorption, it is reasonable to imagine that the collision between hydroxyl radicals and OTC molecules mainly occurs in the bulk solution rather than on the catalyst surface. On the contrary, the adsorbed hydrogen peroxide could readily react with and inactivate hydroxyl radicals generated at TiO_2 surface before they enter the bulk solution. The above two processes may negatively affect the concentration of hydroxyl radicals and the rate of OTC degradation.

4.2. Effect of Water Components on the Photocatalytic Degradation of OTC

Water components like calcium, bicarbonate, sulfate, nitrate, chloride, and humic acid can affect the photocatalytic degradation rate of OTC, because they can be adsorbed onto the surface of TiO_2 (Chen et al., 1997) depending upon the pH of medium and they can compete with the OTC for the active sites. The adsorption of water components can reduce the formation of OH^\bullet radicals at the TiO_2 surface. Moreover, they can react with OH^\bullet radicals. Hydroxyl radical scavenging by the anions, chloride, sulphate, and phosphate produce the corresponding anion radicals which have lower oxidation potential. Consequently, these reactions can influence overall rate of photocatalytic oxidation.

4.2.1. Effect of Calcium ion on the Photocatalytic Degradation of OTC

Ca^{2+} is one of the major hard cation in natural water found in a range of $1.0 - 1,000 \text{ mg L}^{-1}$ (Harter, 2003). Dissolved tetracycline in the environment is found as bounded to Ca^{2+} ,

since it is the divalent metal cation present at the highest concentrations, and the association constant with TCs seems to be large (Werner et al., 2006). OTC aggregation and metal–OTC complexations as $(\text{OTC})_4\text{-CaCl}_2$ and $(\text{OTC})_2\text{-CaCl}_2$ come into existence in the presence of Ca^{2+} (Tongaree et al., 1999). In addition, pK_a values of oxytetracycline are shifted significantly downward upon with the addition of calcium chloride, demonstrating the ability of tetracyclines to bind strongly to divalent metal ions (Regna et al., 1951). Therefore, calcium may also affect the adsorption behavior of OTC onto TiO_2 as well as the photocatalytic oxidation of OTC. Since in polluted water hardness can reach to a value of 300- 10,000 mg/L as CaCO_3 (Peavy et al., 1985), the photocatalytic degradation of 0.1 mM OTC was performed in the presence of 200 and 1,264 mg L^{-1} Ca^{2+} at pH 7. Figure 4.7 represents dark adsorption and photocatalytic degradation of OTC in the presence of two different concentrations of calcium ion.

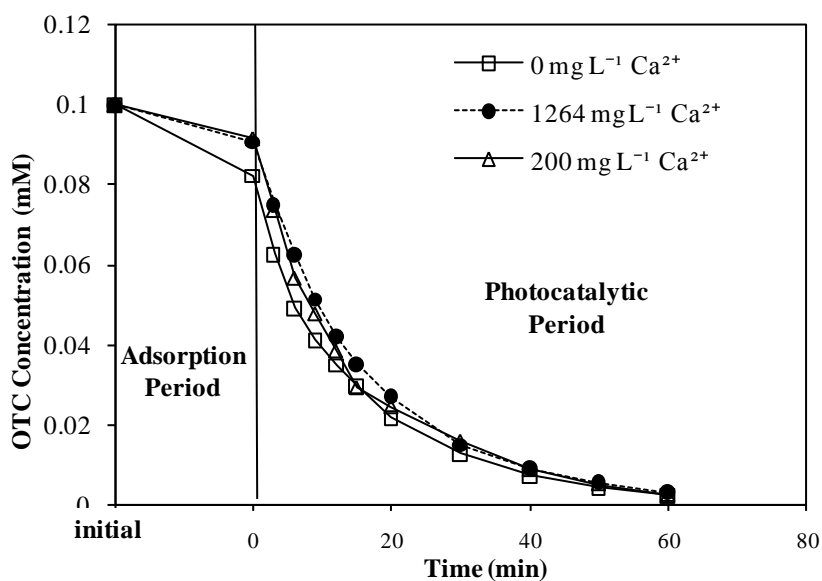


Figure 4.7. Effect of Ca^{2+} concentration on photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1$ mM, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, pH = 7).

The pseudo first-order photocatalytic degradation rate constants calculated from the data in Figure 4.7 and the dark adsorption rate of OTC on TiO_2 in the presence of different concentrations of Ca^{2+} are presented in Table 4.3.

Table 4.3. Pseudo first-order photocatalytic degradation rate constants and dark adsorption rates of OTC with two different concentrations of calcium.

Ca ⁺² (mg L ⁻¹)	Dark Adsorption (%)	k (min ⁻¹)	r ²
0	17.72	0.07	0.99
200	8.43	0.06	0.99
1,264	9.35	0.06	0.99

The results in the table 4.2.1 revealed that the adsorption of OTC on TiO₂ considerably decreased in the presence of calcium ion. The decrease in the adsorption of OTC on TiO₂ can be explained by the formation of soluble calcium–OTC complexes. Although lower adsorption of OTC was achieved photocatalytic degradation rate is almost constant by the addition of calcium ion. This can be the result of the higher photolysis rate of calcium-tetracycline complexes that was proposed in the previous studies (Werner et al., 2006; Bliakova et al., 2003)

4.2.2. Effect of Bicarbonate Ion on the Photocatalytic Degradation of OTC

In natural waters, carbonate ion is found as bicarbonate (pK_a =10.2 for HCO₃⁻/CO₃⁻²) at pH 6.5–8.5 and the presence of this ion in aqueous medium can play an important role in the photocatalytic oxidation of OTC. In this respect, the effect of HCO₃⁻ addition at three different concentrations, 50, 100, and 150 mg L⁻¹ was studied at pH 7 (Figure 4.8). Table 4.4 indicates the variation in the adsorption and photodegradation of OTC by the addition of bicarbonate ion.

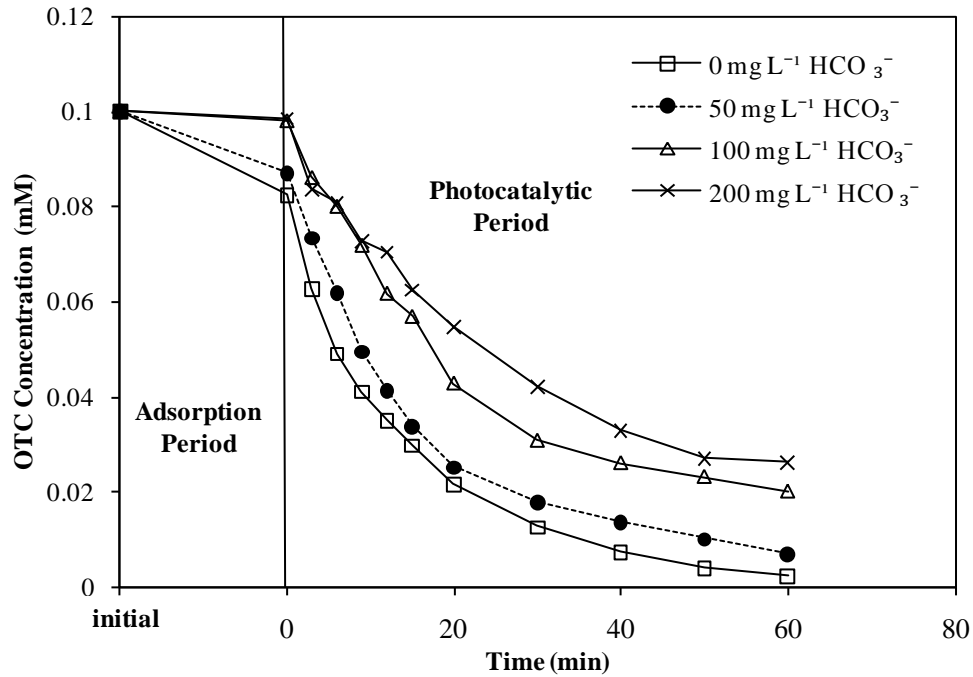


Figure 4.8. Effect of HCO_3^- on the photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

Table 4.4. Pseudo first-order photocatalytic degradation rate constants and dark adsorption rates of OTC in the presence of the different concentrations of bicarbonate.

HCO_3^- (mg L^{-1})	Dark Adsorption (%)	k (min^{-1})	r^2
0	17.71	0.06	0.99
50	13.09	0.05	0.95
100	1.96	0.03	0.93
200	1.53	0.02	0.98

The results showed that both adsorption and photocatalytic degradation of OTC on TiO_2 were decreased by increasing the HCO_3^- concentration. Similarly, some researchers observed a decrease in the photocatalytic degradation rate of organic materials in the presence of bicarbonate ion (Blake, 1991; Bekbolet and Balcioglu, 1996; Lair et al., 2008). However, Hu et al. (2007) observed an increase in the photocatalytic degradation rate of sulphamethaxazole in the presence of bicarbonate ion at pH 9.

Bicarbonate and carbonate ions are usually assumed to decrease photocatalysis efficiency by scavenging hydroxyl radicals according to the following reactions (Espinoza et al., 2007; Bhatkhande et al., 2001).



$$k_{4.11} = 8.5 \times 10^6 \text{ L mol}^{-1} \text{ s}^{-1} \text{ (Dass et al., 2004)}$$



$$k_{4.12} = 3.9 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1} \text{ (Jia et al., 1999)}$$

As a result of reaction between hydroxyl radical and carbonate anion, carbonate ion radical is generated (eq. 4.11 and 4.12). However, carbonate radicals have a lower oxidation potential than hydroxyl radicals ($E_0(\text{CO}_3^{\bullet-}/\text{CO}_3^{2-}) = 1.85 \text{ V}$, $E_0(\text{OH}^\bullet/\text{H}_2\text{O}) = 2.80 \text{ V}$) (Lair et al., 2008). As mentioned with reaction rate constants, compared with carbonates, bicarbonate ions scavenge about 50 times less hydroxyl radicals. However, bicarbonate ions led to a decrease in photocatalytic degradation rate of OTC.

4.2.3. Effect of Phosphate ion on the Photocatalytic Degradation of OTC

By taking into account the usage of manure in farmlands, it is important to examine the effect of phosphate ion on the photocatalytic degradation and adsorption of OTC on TiO_2 . Then, the photocatalytic degradation of OTC was investigated in the presence of four different concentrations of phosphate ion (Figure 4.9 and Table 4.5).

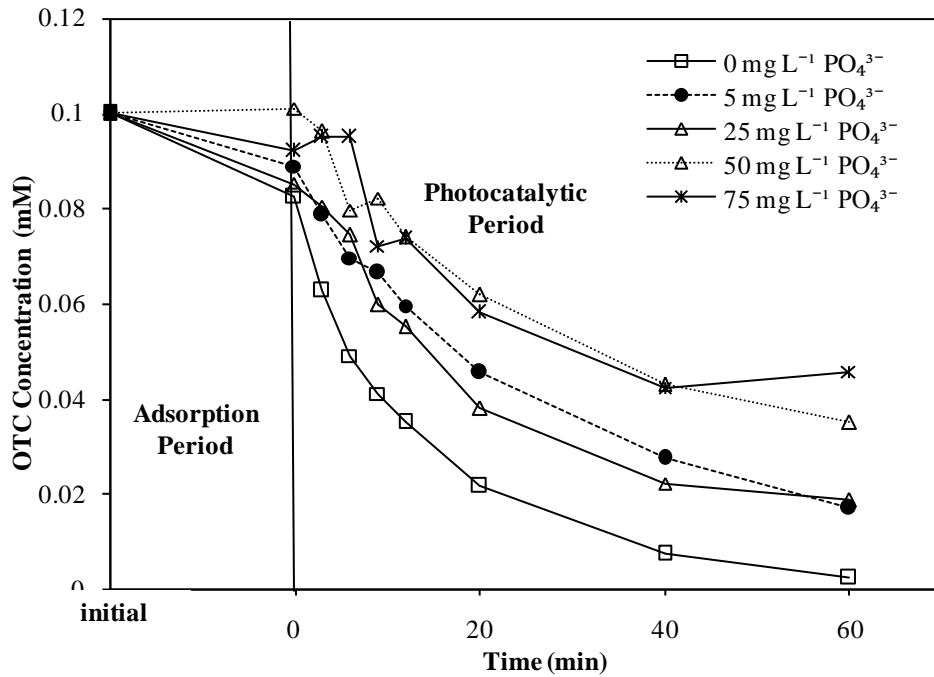


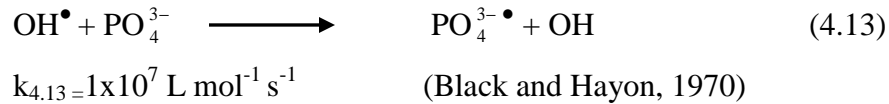
Figure 4.9. Effect of PO_4^{3-} concentration on the photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

Table 4.5. Pseudo first-order photocatalytic degradation rate constants and dark adsorption rates of OTC in the presence of different concentrations of PO_4^{3-} .

PO_4^{3-} (mg L^{-1})	Dark Adsorption (%)	k (min^{-1})	r^2
0	17.71	0.06	0.99
5	11.12	0.03	0.98
25	15.23	0.02	0.96
50	7.95	0.02	0.80
75	1.03	0.02	0.95

Similar to bicarbonate ion, phosphate ion significantly decreased both the adsorption of OTC onto TiO_2 and pseudo first-order photocatalytic degradation rate constant of OTC. As the pH of OTC solution was adjusted to 7, phosphate ion is virtually found as H_2PO_4^- form and its adsorption onto TiO_2 surface was not expected since the ZPC of TiO_2 was reported as 6.3. However, the adsorption of phosphate ions on the Degussa TiO_2 was detected at neutral pH

value (Zhu et al., 2007). It was proposed that adsorption occurs by an exchange between the surface hydroxyl groups of TiO_2 and H_2PO_4^- (Flaig-Baumann et al. 1970). From all these results, it can be inferred that the competition between OTC and H_2PO_4^- resulted in significant abatement in the adsorption of OTC on the photocatalyst surface. Additionally, phosphate ion can act as a scavenger for hydroxyl radicals (eq.4.13.) and this reaction produces less reactive phosphate radicals.



Consequently, lower adsorption of OTC on TiO_2 and the scavenging of hydroxyl radical by the addition of phosphate ion could be the reason of lower degradation rate constant of OTC as observed in the previous studies (Abdullah et al., 1990). The rate constant of reaction between the hydroxyl radical and PO_4^{3-} is higher than that of HCO_3^- . Therefore, PO_4^{3-} can scavenge the hydroxyl radicals more than HCO_3^- .

4.2.4. Effect of Nitrate on the Photocatalytic Degradation of OTC

Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia, should be considered as potential nitrate sources. Primary sources of organic nitrates include human sewage and livestock manure, especially from feedlots. Actually, nitrate in natural waters is known to interact with UV light to produce hydroxyl radicals, which play a significant role during oxidation processes in water. Therefore, it is of interest to know in which way nitrate (NO_3^-) influences the photocatalytic degradation of organic OTC. In this study, four different concentrations of nitrate from 25 to 250 mg L^{-1} were used to investigate its effect on photocatalytic degradation of OTC (Figure 4.10). Table 4.6 presents the pseudo first-order photocatalytic degradation rate constants and dark adsorption rates of OTC as a function of added nitrate ion concentration.

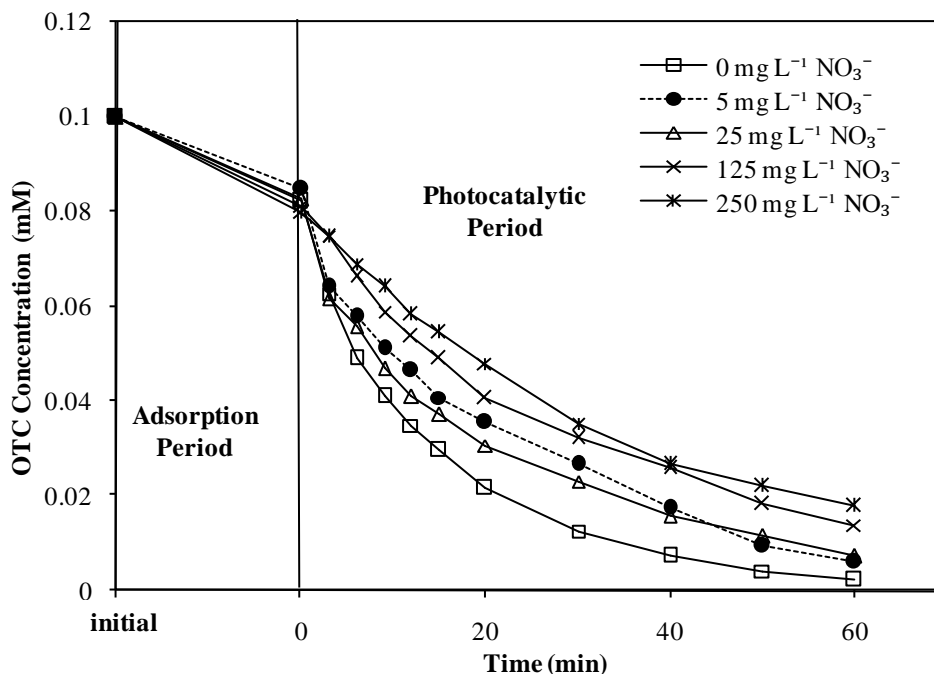


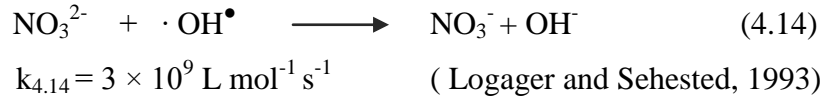
Figure 4.10. Effect of NO_3^- concentration on the photocatalytic oxidation of ($C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

Table 4.6. Effect of nitrate concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC.

NO_3^- (mgL^{-1})	Dark Adsorption (%)	k (min^{-1})	r^2
0	17.71	0.06	0.99
25	17.12	0.04	0.96
125	18.83	0.03	0.99
250	20.11	0.02	0.99

The results showed that nitrate causes a slight increase in the adsorption of OTC onto TiO_2 surface. On the other hand, the photocatalytic degradation rate of OTC decreased significantly with increasing amount of nitrate concentration. This result can be explained by the fact that NO_3^- absorbs light emitted from the lamp and acts as an inner filter for the UV radiation (Zepp et al, 1987). Hence, it decreased photocatalytic degradation of OTC on TiO_2 . In some other studies, it was found that nitrate did not have any effect on the degradation of phenols and hazardous chlorine or nitrogen containing aromatics (Augugliaro et al., 1988;

D'Oliveira et al., 1993). As the case of HCO_3^- and H_2PO_4^- , NO_3^- react with hydroxyl radicals according to the following reaction (eq. 4.14) and rate constant of this reaction is higher than that of other mentioned anions.



Among the investigated anions, nitrate ion has the highest reaction rate constant for hydroxyl radical scavenging and it has a significant effect for decreasing the photocatalytic degradation rate of OTC.

4.2.5. Effect of Sulfate on the Photocatalytic Degradation of OTC

SO_4^{2-} is one of the major natural components of water. In addition, wastewater resulting from fishery industry wastewater may contain antibiotics and sulphate ion ($>1,200 \text{ mg L}^{-1}$) (Alvarez et al., 2000). Therefore, the effect of sulfate on the photocatalytic oxidation process was also investigated in the sulfate ion concentration range of 50–10,000 mg L^{-1} . Figure 4.11 represents the effect of SO_4^{2-} on the photodegradation of OTC on TiO_2 .

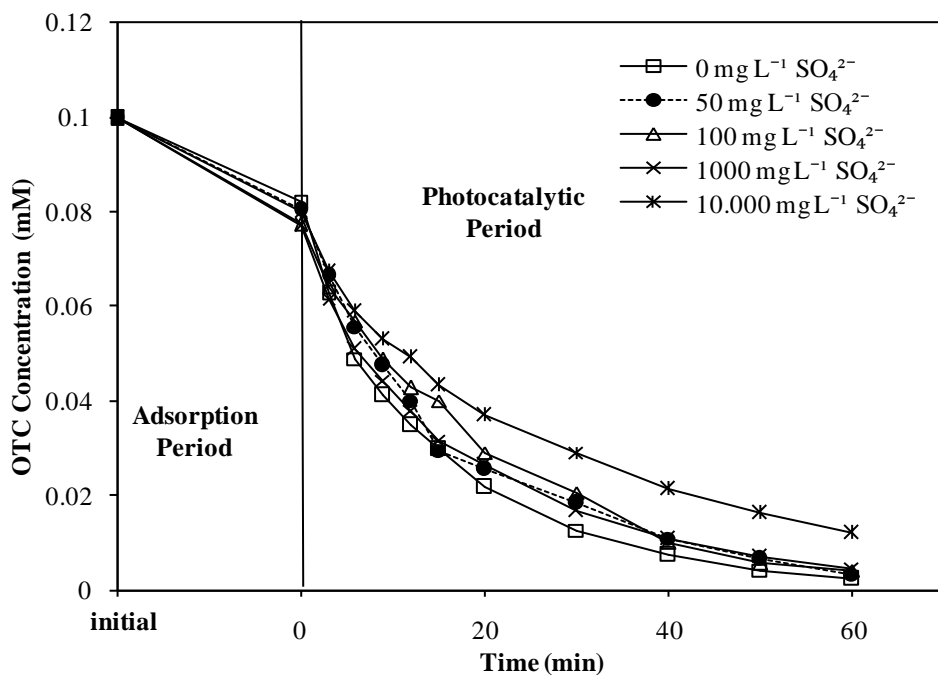


Figure 4.11. Effect of SO_4^{2-} on the photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

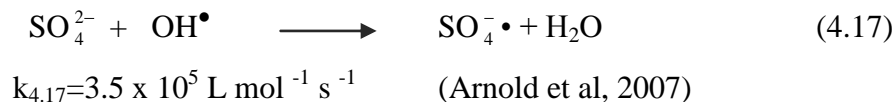
Table 4.7 shows the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC at different concentrations of sulfate ion.

Table 4.7. Effect of sulfate concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC.

SO_4^{2-} (mg L^{-1})	Dark Adsorption (%)	k (min^{-1})	r^2
0	17.71	0.06	0.99
50	19.60	0.05	0.99
100	22.60	0.05	0.99
1,000	22.85	0.05	0.98
10,000	19.77	0.03	0.97

It was also reported that the adsorption of sulfate ion onto TiO_2 is lower than that of H_2PO_4^- at pH 7 (Zhu et al., 2007). The results in Table 4.7 showed that the adsorption of OTC did not decrease by the addition of SO_4^{2-} as in the case of H_2PO_4^- . On the contrary, the adsorption of OTC exhibited a slight increase by the addition of SO_4^{2-} up to $1,000 \text{ mg L}^{-1}$.

Obviously, very little change in the pseudo first-order photocatalytic degradation rate constant of OTC was observed in a wide range of sulfate concentration (50-1,000 mg/L) and photocatalytic degradation rate of OTC was remarkably inhibited only at very high concentrations of SO_4^{2-} . Similar to the other anions, sulphate ion can act as a scavenger for hydroxyl radicals (eq.4.17) and this reaction produces less reactive sulphate radicals.



However, the reaction rate constant of sulphate ion with hydroxyl radical has the lowest value among the all investigated anions. A slight decrease in the photocatalytic degradation

rate constant of OTC in the presence of SO_4^{2-} can be explained by the lower value of $k_{4.17}$ as well as the higher adsorption of OTC on the photocatalyst surface.

4.2.6. Effect of Chloride Ion on the Photocatalytic Degradation of OTC

Chloride ion is another major natural anion and frequently present in natural water and wastewater. It has been found to be a scavenger for the photocatalytic process by competing for surface active sites on the photocatalyst and forming weaker chloride radicals (Chen et al., 1997; Wang et al., 2000; Sökmen et al., 2002) as in the case of carbonate, sulphate, and phosphate ions. The effect of Cl^- was investigated in a considerable wide concentration range considering its high concentration in ground and seawater (Harter, 2003). The results of experiments are presented in Figure 4.12.

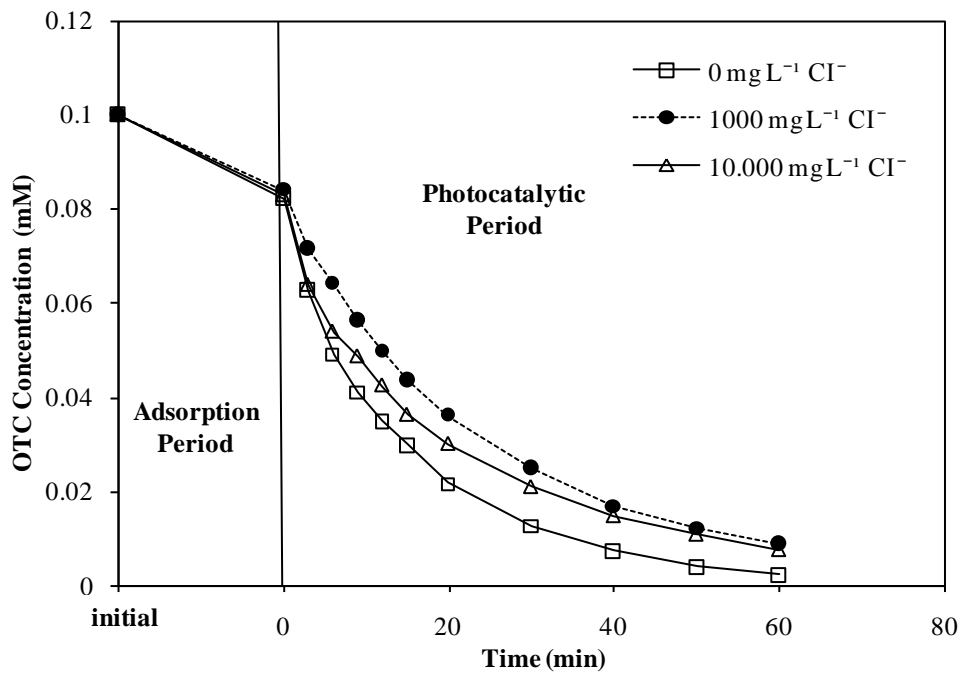


Figure 4.12. Effect of Cl^- concentration on photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1$ mM, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

Table 4.8 shows the effect of chloride concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC.

Table 4.8. Effect of Cl^- concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC.

Cl^- (mg L^{-1})	Dark Adsorption (%)	k (min^{-1})	r^2
0	17.71	0.06	0.99
1,000	16.00	0.04	0.96
10,000	17.11	0.04	0.99

The results showed that there is a negligible decrease of adsorption in the presence of Cl^- at neutral pH. Similarly, the study performed by Wang, et al. (2000) indicated that the addition of chloride ions did not affect the adsorption o-methylbenzoic acid on the surface of TiO_2 at neutral or alkaline conditions.

Similar to the other anions, Cl^- can reduce photocatalytic degradation rate of organics by scavenging the oxidizing radical species; OH^\bullet and HO_2^\bullet radicals. Reaction between chloride ion and hydroxyl radicals is given by the following equation.



Although Cl^- has a high hydroxyl radical reaction rate constant, its effect on the adsorption and photocatalytic degradation rate even at high concentrations is lower than other ions. The effectiveness of Cl^- for the scavenging the hydroxyl radicals can be offset by dissociation of HOCl^\bullet back to OH^\bullet and Cl^- with the dissociation rate constant of $6.1 \pm 0.8 \times 10^9 \text{ s}^{-1}$ (Jayson and Parson, 1973) which is slightly larger than the OH^\bullet scavenging reaction rate constant.

4.2.7. Effect of Humic Acid on the Photocatalytic Degradation of OTC

Humic substances are the common components of natural water and the photosensitizing and photoquenching effects of humic substances on some environmental chemicals are also known (Khan and Gamble, 1983; Zepp et al., 1985; Kochany et al., 1990; Minero et al., 1992). When humic substances absorbs light, reactive oxygen intermediates are formed which may

attack chemicals in the environments and initiate their degradation (Mill, 1999; Konstantinou et al., 2001). In addition, the possibility of an UV screening by humic substances on chemicals cannot be excluded.

The photocatalytic degradation of OTC was performed in the presence of four different humic acid concentrations at pH 7. Since natural color of humic acid prevented the analysis of OTC by spectrophotometer, OTC was quantified by HPLC analysis. Figure 4.13 represents the results of the effect of humic acid concentration on the photocatalytic oxidation of OTC and the inset shows a comparison for the degradation of OTC determined by HPLC and spectrophotometric analysis in the absence of humic acid.

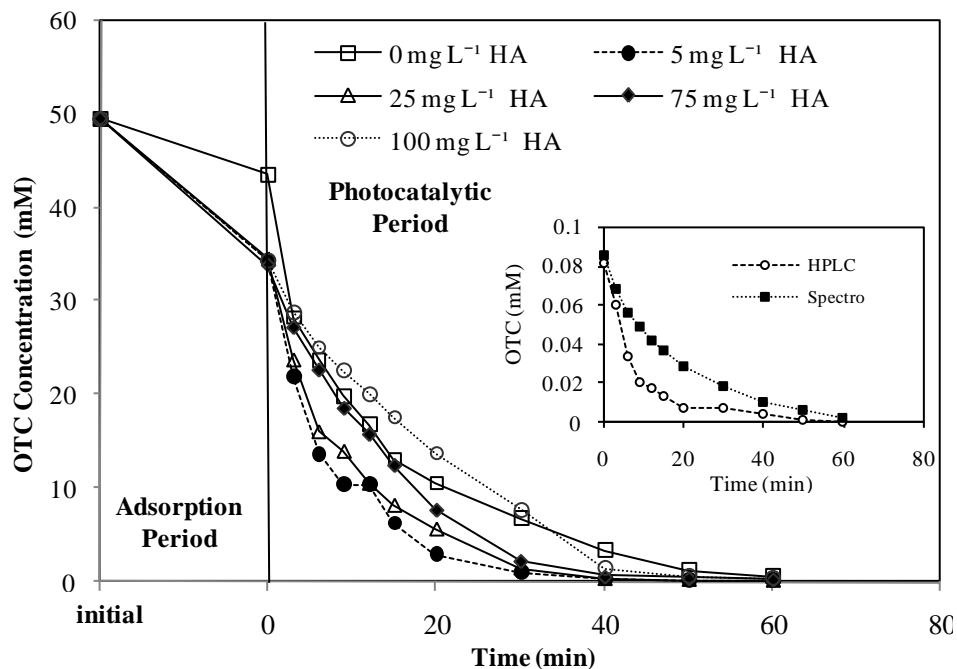


Figure 4.13. Effect of humic acid concentration on the photocatalytic degradation of OTC ($C_{\text{OTC}} = 0.1 \text{ mM}$, $\text{TiO}_2 = 1 \text{ g L}^{-1}$, $\text{pH} = 7$).

As can be seen from Figure 4.14 the amount of OTC determined by spectrophotometer and HPLC is nearly the same within the first 5 minutes of photocatalytic degradation. However, by the progress of reaction degradation products were produced and their presence interfered the determination of spectrophotometric analysis of OTC. Therefore, a difference was observed in the pseudo first-order rate constants and they were calculated as 0.06 min^{-1} and 0.1 min^{-1} with spectrophotometric and HPLC analysis, respectively. Table 4.9 presents the effect of humic acid concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC calculated by the HPLC measurement results.

Table 4.9. Effect of humic acid concentration on the pseudo first-order photocatalytic degradation rate constant and dark adsorption rate of OTC.

Humic Acid (mg L ⁻¹)	Dark Adsorption (%)	k (min ⁻¹)	r ²
0	17.71	0.1	0.96
5	30.62	0.1	0.95
25	30.21	0.09	0.97
75	31.65	0.08	0.97
100	30.56	0.07	0.94

The results showed that the adsorption of OTC on TiO₂ considerably increased in the presence of 5 mg L⁻¹ humic acid and by further increase of humic acid concentration the amount of adsorbed OTC was almost constant.

In literature, it was stated that if the pH is lower than ZPC of TiO₂, the positively charged TiO₂ offers a suitable surface for negatively charged humic acid molecule adsorption, but when pH is higher than ZPC, the surface of TiO₂ becomes negatively charged which provides an unfavorable condition for the HA molecule to approach (Li et al., 2002). Since humic acid has a very high molecular weight, the adsorption of humic acid is expected. Therefore, the surface of TiO₂ is covered with humic acid and it prevents the photocatalytic oxidation of OTC. On the other hand, in literature complexation of OTC with humic substances and the sorption of OTC to humic materials are reported and they depend on the pH value of solution (Gu et al., 2007; MacKay and Canterbury, 2005). It was also stated that, the existence of apparent sorption–desorption retardation behavior may hinder the release of sorbed tetracycline and decrease its bioavailability and degradation.

4.2.8. The Assessment of the Role of Water Components

Since the reaction intermediates interfered the spectrophotometric analysis of OTC the effects of various components of water on the photocatalytic degradation rate of OTC was evaluated in terms initial reaction rate instead of overall reaction rate and the results are summarized in Table 4.10.

Table 4.10. Overall and initial degradation rate constants of OTC in the presence of water components.

Water Components	OTC degradation rate constants	
	k_{initial} (mM min ⁻¹)	k_{overall} (min ⁻¹)
-	0.09	0.07
Ca ²⁺	0.09	0.06
Cl ⁻	0.09	0.04
NO ₃ ⁻	0.07	0.03
SO ₄ ²⁻	0.05	0.05
HCO ₃ ⁻	0.04	0.03
PO ₄ ³⁻	0.03	0.02

The results showed that overall reaction rate constants were generally lower than the initial rate constants for most of the ions due to the intermediates generated during the photocatalytic oxidation. However, the retardation effect of ions on the initial and overall OTC degradation rate constant is not consistent.

4.3. Degradation of OTC by Ozone Oxidation

The degradation of pharmaceuticals in water and wastewater can be also achieved by using ozonation (Ikehata et al., 2006). Ozonation process can either eliminate such pollutants completely through mineralization or convert them into the products that are less harmful to human health and the aquatic environment. In order to compare the performance of photocatalytic process for the treatment of OTC with that of ozonation, experiments were carried out at different pH values and applied ozone doses. Additionally, the effects of Ca²⁺, HCO₃⁻, NO₃⁻, PO₄³⁻, SO₄²⁻, and Cl⁻ ions, and humic acid on the ozonation process were investigated at pH 7.

4.3.1. Effect of Applied Ozone Dose

The effect of applied ozone dose on the degradation of OTC has been studied at three different values (1786, 1086, and 606 mg L⁻¹h⁻¹) at pH 7 and the results are presented in Figure 4.14.

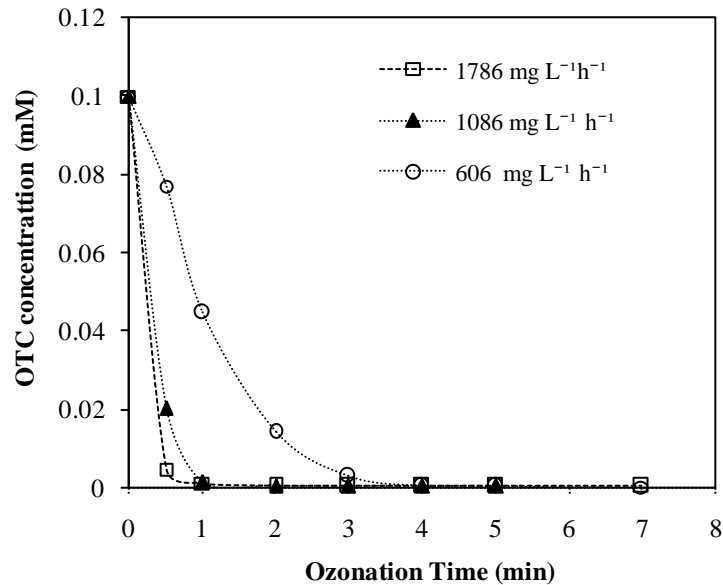


Figure 4.14. Effect of applied ozone dose on the oxidation of OTC (pH =7, C_{OTC}= 0.1 mM).

As can be seen from Figure 4.14, OTC degraded exponentially by the application of ozone and the degradation of OTC obeyed pseudo first-order kinetics as in the case of photocatalytic oxidation. Table 4.11 presents the pseudo first-order rate constants of OTC obtained with different applied ozone doses.

Table 4.11. Pseudo first-order degradation rate constants of OTC with different applied ozone doses.

	Applied ozone dose (mg L ⁻¹ h ⁻¹)		
	1,786	1,086	606
k (min⁻¹)	4.80	4.18	1.67
r²	0.95	0.97	0.99

The results showed that the pseudo first-order degradation rate constant of OTC was increased from 1.67 min^{-1} to 4.18 min^{-1} by raising the applied ozone dose from 606 to 1,086 $\text{mg L}^{-1} \text{ h}^{-1}$. However, a further increase in the applied ozone dose led to only a slight enhancement in the OTC degradation rate. This is probably due to the fact that within a given retention time, OTC solution absorbed the maximum amount of ozone, namely saturation was achieved, and a further increase in the ozone concentration did not cause an increase in the dissolved ozone concentration in the solution. Similar results were obtained for the decolorization of a reactive textile dye (Wu et al., 2007) and for the treatment of a synthetic antibiotic formulation wastewater (Otker, 2002).

4.3.2. Effect of pH on the Degradation of OTC by Ozone

Since pH is one of the important factor affected ozonation pathway, this parameter was selected as the process variable. To elucidate the effect of pH on the ozonation process, experiments were performed at three different initial pH values (4, 7, and 9) of OTC solution with an applied ozone dose of $606 \text{ mg L}^{-1} \text{ h}^{-1}$. As in the case of photocatalytic process, pH was not controlled during ozonation process. The results were evaluated in terms of OTC degradation and aromaticity removal (UV_{254}) as shown in Figure 4.15. During the experiments inlet and outlet ozone concentrations in gas phase were determined to find the amount of absorbed ozone by the solution.

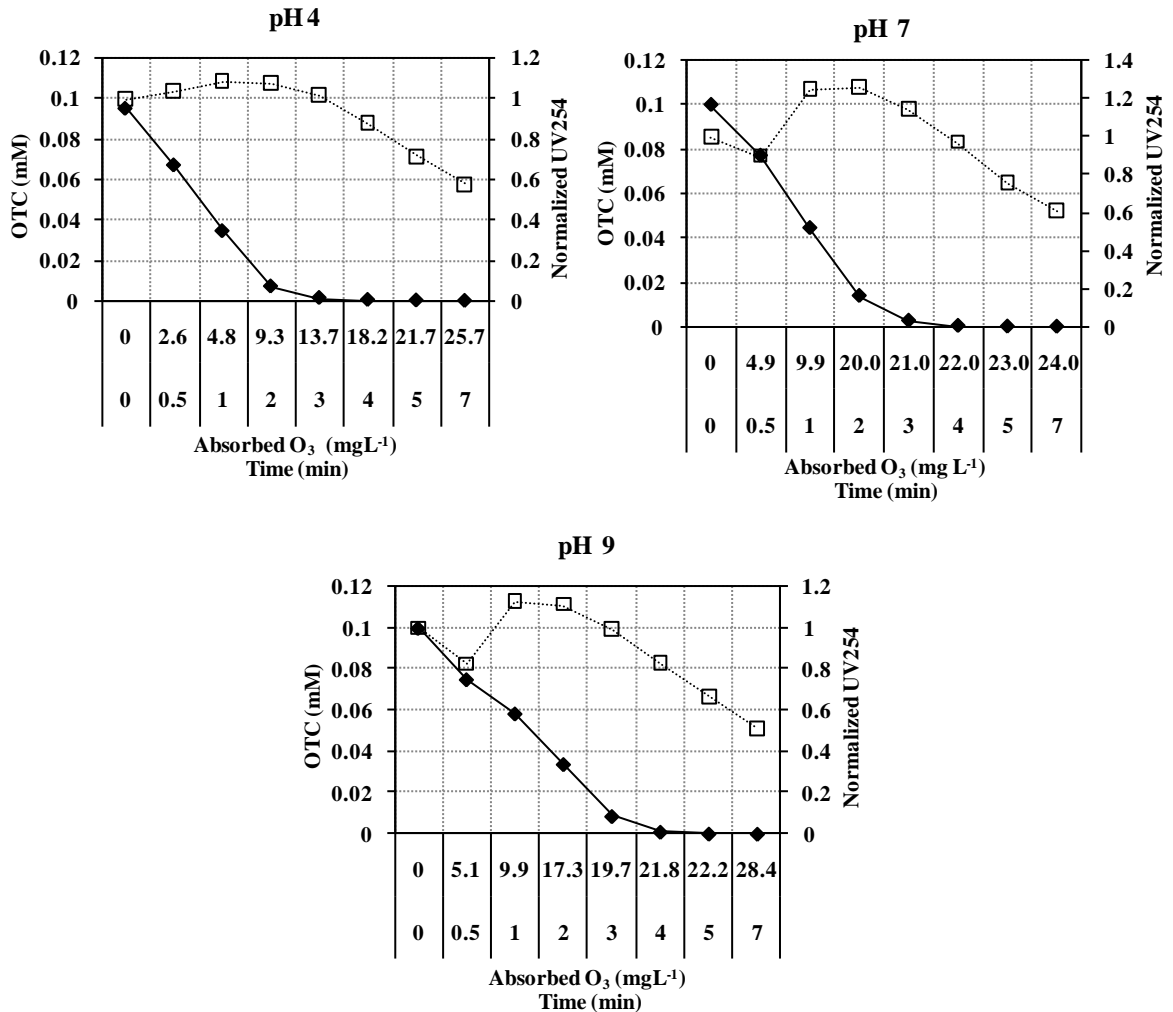


Figure 4.15. Effect of pH on the oxidation of OTC in ozonation process ($C_{\text{OTC}} = 0.1 \text{ mM}$, applied ozone dose= $606 \text{ mg L}^{-1}\text{h}^{-1}$).

The results showed that the aromaticity of the OTC solution increased at the initial period of ozonation and then parallel to OTC degradation it exhibited a decrease at the investigated pH values. The structure of OTC molecule is vulnerable to molecular ozone attack, which caused the rapid degradation of the molecule, but the fragments of molecule may combine causing an increase in the aromaticity. However, these reaction products and remaining OTC in the solution were oxidized with molecular ozone as well as the hydroxyl radicals produced during the ozonation process. Eventually, both parameter decreased by the progress of reaction. On the other hand, absorbance value at the characteristic wavelength of OTC solution steadily decreased during the ozonation period (data not shown). As in the case of photocatalytic oxidation the amounts of OTC determined by spectrophotometric analysis were not consistent with the results obtained by HPLC analysis.

The degradation rates of OTC indicated a good fit to pseudo first-order kinetics with high correlation coefficients at each pH value. The pseudo first-order degradation rate constants and correlation coefficients at different pH values are given in Table 4.12.

Table 4.12. Pseudo first-order rate constants of ozone oxidation of OTC at different pH values.

	Ozonation pH		
	pH 4	pH 7	pH 9
k (min ⁻¹)	1.31	1.67	1.45
r ²	0.99	0.99	0.93

4.3.3. Effect of Water Components on the Oxidation of OTC by Ozone

The results showed that the highest degradation rate was achieved at neutral pH and the lowest degradation rate was obtained at pH 4. In general, the rate of ozone decomposition increases with increasing solution pH since the hydroxyl ions catalyze the decay of ozone to form radicals serving as reactive oxidizing species. Accordingly, it was found by numerous authors (Li et al., 2008; Esplugas et al., 2002; Vogna et al., 2004; Dantas et al., 2007) that the removal of various organic compounds concerned in aqueous solutions was fastest at pH value higher than 7. In this study, by increasing the OTC solution pH from 4 to 9 an increase was observed in the amount of absorbed ozone. However, degradation rate of OTC at pH 9 was slower than that obtained at pH 7. Actually, this unexpected result was observed in some other studies related with the ozonation of antibiotics (Balcioglu and Otker, 2003; Li et al., 2008).

The effects of water components on the ozonation of OTC was investigated at pH 7 and with an applied ozone dose of 1,086 mg L⁻¹ h⁻¹. For each of the ions, 100 mg L⁻¹ was selected as initial concentration. Figure 4.16 represents the removal of OTC as a function of treatment time in the presence of calcium, bicarbonate, phosphate, sulphate, nitrate, and chloride ions, and humic acid.

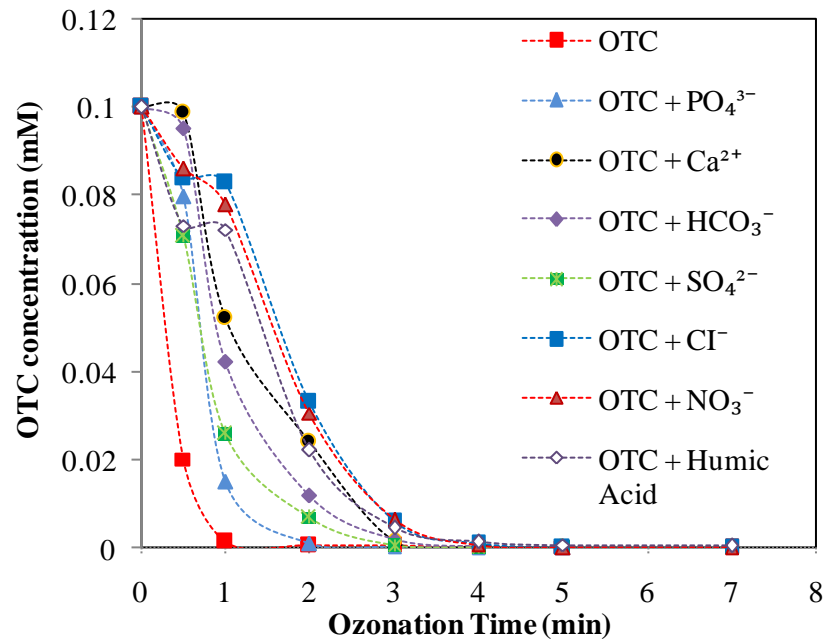


Figure 4.16. Effect of water components of the ozonation of OTC ($C_{\text{OTC}} = 0.1 \text{ mM}$, $C_{\text{O}_3} = 1086 \text{ mg L}^{-1} \text{h}^{-1}$, $\text{pH} = 7$, Concentrations of ions and humic acid = 100 mg L^{-1}).

The pseudo first order degradation rate constants of OTC in the presence of various water components are given in the Table 4.13.

Table 4.13. Pseudo first-order rate constants of ozone oxidation of OTC in the presence of water components.

Water Components	OTC degradation rate constants
	$k_{\text{overall}} (\text{min}^{-1})$
OTC	4.18
OTC + PO_4^{3-}	2.05
OTC + SO_4^{2-}	1.67
OTC + NO_3^-	1.54
OTC + Cl^-	1.53
OTC + HCO_3^-	1.36
OTC + Ca^{2+}	1.32
OTC + Humic Acid	1.13

The results showed that the degradation rate of OTC by ozone oxidation was decreased significantly in the presence of the ions and humic acid. However, the decrease in the degradation rate of OTC was not related with the reaction rate constant of ions with the hydroxyl radical as in the case of photocatalytic oxidation. It was known that some organic and inorganic molecules promote the ozone decomposition while some others exhibit inhibition effect (Langlais et al., 1991). In this study, among the anions, phosphate ion has lower effect for the retardation of OTC degradation and this ion is known as promoter for the formation of reactive free radicals in the ozonation process. Although humic acid is, also known as promoter it caused a decrease in the degradation of OTC most probably due to its high concentration.

5. CONCLUSION

The existence of antibiotics in the environment causes bacterial resistance and toxicity on the organisms. Recent studies revealed that conventional treatment methods are inefficient for the removal of antibiotics, and advanced oxidation methods can be promising for the treatment of water and wastewater containing antibiotics. Water and wastewater composition can significantly affect the performance of advanced oxidation. Therefore, in this study the role of water components on the degradation of tetracycline antibiotic with photocatalytic and ozone oxidation were investigated. In addition, the effects of initial antibiotic concentration, pH, and addition of H₂O₂ on the photocatalytic oxidation and the effects of applied ozone dose and pH on ozone oxidation of OTC were studied.

In the photocatalytic experiments, all investigated anions decreased the photocatalytic degradation rate of OTC depending on the reaction rate constants of them with the hydroxyl radicals. The lower adsorption of OTC on the photocatalyst did not explain the reduction of OTC degradation rate in the presence of various anions. Although, humic acid increased the adsorption of OTC onto TiO₂, increased humic acid concentration decreased the degradation rate of antibiotic probably due to the adsorption of light that is necessary for the production of reactive radicals. The remarkable reducing effects of water components on the photocatalytic degradation rate were observed at the higher concentrations of them found in natural water.

In case of ozone oxidation of OTC, water components significantly decreased the degradation rate and in contrary to the results obtained by photocatalytic oxidation, the effects of ions were not depended on the reaction rates of them with hydroxyl radical.

The effect of initial OTC concentration on the photocatalytic process well fitted to Langmuir- Hinshelwood model. Limited rate constant and equilibrium constants were found as $6.99 \times 10^{-6} \text{ M min}^{-1}$ and $1.613 \times 10^4 \text{ M}^{-1}$, respectively, at a range of 0.05 – 0.5 mM OTC and the presence of 1 g L⁻¹ TiO₂ at pH 7. The degradation rate for the decomposition of OTC increases with increasing OTC concentration and then reaches a saturation value at high concentrations of OTC since the generation of •OH radicals on the surface of catalyst is reduced due to the covering of active sites with OTC.

It was known that higher pH enhances the efficiency of photocatalytic and ozone oxidation. However, in this study, the highest degradation of OTC was achieved at pH 7 by both oxidation processes. It can be concluded that reactivity of intermediates produced during the reaction with the oxidative species depends on the pH of the aqueous medium.

Based on all results, it can be concluded that ozone oxidation can be suggested as an efficient process for the removal of OTC, since it provides rapid and complete removal of OTC even in the presence of water components. On the other hand, photocatalytic oxidation can be suggested as an economic alternative method because solar light can be used as an energy source for this process. However, toxicity of reaction intermediates generated by both photocatalytic and ozone oxidation processes must be investigated for the assessment of process efficiency.

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<http://www.rcdc.nd.edu/compilations/Hydroxyl/OH.HTM>

APPENDIX- A

Calibration Curve of OTC for HPLC Analysis

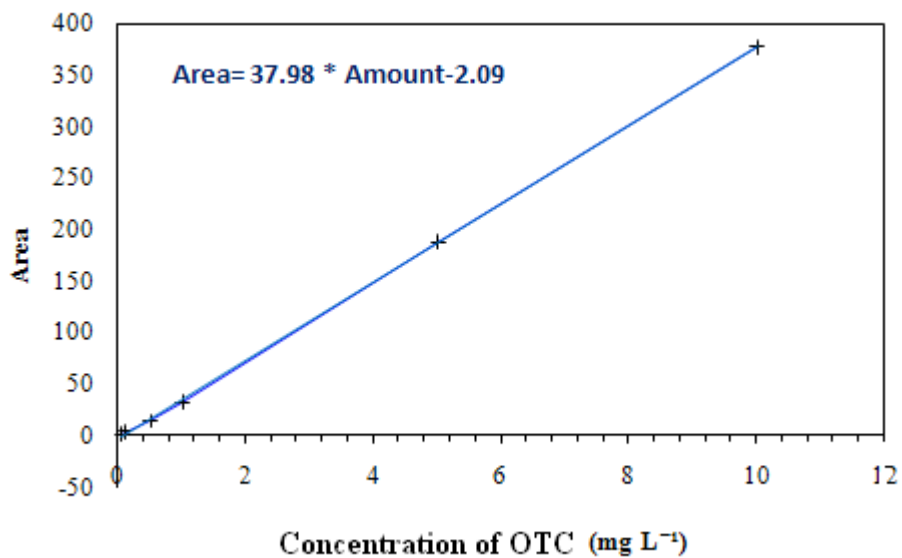


Figure A.1. External calibration curve of OTC

Table A.1. Calibration table

Concentration (mg L ⁻¹)	Area
0.05	1.2
0.1	3.5
0.5	14.5
1	32.4
5	188.1
10	378

Appendix B

Calibration curve of OTC for Spectrophotometric Analysis

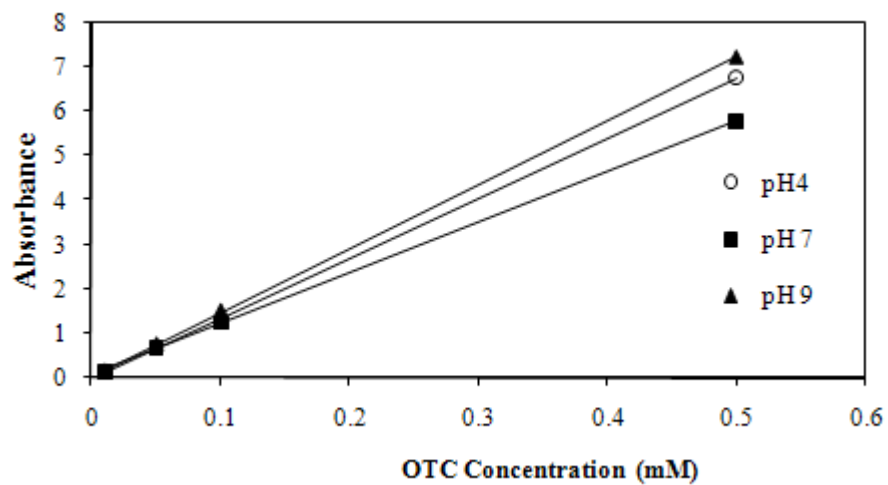


Figure B.1. Calibration curves of OTC at different pH values

Table B. 1. Extinction coefficients of OTC at different pH values

pH	Wavelength (nm)	ϵ (M^{-1})
4	360	13.040
7	368	11.679
9	374	14.510