DESTRUCTION OF ANTIMICROBIAL CONTAMINANTS IN WATER AND WASTE SLUDGE WITH CHEMICAL OXIDATION PROCESSES

by

Nalan Bilgin Öncü

BS. in Chem., Koç University, 2001

M.S. in Mater. Sci., Sabancı University, 2003

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I dedicate this thesis to my niece Bade, who wants to save the world when she's grown up.

I believe in you!

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ABSTRACT

Destruction of antimicrobial contamination in both water and sewage sludge was investigated in the thesis. In the first part of the study, the effectiveness of three oxidation processes –chlorination, ozonation, and heterogeneous photocatalysis– on the destruction of a resistance carrier bacterial plasmid DNA isolated from *E. Coli* were compared and the relative superiority of ozonation or heterogeneous photocatalysis over conventional chlorination was demonstrated. Although the nano-fiber-TiO_2 that was prepared in the study did not provide better plasmid DNA destruction compared to commercial TiO_2-P25, the material is promising for facilitated catalyst separation from the treated water. In the second part of the study, simultaneous degradation of two model antibiotics, ciprofloxacin (CIP) and oxytetracycline (OTC) in secondary sewage sludge, was investigated by the application of (i) ozonation, (ii) hydrogen peroxide oxidation assisted with microwave irradiation (MW/H_2O_2), iii) persulfate oxidation assisted with microwave irradiation ($MW/S_2O_8^{2-}$), and (iv) persulfate oxidation activated with ferrous iron and conventional heating (Fe^{2+}/heat/$S_2O_8^{2-}$). While under appropriate conditions all of the processes provided >95% antibiotic degradation along with sludge solubilization, they offer different benefits for waste sludge treatment. Ozonation was found to be more effective to treat the sludge with low content of total solid (TS=2.5 g/L), while the desorption of the antibiotics was required to achieve high rate of degradation at high solid content (10 g/L) of the sludge. On the other hand, owing to the desorption ability of MW in the MW/H_2O_2 and $MW/S_2O_8^{2-}$ processes, and owing to the complexation of the antibiotics with iron in the Fe^{2+}/heat/$S_2O_8^{2-}$ process, concurrent antibiotic desorption and degradation was achieved in sludge with high TS (10 g/L). $MW/S_2O_8^{2-}$ provided exceptional metal solubilization, considerably enhanced sludge filterability within the shortest treatment time. Along with considerable metal solubilization, the Fe^{2+}/heat/$S_2O_8^{2-}$ process resulted in phosphorus precipitation, which can potentially increase the fertilizer value of the sludge.

ÖZET

Bu çalışmada, gerek suda gerekse atık arıtma çamurunda antimikrobiyal kirletici giderimi araştırılmıştır. Çalışmanın ilk kısmında, üç farklı kimyasal oksidasyon prosesinin –klorlama, ozonlama, ve heterojen fotokataliz– *E. Coli* bakteriden izole edilen ve antibiyotik direnci taşıyan plasmid DNA gideriminde etkileri karşılaştırışmış ve ozonla heterojen fotokataliz proseslerinin konvansiyonel klorlamaya oranla daha üstün olduğu gösterilmiştir. Laboratuvarda hazırlanan nano-fiber-TiO_2 malzeme, ticari TiO_2-P25 malzemeye oranla plasmid DNA gideriminde etkili olmamasına rağmen, arıtılan sudan katalizörün kolay ayrılmasını sağlayabilir. Çalışmanın ikinci kısmında, oksitetrasiklin (OTC) ve ciprofloksasin (CIP) model antibiyotiklerin (i) ozonlama, (ii) mikrodalga ile desteklenen hidrojen peroksit oksidasyonu (MW/H_2O_2), (iii) mikrodalga ile desteklenen persulfat oksidasyonu (MW/$S_2O_8^{2-}$), ve (iv) demir-II-konvansiyonel ısıtma ile aktive edilen persulfat oksidasyonu (Fe^{2+}/ısı/$S_2O_8^{2-}$) prosesleriyle atık arıtma çamurunda giderimleri araştırılmıştır. Tüm proseslerde çamur çözünürleşmesinin yanısıra >%95 antibiyotik giderimi sağlanmasına rağmen, her bir proses çamur arıtımına yönelik farklı yararlar sunmaktadır. Ozonlama, düşük toplam katı miktarı (TK=2.5 g/L) olan çamurun arıtımında daha etkili olurken, yüksek TK (10 g/L) miktarı olan çamurda verimli antibiyotik gideriminin gerçekleştilmesi proses modifikasyonuyla antibiyotiklerin çamurdan dezorpsiyonunu gerektirmiştir. Diğer yandan, MW/H_2O_2 ve MW/$S_2O_8^{2-}$ proseslerinde MW'nin kirleticileri dezorb edebilme özelliği ve Fe^{2+}/ısı/$S_2O_8^{2-}$ prosesinde de antibiyotiklerin demirle kompleks oluşturması sayesinde, yüksek TK miktarı olan çamurda (10 g/L) antibiyotiklerin aynı anda dezorpsiyonu ve giderimi gerçekleşmiştir. MW/$S_2O_8^{2-}$, metal çözünürleşmesi ve çamur süzülebilirliğinde önemli iyileşmeyi kısa artıma süresinde sağlamıştır. Fe^{2+}/ısı/$S_2O_8^{2-}$ prosesi, metal çözünürleşmesinin yanısıra çamurda fosforun çökmesini sağlayarak çamurun gübre değerini artırma potansiyeli sunmaktadır.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Units used
AD	Anaerobic Digestion	
AOP	Advanced Oxidation Process	
AOX	Absorbable Organic Halides	
ATP	Adenosine Triphosphate	
BET	Brunauer–Emmett–Teller	
BLD	Below Limit of Detection	
BOD_5	5-day Biochemical Oxygen Demand	(mg/L)
CHP	Catalyzed Hydrogen Peroxide	
CIP	Ciprofloxacin	
CH	Conventional Heating	
COD	Chemical Oxygen Demand	(mg/L)
CST	Capillary Suction Time	(sec × L/g)
DEHP	Di(2-ethylhexyl)phthalate	
DDD	Defined Daily Dose	
DMP	2,9-Dimethyl-1,10-phenanthroline	
DNA	Deoxyribonucleic Acid	
DS	Dry Solids	(g/L)
EDC	Endocrine Disrupting Compound	
ESI	Elecrospray Ionization	
EtOH	Ethanol	
FQ	Fluoroquinolone	
H_2O_2	Hydrogen Peroxide	
HPLC	High Performance Liquid Chromatography	
HRT	Hydraulic Retention Time	(days)
HT	Heating Rate	(°C/min)
ISCO	In-Situ Chemical Oxidation	
LAS	Linear Alkylbenzene Sulfonates	

LC-MS/MS	Liquid Chromatography-Tandem Mass Spectroscopy	
LOD	Limit of Detection	
Log K_d	Sludge-Wastewater Partitioning Constant	
k	Rate Constant	(M^{-1} s^{-1})
k_e	Equilibrium Constant	
MAP	Magnesium ammonium phosphate	
Me	Metal	
MeOH	Methanol	
MBR	Membrane Biological Reactor	
Me_S	Soluble Metal Concentration	(mg/L)
Me_T	Total Metal Concentration	(mg/L)
MQ	Milli-Q	
MW	Microwave	
NH_4^+	Ammonium	(mg/L)
NPE	Nonylphenol and Nonylphenol Ethoxylates	
O_3	Ozone	
OC	Organic Compounds	
OD_{660}	Optical Density	
ODF	Oxidant Demand Free	
OTC	Oxytetracycline	
P	Power	(W)
PAH	Polynuclear Aromatic Hydrocarbons	
PCB	Polychlorinated Biphenyls	
*p*CBA	Para-Chlorobenzoic Acid	
PCDD/F	Polychlorinated Dibenzo-p-dioxins and Furans	
pK_a	Acid Dissociation Constant	
PO_4^{3-}	Orthophosphate	(mg/L)
PPCP	Pharmaceutical and Personal Care Product	
PS	Primary Sludge	
RT	Ramp Time	(min)
PVP	Polyvinylpyrrolidone	
SBR	Sequencing Batch Reactor	
SCOD	Soluble Chemical Oxygen Demand	(mg/L)

SEM	Scanning Electron Microscopy	
SPE	Solid Phase Extraction	
SRF	Specific Resistance to Filtration	(m/kg)
SS	Suspended Solids	(g/L)
SOC	Super Optimal broth with Catabolite repression	
SV_{60}	Percentage of Settled Sludge Volume in 60 min	(%)
TBA	Tertiary Butyl Alcohol	
TCOD	Total Chemical Oxygen Demand	(mg/L)
TET	Tetracycline	
TGA	Thermogravimetric analysis	
TiO_2	Titanium Dioxide	
TKN	Total Kjeldahl Nitrogen	(mg/L)
TIP	Titanium Isopropoxide	
TN	Total Nitrogen	(mg/L)
TP	Total Phosphorus	(mg/L)
TS	Total Solids	(g/L)
TSS	Total Suspended Solids	(g/L)
T	Temperature	(°C)
TT	Treatment Time	(min)
UV	Ultraviolet	
US	Ultrasound	
VFA	Volatile Fatty Acid	
VS	Volatile Solids	(g/L)
VSS	Volatile Suspended Solids	(g/L)
WAS	Waste Activated Sludge	
XRD	X – Ray Diffraction	

1. INTRODUCTION

1.1. Motivation

Discussions regarding the environmental fate and impact of antibiotics, which constitute one of the most widely utilized group of pharmaceuticals and personal care products (PPCPs) have been continuing for several decades. A considerable amount of research has put forth their presence in different environmental compartments (Snyder et al., 2003; Daughton and Ternes, 1999) and most of the recent debate revolves around a major outcome; the development of antimicrobial resistance. Antimicrobial resistance is a significant issue constituting a challenge both for the human and veterinary therapeutics. During the recent years, researchers have concentrated more in the area of investigating the effectiveness of conventional and non-conventional treatment for the elimination of antimicrobial resistance (Munir et al., 2011a; Oncu-Bilgin et al., 2011; Cengiz et al., 2010; Chen et al., 2010; Diehl and LaPara, 2010; Gomez et al., 2010; Knapp et al., 2010; Balcioglu et al., 2009; Ghosh et al., 2009; Han et al., 2009; Szczepanowski et al., 2009; Templeton et al., 2009; Zhang et al., 2009; Cernat et al., 2007; Peak et al., 2007).

While chlorination as a conventional treatment still continues to be the most commonly preferred alternative due to its economical affordability compared to advanced technologies (AOPs), its inefficiency for the removal of antibiotic resistant bacteria has been shown in some studies (Diao et al., 2004; Shrivastava et al., 2004); in addition, chlorine can even lead to selection of these microorganisms (Shrivastava et al., 2004; Murray et al., 1984; Armstrong et al., 1982). The ability of ozone to remove pathogens that are relatively resistant to chlorine and chloramines is well known (Hunt and Marinas, 1999; Korich et al., 1990). Moreover, the effectiveness of ozone towards antibiotic resistant bacteria has been reported recently (Nielsen et al., 2013). However, destruction of bacteria may not indicate complete elimination of the resistance transfer potential, since resistance can be transferred via carriers such as bacterial plasmid DNA. Considering the penetration of ozone through the bacterial cells and the damaging effect on the DNA (Ishizaki et al., 1987), the destruction of antibiotic resistant bacteria can be well expected. Damage to the bacterial DNA is also known to take place by heterogeneous photocatalysis

with titanium dioxide (TiO_2) (Yang and Wang, 2008; Shen et al., 2008). Therefore, both ozone and heterogeneous photocatalysis can be promising alternatives for the control of antimicrobial resistance contamination in water.

Wastewater is obviously a point source for antimicrobial pollution and some antibiotics, specifically those belonging to tetracycline (TET) and fluoroquinolone (FQ) groups tend to accumulate in sewage sludge mainly by partitioning to the solid phase during treatment (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a; Zhang et al., 2011). In order to prevent dissemination of these pollutants in the environment, they should be treated properly at their source. Currently, with increasing amounts of waste sludge production, the presence of antimicrobial contaminants along different organic and inorganic micro-pollutants in sludge gained considerable interest (Clarke and Smith, 2011; US-EPA, 2009; Lindberg et al., 2007). Beneficial reuse of waste sludge in agriculture has been long applied as an important means of waste sludge handling due to the dual benefit gained from management of surplus sludge and utilization of sludge as fertilizer. On the other hand, concerns have been raised regarding land-application of treated sludge due to a number of hazardous constituents including heavy metals and recalcitrant micro-organics. Specific regulations have been implemented for allowable concentrations of heavy metals, the environmental fate of which is well-known (CEC, 1986; US-EPA, 1993). Limit values for a number of persistent organic compounds with known harmful effects have been set in some countries, including Turkey (Official Gazette, 2010; Schowanek et al., 2004). In addition to these, endocrine disrupting compounds (EDCs) and PPCPs in sludge have recently drawn attention. It has been understood that many of these compounds, which were previously believed to be destructed during wastewater treatment, are not substantially removed (Lindberg et al., 2007). There is limited information regarding their potential harmful effects and/or synergistic side effects in the coexistence of a wide variety of micro-organics. As a result, the management of these contaminants can be an issue that has to be considered for the land-application of biosolids.

Recent reviews of occurrence data have shown that TET and FQ group antibiotics are generally detected in the sludge in the low mg/kg range concentrations; However, concentrations as high as 40.8 mg/kg and 97.5 mg/kg were also reported for ciprofloxacin (CIP) in some reports (US-EPA, 2009; SFT, 2007). Field experiments of sludge

application to soil also revealed the long-term persistence of FQs in soil (Golet et al., 2003). A recent study reported that environmental concentrations of CIP, erythromycin, and TET could exert a selective pressure on microorganisms and increase the prevalence of resistant bacteria in soil (Tello et al., 2012). Although it was pointed out that resistance development under the selective pressure of antibiotics in soil can be of minimal importance, since the resistance will disappear as soon as the selective pressure is removed (Smith, 2009), the long-term persistence potential of some antibiotics indicates that resistance development is plausible. In addition, it was reported that tetracycline resistance genes could persist for long terms even after the selective pressure has been removed (Tamminen et al., 2011).

These facts clearly show that, for safe management of waste sludge, multi-barrier treatment approach has to be implemented. While the number of studies on waste sludge management is growing rapidly, the majority of the literature targets the improvement of the efficiency of biological stabilization processes (Carrere et al., 2010). In addition, considerable number of studies concentrates on nutrient recovery (Tyagi and Lo, 2013) and on sludge conditioning (Li et al., 2008; Wojciechowska, 2005; Park et al., 2003). In these studies various mechanical, thermal or chemical processes have been explored and improved to a great extent. However, the fate of micro-pollutants during these was seldom investigated (Oncu-Bilgin and Balcioglu-Akmehmet, 2013b; Carballa et al., 2006, 2007). Actually, interest in micro-pollutant removal from waste sludge has started quite recently, since up to now the majority of the studies that are concerned with organic compounds in sludge have dealt with the development of efficient analysis methods for the determination of a vast number of micro-pollutants as well as on risk-based analyses.

1.2. Objectives

Based on the aforementioned concerns, this thesis aimed to explore the effects of powerful chemical oxidation processes, with specific emphasis of AOPs on the destruction of antimicrobial contamination in water and waste sludge. The detailed objectives of this research are listed below:

- Evaluation of the effectiveness of ozonation and heterogeneous photocatalytic oxidation processes for the destruction of antibiotic resistance carrier bacterial plasmid DNA in water and comparison of the results with those obtained with conventional disinfectant chlorine.

- Evaluation of the efficiency of a synthesized nano-fiber TiO_2 material as an alternative photocatalyst, for the destruction of antimicrobial resistance carrier plasmid DNA in water.

- Further evaluation of the influences of chlorination, ozonation, and heterogeneous photocatalysis on the risk of antimicrobial resistance proliferation by performing plasmid DNA transformability studies.

- Investigation of ozonation for the degradation of antibiotics from TET and FQ groups in waste sewage sludge and evaluation of the influences of process parameters on the ozonation efficiency.

- Investigation of the combined effect of chemical oxidation and thermal treatment performed by microwave irradiation as an emerging process for sludge treatment, on the destruction of selected antibiotics in waste sewage sludge. Comparison of the effectiveness of hydrogen peroxide and persulfate as the oxidants.

- Investigation of the effects of ferrous iron and conventional heating for the activation of persulfate for the degradation of selected antibiotics in waste sewage sludge.

- Investigation of nutrient, organic matter, and heavy metal solubilizations during treatment of waste sewage sludge with chemical oxidation processes.

- Statistical evaluation of the effects of key process parameters on process efficiency.

1.3. Thesis Structure

The thesis consists of eight sections. The background and the experimental methods are described in Sections 2 and 3, respectively. Section 4 explores the effects of chlorination, ozonation, and heterogeneous photocatalysis with commercial and nano-fiber TiO_2 photocatalysts on antimicrobial resistance carrier plasmid DNA in water. Sections 5, 6, and 7 deal with treatment of waste sludge with the purpose of simultaneous antibiotic degradation and sludge solubilization. Ozonation of waste sewage sludge is described in Section 5, microwave assisted hydrogen peroxide and persulfate oxidation of sludge are described in Section 6, and ferrous iron and heat activated persulfate oxidation of waste sewage sludge is described in Section 7. The major outcomes of the thesis are summarized in Section 8.

2. THEORETICAL BACKGROUND

2.1. Antimicrobial Pollution in the Environment

2.1.1. Antibiotic Consumption Rates

Antibiotics are utilized both as therapeutics for human and animal medication and as growth promoters in animal farming practices (Thiele-Bruhn, 2003). In Turkey, it was reported that the total antibiotic utilization rate increased from 14.6 to 31.4 defined daily dose (DDD)/1,000 inhabitants-days between the years 2001 and 2006 (Karabay and Hosoglu, 2008). While utilization rates of different antibiotic groups in the EU are listed in Table 2.1 (ESAC, 2010), some major antibiotics utilized for human and animal medication are listed in Table 2.2 (Kemper, 2008). The total rate of antibiotics utilized in the EU countries increased from 38.6 to 506.7 DDD/1000 inhabitants-days between 2009 (Adriaenssens et al., 2010) and 2010 (ESAC, 2010). A recent study, which reported antibiotic utilization rates from eight Latin American countries during the years 1997–2007, showed that consumption rates in half of these countries increased during the studied period (Wirtz et al., 2010).

Table 2.1. Utilization rates of some antibiotics in the EU in 2010.

Antibiotic Class	**DDD/1,000 inhabitants-days**	**%**
Tetracyclines	55.8	11.0
β-Lactams: penicillins	243.9	48.1
β-Lactams: other	48.4	9.5
Sulfonamides	15.1	3.0
Macrolides	74.6	14.7
Quinolones	40.1	7.9
Others	28.8	5.7

Table 2.2. Some major antibiotics utilized for human and animal medication.

Antibiotics	Utilized to treat	Antibiotics	Utilized to treat
Amynoglycosides		***Fluoroquinolones***	
Apramycin	Pigs only	Ciprofloxacin	Humans
Gentamycin	All animas, humans	Enrofloxacin	All animals
Kanamycin	Dogs, pigs, cattle, horses	Marbofloxacin	All animals
Sisomycin	Humans only	Flumequin	Humans
Neomycin	All animals	Ofloxacin	Humans
Spectinomycin	Humans only		
Streptomycin	Pigs, cattle, poultry, sheep	***Lincosamides***	
	Obsolete	Clindamycin	Dogs, humans
		Lincomycin	Pigs, cats, dogs, cattle
β-Lactams: penicillins			
Amoxicillin	All animals	***Macrolides***	
Ampicillin	All animals	Azithromycin	Humans
Azlocillin	Humans	Clarithromycin	Humans
Benzylpenicillin	All animals	Erythromycin	Humans, cattle, chicken
Coxacilin	Cattle	Roxithromycin	Humans
Dicloxacilin	Cattle	Spiramycin	All animals
Flucloxacillin	Humans		
Methicillin	Humans	Tylosin	Animals only
Mezlocillin	Humans	Vancomycin	Humans
Nafcillin	Humans		
Oxacilin	Cattle	***Sulfonamides***	
Piperacillin	Humans	Sulfanilamide	Humans
Phenoxymethylcillin	Humans	Sulfadimethoxine	Cattle, pigs, chicken
Penicillin G	Humans	Sulfadimidine	Cattle, sheep, chicken
		Sulfamethoxazole	Humans
Cephalosporines		Sulfapyridine	Pigs
Cefalexin	Dogs	Sulfathiazole	Humans
Cefalotin	Humans		
Cefazolin	Humans	***Trimethoprim***	In combination with sulfonamides
Ceftiofur	Cattle, pigs		
Cefotaxim	Humans	***Tetracyclines***	
Cefotiam	Humans	Chlorotetracycline	Cattle, pigs
Cefquinom	Cattle, pigs		
		Doxycycline	Humans, cats, dogs
Fenicoles		Oxytetracycline	Humans, cattle, sheep, pigs
Chloramphenicole	Cats, dogs	Tetracycline	Humans, horse, sheep, pigs

2.1.2. Environmental Occurrence of Antibiotics

One of the major pathways for antibiotics to reach the environment is through animal manure and human wastes in which significant concentrations of non-metabolized antibiotics can be present (Stuart et al., 2012; Lapworth et al. 2012; Haller et al., 2002; Halling-Sorensen, 2001). It has been estimated that manure application as fertilizers may exert loads as high as kilograms per hectare (Winckler and Grafe, 2000).

Behavior of antibiotics in the environment depends on their physical-chemical and biological properties (Ingerslev and Halling Sørensen, 2001). Biodegradation of antibiotics varies according to their properties as well as the present environmental conditions. For example while 25-day half-life in soil was reported for chlortetracycline (Halling-Sorensen et al., 2005), FQs were reported to have long-term persistence in soil in a study conducted for a 21 month period (Golet et al., 2003).

While some antibiotics such as TET and tylosin are easily attained in soils by sorption (Thiele-Bruhn, 2003) and can be protected from photodegradation (Sengelov et al., 2003), others such as olaquindox are attained to a lesser extent and tend to leach in the soil (Rabolle and Spliid, 2000). Transport of antibiotics in subsoil depends on various factors. For example, while complexation of antibiotics with metal cations present in the soil is expected to increase their adsorption by cation bridge formation, changes in environmental factors such as soil pH (Teixido et al., 2012) or interaction with other micro-pollutants such as surfactants (ElSayed et al., 2013) can influence their mobility in soil. Consequently, residual amounts of persistent antibiotics can reach water sources.

Antibiotics have been detected in surface waters, ground water, and soils as well as in treatment plant effluents, sludges, and biosolids at concentrations from μg/L (waters) to mg/kg (dry solids) (Table 2.3, Table 2.4).

Table 2.3. Maximum antibiotic concentrations detected in water and wastewater.

Source	Antibiotic	Amount (ng/L)	References
Natural Waters	***β-Lactams***		
	Penicillin G	250	Watkinson et al., 2009
	Tetracyclines		
	Chlortetracyclin	600	Watkinson et al., 2009
	Doxycycline	400	Watkinson et al., 2009
	Oxytetracycline	68,000	Matsui et al., 2008
	Sulfonamides		
	Sulfadiazine	4,130	Boxall et al., 2005
	Sulfamethoxazole	2,000	Watkinson et al., 2009
	Sulfachloropyridazine	350	Luo et al., 2011
	Macrolides		
	Lincomycin	21,000	Boxall et al., 2005
	Erythromycin	1,700	Kolpin et al., 2002
	Tylosin	280	Kolpin et al., 2002
	Roxithromycin	350	Watkinson et al., 2009
	Quinolones		
	Ciprofloxacin	150	Luo et al., 2011
	Norfloxacin	120	Kolpin et al., 2002
	Ofloxacin	200	Luo et al., 2011
	Trimethoprim	212	Wu et al., 2008
Treatment Plant Effluents	***β -Lactams***		
	Cefaclor	1,800	Watkinson et al., 2009
	Penicillin G	300	Watkinson et al., 2009
	Penicillin V	2,000	Watkinson et al., 2009
	Cloxacillin	700	Watkinson et al., 2009
	Cephalexin	250	Watkinson et al., 2009
	Tetracyclines		
	Tetracycline	3,600	Karthikeyan and Meyer, 2006
	Oxytetracycline	4,200	Karthikeyan and Meyer, 2006
	Chlortetracyclin	250	Watkinson et al., 2009
	Doxycycline	150	Watkinson et al., 2009
	Sulfonamides		
	Sulfamethoxazole	500	Martinez Bueno et al., 2012
	Sulfathiazole	600	Watkinson et al., 2009
	Sulfasalazine	150	Watkinson et al., 2009
	Macrolides		
	Erythromycin	1,100	Karthikeyan and Meyer, 2006
	Roxithromycin	870	Karthikeyan and Meyer, 2006
	Tylosin	3,400	Watkinson et al., 2009
	Lincomycin	510	Karthikeyan and Meyer, 2006
	Quinolones		
	Nalidixic acid	450	Watkinson et al., 2009
	Norfloxacin	330	Karthikeyan and Meyer, 2006
	Clindamycin	110	Alexy and Kummerer, 2006
	Ciprofloxacin	1,900	Martinez Bueno et al., 2012
	Ofloxacin	2,800	Martinez Bueno et al., 2012
	Trimethoprim	250	Watkinson et al., 2009

Table 2.4. Maximum antibiotic concentrations detected in solid matrices.

Source	Antibiotic	Amount (μg/kg)	References
Soil	***Tetracyclines***		
	Tetracycline	199	Hamscher et al., 2002
	Oxytetracycline	305	Boxall et al., 2005
	Chlortetracycline	70	Hamscher et al., 2002
	Sulfonamides		
	Sulfadiazine	0.8	Boxall et al., 2005
	Sulfamethazine	2	Hamscher et al., 2005
	Sulfadimidine	11	Hoper et al., 2002
	Macrolides		
	Lincomycin	8.5	Boxall et al., 2005
	Tylosin	57	Jacobsen et al., 2004
	Trimethoprim	0.5	Boxall et al., 2005
Sediments	***Tetracyclines***		
	Tetracycline	5	Luo et al., 2011
	Oxytetracycline	1	Luo et al., 2011
	Sulfonamides		
	Sulfadiazine	30	Luo et al., 2011
	Sulfachloropyridazine	25	Luo et al., 2011
	Sulfamethoxazole	25	Luo et al., 2011
	Macrolides		
	Erythromycin	5	Luo et al., 2011
	Roxithromycin	6	Luo et al., 2011
	Quinolones		
	Ciprofloxacin	35	Luo et al., 2011
	Ofloxacin	43	Luo et al., 2011
	Trimethoprim	20	Luo et al., 2011
Sludge	***Tetracyclines***		
Biosolids	Tetracycline	5,270	US-EPA, 2009
	Doxycycline	5,090	US-EPA, 2009
	4-Epitetracycline	4,380	US-EPA, 2009
	Sulfonamides		
	Sulfisoxazole	22	Spongberg and Witter, 2008
	Sulfapyridine	197	Gobel et al., 2005
	Sulfamethazine	113	Gobel et al., 2005
	Sulfamethoxazole	160	Kinney et al., 2006
	Sulfathiazole	37	Nieto et al., 2010
	Macrolides		
	Azithromycin	5,205	US-EPA, 2009
	Clarithromycin	63	Gobel et al., 2005
	Roxithromycin	1,800	Nieto et al., 2007
	Tylosin	4,000	Nieto et al., 2007
	Quinolones		
	Ciprofloxacin	97,500	SFT, 2007
	Norfloxacin	11,000	Lindberg et al., 2005
	Ofloxacin	58,100	US-EPA, 2009
	Trimethoprim	133	Gobel et al., 2005

Although varying analytical methods are used and wide variation in antibiotic concentrations are reported in the literature, water sources generally harbor high concentrations of some sulfonamides such as sulfamethoxazole, while TETs and FQs are attained primarily in the solid matrices. Significant correlation between the sorption and the sludge-wastewater partitioning constant values of the FQs was reported in sewage sludge (Jia et al., 2012). On the other hand, reported concentrations of sulfonamides found in solid matrices are relatively low and maximum detected concentrations are in the range of 0.8–197 μg/kg dry matter as seen from Table 2.4. However, the figures may not represent the real concentrations of the antibiotics, since applied extraction methods cannot effectively recover all antibiotics present in solid matrices. For instance, it was shown that a major portion of sulfadiazine and sulfamethoxazole was non-extractable (Heise et al., 2006).

2.1.3. Environmental Occurrence of Antibiotic Resistance Carriers

The presence of antibiotics in various environmental matrices constitutes a contamination potential resulting in a risk for development of antibiotic resistance in the local bacterial communities (Kummerer and Henninger, 2003; Chee-Sanford et al., 2001; Guardabassi et al., 1998). Resistance can be developed naturally by mutation or selection as well as by uptake of mobile genetic elements such as plasmids. Selective pressure exerted by the presence of one group of antibiotic may lead to the acquirement of genetic mobile cassettes that carry genes encoding resistances to other antibiotics along with the selective resistance. As a result, resistance to antibiotics, which may not be present at significant environmental concentrations, can be also acquired through this path (Chiew et al., 1998). In a recent study, it was reported that resistance towards non-consumed antibiotics could be acquired through the transfer of multiple antibiotic-resistance genes via class 1 integrons (Oberle et al., 2012). Mobile genetic elements can be also exchanged among members of different classes of bacteria (Lorenz and Wackernagel, 1994). Through exchange of these mobile genetic elements, resistance can be transferred for example from non-pathogenic to pathogenic microorganisms. Antibiotic resistant bacteria or antibiotic resistance genes have been detected almost everywhere (Table 2.5). The presence of antibiotics and antibiotic resistance genes in treatment plant effluents, manure, soil, and

sludge necessitates addressing the effectiveness of currently applied wastewater treatment technologies for the removal of these.

Table 2.5. Occurrence of antibiotic resistance in various environmental matrices.

Resistant Bacteria and Genes	Source	References
Tetracycline Resistance	Natural Water	Harnisz et al., 2011; Agerso and Petersen, 2007;
	Drinking Water	Cernat et al., 2007
	Sludge/Biosolids	Munir et al., 2011a; Zhang et al., 2009
	Wastewater	Munir et al., 2011a; Chen et al., 2010; Peak et al. 2007
	Soil/Manure	Munir et al., 2011b; Szczepanowski et al., 2009; Han et al., 2009; Peak et al. 2007
	Sediments	Knapp et al., 2010
Macrolide Resistance	Sludge/Biosolids	Szczepanowski et al., 2009
	Wastewater	Chen et al., 2010; Szczepanowski et al., 2009
	Soil/Manure	Chen et al., 2010; Knapp et al., 2010; Han et al., 2009
Aminoglycoside Resistance	Natural Water	Barker-Reid et al., 2010; Mohapatra et al., 2008
	Drinking Water	Cernat et al., 2007
	Sludge/Biosolids	Szczepanowski et al., 2009
	Wastewater	Szczepanowski et al., 2009
Trimethoprim Resistance	Natural Water	Mohapatra et al., 2008; Cernat et al., 2007
	Drinking Water	Cernat et al., 2007
	Sludge/Biosolids	Szczepanowski et al., 2009
	Wastewater	Szczepanowski et al., 2009
Sulfonamide Resistance	Natural Water	Barker-Reid et al., 2010; Mohapatra et al., 2008; Cernat et al., 2007
	Drinking Water	Cernat et al., 2007
	Sludge/Biosolids	Munir et al., 2011a; Munir et al., 2011b; Szczepanowski et al., 2009
	Wastewater	Munir et al., 2011a; Szczepanowski et al., 2009
	Soil/Manure	Munir et al., 2011b
β-Lactam Resistance	Natural Water	Mohapatra et al., 2008
	Drinking Water	Cernat et al., 2007
	Sludge/Biosolids	Szczepanowski et al., 2009
	Wastewater	Han et al., 2009
	Soil/Manure	Demaneche et al., 2008
	Sediments	Knapp et al., 2010
Vancomycin Resistance	Natural Water	Barker-Reid et al., 2010
	Sludge/Biosolids	Sahlstrom et al., 2009
	Wastewater	Gomez et al., 2010

2.2. Theories of Chemical Oxidation Processes

During the application of chemical oxidation processes, different oxidizing species can be responsible for micro-pollutant degradation depending on oxidation system. Although hydroxyl radical ($OH^{\bullet}$) is known as the most powerful oxidant specie having a standard oxidation potential of 2.6 V, in treatment of various environmental matrices the degradation rates of pollutants do not only depend on the oxidation potential of the chemical oxidant, but are also influenced by the composition of treated matrix and by many operational variables. Therefore, various chemical oxidants have been utilized and developed to treat different environmental matrices depending on the properties of the oxidant, the targeted environmental matrix, and the targeted organic contaminants.

Chlorination, ozonation, and photocatalytic oxidation processes are widely investigated for the treatment of various pollutants in different environmental matrices. These oxidants are also used for disinfection purposes. The action of each oxidation system on microorganisms is different and each oxidant offers different advantages. Chlorine has been utilized for more than 100 years and is still one of the most commonly applied oxidant for water disinfection. While chlorine is cheap and an effective disinfectant for various pathogens, its ineffectiveness towards some more resistant microorganisms and the formation of carcinogenic disinfection by-products such as trihalomethanes have increased interest in alternative disinfectants (Qasim, 2000). Ozone is a more powerful disinfectant than chlorine (Von Gunten, 2003) and is more effective towards microorganisms such as protozoa that are more resistant to chlorine. Depending on the conditions, ozone can decompose to produce more powerful hydroxyl radical. In addition, ozone can destroy the cell wall of the microorganisms leading to the leakage of the cell ingredients, while cell disruption and DNA leakage with chlorine can take place only at high dosages (Venkobachar, 1977). However, disinfection by-product formation can be an issue in ozonation; especially the formation of carcinogenic bromate in the presence of bromide and natural organic material (NOM) is problematic (Von Gunten, 2003; Siddiqui, 1995). Heterogeneous photocatalytic oxidation with TiO_2 as the most widely investigated photocatalyst involves the action of hydroxyl radicals and is thus an AOP. This process is expected to result in minimal or no by-product formation (Richardson et al., 1996). There is no chemical requirement as activation of the photocatalyst can be acquired with light and

after treatment the photocatalyst can be recovered from the system. However, the difficulty in recovering TiO_2 from the treated water and the limited efficiency due to the catalyst's wide band gap are the main disadvantages of photocatalysis (Chen and Mao, 2007).

2.2.1. Photocatalytic oxidation

As mentioned previously, photocatalytic oxidation by titanium dioxide involves the generation of hydroxyl radicals ($OH^{\bullet}$) and is thus classified as an AOP. Photocatalytic process involves light-absorbing semiconductor materials, the electronic structure of which consists of a valence band (VB) full with electrons and an empty conductance band (CB). The energy gap between these bands is called the band-gap energy (E_g). Upon illumination with light having energy greater than E_g, electron-hole pairs (h^+- e^-) are produced (Figure 2.1).

$$TiO_2 + h\nu \rightarrow TiO_2 + h^+ + e^- \quad (2.1)$$

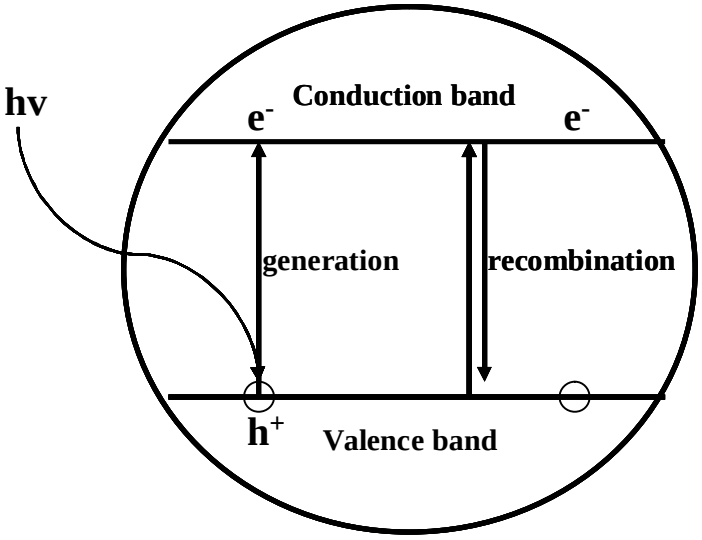


Figure 2.1. Schematic illustration of photocatalytic activation of a semiconductor material (adapted from Mahmood et al., 2012).

Electron-hole pairs can react through several mechanisms (Parsons and Williams, 2004):

i. $h^+ e^-$ pairs can combine to release heat,

$$h^+ + e^- \rightarrow \text{heat} \quad (2.2)$$

ii. h^+ can react with electron donors (water and pollutants) to generate oxidized products,

$$H_2O + h^+ \rightarrow OH^{\bullet} + H^+ \quad (2.3)$$

iii. e^- can react with electron acceptors (dissolved oxygen),

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (2.4)$$

The penetration depth of light limits the application of photocatalysis to matrices with high concentrations of suspended material. In addition, it was reported that the process is not effective when the chemical oxygen demand (COD) of the treated matrix exceeds 800 mg/L (Gogate and Pandit, 2004). Therefore, the heterogeneous photocatalysis process can be more suitable for water treatment.

While heterogeneous photocatalysis is applicable over a wide pH range, the pH influences reaction mechanism in two major ways. The first influence of pH is on the valence and conduction band potentials, which in turn influences the oxidation or reduction efficiency. For example, an increase in the pH enhances the reduction potential of the catalyst. The second influence of pH is on the surface charge of TiO_2, which can influence the adsorption-desorption behavior of micro-pollutants that are also influenced by pH dependent speciation. It is known that degradation of micro-pollutants can be more effective when they are adsorbed on the surface of the photocatalyst, where higher contact with reactive species can be provided (Tan, 2003).

As mentioned previously, one of the major problems related to the utilization of TiO_2 is the difficulty in photocatalyst separation from the treated matrix. Different approaches

have been adapted to prepare supported system photocatalysts to solve this problem. One promising method is utilization of electrospinning technology, by which porous structured fibers with diameters in the nanometer range can be prepared (Reneker et al., 2000) and deposited on a desired surface as a supported system. Few works have tested the photocatalytic performance of electrospinned TiO_2 fibers and mainly with relatively simple organic molecules such as methylene blue (Wang et al., 2013).

2.2.2. Ozonation

Similar to photocatalytic oxidation, ozone oxidation can provide both the disinfection and oxidation of pollutants. Highly reactive ozone molecule possesses both electrophilic and nucleophilic properties due to its electronic configuration (Beltran, 2004). Ozone can act on various components of a treated matrix by direct reaction with molecular ozone or by indirect reaction with radical species that are formed during the decomposition of ozone in a chain process (Langlais et al., 1991).

Along with its high oxidation potential, non-selectivity of hydroxyl radical is important in ozonation process and the radical oxidation mechanism is faster than direct oxidation.

pH can influence both direct and indirect reactions of ozone. However, in the presence of high concentrations of pollutants that are reactive to molecular ozone, ozone will be consumed by direct reactions without producing reactive radicals regardless of pH. It should be noted that pH can also affect the reactivity of these compounds with ozone by influencing their dissociation. In the presence of non-reactive compounds to ozone, increased pH results in the decomposition of ozone to generate hydroxyl radicals (Beltran, 2004). Ozone decomposition initiates various propagation reactions (Siegrist et al., 2011; Kasprzyk-Hordern et al., 2003):

$$O_3 + OH^- \rightarrow HO_2^{\bullet} + O_2^{\bullet -} \qquad k = 70 \pm 7\ M^{-1}\ s^{-1} \qquad (2.5)$$

$$HO_2^{\bullet} \leftrightarrow O_2^{\bullet -} + H^+ \qquad pKa = 4.8 \qquad (2.6)$$

$$O_3 + H_2O \rightarrow 2OH^{\bullet} + O_2 \qquad k = 1.1 \times 10^{-4}\ M^{-1}\ s^{-1} \qquad (2.7)$$

$$O_3 + HO_2^{\bullet} \leftrightarrow 2O_2 + OH^{\bullet} \qquad k = 1.6 \times 10^{9}\ M^{-1}\ s^{-1} \qquad (2.8)$$

$$2HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \quad (2.9)$$

At acidic pH, formation of hydroxyl radicals depends on the presence of catalysts. Generation of free radicals from ozone can be initiated both homogeneously by dissolved metal catalysts and heterogeneously by metal oxides or supported metal catalysts (Legube and Leitner, 1999). The mechanism depends on the nature of the catalyst and the reaction conditions (Beltran, 2004). A typical example for homogeneous catalysis is the reaction of ferrous iron with ozone (Kasprzyk-Hordern et al., 2003):

$$Fe^{2+} + O_3 \rightarrow FeO^{2+} + O_2 \quad (2.10)$$

$$FeO^{2+} + H_2O \rightarrow Fe^{3+} + OH^{\bullet} + OH^- \quad (2.11)$$

The catalytic action of metals can be important in the ozonation of complex environmental matrices. Inefficient ozone consumption can require higher ozone dose application for such complicated matrices.

Bicarbonate and carbonate species are well known scavengers for hydroxyl radical Eqns. 2.12 and 2.13). Although they can produce carbonate and bicarbonate radicals, which are known to react with some organic compounds (Umschlag and Herrmann, 1999), carbonate species generally tend to slow down the degradation rates of target compounds since the reactivity of carbonate radicals is lower compared to those of the hydroxyl radicals (Buxton et al., 1988).

$$OH^{\bullet} + HCO_3^- \rightarrow OH^- + HCO_3^{\bullet} \qquad k = 8.5 \times 10^6\ M^{-1}\ s^{-1} \quad (2.12)$$

$$OH^{\bullet} + CO_3^{2-} \rightarrow OH^- + CO_3^{\bullet -} \qquad k = 3.9 \times 10^8\ M^{-1}\ s^{-1} \quad (2.13)$$

Besides the carbonates, other anions commonly found in the composition of environmental matrices can act as scavenger for hydroxyl radical. In addition, consumption of ozone with hydroxyl radicals can be an important scavenging reaction due to the high reaction constant:

$$OH^{\bullet} + O_3 \rightarrow HO_2^{\bullet} + O_2 \qquad k = 3.0 \times 10^9\ M^{-1}\ s^{-1} \quad (2.14)$$

2.2.3. Hydrogen Peroxide Oxidation

While the direct reaction of hydrogen peroxide with some biological enzymes can be important, generally this reaction with organic contaminants is of minor importance (Watts and Teel, 2005) and the degradation of these requires the catalytic decomposition of hydrogen peroxide to produce a variety of oxidizing and reducing species. On the other hand, hydrogen peroxide is very reactive with reduced metal species that can catalyze its decomposition. The reactions of hydrogen peroxide involving its catalysis to generate reactive species are called catalyzed hydrogen peroxide (CHP) reactions. The most known catalytic reaction of dilute hydrogen peroxide is that with ferrous iron at pH 3−5. This reaction is known as the Fenton reaction, which has been utilized to treat wastewater, groundwater, and contaminated soil for many years (Siegrist et al., 2011). Major reactions for the formation of reactive radicals from hydrogen peroxide are listed below:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$	$k = 6.3 \times 10^1\ M^{-1}s^{-1}$	(2.15)
$OH^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet}$	$k = 3.3 \times 10^7\ M^{-1}s^{-1}$	(2.16)
$HO_2^{\bullet} + H_2O_2 \rightarrow H_2O + O_2 + OH^{\bullet}$		(2.17)
$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^-$	$k = 3.2 \times 10^8\ M^{-1}s^{-1}$	(2.18)
$HO_2^{\bullet} \leftrightarrow O_2^{\bullet-} + H^+$	pKa = 4.8	(2.19)
$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+$	$k < 2 \times 10^3\ M^{-1}s^{-1}$	(2.20)
$Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + HO_2^{\bullet}$	$k = 2.7 \times 10^{-3}\ M^{-1}s^{-1}$	(2.21)
$Fe^{3+} + H_2O_2 \rightarrow Fe(HO_2)^{2+} + H^+$	$k_e = 3.1 \times 10^{-3}$ (unitless)	(2.22)
$Fe(HO_2)^{2+} \rightarrow Fe^{2+} + HO_2^{\bullet}$	$k_e = 2.7 \times 10^{-3}\ M^{-1}s^{-1}$	(2.23)

Excess hydrogen peroxide can also act as a scavenger due to excessive consumption of radicals via Eqns. 2.16 and 2.17 (Siegrist et al., 2011). Since no radical specie is produced in reactions 2.18 and 2.20, these reactions are known as termination reactions in hydrogen peroxide oxidation process. These reactions also indicate that iron doses have to be controlled, since excess iron can act as a radical scavenger. Other important scavenging reactions can be those of carbonate and bicarbonate as was also shown for the ozone oxidation mechanism (Eqns. 2.12 and 2.13). However, some studies have also reported that

carbonate radicals can contribute positively on the process efficiency (Umschlag and Herrmann, 1999).

As shown in the equations above, the reaction mechanism with hydrogen peroxide is very complex and involves different oxidizing and reducing species which are listed in Table 2.6.

Table 2.6. Potential reactive species that participate in CHP reactions (Siegrist et al., 2011).

Reactant species	Standard reduction potential (V)	pH range
Hydrogen peroxide (H_2O_2)	1.8	< 11.6
Hydroxyl radical ($OH^{\bullet}$)	2.6	< 11.9
Superoxide anion ($O_2^{\bullet -}$)	-0.3	> 4.8
Perhydroxyl radical ($HO^{\bullet}$)	1.5	< 4.8
Hydroperoxide anion (HO_2^-)	0.9	> 11.6
Ferryl ion (FeO^{2+})	Not known	Not known
Solvated electrons (e^-)	-2.8	> 7.8
Singlet oxygen ($^1O_2$)	Not applicable	Not known
Triplet oxygen (O_2)	1.2	All

Among the oxidizing species given in the table, the superoxide anions are held responsible for contaminant desorption in CHP systems (Watts et al., 1999). It has been shown that especially at high hydrogen peroxide concentrations (0.1−10 M) the rate of superoxide anion generation and the reactivity of the superoxide anion is increased to a great extent due to improved solvation (Siegrist et al., 2011; Smith et al., 2004). Contaminant sorption and desorption mechanism of zwitterionic compounds can be also influenced by pH. In addition, similar to ozonation, pH can also influence the reactions of hydrogen peroxide due to an influence on contaminants' reactivity as a result of changes in their protonated/deprotonated forms at different pH values.

2.2.4. Persulfate Oxidation

Utilization of persulfate (or peroxydisulfate) oxidation as a treatment technology is relatively new compared to other chemical oxidation processes. Persulfate oxidation can take place either directly with the persulfate anion or by activation to produce reactive radicals. While the persulfate anion has a standard reduction potential of about 2 V (Siegrist et al., 2011), its direct reactions are relatively slow and the production of radical

species by the activation of persulfate is required to provide effective degradation of target pollutants. Activation of persulfate can generate mainly the highly reactive sulfate radical that has a standard reduction potential of 2.5 V (Siegrist et al., 2011), which is close to that of the hydroxyl radical.

Persulfate can be activated by different activation agents such as heat, UV irradiation, and transition metals. Depending on the type of activation and the reaction conditions, various other radicals can be produced in addition to the sulfate radical. Some typical activation reactions for persulfate are given below:

$$S_2O_8^{2-} + \text{heat/UV irradiation} \rightarrow 2SO_4^{-\bullet} \quad (2.24)$$

$$S_2O_8^{2-} + Me^{n+} \rightarrow Me^{(n+1)+} + SO_4^{2-} + SO_4^{-\bullet} \quad \text{(Metal-M}_e\text{ activation)} \quad (2.25)$$

$$S_2O_8^{2-} + H^+ \rightarrow HS_2O_8^- \rightarrow SO_4^{-\bullet} + SO_4^{2-} + H^+ \quad \text{(Acid activation)} \quad (2.26)$$

$$S_2O_8^{2-} + OH^- \rightarrow SO_4^{2-} + OH^{\bullet} \quad \text{(Alkaline activation, pH>11)} \quad (2.27)$$

Persulfate activation by hydrogen peroxide during soil treatment has been also shown. Although the exact mechanism is not known, it was proposed that persulfate is activated by the hydroxyl radical that is produced as a result of catalytic decomposition of hydrogen peroxide by soil minerals (Siegrist et al., 2011):

$$S_2O_8^{2-} + OH^{\bullet} \rightarrow SO_4^{-\bullet} + SO_4^{2-} + 1/2O_2 + H^+ \quad \text{(catalyzed } H_2O_2 \text{ activation)} \quad (2.28)$$

Radical propagation reactions of persulfate are very complex and the role of many species in these reactions is not well understood. Similar to ozone and CHP, the radical oxidation performance of persulfate is also influenced by scavenging reactions. Some these are given below (Siegrist et al., 2011):

$$SO_4^{-\bullet} + CO_3^{2-} \rightarrow SO_4^{2-} + CO_3^{\bullet-} \quad k = 6.1 \times 10^6\ M^{-1}s^{-1} \quad (2.29)$$

$$SO_4^{-\bullet} + HCO_3^- \rightarrow SO_4^{2-} + HCO_3^{\bullet} \quad k = 1.6 \times 10^6\ M^{-1}s^{-1} \quad (2.30)$$

In addition to these reactions, self scavenging of sulfate radicals can take place via Eqns 2.31−2.33 (Liang and Su, 2009).

$SO_4^{-\bullet} + SO_4^{-\bullet} \rightarrow S_2O_8^{2-}$ $k = 4 \times 10^8\ M^{-1}s^{-1}$ (2.31)

$SO_4^{-\bullet} + S_2O_8^{2-} \rightarrow SO_4^{2-} + S_2O_8^{-\bullet}$ $k = 6.1 \times 10^5\ M^{-1}s^{-1}$ (2.32)

$OH^{\bullet} + S_2O_8^{2-} \rightarrow OH^{-} + S_2O_8^{-\bullet}$ $k = 1.2 \times 10^7\ M^{-1}s^{-1}$ (2.33)

Self scavenging reactions of persulfate may become more important at very high temperatures (Huie and Clifton, 1989). Nevertheless, Peyton et al. (1993) stated that these scavenging reactions exert minor influence in the presence of other constituents that are highly reactive with the sulfate radical. In addition, the reactivity of $S_2O_8^{-\bullet}$ is not known and its generation may not be necessarily unproductive (Peyton, 1993).

Compared to hydroxyl radicals, sulfate radicals are relatively more selective and thus can result in more effective contaminant degradation in the presence of competing constituents. The higher non-selectivity of hydroxyl radicals can be explained by their various reaction mechanisms including direct electron transfer, hydrogen abstraction, and addition to double bonds. On the other hand, sulfate radicals mainly react by direct electron transfer reactions (Siegrist et al., 2011). In addition to these, persulfate can be more advantageous than ozone since oxidant solubility is not limited when persulfate is applied in the sodium form and can be more advantageous than hydrogen peroxide due to its higher oxidant stability (Siegrist et al. 2011, Tsitonaki et al., 2010). The mentioned advantages of persulfate resulted in increased number of studies exploring degradation of various organic pollutants both in water and in solid matrices in the literature (Liang et al., 2003; 2004; 2008; 2009; Liang and Su, 2009; Oh et al., 2009; Teel et al., 2009; Uslu-Otker and Balcioglu-Akmehmet, 2009a; Zhen et al., 2012a, b, c).

2.3. Effect of Conventional and Non-Conventional Processes on Antibiotic Degradation in Different Environmental Matrices

Reported antibiotic removal rates during conventional wastewater treatment processes exhibit wide variation depending on the compound properties and the operational conditions of the applied treatment processes, although low removal rates are common (e.g. for high strength pharmaceutical wastewater (Zhou et al., 2006)). Nevertheless, Miege et al. (2009) stated that drawing out a general conclusion regarding the effect of operating

conditions on PPCP removal is challenging because of difficulty in testing these individually.

During biological wastewater treatment, antibiotics can exert toxicity on the microbial community (Carucci et al., 2006) and may also lead to resistance development especially in biofilms (Schwartz et al, 2003). Although lab scale experiments did not reveal toxic effects of antibiotics on activated sludge (Prado et al., 2010), these may not represent real scale conditions (Stone et al., 2009).

Regarding pharmaceutical removal in a wastewater treatment plant, improvement of effluent quality can be achieved by inclusion of tertiary treatment as proposed by Le-Minh et al. (2010). Advanced treatment technologies such as membrane filtration (nanofiltration and ultrafiltration) can be applied to wastewater (Li et al., 2004; Kim et al., 2007a). However, application of these technologies results in the transfer of the pollutant from one phase to another without destruction. In addition, membrane technology produces secondary waste that requires further treatment. Other conventional processes applied to wastewater to increase the quality of effluent include chemical oxidation such as ozonation and chlorination. The major problem with chlorine application to wastewater is the requirement of high doses of oxidant in order to achieve considerable treatment performance for recalcitrant contaminants and these lead to the formation of carcinogenic by-products (Singer, 1993). For example, to treat swine wastewater a chlorine dose as high as 100 mg/L was required for efficient removal of sulfonamide antibiotics, which are mainly present in the aqueous phase (Macauley et al., 2006). Ozone and ozone based AOPs, on the other hand, have proven to be effective for the removal of a wide range of antibiotics (Dodd et al., 2006; Balcioglu-Akmehmet and Otker, 2003). For this oxidation process, by-product formation in the presence of bromide ions (Von Gunten, 2003; Siddiqui, 1995) as mentioned previously and the technology costs constitute the major challenges, which necessitate optimization of oxidant dosages. Other AOPs applied to wastewater to degrade antibiotics include Fenton or photo-Fenton processes (Gonzalez et al., 2007; Elmolla and Chaudhuri, 2009). The major drawback of the Fenton process is the requirement of iron post-removal and the acidic pH.

During biological treatment of wastewater, the sorption of contaminants on sludge can be the reason for the observed high removal rates of antibiotics (Jia et al., 2012; Kim et

al., 2005; Giger et al., 2003). The recent findings for the occurrence of antibiotics in manure and sludge have pointed out the need for the treatment of these matrices as well. Although less commonly investigated, the removal of antibiotics from complex solid matrices with both conventional and non-conventional processes has been shown. The results of investigations on the anaerobic digestion (AD) of animal manure to degrade tetracycline antibiotics indicated 75% removal of extractable chlortetracycline and 59% removal of oxytetracycline in 64 days (Arikan, 2008; Arikan et al., 2006). However, in these studies the removal mechanism of antibiotics was not explored. The long digestion periods of manure in the presence of antibiotics pose risk of antibiotic resistance development as represented by the high rates of resistant determinants found in these matrices (Table 2.5). Therefore, evaluation of AOPs must be considered for solid matrices, as well.

AOPs were applied to the slurries of manure and sludge in order to solubilize the nutrients or to remove some specific pollutants; however, pharmaceutical removal was targeted in a limited number of studies. Various AOPs were investigated by Uslu-Otker and Balcioglu-Akmehmet (2009a; 2009b; 2008) for the removal of oxytetracycline and sulfamethazine from manure. These processes were also applied to spent adsorbents containing tetracycline group antibiotics (Ozbarli et al., 2010). A pretreatment carried out with magnesium salt promoted the extraction of oxytetracycline into the aqueous phase and improved the applied oxidation process efficiency. Carballa et al., (2007) investigated the removal of PPCPs including the antibiotic sulfamethoxazole, from ozone pretreated sludge during AD. The antibiotic was completely removed during the AD process and the effect of ozone could not be reported. However, as mentioned before, non-extractable concentrations of sulfamethoxazole may lead to underestimation of the actual removal efficiency. Oncu-Bilgin and Balcioglu-Akmehmet (2013b) investigated the effect of ozone on the degradation of oxytetracycline (OTC) and ciprofloxacin (CIP) from synthetically contaminated activated sludge. The process resulted both in sludge solubilization accompanied with a decline in the antibiotic concentrations in the sludge. Some examples to treatment processes applied to wastewater, manure, and sludge for the degradation of antibiotics are listed in Table 2.7.

Table 2.7. Effect of conventional and non-conventional treatment processes on antibiotic removal from various environmental matrices.

Treated Medium	Treatment Technology	Examples	References
Wastewater	Biological treatment	Activated sludge, anaerobic-aerobic sequential treatment, anaerobic treatment, membrane bioreactor	Le-Minh et al., 2010; Carucci et al., 2006; Zhou et al., 2006
	Membrane filtration processes	Microfiltration, nanofiltration, ultrafiltration, reverse osmosis, activated carbon	Kim et al., 2007a; Li et al., 2004
	Chemical oxidation	Ozonation, chlorination	Uslu-Otker and Balcioglu-Akmehmet, 2008; Macauley et al., 2006; Dodd et al., 2006; Von Gunten, 2003
	Advanced oxidation	O_3/H_2O_2, O_3/UV, Fenton and photo-Fenton processes	Elmolla and Chaudhuri, 2009; Gonzalez et al., 2007; Von Gunten, 2003
Manure	Biological treatment	AD	Arikan, 2008; Arikan et al., 2006
	Chemical oxidation	Ozonation, persulfate oxidation, Fenton process	Uslu-Otker and Balcioglu-Akmehmet, 2009a; Uslu-Otker and Balcioglu-Akmehmet, 2009b; Uslu-Otker and Balcioglu-Akmehmet, 2008
Sludge	Biological treatment	AD, anaerobic digestion with ozonation pretreatment	Carballa et al., 2007; Lindberg et al., 2006
	Chemical oxidation	Ozonation	Oncu-Bilgin and Balcioglu-Akmehmet, 2013b

AD: anaerobic digestion; O_3: ozonation, H_2O_2: hydrogen peroxide, UV: ultraviolet

2.4. Effect of Conventional and Non-Conventional Processes on Antibiotic Resistance Carriers

The results of the studies regarding the biological treatment of wastewater revealed that increased influent antibiotic concentrations (Kim et al., 2007b) and longer hydraulic retention times (HRTs) during activated sludge treatment (Manaia et al., 2010) promoted the development of resistance significantly. These findings evidently suggest the need for AOPs as opposed to the arguments promoting the application of longer HRTs for the removal of micro-pollutants from wastewaters (Jones et al., 2007). On the other hand,

advanced membrane biological reactor (MBR) technology was proposed by Munir et al. (2011a) as an effective process for the removal of tetracycline and sulfonamide genes from wastewater.

Biological treatment processes applied to investigate the fate of resistance carriers in manure include aerobic thermophilic digestion, mesophilic AD, and aerobic biofiltration (Chen et al., 2010; Han et al., 2009; Munir et al., 2011a; Ghosh et al., 2009; Diehl and LaPara, 2010). Chen et al. (2010) have shown the ineffectiveness of aerobic biofiltration, mesophilic AD, and lagoon treatment applications for the elimination of antibiotic resistance and proposed the requirement of more effective treatment methods. On the other hand, Han et al. (2009) proposed autothermal aerobic digestion as a suitable method to remove antibiotic resistant microorganisms from swine manure; no resistant microorganisms were detected after treatment at 60−65ºC for 3 days. Munir et al. (2011a) proposed that AD and lime stabilization of biosolids removes resistance genes more effectively than conventional dewatering and gravity thickening. Ghosh et al. (2009) reported thermophilic AD to be more effective than mesophilic AD and Diehl and LaPara (2011) showed that AD at high temperatures was significantly more effective than aerobic digestion for the removal of several tetracycline genes from sewage sludge. Although high temperatures can provide significant reduction of resistant bacteria, these can be still present as demonstrated by Chenier and Juteau (2009) for treatment of swine manure by an aerobic thermophilic sequencing batch reactor (SBR).

Advanced oxidation technologies namely, ozone and Fenton processes have also been applied to manure containing ampicillin resistant *E. Coli* by Cengiz et al. (2010). To eliminate the ampicillin resistance carrier plasmid DNA from cow manure, which was demonstrated by the removal of a *tet*(M) gene, high doses of ozone and Fenton reagent were required within a prolonged treatment period. The recent literature covering the mentioned processes that target removal of antibiotic resistance elements from wastewater, manure, and sludge is listed in Table 2.8. The presence of antibiotic contamination along with antibiotic resistance bacteria and genes in the studied matrices suggests that the risk of antimicrobial resistance is real. Therefore, proper methods are needed to eliminate this risk.

Table 2.8. Effect of conventional and non-conventional treatment processes on antibiotic resistance carriers in various environmental matrices.

Treated Medium	Treatment Technology	Examples	Monitored Resistant Bacteria/Resistance Determinants	References
Wastewater	Biological treatment	Activated sludge, wastewater lagoons, membrane biological reactor, conventional activated sludge, oxidative ditch, rotatory biological contactors, AD, lime stabilization	Tetracycline, sulfonamide resistant bacteria and genes, ciprofloxacin resistance	Munir et al., 2011a; Chen et al., 2010, Gomez et al., 2010; Manaia et al., 2010; Szczepanowski et al., 2009; Zhang et al., 2009; Kim et al., 2007b; Peak et al., 2004;
	Physical treatment	UV	Tetracycline, sulfonamide resistant bacteria and genes	Munir et al., 2011a
	Chemical treatment	Chlorine	Tetracycline, sulfonamide resistant bacteria and genes	Munir et al., 2011a
Drinking Water	Physical Treatment	UV	Ampicillin resistant and trimethoprim resistant *Escherichia Coli*	Balcioglu-Akmehmet., 2009; Templeton et al., 2009
	Chemical treatment	Chlorine	Ampicillin resistant and trimethoprim resistant *Escherichia Coli*	Templeton et al., 2009
Manure	Biological treatment	Autothermal thermophilic aerobic digestion, Aerobic biofiltration, mesophilic AD	Tetracycline, kanamycine, ampicillin, rifampicin resistant bacteria, macrolide and tetracycline resistance genes	Chen et al., 2010; Han et al., 2009
Sludge	Biological treatment	Aerobic/anaerobic thermophilic/mesophilic digestion	Tetracycline resistance genes	Diehl and LaPara, 2010; Ghosh et al., 2009
Manure	Chemical treatment	Fenton and ozone	Ampicillin resistant *Escherichia Coli* and resistance carrier plasmid DNA (by *tet*(M) gene removal monitoring)	Cengiz et al., 2010; Balcioglu et al., 2009

2.5. Management of Waste Sewage Sludge

As mentioned previously, sludge can concentrate various pollutants including antibiotics during wastewater treatment. Up to date, over 360 organic compounds (OCs) have been identified in sewage sludge (Eriksson et al., 2008) and the list is expected to grow as a result of rapid developments in analytical techniques. Handling of excess sludge, which is produced in considerably high amounts during wastewater treatment is one of the major challenges faced by wastewater treatment facilities owing mainly to the high costs associated with sludge treatment (up to 60% of total operation costs) (Liu, 2003). New stringent regulations regarding sludge production and disposal have contributed to this challenge. The European Commission's Urban Waste Water Treatment Directive 91/271/EEC sets requirements that bring about higher quantities of sludge production, while also limiting land-application only to treated sludge in order to minimize adverse effects of untreated sludge (CEC, 1991).

2.5.1. Brief Review of Current Regulations Regarding the Land-application of Biosolids

In order to minimize potential negative environmental impacts, the agricultural use of waste sludge has been limited by specific regulations that mainly monitor heavy metal concentrations and pathogens. Limit values for heavy metals are listed in Table 2.9 (Official Gazette, 2010, 2010; US-EPA, 1993; CEC, 1986).

Table 2.9. Limit values of heavy metals for the land-application of sludge.

	Heavy Metals (mg/kg DS)							
	As	Cd	Cu	Pb	Hg	Ni	Se	Zn
US	41	39	1,500	300	17	420	100	2,800
EU	30	20−40	1,00−17,500	750−1,200	16−25	300−400		2,500−4000
Turkey		10	1,000	750	10	300		2,500

Due to increased environmental concerns, some countries have also adapted standards for the regulation of several OCs (Schowanek et al., 2004; Official Gazette, 2010, 2010) based mainly on the latest EC Working Document on Sludge (CEC, 2000) (Table 2.10).

Table 2.10. Limit values of organic contaminants for the land-application of sludge.

	Organic Contaminants(mg/kg DS)						
	AOX	LAS	DEHP	NPE	PAH	PCDD/F	PCB
Turkey	500	2,600	100	50	6	100 ng	0.8
Austria	500				6	100 ng	0.2−1
Denmark		1,300	50	10	3		
Germany	400					30 ng	0.1/congener

AOX: absorbable organic halides; LAS: linear alkylbenzene sulfonates; DEHP: di(2-ethylhexyl)phthalate; NPE: nonylphenol and nonylphenol ethoxylates; PAH: polynuclear aromatic hydrocarbons; PCDD/F: polychlorinated dibenzo-p-dioxins and furans; PCB: polychlorinated biphenyls.

2.5.2. Conventional Treatment Processes

The purpose of common sludge treatment methods is mainly reduction of water and pathogen content. Sludge treatment includes thickening, dewatering, stabilization, and disinfection, and thermal drying (Andersen, 2001). After treatment, the sludge is disposed or recycled. Methods of sludge disposal and recycling include landfilling, incineration, and application to soil as biosolids (Andersen, 2001). Landfilling has been historically considered one of the cheapest methods for sludge handling. However, it is not an environmentally sustainable solution and is not one of the most preferred options for sludge management in many developed countries. One of the major objections regarding landfilling is its contribution to the greenhouse gas effect by methane release. Incineration can effectively reduce sludge volumes, but has disadvantages such as increased cost due to emission regulations. Moreover, the technology makes use of fossil fuels and 30% of the solids are converted to ash with high heavy metal content (Fytili and Zabaniotou, 2008). Currently, the beneficial use of waste sludge in agriculture has gained higher importance as the waste sludge volumes generated from wastewater treatment increased considerably (Figure 2.2).

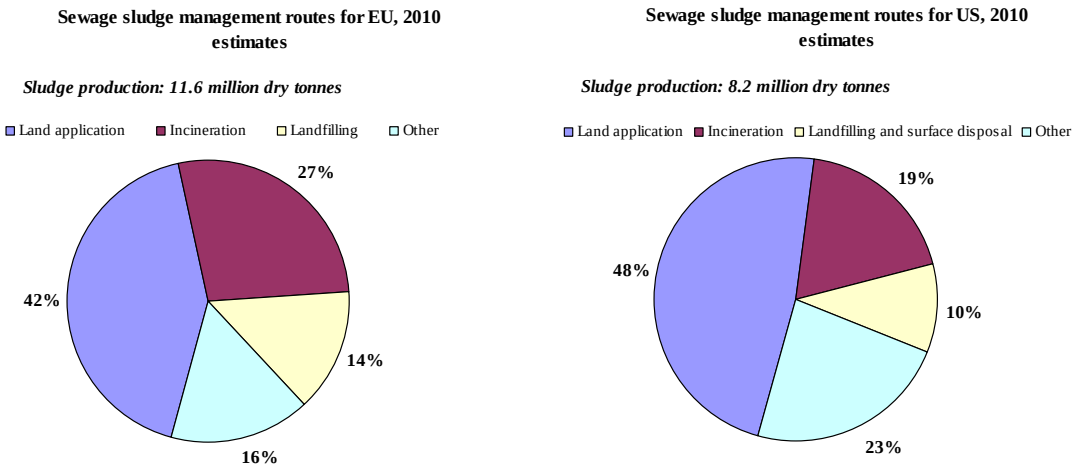


Figure 2.2. Estimates of sewage sludge production and management routes for 2010. (adapted from Tyagi et al., 2009; Milieu/WRc/RPA, 2008)

Sludge management in Turkey is mainly carried out by landfilling and land-application of biosolids. Incineration is not very common and the only large-scale incineration plant is handling industrial and hazardous sludge, while currently there is no incineration plant for the disposal of domestic and municipal sludge (Filibeli and Ayol, 2007; TUIK, 2007).

2.5.3. Application of Chemical Oxidation Processes for Sludge Treatment

2.5.3.1. Ozonation of Sewage Sludge. Among several treatment technologies applied to sludge, ozonation has gained acceptance as one of the most effective sludge disintegration technologies and has been utilized successfully in full-scale applications (Chu et al., 2009). Application of ozone at the recycle line of the activated sludge treatment process can reduce sludge generation up to 70%, which requires high ozone dose e.g 50 mg/g volatile suspended solids (VSS) (Deleris et al., 2002). Furthermore, lower dose of ozone can improve the biodegradability of sludge in a subsequent biological digestion process to a great extent (Bourgier et al., 2007; Yeom et al., 2002). The benefits of ozonation in sludge treatment are not limited with these applications. Ozonation has also been utilized for sludge stabilization and conditioning (Park et al., 2003). Review of literature covering the application of ozone for sludge treatment is given in Table 2.11.

Table 2.11. Applications of ozone for sludge treatment.

Source of sludge	Solid content of sludge	Applied ozone dose	Results	Reference
Secondary sewage sludge	0.2 g/L–2 g/L, TS	8−9 g O_3/g SS	40−90% oxidation of organic carbon, >50% of soluble carbon is biodegradable, >95% of organic nitrogen mineralization to ammonia	Deleris et al., 2000
Mixed sewage sludge	~9.6 g/L, SS	0.05−0.2 g O_3/g COD	38% oxidation of organic carbon, 29% of organic carbon solubilization, 1.8-fold enhancement in methane production in subsequent digestion	Weemaes et al., 2000
Secondary sewage sludge	2.1 g/L, VSS	0.01−0.03 g O_3/g SS	50% sludge volume reduction	Huysmans et al., 2001
Secondary sewage sludge	2 g/L, SS	0.05 g O_3/g VSS	70% sludge volume reduction, improvement in subsequent nitrification efficiency, improvement in sludge settling characteristics and reduction in effluent SS	Deleris et al., 2002
Secondary sewage sludge	12 g/L, TS	0.02−5 g O_3/g SS	2−3 fold enhancement in aerobic and anaerobic biodegradability with 0.1g O_3/g SS, decrease of solubilization above 0.5 g O_3/g SS	Yeom et al., 2002
Secondary sewage sludge	2 g/L, TS	10 mg O_3/min	Preferential attack of ozone on soluble material and influence of sludge particle size	Cesbron et al., 2003
Secondary sewage sludge	8−12 g/L, SS	0.1–5 g O_3/g DS	70% mass reduction and 85% volume reduction of sludge with 0.5 g O_3/g DS, improvement in settleability and dewaterability, improvement in filterability above 0.2 g O_3/g DS	Park et al., 2003
Mixed sewage sludge	12.9 g/L , TS	0–5 g O_3/L	50% improvement of PAH removal by ozonation of digested sludge up to 1.5 g O_3/L TS (0.12 g O_3/g TS), up to 81% removal with addition of peroxide or surfactants	Bernal-Martinez et al., 2005
Secondary sewage sludge	3−5 g/L, SS	0.05 g O_3/g SS	Zero sludge production	Lee et al., 2005
Secondary sewage sludge	1.2−4 g/L, SS	0.19–0.58 g O_3/g SS	Phosphorus solubilization, biomass inactivation, 30% solubilization of organic particulate carbon at 0.03 g O_3/g SS	Saktaywin et al., 2005

Table 2.11 Applications of ozone for sludge treatment (cont.).

Source of sludge	Solid content of sludge	Applied ozone dose	Results	Reference
Secondary sewage sludge	20 g/L, TS	0.1–0.16 g O_3/g TS	15% improvement in sludge biodegradability, 1.25 fold enhancement in biogas production	Bourgrier et al., 2006
Digested sludge	12.9 g/L, TS	0.5-4 gO_3/L	Partial degradation of PAHs by ozonation. Decrease in removal at higher ozone doses due to competitive influence of solubilized organic matter. Parameters most influential in PAH removal found as water solubility and number of 5-carbon rings.	Carrere et al., 2006
Mixed sewage sludge	15.6 g/L, TS	0.1 g O_3/g TS	Ozonation improved PAH removal during AD. Greater improvement was observed for PAH with higher water solubility. Surfactant addition improved PAH degradation, but negatively influenced the anaerobic process	Bernal-Martinez et al., 2007
Secondary sewage sludge	16.1 g/L, SS	0.015−0.200 g O_3/g TS	Optimal biogas production found at an ozone dose of 0.15 g O_3/g TS (144% increase in specific production)	Bourgier et al., 2007
Mixed sewage sludge	35−110 g/L, TS	0.02 g O_3/g SS	PPCP degradation rate in the range of 20−80% was achieved, depending on specific compound; 60% COD solubilization and increased biogas production	Carballa et al., 2007
Secondary sewage sludge	–	0.006–0.030 g O_3/g SS	Improved sludge solubilization, 60% improvement in denitrification	Dytczak et al., 2007
Secondary sewage sludge	1.8 g/L, SS	0.007–0.033 g O_3/g SS	Stronger floc structure, more resistant to ozone after prolonged ozonation, anticipated increase in required ozone doses and costs in the long term	Dytczak and Oleszkiewicz., 2008
Secondary sewage sludge	3.5−5.0 g/L, SS	0.06–0.16 g O_3/ g SS	Compared to bubble contractor, 30% higher microorganism inactivation, > 2 fold higher COD and TN solubilization, 8 fold higher TP solubilization. >99% ozone utilization efficiency	Chu et al., 2008
Secondary sewage sludge	4.18 g/L, SS	0.010–0.035 g O_3/g SS	Organic matter solubilization increased up to a maximum and did not improve Further; ammonia solubilization increased above a threshold ozone dosage; sludge solid content influenced solubilization only at low ozone dosages	Manterola et al., 2008
Mixed sewage sludge	35−110 g/L, TS	0.02 g O_3/g SS	Improved pathogen reduction and LAS removal with combined ozonation pretreatment and AD	Carballa et al., 2009

Table 2.11 Applications of ozone for sludge treatment (cont.).

Source of sludge	Solid content of sludge	Applied ozone dose	Results	Reference
Secondary sewage sludge	4.6 g/L, SS	0.005–0.02 g O_3/g SS	60% decrease in intracellular ATP at 0.02 g O_3/g TS, floc destruction and decrease in enzymatic activity followed by decrease in cell viability, enrichment of most metals released from flocs in micro-solids	Chu et al., 2009
Secondary sewage sludge	3.2−5.0 g/L, SS	0.03–0.27 g O_3/g SS	Below 0.02 g O_3/g TSS, the main mechanism was sludge solubilization, while higher doses influenced the microorganism population types in the reactor leading to the growth of predators. Decline in disintegration above 0.1 g O_3/g TSS, decline in oxidation due to increase in radical scavengers above 0.14 g O_3/g TSS	Yan et al., 2009
Secondary sewage sludge	10 g/L, TS	0.05–1 g O_3/g TS 5–20 mg metal catalyst/g TS	2-fold improvement in sludge reduction with Mn catalyst (10 mg/g TS)	Lee et al., 2010a
Digested sludge	2.04 %, TS	0.1 g O_3/kg TS	11.3 % improvement in VSS reduction of ozonated sludge during digestion	Erden et al., 2010
Secondary sewage sludge	14.26 g/L, TS	0.1 g O_3/g TS	Highest TSS removal and reduction in required specific energy was obtained by ozone and ultrasound pretreatment, 15% sludge volume reduction was obtained with ozone	Salsabil et al., 2010
Secondary sewage sludge	1.87−8.53 g/L, SS	0.120–0.175 gO_3/g SS	Negative influence of high iron content in sludge on solubilization (50% decrease at 80−120 mg Fe/g SS), release of phosphorus and metals by ozonation. Release of Cu, Ni and Zn is correlated with COD solubilization	Sui et al., 2011
Secondary sewage sludge	3.1−3.5 g/L, SS	0.01 g O_3/g SS With 1040 kPA pressure application	Much improved sludge solubilization with ozonation in pressure cycles, compared to bubble contractor system. 37-fold increase in SCOD/TCOD and 25% reduction in TSS; solubilization of phosphorus, nitrogen and protein	Cheng et al., 2012

Investigation of ozonation technology for the removal of emerging organic micro-pollutants from sludge has considerably increased in the last years. Gupta et al. (2013) reported 23% removal of adsorbable organic halides and 26% removal of extractable organic halides. Qiang et al (2013) reported the removal of several endocrine disruptor compounds in sludge with ozonation; among the studied compounds, the strongly hydrophobic nonylphenol was resistant to degradation both during ozonation at high pH and with combined application of O_3/H_2O_2. Bernal-Martinez et al. (2007) reported improved removal of PAH during AD of ozone pretreated sludge. Ozonation was more effective on PAH with higher solubility in sludge. Addition of surfactant improved PAH removal, but negatively influenced the efficiency of the anaerobic process. Carrere et al., (2006) also reported that removal of PAH during sludge ozonation was dominated by their solubility. However, as stated previously investigation of sludge ozonation on the removal of sorbed antibiotics in sludge has not been reported.

2.5.3.2. Combined Microwave and Oxidative Treatment of Sewage Sludge. Among promising sludge treatment methods, microwave (MW) irradiation, which is basically electromagnetic radiation in the microwave frequency range, has gained interest as a relatively new technology for sludge solubilization. Due to its water content, sludge can absorb MW energy. The advantage of MW compared to conventional heating is that it is less energy and time consuming (Jones et al., 2002). During MW treatment, hot-spot regions can be formed in the sludge as a result of non-homogeneous temperature distribution (Kington and Jassie, 1988). MW irradiation can induce solubilization of nutrients and enhance the hydrolysis step in a subsequent digestion process of waste sludge. Solubilization of sludge mainly depends on the irradiation temperature in MW treatment. Toreci et al. (2009a) reported that MW irradiation at temperatures as high as 175°C resulted in the highest biogas production enhancement. However, at temperatures above 180°C, the recalcitrant products that can form as a result of Maillard reaction can induce a negative influence on a subsequent AD process (Pinnekamp, 1989). MW treatment can also provide improved pathogen reduction especially above 85°C. When combined with a subsequent AD process, exceptional quality (Class A) biosolids with no detectible pathogen levels can be produced (Hong et al, 2006).

Combined application of MW irradiation and chemical oxidation was also used to enhance the solubilization of waste sludge and to improve the sludge biodegradability for a subsequent AD process. The most frequently applied oxidant was hydrogen peroxide. Although the addition of peroxide to WAS during MW pretreatment was shown to considerably increase the release of soluble COD (SCOD), PO_4^{3-} (orthophosphate), nitrate (NH_3), and volatile fatty acid (VFA), generally a negative influence on biogas production during AD of pretreated waste sewage sludge was reported even at H_2O_2 doses as low as 1 g H_2O_2/g TS (Eskicioglu et al., 2008b) due to production of recalcitrant products. Literature for MW pretreatment and combined thermo-oxidation treatment of sludge with MW and chemical oxidants are listed in Table 2.12 and Table 2.13, respectively. Currently there are no studies investigating the fate of micro-pollutants in sludge during MW treatment.

Table 2.12. Applications of MW for sludge treatment.

Source of sludge	Solid content of sludge	Process conditions	Results	Reference
Secondary sewage sludge	30.07 g/L, TS	P= 700 W T=59 –100°C TT=3–15 min	Improved biogas production, improved VSS reduction, and decrease of AD HRT from 15 to 8 days as a result of MW pretreatment of sludge.	Park et al., 2004
Secondary sewage sludge	0.36−0.39 %, TS	P=1000 W T=60−170°C TT= 5 min	76% of the TP released in 5 min by MW treatment. Sludge stabilization took place above 100°C.	Liao et al., 2005a
Primary, secondary, mixed, and digested sewage sludge	4.5 %, TS (primary and mixed) 3.3 % (digested)	P=550 W TT=0.5−2	Improvement in dewaterability of sludge (as measured by SRF, CST, and dry matter content in sludge dewatered in laboratory centrifuge). Solubilization of nutrients, COD, and BOD_5. Athermal effect of MW observed along with thermal effect. Influence of sludge type on dewaterability observed.	Wojciechowska, 2005
Primary, secondary, and digested sewage sludge	29.8 g/L (PS) 41.3 g/L (WAS)	P=425 W (WAS) 576.6 (PS) TT=0.5−1.5 min	Highest solubilization at 70°C was obtained for WAS (125% SCOD increase). Better performance of digester in fecal coliform inactivation observed with MW pre-treated sludge and Class A sludge could be generated.	Hong et al., 2006
Secondary sewage sludge	5.9 %, TS	T=96°C TT=5 min	Greater WAS solubilization achieved by conventional heating due to longer exposure to reach the target temperature. Two-fold higher biogas production obtained with WAS pretreated conventionally. Slower digestion of higher molecular weight fractions by microorganisms observed.	Eskicioglu et al., 2006
Secondary sewage sludge	38−49 g/L, TS	P= 400 W TT= 4–10 min PP=800 W TT=3–5 min	MW did not influence biogas production in thermophilic AD. Only low temperature (70°C) CH had positive influence on biogas production.	Climent et al., 2007

Table 2.12. Applications of MW for sludge treatment (cont.).

Source of sludge	Solid content of sludge	Process conditions	Results	Reference
Secondary sewage sludge	1.4 and 5.4 %, TS	P=400 W T=50–96°C TT=0.5–5 min	3.2–3.6 fold increase in SCOD/TCOD by MW irradiation. Highest biogas production observed with WAS treated at 96°C. Short-term inhibition of MW on digestion was found. Improved dewaterability of pretreated WAS after AD.	Eskicioglu et al., 2007a,b
Secondary sewage sludge	4.6−5.5 %, TS	F=2,450 MHz P=1,250 W T=50–96°C	Athermal effects of MW were found to be minimal for WAS solubilization. 2.7 fold SCOD/TCOD increase was obtained by MW pretreatment at 96°C. Non-acclimated inoculum was negatively influenced by MW pretreatment.	Eskicioglu et al., 2007b
Primary, secondary sewage sludge, and biocake	1.4−30 %, TS	P=1,460 W T=20–90°C P=1,250 W T=125–175°C	Sludge solubilization increased with sludge TS content and decreased with rate of heating. Pretreated sludge type influenced the anaerobic biodegradability. Higher sludge age was suspected to decrease solubilization.	Eskicioglu et al., 2008a
Secondary sewage sludge	4.6 %, TS	P= 1,250 W T=50–175°C	COD solubilization and biogas production increased with MW pretreatment temperature. 31% higher biogas production in digestion utilizing acclimated inoculum was achieved with sludge pretreated at 175°C.	Eskicioglu et al., 2009
Secondary sewage sludge	25.7 g/L, TS	P=700 W T=59°C−100°C TT=3–15 min	SCOD, proteins, lipids and carbohydrate were released by sludge solubilization. Treated sludge SCOD/TCOD reached 22%. Treatment time between 5-15 min did not influence the biochemical acidogenic potential.	Ahn et al., 2009
Secondary sewage sludge	4−5 %, TS	P=1200 W T=175°C TT=40–120 min	SCOD/TCOD reached 25% and 30% at 175°C and at heating rates of 1.25 and 3.25 °C/min, respectively. However lower heating rate had negative influence on biogas production in the early stages of AD. Better performance for single stage digesters was observed compared to two-stage digesters with MW pretreated WAS.	Toreci et al., 2009a

Table 2.12. Applications of MW for sludge treatment (cont.).

Source of sludge	Solid content of sludge	Process conditions	Results	Reference
Secondary sewage sludge	5.6 %, TS	P=1200 W T=175°C TT=40–120 min	Lower heating rate did not significantly increase SCOD release; however molecular weight distribution of soluble fractions were influenced significantly. High temperature MW treatment had positive effect on biodegradability. Athermal effect of MW was observed at lower temperature.	Toreci et al., 2009b
Primary sewage sludge	1−4 %, TS	P=1460 W T=35−90°C	Higher sludge solubilization was achieved at higher applied MW temperature and at higher sludge TS, however ultimate biodegradability of PS was not influenced. No influence of heating intensity was observed.	Zheng et al., 2009
Secondary sewage sludge	1−3 %, TS	P= 400−1660 W, T=60−120°C	Significant influence of MW output power, MW target temperature and TS concentration on WAS solubilization was observed.	Park et al., 2010
Secondary sewage sludge	6.0 %–11.9, TS	P=1200 W T=110−175°C TT=40–120 min	Higher solubilization at higher TS was observed with MW pretreatment above the boiling temperature. The difference became more pronounced when lower heating rate was applied. Higher release of proteins and sugar was observed at lower heating rate, while increase in WAS TS negatively influenced the release of these components. VFA release was greatly improved at high temperature MW pretreatment.	Toreci et al., 2010a
Secondary sewage sludge	5 %, TS	P=1200 W T=110−175°C TT=40–120 min	Low influence of WAS TS and heating rate on dewaterability after MW pretreatment was observed, while temperature had more significant influence. Higher effect of acute inhibition of anaerobic inoculum was observed for pretreated high TS.	Toreci et al., 2010b
Secondary sewage sludge	1.44%, TS	P=500−900 W TT=0.3−2.3 min	At high temperatures and long treatment times the settling of WAS was negatively influenced. Turbidity increased after MW pretreatment. Solubilization of COD, nitrogen, polysaccharides, and proteins was observed with increased temperature and contact time.	Yu et al., 2010a

Table 2.13. Applications of combined MW and chemical oxidation processes for sludge treatment.

Source of sludge	Solid content of sludge	Process conditions	Oxidant dose	Results	Reference
Secondary sewage sludge	0.61−0.64 %, TS	T=60−170°C RT=2−15 min TT=5 min	2.6−2.7 g H_2O_2/g TS	>84% of TP released at 170°C, 5 min treatment	Liao et al., 2005b
Secondary sewage sludge	0.35−0.40 %, TS	T=60−120°C RT=2−5 min T=5 min	3.1 and 6.3 g H_2O_2/g TS	Increase in PO_4^{3-} release >80°C, increase in nutrient solubilization with peroxide dose. ~100% of COD in soluble form after treatment at 80°C, for 5 min with 0.66 M H_2O_2, no significant effect on metal solubilization	Wong et al., 2006a and 2006b
Secondary sewage sludge	0.35−0.40 %, TS	T=60−200°C TT=5−20 min	3.1 and 6.3 g H_2O_2/g TS	Most significant factors for PO_4^{3-} and NH_3 release are temperature and H_2O_2 addition, respectively. H_2O_2 and acid addition improves nutrient solubilization; increases COD solubilization during treatment up to 80°C	Wong et al., 2007, Chan et al., 2007
Secondary sewage sludge	6.4 %, TS	T=60−120°C RT= 3−5 min TT=5 min	1 g H_2O_2/g TS	MW combined with H_2O_2, resulted in sludge oxidation, increased sludge disintegration; methane production was negatively influenced by H_2O_2 addition	Eskicioglu et al., 2008b
Secondary sewage sludge	1 %, TS	T=40−80°C RT=2−4 TT=3 min	3.3 g H_2O_2/g TS, $FeSO_4/H_2O_2$=0.4 (w/w)	In general, addition of ferrous iron was not advantageous in means of COD and nutrient solubilization, while release of VFA improved considerably above 60°C	Lo et al., 2008
Secondary sewage sludge	0.51–2.30 %, TS	T=80−120°C TT=1.5−5 min	0.5 – 4.7 g H_2O_2/g TS	Maximum sludge disintegration achieved at the highest TS and with addition of H_2O_2	Yin et al., 2008a
Secondary sewage sludge	2.63 %, TS	T=60−120°C TT=3 min	1.08–5.28 mg O_3/mL; 0.38 and 0.76 g H_2O_2/g TS	Pre-ozonation of sludge improved sludge solubilization during the MW + H_2O_2 process	Yin et al., 2008b

Table 2.13. Applications of combined MW and chemical oxidation processes for sludge treatment (cont.).

Source of sludge	Solid content of sludge	Process conditions	Oxidant dose	Results	Reference
Secondary sewage sludge	1.2–2.8 %, TS	T=60−120°C RT=3−6 min TT=5 min	0.54 – 1.14 g H_2O_2/g TS	Among investigated parameters, mixing of sludge was least influential on sludge solubilization. Most influential factors were temperature and H_2O_2 dose. Mixing had negative influence on NH_3 and VFA release at high temperatures.	Kenge et al., 2009
Secondary sewage sludge	15 g/L, SS	T=15−100°C	0.5 – 4.0 g H_2O_2/g TOC	Loss of catalase activity above 45°C. Dosing of H_2O_2 performed to preheated WAS to decrease H_2O_2 decomposition by catalase. 282.5% SCOD release achieved at 4 g H_2O_2/TOC. Optimum oxidant dose was found as 0.1–1.0 g H_2O_2/TOC.	Wang et al., 2009
Secondary sewage sludge	0.32 %, TS	T=55°C–70°C, RT=0.3−3.5 min TT= 5 min	0.06 – 0.31 g H_2O_2/g TS	Complete destruction of fecal coliforms at 70°C and >0.08% H_2O_2. Better sludge solubilization was observed at higher temperature.	Yu et al., 2010b
Secondary sewage sludge and EPS-free sludge	0.37–0.41 %, TS	T=60−120°C	0.1 – 0.5 % H_2O_2 (w/w)	Higher release of PO_4^{3-} observed from extracted sludge. Carbohydrate release increased up to a certain H_2O_2 dose and then decreased at higher doses.	Yu et al., 2010c

2.5.3.3. Persulfate Treatment of Sewage Sludge. Among chemical oxidation processes applied for sewage sludge treatment, persulfate oxidation is a relatively novel process. Although persulfate oxidation has been accepted as an effective technology for the in-situ chemical oxidation (ISCO) treatment of contaminated soil and ground water (Siegrist et al., 2011), for the sludge only three recent studies have been published by Zhen et al. (2012a, b, c). In these studies, the authors presented the enhanced dewaterability of sludge treated with ferrous iron activated persulfate.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals, Antibiotics, and Radical Probe Compounds

All chemicals, antibiotics, and the radical probe compounds that were utilized throughout the study are listed in Table 3.1.

3.1.2. Bacterial Strain

The bacterial strain that was utilized for plasmid DNA isolation is an ampicillin resistant *E. coli* HB101 and was obtained from the Pasteur Institute (France). Bacterial stock glycerol solutions were stored at -80°C till use.

3.1.3. Secondary Sewage Sludge Samples

The secondary sewage sludge was obtained from a municipal wastewater treatment plant located in Pasakoy-Istanbul/Turkey that receives wastewaters from the Omerli reservoir catchment. The plant has a capacity to treat wastewater of 500,000 population equivalent with an average flow of 100,000 m^3/d and adopts an advanced biological treatment with an A2/O (anaerobic-anoxic-oxic) process for carbon, nitrogen, and phosphorus removal. The resulting effluent is discharged to the Riva River. The sludge age in the treatment plant is about 20 days.

Sludge characteristics were evaluated at different sampling campaigns was carried during the course of the study: Sampling Campaign one –SC-1 (December 2010-June 2011); Sampling Campaign two –SC-2 (October, 2012), and Sampling Campaign three –SC-3 (March, 2013) (Sections 5, 6, and 7. Before use, the sludge was concentrated up to 25±5 g/L TS by settling for 20 h followed by subsequent decantation of the top liquid layer. The sludge was stored at 4ºC and utilized within no longer than 1 week of collection.

Table 3.1. List of chemicals used in the experiments.

Chemical Name	Chemical Formula	CAS Number	Experimental Use	Supplier Company
Oxytetracycline-HCl	$C_{22}H_{24}N_2O_9HCl$	2058-46-0	Model antibiotic	Sigma
Ciprofloxacin-HCl	$C_{17}H_{18}FN_3O_3HCl$	86393-32-0	Model antibiotic	Mp. Biomedicals
Para-chlorobenzoic acid (*p*CBA)	$C_7H_5ClO_2$	74-11-3	Radical probe	Aldrich
Anisole	$CH_3OC_6H_5$	100-66-3	Radical probe	Fluka
Tertiary-butanol	$C_4H_{10}O$	75-65-0	Radical scavenger	Sigma-Aldrich
Ethanol	CH_3CH_2OH	64-17-5	Radical scavenger	Sigma-Aldrich
Sodium hypochlorite solution	NaClO	7681-52-9	Chlorine treatment	Sigma-Aldrich
Titanium dioxide	TiO_2	13463-67-7	Heterogeneous photocatalyst	Evonik
Hydrogen peroxide (30%)	H_2O_2	7722-84-1	Hydrogen peroxide treatment	Sigma-Aldrich
Sodium persulfate	$Na_2S_2O_8$	7775-27-1	Persulfate treatment	Sigma-Aldrich
Ferrous sulfate	$FeSO_47H_2O$	7782-63-0	Persulfate treatment	Sigma-Aldrich
Ferric sulfate	$Fe_2(SO_4)_3xH_2O$	15244-10-7	Persulfate treatment	Sigma-Aldrich
Ampicillin	$C_{16}H_{19}N_3O_4S3H_2O$	7177-48-2	Bacteria growth	Sigma-Aldrich
Casein Enzyme Hydrolysate, T1			Bacteria growth	HIMEDIA
Sodium chloride	NaCl	7647-14-5	Bacteria growth	Riedel-de-Haën
Yeast extract		8013-01-2	Bacteria growth	BioChemica
Potassium dihydrogen phosphate	KH_2PO_4	7778-77-0	Bacteria growth, Ultrasonic extraction, Oxidant analysis	Merck
Disodium hydrogen phosphate	$Na_2HPO_4H_2O$	7558-79-4	Bacteria growth, Ultrasonic extraction, Oxidant analysis	Fisher Scientific
Citric acid monohydrate	$HOC(COOH)(CH_2COOH)_2H_2O$	5949-29-1	Ultrasonic extraction	Merck
Disodium ethylene diaminetetraacetic acid	Na_2EDTA	139-33-3	Ultrasonic extraction	Sigma-Aldrich
85 % Ortho-phosphoric acid	H_3PO_4	7664-38-2	Ultrasonic extraction	Merck
25 % Ammonia solution	NH_3	7664-41-7	Solid phase extraction	Merck
Magnesium nitrate	$Mg(NO_3)_26H_2O$	13446-18-9	Ultrasonic extraction	Sigma-Aldrich
Magnesium sulfate	$MgSO_47H_2O$	7487-88-9	Sludge pretreatment	Sigma-Aldrich

Table 3.1. List of chemicals used in the experiments (cont.)

Chemical Name	Chemical Formula	CAS Number	Experimental Use	Supplier Company
Formic acid	HCOOH	64-18-6	HPLC analysis	Sigma-Aldrich
HPLC grade methanol	CH_3OH	67-56-1	HPLC analysis	Sigma-Aldrich
HPLC grade acetonitrile	CH_3CN	75-05-8	HPLC analysis	Sigma-Aldrich
Potassium iodide	KI	7681-11-0	Oxidant analysis	Sigma
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}4H_2O$	12054-85-2	Oxidant analysis	Aldrich
Sodium thiosulfate	$Na_2S_2O_3$	7772-98-7	Oxidant analysis	Sigma-Aldrich
Potassium indigo trisulphonate	$C_{16}H_7K_3N_2O_{11}S_3$	67627-18-3	Oxidant analysis	Acros Organics
Copper(II) sulfate	$CuSO_45H_2O$	7758-98-7	Oxidant analysis	Riedel-de Haen
2,9-Dimethyl-1,10-phenanthroline	$C_{14}H_{12}N_2$	484-11-7	Oxidant analysis	Aldrich
Free DPD Kit			Oxidant analysis	Hach
Sodium Bicarbonate	$NaHCO_3$	144-55-8	Oxidant analysis	Sigma-Aldrich
Ferrozine	$C_{20}H_{13}N_4NaO_6S_2$	69898-45-9	Iron analysis	Fluka
Ammonium acetate	$CH_3CO_2NH_4$	631-61-8	Iron analysis	Sigma
Hydroxylamine hydrochloride	NH_2OHHCl	5470-11-1	Iron analysis	Sigma-Aldrich
Titanium (IV) isopropoxide	$Ti[OCH(CH_3)_2]_4$	546-68-9	Nano-fiber-TiO_2 preparation	Sigma-Aldrich
Polyvinylpyrrolidone	$(C_6H_9NO)_n$	9003-39-8	Nano- fiber-TiO_2 preparation	ISP
Acetic acid	CH_3CO_2H	64-19-7	Nano- fiber-TiO_2 preparation	Merck
PhosVer 3 Kit			Ortho-phosphate analysis	Hach
Nitrogen-Ammonia Reagent Set			Ammonia and TKN analysis	Hach
NitraVer 6 Kit			Nitrate analysis	Hach
Potassium dichromate	$K_2Cr_2O_7$	7778-50-9	Chemical oxygen demand analysis	Sigma-Aldrich
Sulfuric acid	H_2SO_4	7664-93-9	Chemical oxygen demand analysis	Aldrich
Silver sulfate	Ag_2SO_4	10294-26-5	Chemical oxygen demand analysis	Sigma-Aldrich
Mercury(II) sulfate	$HgSO_4$	7783-35-9	Chemical oxygen demand analysis	Fluka

3.2. Methods

3.2.1. Preparation of Nano-Fiber-TiO_2

The preparation of the nano-fiber-TiO_2 material was performed with a sol-gel electrospinning combined procedure according to Li and Xia (2003) as described schematically in Figure 3.1.

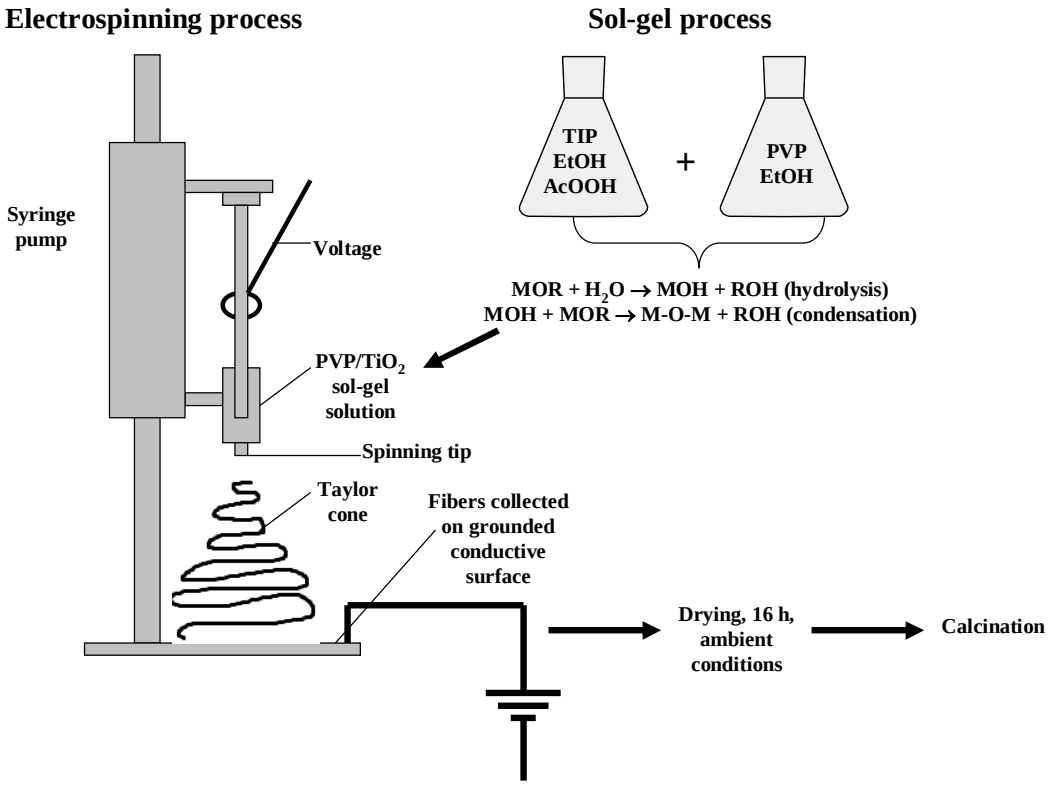


Figure 3.1. Schematic illustration of the sol-gel process and the electrospinning apparatus utilized to generate nano-fiber-TiO_2.

The stages of the procedure performed at room temperature can be listed as follows.

i. An alcohol solution of a metal alkoxide (MOR) is mixed with a polymer solution and a catalyst. The polymer aids as a supporting material during the electrospinning process.

The catalyst controls the hydrolysis reaction to produce metal hydroxide (MOH) and alcohol (ROH):

$$MOR + H_2O \rightarrow MOH + ROH \quad (3.1)$$

ii. A condensation (polymerization) reaction that is allowed to proceed for one hour results in gradual formation of a metal oxide (M-O-M) network (gel) (Brinker and Scherer, 1990):

$$MOH + MOR \rightarrow M\text{-}O\text{-}M + ROH \quad (3.2)$$

Titanium (IV) isopropoxide (TIP) dissolved in ethanol (EtOH) was used as the precursor alkoxide and acetic acid (AcOOH) was used as the catalyst. Polyvinylpyrrolidone (PVP) was used as the supporting polymer for fiber formation by electrospinning.

iii. The sol-gel mixture is electrospinned to produce the nano-fibers (Li and Xia, 2003; 2004). The process constitutes of applying high voltage to the polymer-sol mixture, which is passed trough a syringe at a controlled flow rate. Fibers are formed by the charging of a solution droplet that is generated at the tip of the syringe, which becomes stretched and finally produces a Taylor cone upon eruption. During the process of fiber formation, the organic solution is partially evaporated and the formed fibers are collected on a grounded surface.

iv. The resulting nano-fibers are first dried for at ambient conditions 16 h to complete the polymerization process and further evaporate the organic solvent.

v. The dry material is calcinated to remove the polymer, leaving back fibers consisting of only metal-oxide network.

3.2.2. Plasmid DNA Isolation and Chemical Oxidation

Plasmid DNA that was utilized in the experiments described in Section 4 was isolated from ampicillin resistant *E. Coli* strain according the procedure described schematically in Figure 3.2.

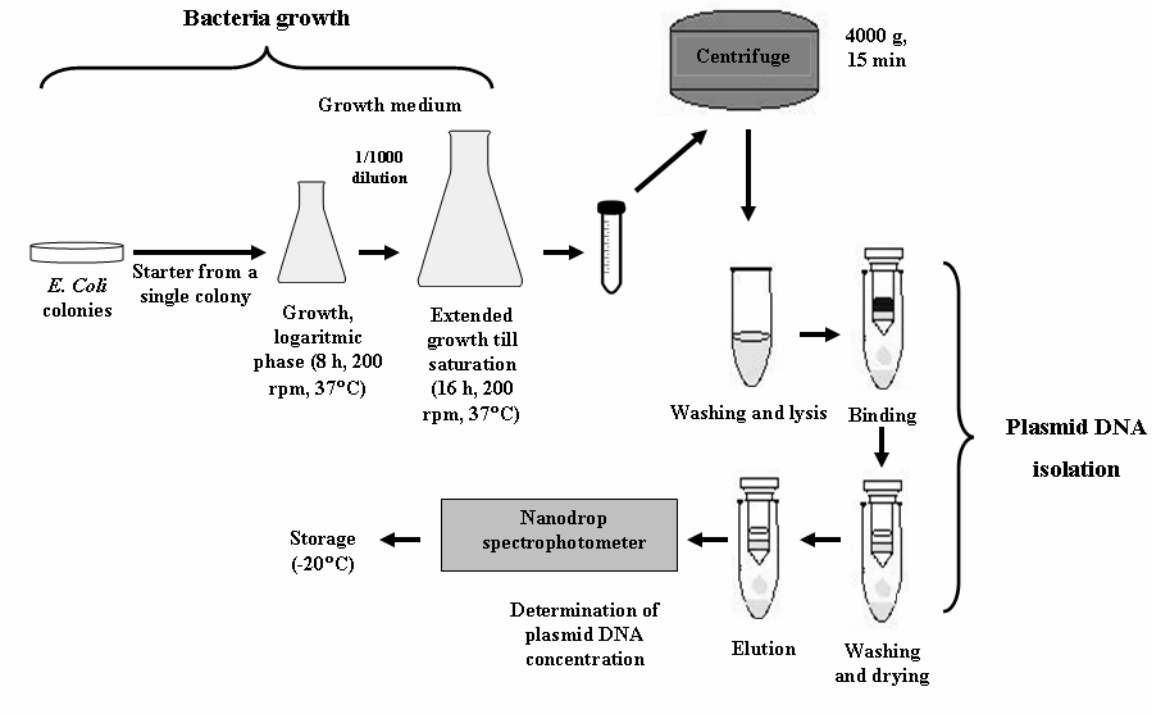


Figure 3.2. Isolation of plasmid DNA.

The isolated plasmid DNA was treated with three different oxidation processes the details of which are illustrated in Figure 3.3.

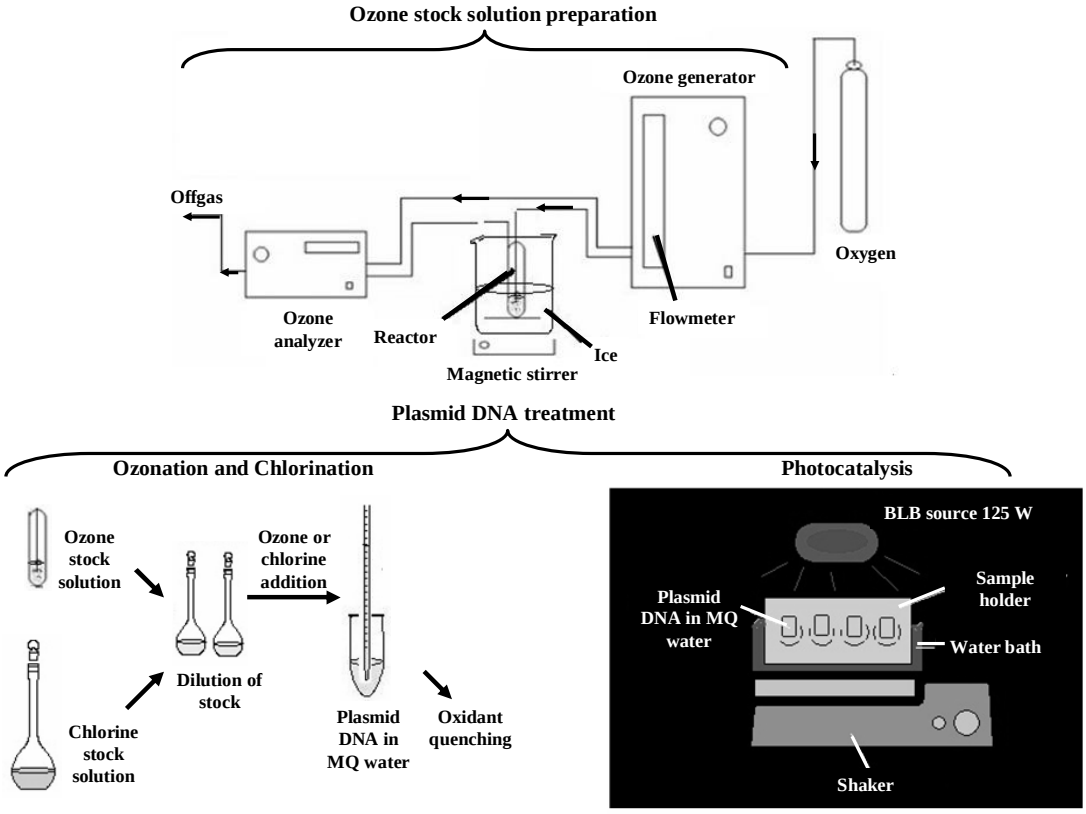
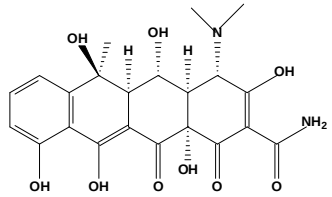
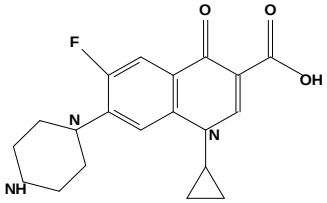


Figure 3.3. Schematic illustration of plasmid DNA treatment by ozonation, chlorination, and TiO_2 photocatalysis.

3.2.3. Contamination of Secondary Sewage Sludge with Model Antibiotics

Two model antibiotics, OTC and CIP were utilized in the study and their molecular structures and some physicochemical properties are given in Table 3.2.

Table 3.2. Physicochemical properties of OTC and CIP.

Antibiotic	**log K_d**	**Molecular Weight (g/mol)**	**Water solubility (mg/L)**	**pK_a**
Oxytetracycline (OTC) 	3.48 (Stuer-Lauridsen et al., 2000)	460.4	1000 (Sarmah et al., 2006)	3.2, 7.5, 8.9 (Qiang and Adams, 2004)
Ciprofloxacin (CIP) 	4.3 (Golet et al., 2003)	331.4	n.a.*	3.0, 6.1, 8.7, 10.6 (Qiang and Adams, 2004)

* n.a.: not-available

Appropriate amounts from aqueous antibiotic stock solutions of 62.5 mg/L and 2.5 mg/L were added to concentrated sludge two provide two different antibiotic concentrations of 20 mg/L and 0.8 mg/L, respectively and were simultaneously used to adjust the sludge TS to the desired value. The contaminated sludge was equilibrated over-night (16±1 h) at 25±5 ºC in a temperature controlled water bath at 200 rpm.

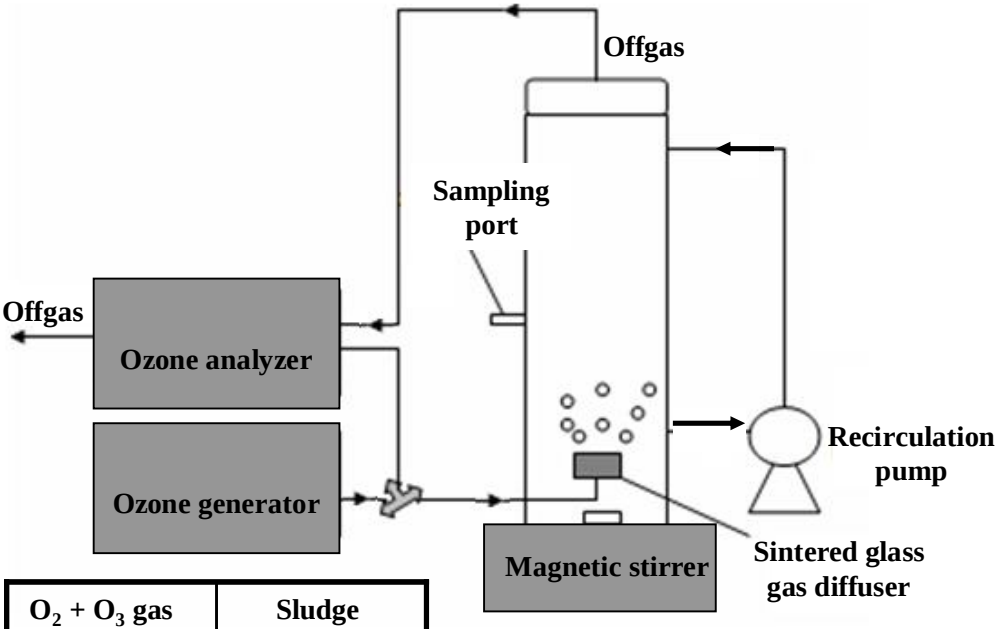
3.2.4. Pretreatment of Secondary Sewage Sludge

Antibiotic spiked waste sewage sludge was pretreated prior ozonation experiments by the addition of appropriate volumes of 1M $MgSO_4$ stock solution to provide 0.91 g/L (75.2 meq/L) Mg^{2+}, followed by adjustment of sludge pH to 8 and equilibration for one h at 25±5 ºC in a temperature controlled water bath at 200 rpm.

3.2.5. Chemical Oxidation of Secondary Sewage Sludge

3.2.5.1. Ozonation. Ozonation of sludge was carried out in a 2 L cylindrical glass column reactor (Φ = 6 cm, H = 78 cm). Ozone was produced by a laboratory scale ozone generator (Fisher OZ 500) from dry and pure oxygen and was continuously supplied through a sintered

glass gas diffuser located at the bottom of the reactor. The sludge in the reactor was mixed by recycling the sludge at a rate of 0.30 L/min (countercurrent to gas flow) and also with the aid of a magnetic stirrer (100 rpm). Schematic illustration of the reactor is given in Figure 3.4.



$O_2 + O_3$ gas flow rate	Sludge recirculation rate
1.83 L/min	0.3 L/min

Figure 3.4. Schematic illustration of sewage sludge treatment by ozonation.

3.2.5.2. MW/H_2O_2 and $MW/S_2O_8^{2-}$. Microwave (MW) irradiation was used to assist chemical oxidation. MW treatment of sewage sludge was performed with a bench scale microwave irradiation system (Berghof, Speedwave MWS-3, 2.54 GHz). Either hydrogen peroxide or sodium persulfate was added to the sludge to assist the MW treatment. A total of 27.5 mL sludge was treated in closed TFM vessels each with a volume of 60 mL.

3.2.5.3. $Fe^{2+}/Heat/S_2O_8^{2-}$. Activation persulfate oxidation by ferrous iron and conventional heating for the treatment of sewage sludge having a total volume of 27.5 mL was performed in closed TFE tubes of 50 mL capacity. The reactions were initiated by successive addition

of ferrous iron and sodium persulfate. Heating and mixing of sludge samples was provided in a temperature controlled water bath at 200 rpm.

3.2.6. Analytical Methods

3.2.6.1. Characterization of Nano-Fiber-TiO_2. Characterization of the nano-fiber-TiO_2 material was performed with the methods listed in Table 3.3.

Table 3.3. Analytical methods used for nano-fiber-TiO_2 characterization.

Test	Analysis Method	Instrument Model/Company
Fiber morphology	Scanning Electron Microscopy (SEM)	Supra Gemini 35 VP Field Emission SEM/ Leo
Mineral structure	X – Ray Diffraction (XRD)	D8 Advance Type/ Bruker–AXS
Surface area and pore size	Brunauer–Emmett–Teller (BET)	Nova 2200e/ Quantachrome
Thermal analysis	Thermogravimetric analysis (TGA)	STA449C/Netzch

3.2.6.2. Characterization of Secondary Sewage Sludge Samples. Assessment of sludge solid content (total solids – TS, suspended solids – SS, and volatile solids – VS), chemical oxygen demand (SCOD and total COD – TCOD) sludge nutrient components and dewatering related properties (settling and filterability) was performed according to the standard methods (APHA, 2005). The soluble components of the sludge were determined after filtering the centrifuged samples (5,000 *g* for 10 minutes; Eppendorf Centrifuge 5804) through 0.45 μm filter (Sartorius, Germany). In order to determine TCOD, prior to COD analysis homogenized sludge samples were digested with 0.5 M NaOH by the aid of mixing at 150 rpm for 24 h (Tiehm et al., 2001). Sludge settleability was studied by measuring the sludge volume after settling sludge with a total volume of 1 L in an Imhoff cone for 60 min (SV_{60}) and was expressed as the percent fraction of the total volume. Sludge dewaterability was assessed with capillary suction time (CST). TP (total phosphorus), PO_4^{3-}, TKN (total kjeldahl nitrogen), NH_3, and NH_4^+ (ammonium) were assessed spectrophotometrically with analytical test kits (Hach Company) according to the procedures of the manufacturer, utilizing the ascorbic acid method for TP and PO_4^{3-} analysis, the cadmium reduction method for NH_3

analysis and the Nessler method for TKN and NH_4^+ analysis, respectively. Prior analysis of TKN and TP, sludge was acid-digested with the aid of a Digestion Apparatus (Digesdahl, Hach) according to the procedures of the manufacturer. Total metal concentrations in the sludge were analyzed after acid digestion of the raw sludge in the MW system according to the EPA Method 3052 (US-EPA, 1995) and the metals released in the supernatant of the treated samples were detected by ICP-OES (Perkin-Elmer Optima 2100 DV). pH was measured in filtered sludge samples with a pH probe (WTW pH 330 pH meter, Weilheim, Germany).

3.2.6.3. Oxidant Analysis. Chlorine was determined by Free DPD Kit purchased from Hach Company.

The ozone concentration in the stock solution prepared for plasmid DNA treatment was determined with the indigo trisulphonate (Acros Organics) spectroscopic method (APHA, 2005). For this purpose, indigo stock solution was prepared by dissolving 770 mg potassium indigo trisulphonate in a 1 L volumetric flask containing 500 mL distilled water and 1 mL concentrated phosphoric acid, and then by diluting with distilled water to the volume. The stock solution was stored in dark for no more than 4 months. Indigo reagent II was prepared by adding 100 mL stock indigo solution, 10 g NaH_2PO_4, and 7 mL concentrated phosphoric acid to a 1 L volumetric flask and diluting with distilled water to the volume. The reagent was stored in the dark for no more than one week and discarded when the absorbance of a 1/100 dilution fell below 0.16/ cm at 600 nm. To measure residual ozone concentrations, 10 mL of indigo reagent II and appropriate amounts of samples were measured into 100 mL volumetric flasks and diluted to the volume with distilled water. Then the absorbance at 600 nm was measured as soon as possible. The ozone concentration in the samples was calculated according to the following equation:

$$O_3\ (mg/L) = (100 \times \Delta A)/(f \times b \times V) \qquad (3.1)$$

where, ΔA is difference in absorbance between sample and blank (prepared by diluting 10 mL of indigo reagent II to 100 mL in a volumetric flask), *b* is the path length of the cell (1 cm), *V* is the sample volume in mL, and the factor *f*, which is based on a sensitivity factor of 20 000/cm for the change of absorbance (600 nm) per mole of added ozone per liter, is 0.42.

Absorbed ozone concentrations during sewage sludge treatment were calculated by the difference in concentrations in the feed and off gas, which were measured with a UV ozone analyzer (Fisher Ozotron 23) in time intervals of 30 s:

$$O_{3_{abs}}\ (mg\ O_3 / g\ TS) = \frac{O_3\ flow\ rate\ (L/\min) \times \int([O_3]_{in} - [O_3]_{out}\ (mg/L)\ dt(\min)}{initial\ TS\ (g/L) \times Sludge\ volume\ (L)} \qquad (3.2)$$

Hydrogen peroxide was determined both by the modified iodometric method of Kolthoff and Carr (1953) in the presence of molybdate catalyst and by the photometric method of Kosaka et al. (1998) for much sensitive analysis of low concentrations. The iodometric method consisted of weighting a sample into a 500 mL flask. Then adding 200 mL of deionized water, 20 mL of potassium iodide solution (10%; w/v), and 25 mL of acid mixture (0.18 g of ammonium molybdate, 300 mL sulfuric acid, and 700 mL deionized water), respectively. The flask was stopperred and allowed to stand for 5 min. The mixture was titrated with 0.1 N or 0.01 N standard thiosulfate solution to the starch indicator end point. The concentration of hydrogen peroxide was calculated by the following equation:

$$H_2O_2\ (\%, w/w) = (A - B) \times N \times 1.7007/\omega \qquad (3.3)$$

where, A and B are the titration volumes for sample and blank, respectively; N is the normality of the sodium thiosulphate solution, and ω is the weight of the sample used.

The photometric method involves the reduction of copper (II) with hydrogen peroxide, which then is reacted with 2,9-dimethyl-1,10-phenanthroline (DMP) reagent to form a bright yellow colored complex that gives absorbance at 454 nm by the reaction:

$$2Cu^{2+} + 4DMP + H_2O_2 \rightarrow 2Cu(DMP)^{2+} + O_2 + 2H^+ \qquad (3.4)$$

The method is suitable to measure low residuals of hydrogen peroxide (0.8 μM) in samples containing humic substances up to 10 mg C/L.

While persulfate in MW treated sludge samples was determined by the iodometric method of Kolthoff and Carr (1953), in the presence of iron, persulfate analysis was performed with the photometric method of Liang et al. (2008) that is a modification of the iodometric method.

For iodometric persulfate analysis, 2 mL of the sample was taken into a 500 mL flask, then was diluted to 30 mL with deionized water and 4 g of potassium iodide was added. The stoppered flask was allowed to stand for 15 min. Afterwards the sample was acidified with 1 mL of 6 N acetic acid and the iodine was titrated with either 0.1 N or 0.01 N standard thiosulphate solution to the starch end point. The concentration of sodium persulfate was determined by the following equation:

$$Na_2S_2O_8^{2-}\ (mg/mL) = 119 \times (A - B)\ N/v \qquad (3.5)$$

where A and B are the titration volumes for sample and blank, respectively; *N* is the normality of the sodium thiosulphate solution, and ν is the volume of the sample.

The photometric method of Liang et al. (2008) involves reacting residual persulfate with potassium iodide in the presence of sodium bicarbonate, which is added to avoid oxygen-oxidation of iodide. The yellow color is measured photometrically. Absorbance is maximum at 352 nm, however to eliminate the interference of dissolved iron that is utilized as activator, absorbance was determined at 400 nm.

3.2.6.4. Iron Analysis. Discrimination between ferrous (Fe^{2+}) and ferric forms (Fe^{3+}) of iron was made according to the modified Ferrozine method of Viollier et al. (2000). Fe^{2+} in the supernatants was quantified by the Ferrozine assay, where 0.01 M ferrozine (in 0.1 M ammonium acetate) was reacted with Fe^{2+} to form a stable colored complex giving absorbance at 562 nm. For Fe^{3+} determination, the sample was first reduced with 1.4 M hydroxylamine hydrochloride (in 2M HCl) and then reacted with ferrozine. Ferric iron was determined by the difference between final ferrous and initial ferrous iron in the samples.

3.2.6.5. Analysis Methods for the Evaluation of Plasmid DNA Treatment Performance. The influence of the oxidation processes on plasmid DNA structure was investigated by running treated plasmid DNA samples on gel electrophoresis system.

The ability of treated plasmid DNA to transform antibiotic resistance was investigated by transforming the plasmid DNA to competent XL blue cells (kindly provided by Sabanci University, Biological Sciences and Bioengineering Department).

3.2.6.6. Analysis Methods for the Evaluation of Sludge Treatment Performance. Sludge treatment performance was analyzed by evaluating antibiotic degradation and sludge solubilization.

Antibiotics were recovered from secondary sludge samples by ultrasonic solvent extraction followed by solid phase extraction (SPE) (US-EPA, 2007) performed for the cleaning of extracts and concentration of the antibiotics. Ultrasonic extraction of OTC and CIP from raw and treated sludge samples was performed according to the methods of Blackwell et al. (2004) and Turiel et al. (2006), respectively. For this purpose, sludge samples of 10−30 mL were mixed with an aqueous solution of $Mg(NO_3)_2$ (1M, pH 8.1) for CIP and with an extraction buffer consisting of methanol (MeOH): 0.1 M EDTA: McIlvaine buffer (60 ml of 0.2 M citric acid + 40 ml of 0.4 M Na_2HPO_4), 50:25:25) for OTC, then vortexed for 30 s and extracted with the aid of ultrasonic bath (Sonorex super RK 510 – 640 W, Morfelden-Walldorf, Germany) for 30 min. Then the samples were centrifuged at 5,000 *g* for 10 min and the resulting supernatants were passed through 0.45 μm filters (regenerated cellulose, Sartorius, Germany). For OTC analysis, the collected supernatants were concentrated and purified by solid phase extraction, where SAX (6 ml/500 mg, Phenomenex, USA) and Oasis HLB cartridges (6 ml/200 mg, Waters, Milford, MA, USA) were utilized in tandem. Elution was performed by 2 mL methanol. The extraction procedure is illustrated in Figure 3.5.

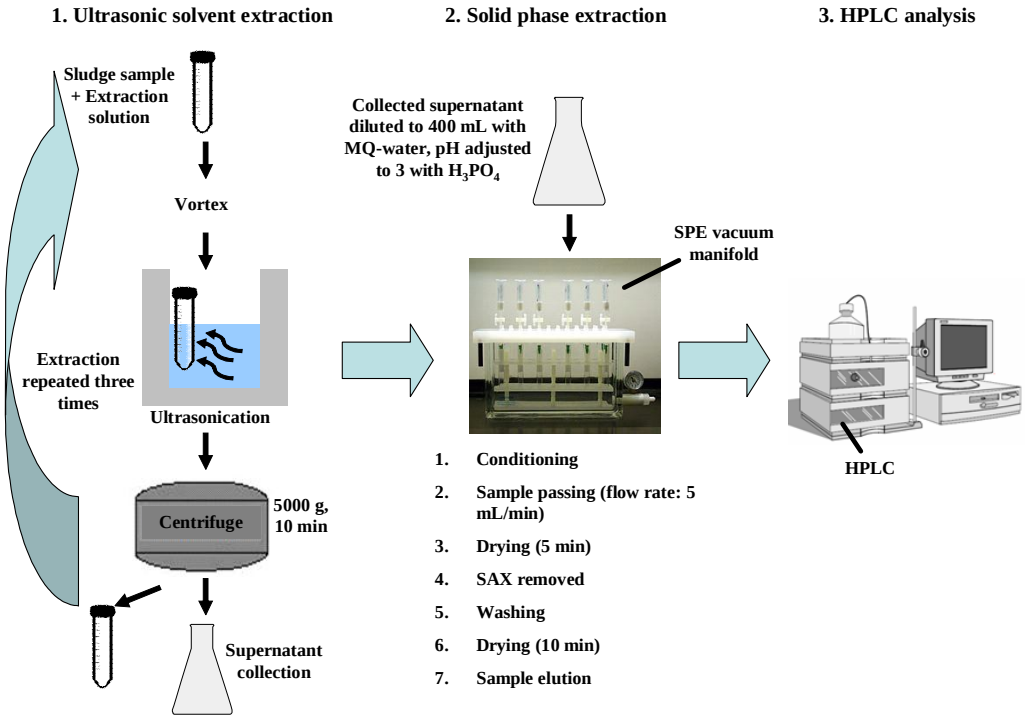


Figure 3.5. Schematic illustration of ultrasonic solvent extraction and solid phase extraction.

Analysis of antibiotics was performed with high performance liquid chromatography (HPLC) (Agillent Technol. 1100 series) utilizing YMC-Pack ODS-AQ column (3 µm, 50 x 4.0 mm). Gradient elusion was performed by acetonitrile and water (MQ), both containing 0.1% formic acid (98%, Fluka). OTC was detected at 360 nm with a diode array detector whereas CIP was detected with a fluorescence detector at excitation and emission wave-lengths of 280 nm and 450 nm, respectively. Limits of detection (LOD) for the antibiotics were estimated using the signal-to-noise ratio as S/N=3. A typical HPLC chromatogram for OTC and CIP antibiotics recovered from secondary sludge is given in Figure 3.6.

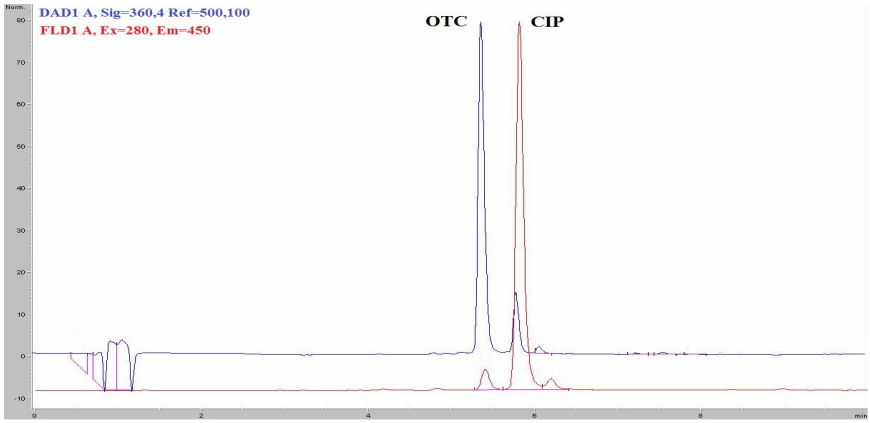


Figure 3.6. HPLC chromatogram of OTC and CIP from extracted sewage sludge samples.

Analysis of low antibiotic concentrations in some samples was carried out with qualitative Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS) analysis, performed with Triple Quadrupole mass spectrometer (Agilent Technol. 6460 series) at the Pendik Veterinary Control and Research Institute. The mass spectrometer was operated in the positive electrospray ionization (ESI) mode. Gradient elution at a flow rate of 0.5 mL/min was performed with oxalic acid solution (0.002 M) and acetonitrile, both containing formic acid of 0.2% and 0.1%, respectively. Chromatographic separation was performed with a Zorbax SB-C18 column (3.5 µm, 3 x 75 mm) operated at a temperature of 40°C. The parent mass and product mass range values were 461 and 426–444 for OTC, and 332 and 288–314 for CIP, respectively. A typical LC-MS/MS chromatogram is given in Figure 3.7.

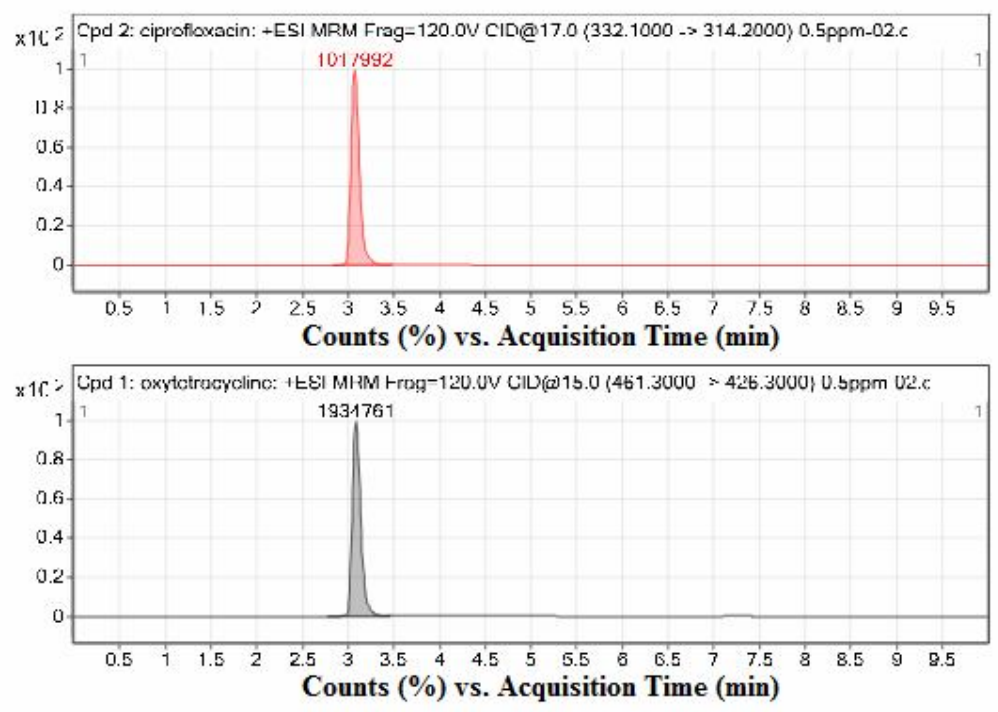


Figure 3.7. LC-MS/MS chromatograms of OTC and CIP from extracted sewage sludge samples.

As can be seen from the chromatograms, OTC and CIP could be separated and analyzed efficiently. The CIP and OTC recovery rates from the sludge were 85±5% (LOD=0.08 mg/kg, n=15 samples, SD=1.5) and 88±2% (LOD=0.04 mg/kg, n=25 samples, SD=6.3), respectively.

Sludge solubilization performance was analyzed by evaluating organic carbon, nitrogen, and phosphorus solubilization, respectively. Solubilization of organic carbon was expressed as the percentage of the ratio of soluble SCOD to TCOD of the treated sludge. In order to obtain comparative results with the literature, in ozonation experiments, the solubilization from particulate matter was calculated according to the following equation:

$$\% \ Solubilization = (SCOD_{final} - SCOD_{initial}) \times 100/TCOD_{particulate} \quad (3.6)$$

For the other chemical oxidation processes applied to waste sludge, the solubilization of organics was expressed as the percent ratio of SCOD to TCOD:

$$\% \ Solubilization = SCOD/TCOD \times 100 \quad (3.7)$$

Solubilizations of phosphorus and nitrogen were defined as the percentage amount of PO_4^{3-} and NH_4^+ released from TP and TKN, respectively. Metal solubilization was defined as the percentage concentration of metals released to the dissolved phase (Me_s) from the total metal concentration (Me_t).

3.2.6.7. Analysis of Radical Probe Compounds. Radical probe compounds para-chlorobenzoic acid (*p*CBA) and anisole were analyzed by HPLC utilizing YMC-Pack ODS-AQ column (3 µm, 50 x 4.0 mm) after the centrifugation and filtration of sludge samples. The mobile phase in HPLC was a mixture of acetonitrile and water (60:40 v/v). While *p*CBA was detected at 234 nm, anisole was detected at 254 nm with a diode array detector. *p*CBA recovery in the supernatant was >90% and the recovery rate of anisole in the supernatant was 67±0%.

3.2.6.8. Statistical Analysis and Experimental Design. Statistical analysis in sludge ozonation experiments was carried out with SPSS version 11.5 to test for significant effects of the investigated process parameters on the antibiotic removal efficiency. Testing was performed by one-way analysis of variance (ANOVA). The significance level was set at $p \leq 0.05$ for each analysis. The Bonferroni post-hoc test was utilized for multiple comparisons. The analyses were obtained from the results of parallel experiments run successively in order to prevent significant variation due to change in sludge characteristics.

For other chemical oxidation processes applied to waste sludge, experimental full factorial design was utilized in order to explore the main and significant effects of the selected variables and their interactions by conducting a minimal number of experiments with real sludge samples. The design matrix was established and the testing was performed with Minitab 15 (Minitab Inc.).

4. EFFECTS OF CHLORINE, OZONE AND HETEROGENEOUS PHOTOCATALYSIS ON PLASMID DNA

4.1. Introduction

Currently, biological treatment remains to be the most commonly applied wastewater treatment technology, but has proven to be incapable for the degradation of recalcitrant contaminants including many antibiotics (Daughton and Ternes, 1999; Holm et al., 1995). As a result, antibiotics are released into the environment via effluents of domestic wastewater treatment plants (Yang et al., 2005) and wastewaters of intensive animal feeding operations (Halling-Sorensen et al., 1998; Migliore et al., 1993). The release of these can lead to development of antibiotic resistance in local bacterial communities (Kummerer and Henninger, 2003; Chee-Sanford et al., 2001, Guardabassi et al., 1995). Microorganisms can bear natural primary resistance, which can be inherited and passed through generations by vertical gene transfer; however, secondary resistance transferred by conjugation is also common due to antibiotic exposure. In the latter, genetic elements such as plasmids can be exchanged among members of different classes of bacteria (Lorenz and Wackernagel, 1994). Through exchange of these mobile genetic elements, resistance can be transferred for example from non-pathogenic to pathogenic microorganisms. Antibiotics can lead to resistance development even at very low concentrations in the environment (Laengin et al., 2009). Antibiotic resistant bacteria have been detected in aquatic environments (Laengin et al., 2009; Campeau et al., 1996) and in drinking water (Schwartz et al., 2003). While current advanced treatment technologies have been improved for the elimination of micro-pollutants, conventional chlorination, which is especially preferred due to its economical affordability compared to advanced treatment technologies, continues to be the most commonly applied technology for water and wastewater treatment, However, studies show that chlorine is not very effective in the removal of antibiotic resistant bacteria (Diao et al., 2004; Shrivastava et al., 2004) and sometimes even leads to selection of these (Shrivastava et al., 2004; Murray et al., 1984). Ozone on the other hand is a powerful disinfectant, which has been shown to remove pathogens that are relatively resistant to chlorine and chloramine (Macauley et al., 2006; Korich et al.,

1990). Studies on the mechanism of ozone disinfection showed that ozone can penetrate the bacterial cells, reacting with cell ingredients (Hunt and Marinas, 1993) and causing damage to the DNA (Ishizaki et al., 1987). The ability of oxidant to cause DNA damage can be considered an important feature when transfer of antibacterial resistance through genetic materials is considered. The damaging effect on DNA has also been shown with heterogeneous photocatalysis by TiO_2-photocatalyst (Yang and Wang, 2008; Shen et al., 2008). Although TiO_2-photocatalysis is a less commonly applied technology due to the limited performance of TiO_2 owing to its wide band gap (Chen and Mao, 2007), this technology can still be suitable as a post-treatment process in drinking water treatment.

The purpose of this part of the thesis is to compare the effectiveness of chlorination, ozonation, and heterogeneous photocatalysis in reducing the risk of antibiotic resistance pollution by resistance carriers, which inevitably reach drinking water sources. Although the disinfection kinetics with the three oxidation processes has been deeply investigated (Korich et al., 1990, Haas and Karra, 1984; Wei et al., 1994) and the effect of the oxidants on the DNA structure have also been shown in separate studies, there is no work comparing their effect on eliminating the risk of antibiotic resistance transfer. Here, the approach followed for this purpose involves isolation of the plasmid DNA of a multi-resistant *E. coli* HB101 and its treatment by various oxidant doses and/or treatment periods of chlorine, ozone, and heterogeneous photocatalysis followed by transformation of competent cells by the treated plasmid DNA in order to understand the effect of treatment on resistance transfer potential. The effect of plasmid DNA concentration on oxidation performance has also been investigated.

Heterogeneous photocatalysis was applied with a commercial powdered form of TiO_2 and a nano-fiber TiO_2-material that was prepared in the laboratory by electrospinning-sol-gel method. Nano size can provide larger surface area per volume of catalyst (Theron et al., 2008). Electrospinning is a relatively novel method that can be utilized to obtain porous fiber system in the nanometer range (Aryal et al., 2008; Li and Xia, 2003). An advantage of such a system is the nano-porous fiber structure providing high surface area and its supported nature for easy removal of the catalyst that is a common problem in treatment operations utilizing nano-materials.

4.2. Materials and Methods

4.2.1. Chemicals

TiO_2-P25 was used as the commercial photocatalyst. The nano-fiber-TiO_2 was prepared from a titanium isopropoxide precursor (TIP, $Ti(OiPr)_4$) and polyvinylpyrrolidone (PVP, *MW* = 1,570,000). All solutions were prepared with Milli-Q (MQ) water (Millipore, Milford, MA). Sodium hypochlorite solution (~ 10% RT) was used to prepare chlorine stock solutions (Free Cl^- = 20,000 mg/L, as determined by Free DPD Kit purchased from Hach). Sodium thiosulphate was utilized to quench the oxidants.

4.2.2. Preparation of Nano-Fiber-TiO_2

The nano-fiber-TiO_2 was prepared by the sol-gel electrospinning combined method as described by Li and Xia (2003) and from a sol-gel precursor TIP and PVP as described in Section 3.2.1. TIP and PVP were dissolved in various w/v (weight/volume) ratios in ethanol (EtOH). To make TIP/PVP = 4.83 (w/w) for example, 0.29 g PVP was dissolved in 7.8 g ethanol and 1.4 g TIP was dissolved in 1 g ethanol and 3.14 g acetic acid. Then these solutions were stirred by slow addition of the TIP solution into the polymer solution and allowed to mix for one hour. Afterwards, the mixtures were loaded into a plastic syringe having a 0.80 mm needle, which was connected to High Voltage Power Supply (Gamma High Voltage Research). The mixture was electrospinned at a constant flow (set to 50, 100, and 200 µL/h, respectively) applied by a syringe pump that was controlled with a syringe pump software – WinPumpTerm (New Era Pump Systems, Inc.). The voltage of the supply was set to 10 kV. The generated fibers were collected on an aluminum sheet attached onto a grounded surface. Distance between the syringe tip and the grounded alumina surface was 15 cm. After electrospinning, the fiber sheets were left under open air overnight to allow completion of the sol-gel reaction. The fibers were then calcinated at various temperatures (400°C, 430°C, and 500°C) to remove the organic phase and to obtain polycrystalline TiO_2 anatase. The schematic illustration of the electrospinning apparatus was shown previously in Section 3.2.1., Figure 3.1.

4.2.3. Characterization of Nano-Fiber-TiO_2

The TiO_2 nanofibers were characterized with Scanning Electron Microscopy (SEM, Supra Gemini 35 VP Field Emission Scanning Electron Microscope, Leo) to demonstrate fiber formation and to measure the fiber dimensions. Samples were coated with carbon by a T950x Turbo Evaporate Instrument (Emitech) prior to analysis. Mineral structure of prepared photocatalyst was determined by X–Ray Diffractometer (D8 Advance Type, Bruker–AXS). BET surface area and pore size analysis of photocatalyst samples was performed with Quantachrome Nova 2200e, and the thermogravimetric analysis (TGA) was carried out with Netzch STA449C.

4.2.4. Plasmid DNA Isolation

Plasmid DNA isolation was carried out by Plasmid Plus Maxi Kit (Qiagen) following the procedures described in Section 3.2.2. Briefly, a bacteria starter growth solution was initiated by inoculating a single ampicillin resistant *E. coli* HB101 colony (Pasteur Institute, France) from a previously prepared plate in 100 mL growth medium (1 L aqueous solution containing 10 g tryptone, 10 g sodium chloride, 5 g yeast extract, 15 g agar and 0.1 mg/mL ampicillin), which was grown for 8 h at 37°C, 200 rpm (logarithmic growth). Then a portion of this was transferred into a higher volume of growth medium (1/1000 dilution) and the growth was extended for another 16−18 h till the optical density at 660 nm (OD_{660}) reached approximately four (measured by Shimadzu UV-1208 model spectrophotometer with 1 cm optical path at 600 nm). The bacteria were pelleted by centrifugation at 4000 g for 15 min and plasmid DNA was extracted from the pellets utilizing the Plasmid Plus Maxi Kit, according to the procedures of the manufacturer. The plasmid DNA concentration was determined by Nanodrop Spectrophotometer (ND-1000, NanoDrop) and the plasmid DNA extracts were stored at -20 °C until further use. Plasmid DNA was prepared in two concentrations by dilution with autoclaved MQ water.

4.2.5. Oxidation of Plasmid DNA

Chlorine stock solution was prepared from sodium hypochlorite solution, which was diluted to different chlorine concentrations (0.5–5 mg/L). The treatment was carried out by the addition of 350 µL chlorine solution to eppendorf tubes containing 350 µL sample solution with 80 µL of 200 mg/L or 100 mg/L plasmid DNA and 270 µL MQ water to prepare two plasmid DNA concentrations of 12.8 mg/L and 6.4 mg/L). The treatment period was one minute. Chlorine in the treated samples was quenched by addition of 550 µL, 0.4% (w/w) sodium thiosulphate.

Ozonation experiments were carried out with a batch system and with an ozone stock solution with oxidant demand free (ODF) water which was prepared according to the standard methods (APHA, 2005). Ozone stock solution was prepared by using a laboratory scale ozone generator unit (Fisher OZ 500) operated with dry and pure oxygen. In order to prevent ozone decomposition, the temperature of the stock ozone solution was maintained at 4ºC ± 2 by ice bath. The ozone stock solution concentration was determined by the indigo trisulphonate (Acros Organics) spectroscopic method (APHA, 2005). The ozonation experiments were carried out in eppendorf tubes containing 0.5 mL sample solution (80 µL of plasmid DNA with concentration of 200 mg/mL or 100 mg/mL and 420 µL MQ water). 0.5 mL ozone solution with known concentration was slowly pipetted into the samples to obtain desired ozone doses (1–4.42 mg/L) and ozonation was carried out for one min. Remaining ozone from the treated samples was quenched by addition of 250 µL, 0.4% (w/w) sodium thiosulphate (to make a total of 1.25 mL of sample as in the experiments with chlorine and TiO_2).

Photocatalytic treatment was carried out in 4 mL borosilicate glass tubes designed specifically for the process. 0.2 mg commercial photocatalyst (TiO_2-P25) or nano-fiber-TiO_2 was suspended in 1.17 mL MQ by applying sonication for one min. Then 80 µL of plasmid DNA with concentration of 200 mg/L or 100 mg/L was added and the sample was immediately covered with aluminum foil and sonicated for another minute. The suspension was illuminated by a 125W BLB Lamp, the quantum yield of which was measured by ferrioxalate chemical actinometry as $\phi = 6.5 \times 10^{-6}$ quanta/s. During treatment, the samples were placed in a water bath with temperature control and

were slightly mixed at 40 rpm on a mini orbital shaker (Stuart, SSM1). Treatment periods ranged between 5−75 min. Dark control experiments and control experiments under black-light irradiation were also carried out. Method of plasmid DNA recovery from TiO_2 suspension was determined by applying a procedure that was developed by Suzuki et al. (2008). The procedure involved the following steps: A sample solution of 1.25 mL, containing 0.2 mg TiO_2 and 80 µL of 200 mg/mL plasmid DNA in MQ water was sonicated in ultrasonic bath for one min. The sample was immediately covered and slightly mixed on the mini orbital shaker for 75 min in dark. Then, it was centrifuged at 12,000 g for 10 min. The first supernatant (S-1) was separated and preserved for analysis. The pellet was resuspended in 1.25 mL MQ water, sonicated for one min and again centrifuged at 12,000 g for 10 min. The secondary supernatant (S-2) was separated. 100 µL, 0.25 N standard NaOH solution (pH 13) was added to the pellet followed by one min sonication and then the sample was inserted into water bath at 98ºC for 10 min. Following a final sonication for one min and centrifugation for 10 min at 12,000 g the final supernatant (S-3) was separated. The treatment involving the addition of NaOH was applied in order to remove remaining plasmid DNA that might have adsorbed onto TiO_2. S-1, S-2, and S-3 were separately evaluated and the recovery of plasmid DNA during the centrifugation and separation steps and the alkaline treatment steps were compared. Samples prepared in these experiments were also utilized as dark control experiments.

4.2.6. Evaluation of the Oxidation Process Performance

Plasmid DNA samples treated by the three oxidation processes were analyzed on a 1% (wt/v) agarose gel (Basica Le Agarose, Prona and 0.5X TBE prepared from 50X TBE, FERMENTAS) and run on gel electrophoresis system (Gel XL Ultra V-2, Colepalmer, Labnet International, Inc.). Plasmid DNA (20 µL + 4 µL 6X loading dye) was loaded to the gel and 90−100 V was applied for 45−60 min. The gel was stained with ethidium bromide (5 mg/L). MassRuler DNA Ready-to-use Ladder Mix 103 mg/L (FERMENTAS) (3.5 µL) was utilized as the ladder.

The effect of treatment on the ability of plasmid DNA to transform competent cells was investigated. Frozen XL blue competent cells were utilized as transformants.

The transformation process briefly included the following steps: 100 µL of competent cell suspension was placed in pre-chilled plastic micro-centrifuge tubes containing 0.5 µL plasmid DNA. One sample containing the competent cell suspension and PUC19 (a control plasmid that carries ampicillin resistance) were prepared as a positive control. The tubes containing the cell and DNA mixtures were chilled for 20−30 min in ice and then placed in a 42°C heating block for 1−1.5 min. SOC (Super Optimal broth with Catabolite repression) media was added into the cell samples to make a total of 1 mL and these were incubated at 37°C, 240 rpm for 60 min (post-incubation). The cells were pelleted at 12,000 g for two min. The pellet was resuspended in 100 µL sterile MQ water. Then 100 µL of the samples were plated on selective agar containing ampicillin and were incubated at 37°C overnight. A negative control plate containing only XL-blue cells and no plasmid DNA was also plated. Plasmid DNA activity was measured by the number of cells transformed (number of cells counted on agar plates).

4.3. Results and Discussion

4.3.1. Characterization of Nano-Fiber-TiO_2

Electrospinning conditions and TIP/PVP ratio had considerable influence on the fiber diameter (Table 4.1) as well as on the fiber morphology of TiO_2. Decrease in the TIP/PVP (w/w) ratio resulted in increase in the fiber diameter. The most uniform fibers were obtained with TIP/PVP (w/w) = 4.83 (0.02 (w/v) PVP, 0.10 (w/v) TIP).

Table 4.1. Fiber diameter variation at different electrospinning conditions.

TIP/PVP (w/w)	Flow rate (µL/h)	Fiber diameter (nm)
4.83	50	~200
4.67	50	~135−166
3.11	50	~230−370
2.46	50	~600−1200
3.11	100	~300−600
3.11	200	~350−600

Increase in the electrospinning syringe flow rate resulted in uneven fibers with varying size distribution and material droplets were observed in the SEM images. The

SEM image of the as electrospinned sample having TIP/PVP (w/w) = 4.83 and prepared at electrospinning flow rate of 50 μL/h is presented in Figure 4.1.

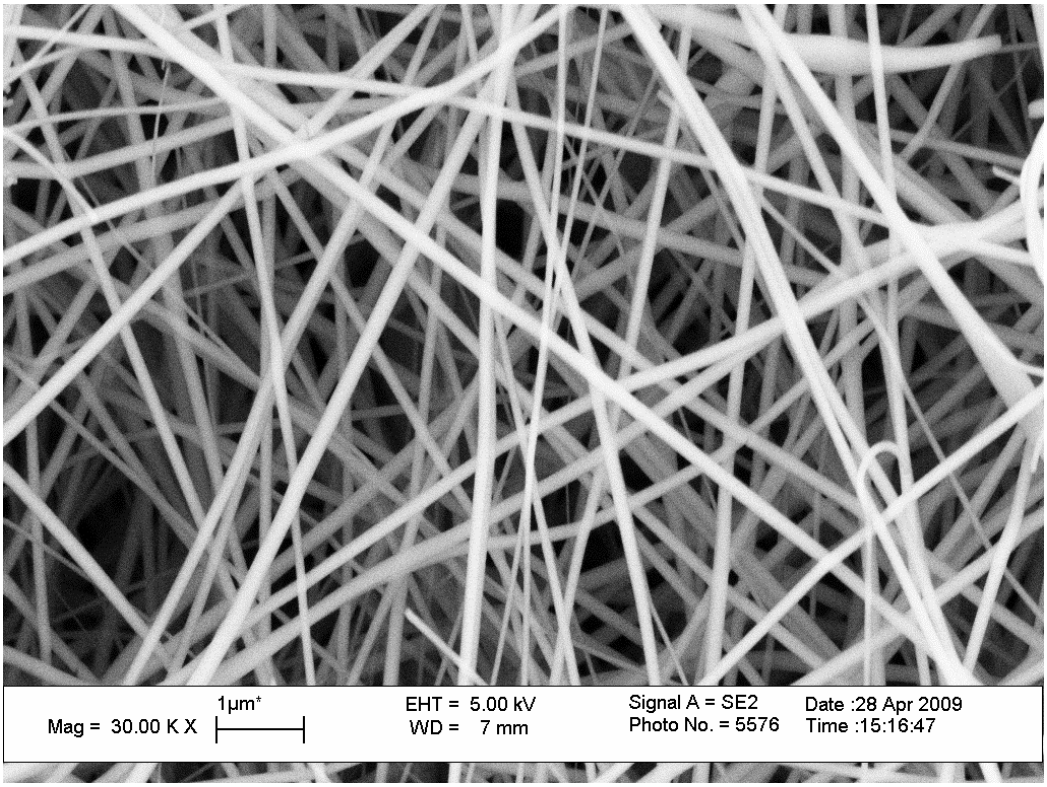
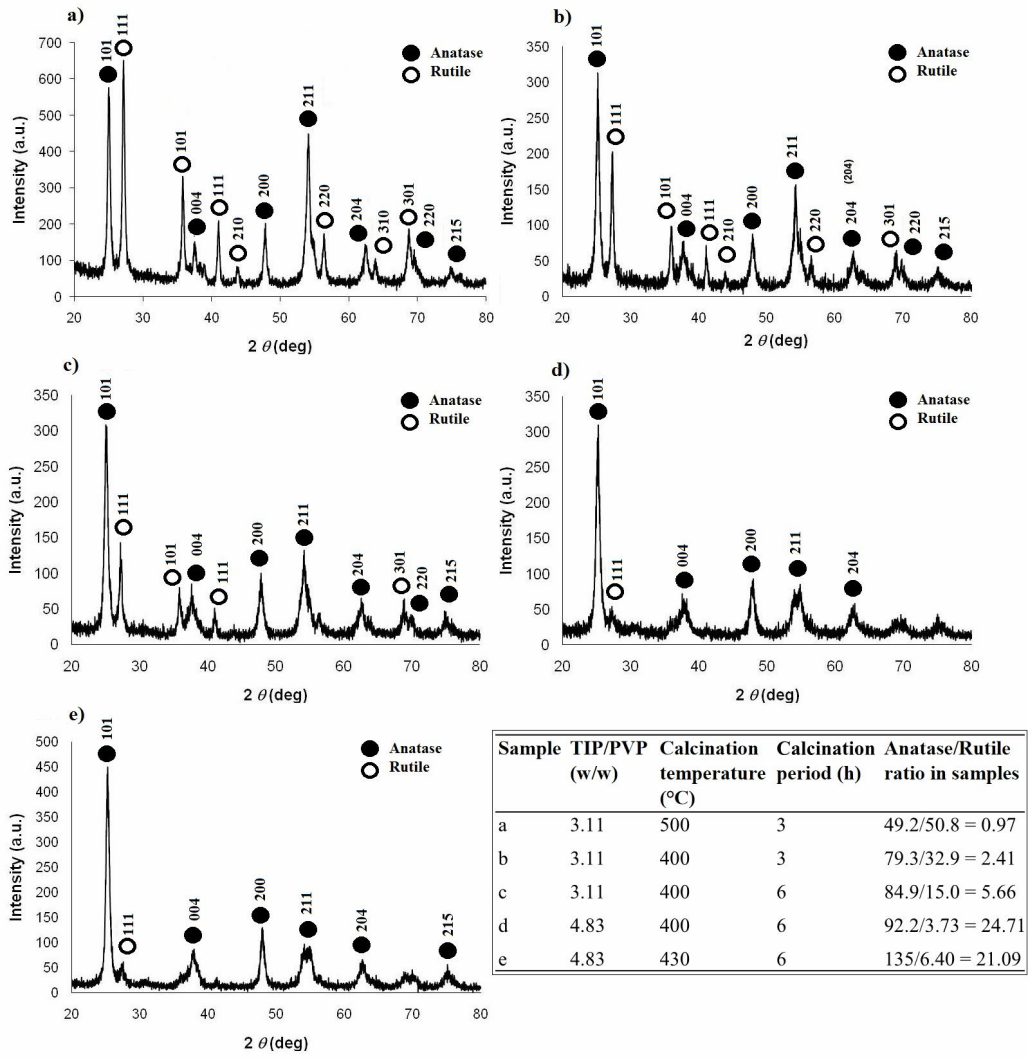


Figure 4.1. SEM image of TIP/PVP (w/w = 4.83).

The fine fibrous structure of the material can be observed in the figure.

The ratio of anatase and rutile in the samples calcinated at different temperatures and different durations was studied with XRD (Figure 4.2 (a)-(e)). Anatase/rutile ratio was estimated with DIFFRAC^{plus} EVA software (Bruker).



Sample	TIP/PVP (w/w)	Calcination temperature (°C)	Calcination period (h)	Anatase/Rutile ratio in samples
a	3.11	500	3	49.2/50.8 = 0.97
b	3.11	400	3	79.3/32.9 = 2.41
c	3.11	400	6	84.9/15.0 = 5.66
d	4.83	400	6	92.2/3.73 = 24.71
e	4.83	430	6	135/6.40 = 21.09

Figure 4.2. XRD Patterns of calcinated samples (a) TIP/PVP = 3.11 (w/w), 500 °C, 3 h calcinated (b) TIP/PVP = 3.11 (w/w), 400 °C, 3 h calcinated (c) TIP/PVP = 3.11 (w/w), 400 °C, 6 h calcinated (d) TIP/PVP = 4.83 (w/w), 400 °C, 6 h calcinated (e) TIP/PVP = 4.83 (w/w), 430 °C, 3 h calcinated.

As deduced from Figure 4.2 (e), the highest anatase/rutile ratio (~92/4) was obtained for the sample having TIP/PVP = 4.83 (w/w), calcinated at 400°C for 6 h, showing that the anatase/rutile ratio increased when the temperature was dropped from 500 to 400°C and when the calcination duration was increased to 6 h. Considering the presence of uniform fibers and the higher anatase to rutile ratio obtained after calcination, TIP/PVP (w/w) 4.83 calcinated at 400ºC for 6 h was selected as the

photocatalyst to be utilized for the photocatalytic experiments. TGA analysis of this sample (Figure 4.3) showed that calcination at 400°C for 6 h caused 50% decrease in the mass of the electrospinned and air dried sample.

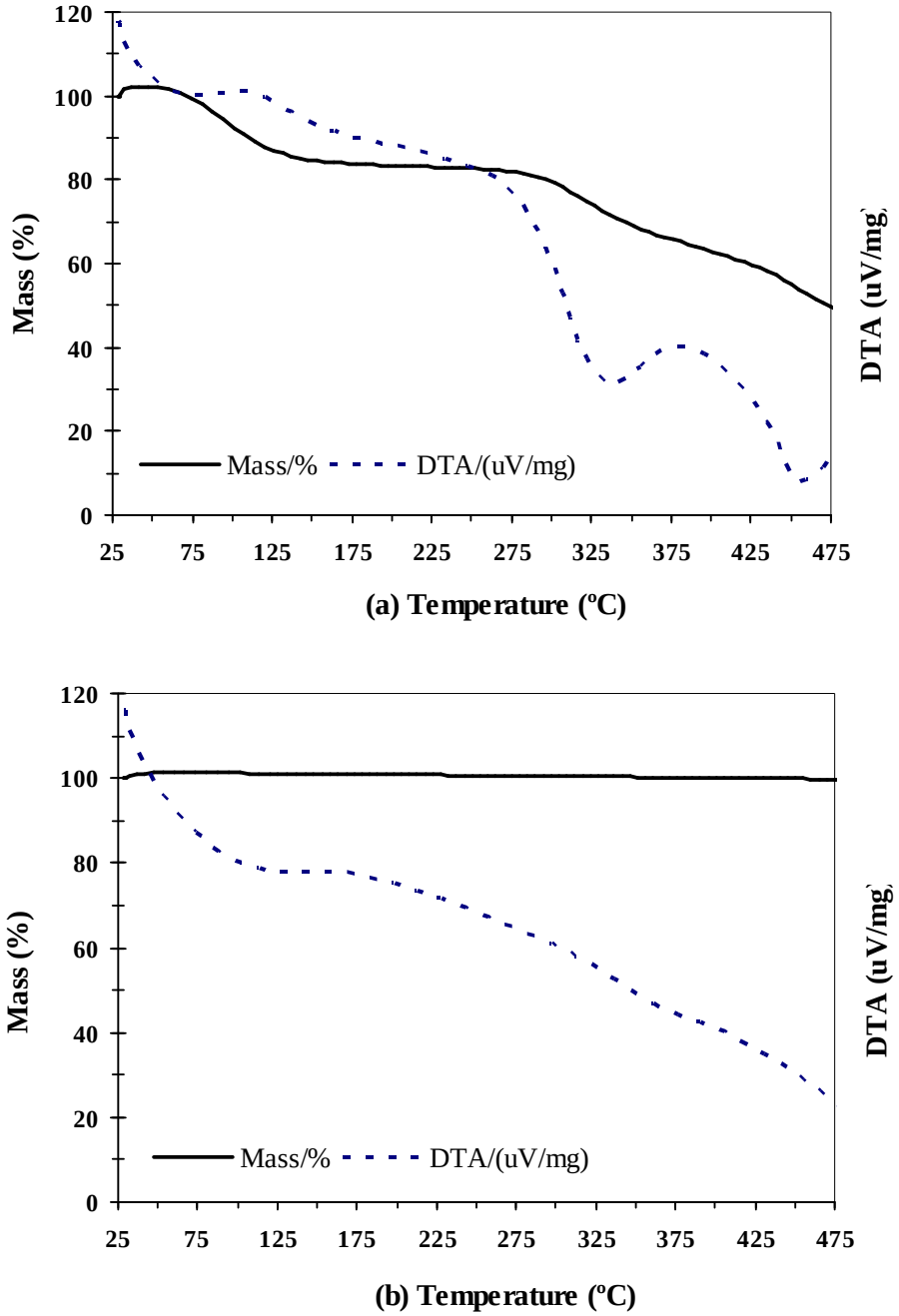
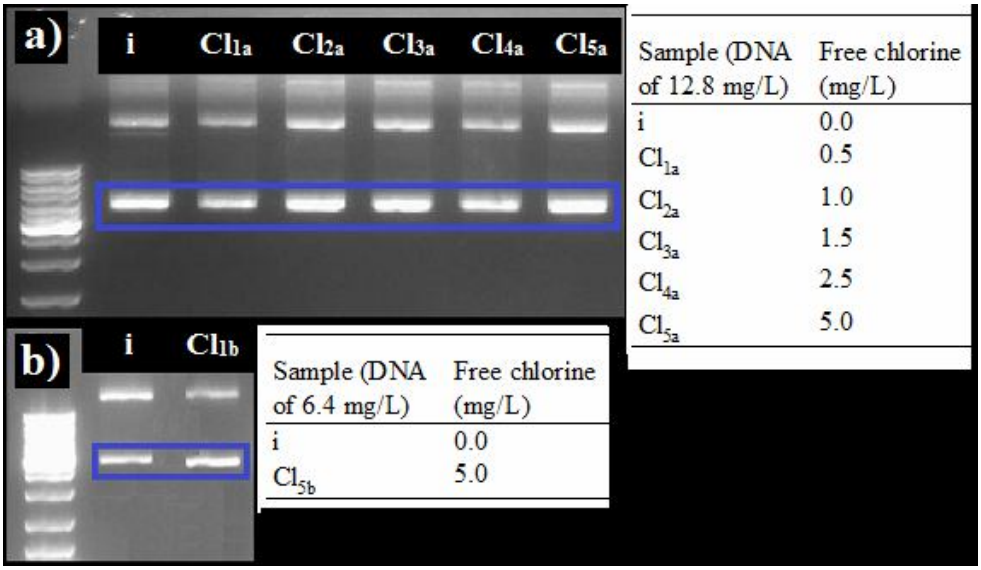


Figure 4.3. TGA diagrams TIP/PVP = 4.83 (w/w) (a) after electrospinning and drying in air overnight (b) after calcination at 400°C for 6 h.

Organic content was completely removed from this sample leaving behind TiO_2 which is mainly in the anatase form as shown in the XRD analysis (Figure 4.2 (e)). BET analysis showed that the surface area of the nano-fiber-TiO_2 (53 m^2/g) photocatalyst was comparable to that of commercial TiO_2-P25 (51 m^2/g).

4.3.2. Oxidation of Plasmid DNA

4.3.2.1. Influence on Plasmid DNA Structure. No effect of chlorine on plasmid DNA structure was observed in this study, where gel electrophoresis was utilized in order to analyze oxidant effect on plasmid DNA structural integrity. The gel images of chlorine treated plasmid DNA having concentration of 12.8 mg/L and 6.4 mg/L are displayed in Figure 4.4 (a) and (b), respectively. The band that belongs to the supercoiled (sc) or closed-circular plasmid DNA structure has been marked on the gel images. Disruption of plasmid DNA integrity and formation of other plasmid DNA conformations is expected to result in decrease in the band intensity of supercoiled form with subsequent increase in band intensities of other forms.



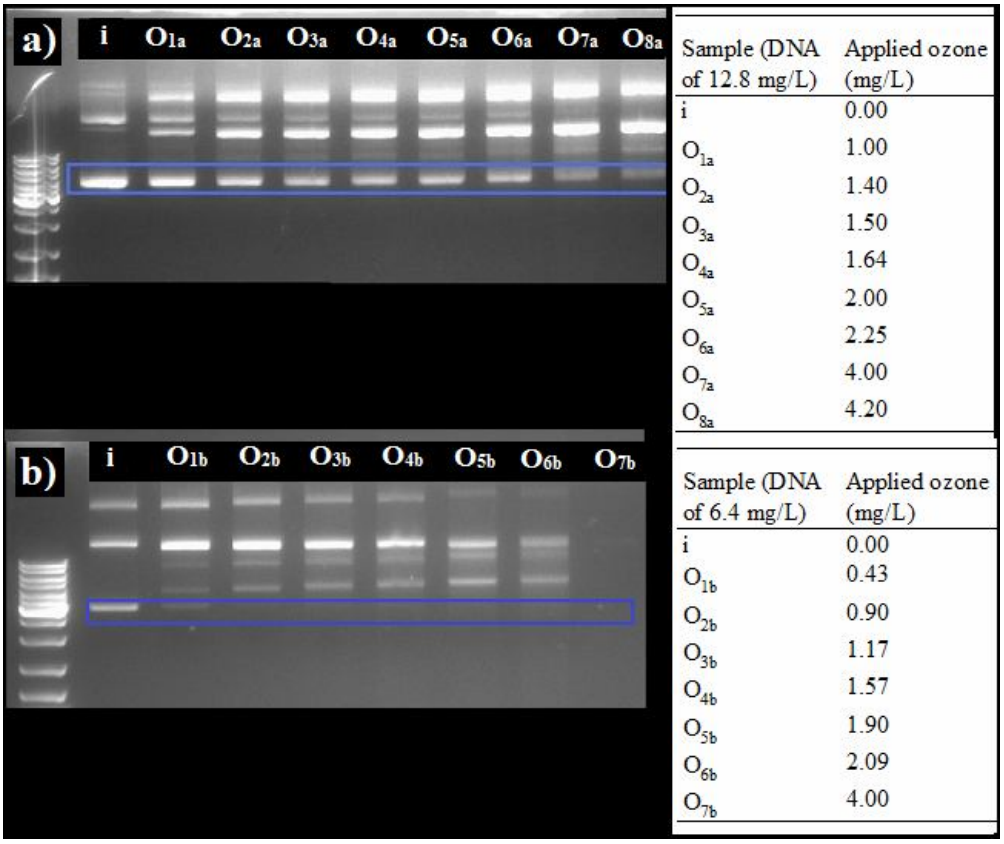
Sample (DNA of 12.8 mg/L)	Free chlorine (mg/L)
i	0.0
Cl_{1a}	0.5
Cl_{2a}	1.0
Cl_{3a}	1.5
Cl_{4a}	2.5
Cl_{5a}	5.0

Sample (DNA of 6.4 mg/L)	Free chlorine (mg/L)
i	0.0
Cl_{5b}	5.0

Figure 4.4. Agarose gel electrophoresis of chlorine (Cl) treated plasmid DNA (a) high concentration – 12.8 mg/L (b) low concentration – 6.4 mg/L. Marked bands: supercoiled conformation.

It can be viewed from the images that band intensities of supercoiled plasmid DNA treated at different concentrations of chlorine (0.5 – 5.0 mg/L), are similar to those of the untreated control sample (Figure 4.4). A two-fold decrease in plasmid DNA concentration did not change this observation and again plasmid DNA integrity was not effected (Figure 4.4). The proposed most dominant mechanism during chlorine disinfection is the reaction of chlorine with the cell membrane lipids which results in change in cell membrane permeability (Venkobachar et al., 1977) among various disinfection mechanisms (US-EPA, 1999). Considering the possible leakage of resistance carrier elements such as plasmid DNA and their presence in free forms and in the light of the findings in this work and those reported in the literature, the relative ineffectiveness of chlorine in antibiotic resistance management cannot be ignored. Nonetheless, it should also be noted that in some previous studies in which the effect of generated hypohalites at inflammation sites on the cell components was investigated, oxidative damage and base modifications of DNA by endogenous hypochlorite has been reported (Ohnishi et al., 2002; Hawkins and Davies, 2002). The contradictory finding in the present work can be attributed to the higher plasmid DNA concentration. Quantities of environmental extracellular DNA detected in marine and freshwater have been reported in the range of 0.0002 – 0.044 mg/L (Lorenz and Wackernagel, 1994). However, higher concentrations may be released by leakage after cell lysis as in the case of disinfection of wastewater. Also, it is possible that released intact plasmid DNA and extracellular DNA may be protected by binding to particulate or suspended matter in water (DeFlaun et al., 1987), and then become available under appropriate conditions. The reason for selecting higher concentrations of plasmid DNA concentrations in this work was to facilitate the analyses regarding the oxidant effect on plasmid DNA structure.

Gel electrophoresis images of ozone treated plasmid DNA at the high and low concentrations are displayed in Figure 4.5 (a) and (b), respectively. Relaxed, linear, and supercoiled plasmid DNA conformations can be viewed in the gel images in the order of slowest migrating to the fastest migrating bands, respectively.



Sample (DNA of 12.8 mg/L)	Applied ozone (mg/L)
i	0.00
O_{1a}	1.00
O_{2a}	1.40
O_{3a}	1.50
O_{4a}	1.64
O_{5a}	2.00
O_{6a}	2.25
O_{7a}	4.00
O_{8a}	4.20

Sample (DNA of 6.4 mg/L)	Applied ozone (mg/L)
i	0.00
O_{1b}	0.43
O_{2b}	0.90
O_{3b}	1.17
O_{4b}	1.57
O_{5b}	1.90
O_{6b}	2.09
O_{7b}	4.00

Figure 4.5. Agarose gel electrophoresis of ozone (O) treated plasmid DNA (a) high concentration –12.8 mg/L (b) low concentration – 6.4 mg/L. Marked bands: supercoiled conformation.

As opposed to the results obtained by chlorine, increasing oxidant doses resulted in gradual increase in the intensity of bands belonging to other DNA conformations (i.e. relaxed and linear forms) accompanied with decrease in the supercoiled DNA bands. Progress in this effect as the dose of ozone increases can be viewed in the gel image of the plasmid DNA having higher concentration, and at an ozone dose of 4.20 mg/L the band intensity of the supercoiled DNA is minimal (Figure 4.5 (a)). When plasmid DNA with lower concentration (Figure 4.5 (b)) was treated with ozone, an ozone dose of 0.90 mg/L resulted in complete disappearance of the supercoiled DNA band. As the ozone dose increased, intensity of other DNA bands increased initially showing conversion of supercoiled DNA structure, but decreased at higher doses. Complete disappearance of plasmid DNA bands was observed at an ozone dose of 4.00 mg/L. Ozone has been reported to damage DNA by causing strand breaks as well as

base damage and protein cross-linking (Sawadaishi et al., 1985; Hamelin et al., 1977). Sawadaishi et al. (1985) explained strand-break formation by the conversion of supercoiled plasmid DNA to open circular plasmid DNA in ozone treated samples initially containing relatively high concentration of plasmid DNA (50 μg). Also, Ishizaki et al. (1987) have shown immediate strand scission of chromosomal DNA of *E. coli* upon treatment by ozone. Similarly here, ozone treatment resulted in strand scission converting the supercoiled plasmid DNA to relaxed form causing subsequent linearization. Increase of intermediate bands in the gel image indicated degradation of plasmid DNA and formation of smaller fragments.

Light control experiments for photocatalytic treatment are shown in Figure 4.6.

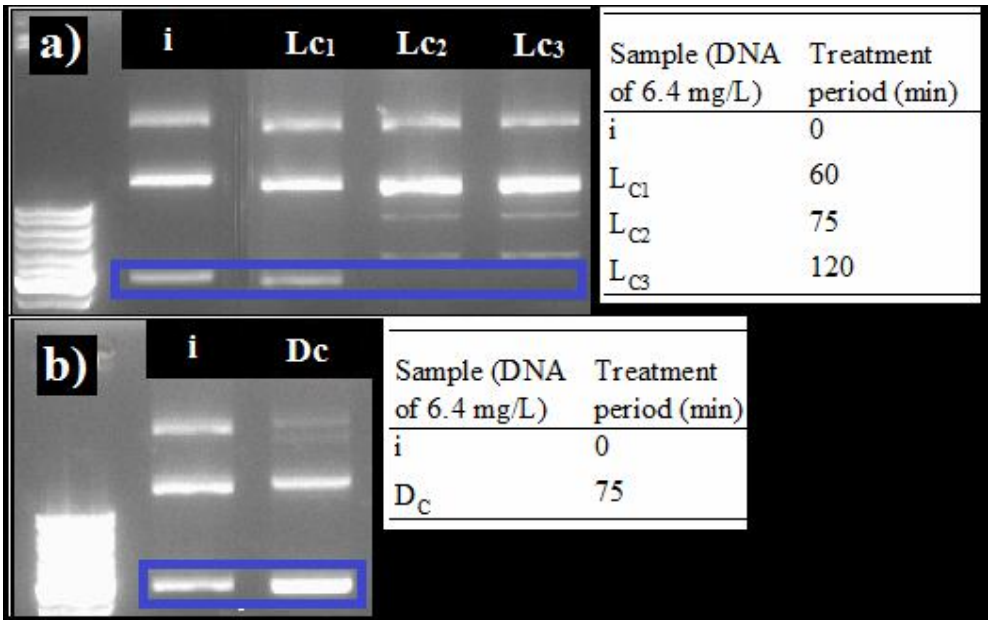


Figure 4.6. Agarose gel electrophoresis of light (L_C) (a) and (b) dark control (D_C) treated plasmid DNA at low concentration – 6.4 mg/L. Marked bands: supercoiled conformation.

The light control experiments indicated that in the absence of the photocatalyst, no major effect of illumination occurred on the plasmid DNA having high concentration; while for plasmid DNA of low concentration minimal conformational change took place at the 75th min (Figure 4.6 (a)). Extended treatment under illumination for 120

min did not lead to any further change in DNA band intensities. Plasmid DNA was effectively recovered by the initial centrifugation step carried out during the dark control experiments. Intensities of plasmid DNA bands from S-1 were similar to those of the untreated control sample and no bands were detected in S-2 and S-3. The plasmid DNA bands of S-1 are displayed in 4.6 (b), along with the untreated control sample. Therefore, dark control experiments carried out for 75 min showed that plasmid DNA could be effectively recovered from TiO_2 by a simple centrifugation step.

Treatment of plasmid DNA with commercial TiO_2-P25 caused conformational change in plasmid DNA bands as in the ozone treatment experiment (Figure 4.7 (a) and (b)).

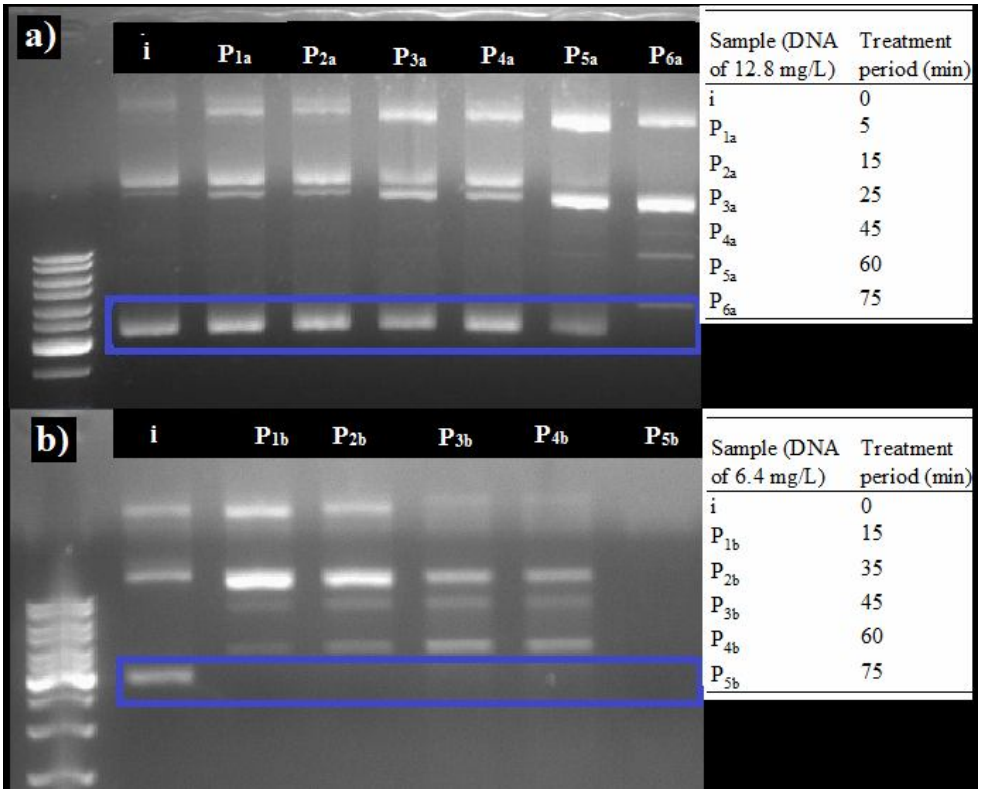


Figure 4.7. Agarose gel electrophoresis of commercial TiO_2-P25 (P) treated plasmid DNA (a) high concentration – 12.8 mg/L (b) low concentration– 6.4 mg/L. Marked bands: supercoiled conformation.

For the higher concentration plasmid DNA (Figure 4.7 (a)) initially, change in the intensity of the band belonging to the supercoiled structure was not apparent as for the ozone experiment, however conversion to other conformations was evident by the increase in their band intensities. Decrease in the supercoiled band intensity occurred at the 60 min treatment period, which disappeared completely at the 75 min treatment period. For the sample with lower plasmid DNA concentration, the supercoiled DNA band disappeared within 15 min of treatment. Similar to the effect observed with ozone treated sample having lower plasmid DNA concentration, with shorter treatment periods, intensities of other plasmid DNA bands increased. After 45 min treatment, bands intensities of other conformations decreased as well. All plasmid DNA bands were completely removed from the gel image with 75 min treatment. Similar to ozone, photocatalytic treatment with the commercial TiO_2-P25 caused strand breaks resulting in conformational change. In addition, bands belonging to smaller fragments indicated the degradation of DNA as was also observed during ozone treatment. Dunford et al. (1997) have shown that strand breakage in plasmid DNA during TiO_2 photocatalysis took place by direct oxidation via hydroxyl radicals and Shen et al. (2008) have confirmed conformational changes in plasmid DNA upon treatment with photosensitized nano-TiO_2 material structure by atomic force microscopy (AFM).

The influence of nano-fiber-TiO_2 photocatalyst on the plasmid DNA is shown in Figure 4.8.

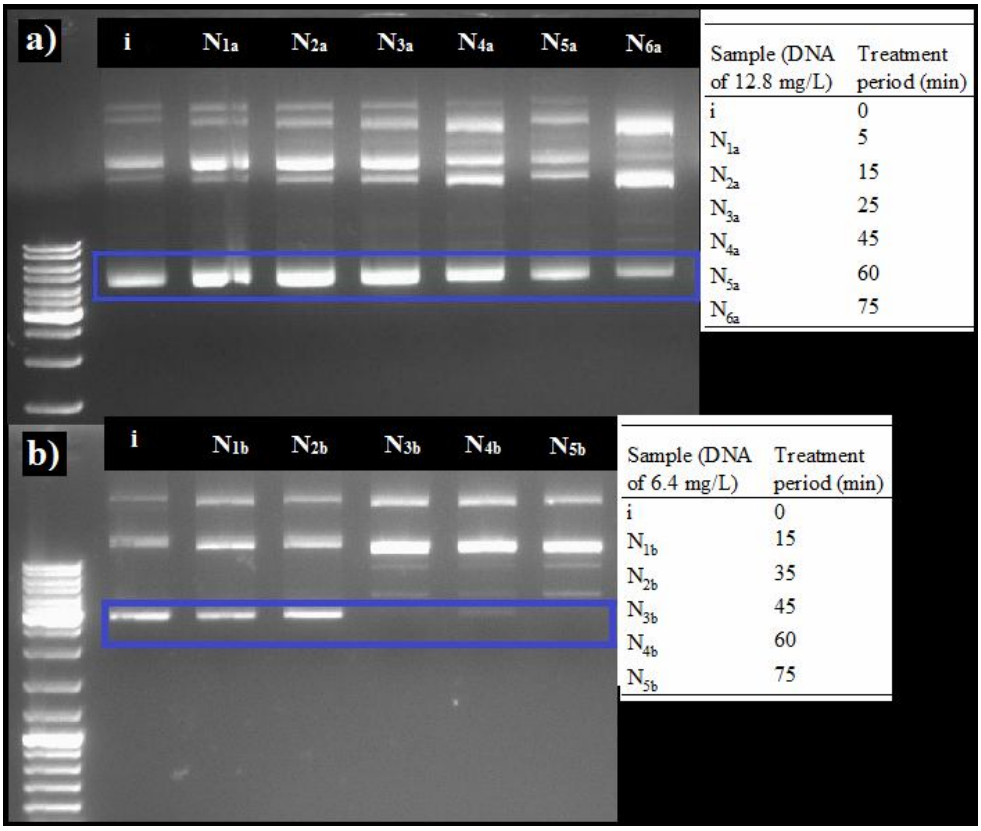


Figure 4.8. Agarose gel electrophoresis of nano-fiber-TiO_2 (N) treated plasmid DNA (a) high concentration – 12.8 mg/L (b) low concentration plasmid DNA – 6.4 mg/L. Marked bands: supercoiled conformation.

Treatment with the nano-fiber-TiO_2 photocatalyst also caused conformational changes in plasmid DNA and the effect became more pronounced with increasing treatment periods. The disappearance of the supercoiled Plasmid DNA band after 45 min treatment period can be observed for plasmid DNA having lower concentration. No major change occurred after 45 min and the bands belonging to other conformations of the plasmid DNA remained unchanged even after 120 min (data not shown). Even though the surface areas of the two photocatalysts were comparable, the commercial photocatalyst was more effective on plasmid DNA. These indicate that the material properties of the nano-fiber-TiO_2 should be further improved. For instance the rutile and anatase ratios can be of critical importance as it has been shown that some specifically arranged mixtures of anatase and rutile can be more active than either form (Hurum et al., 2003).

4.3.2.2. Influence on Transformability of Competent Cells. Transformability of competent cells with treated plasmid DNA was investigated in order to correlate the damaging effect of oxidants with their ability to reduce the potential of the plasmid DNA to transfer antibiotic resistance. For this purpose, samples of plasmid DNA treated at several doses of oxidants (typically the lowest and the highest oxidant doses) were selected. Decrease in the transformation ability of treated plasmid DNA was calculated relative to the transformation ability of non-treated plasmid DNA as $\log(N/N_o)$, where N and N_o are numbers of colony forming units counted on the plates containing competent cells combined with treated and non-treated samples, respectively. Transformation ability of plasmid DNA at both concentrations was not affected even with the highest chlorine dose (5 mg/L) and thus the data was not plotted. Similarly, data for the nano-fiber-TiO_2 photocatalyst was not plotted, since the material did not considerably influence plasmid DNA and its transformation ability. The results only for ozone treatment and photocatalytic treatment with commercial TiO_2-P25 are displayed in Figure 4.9 as $\log(N/N_o)$ plot against ozone dose and treatment period for TiO_2 photocatalysis, respectively.

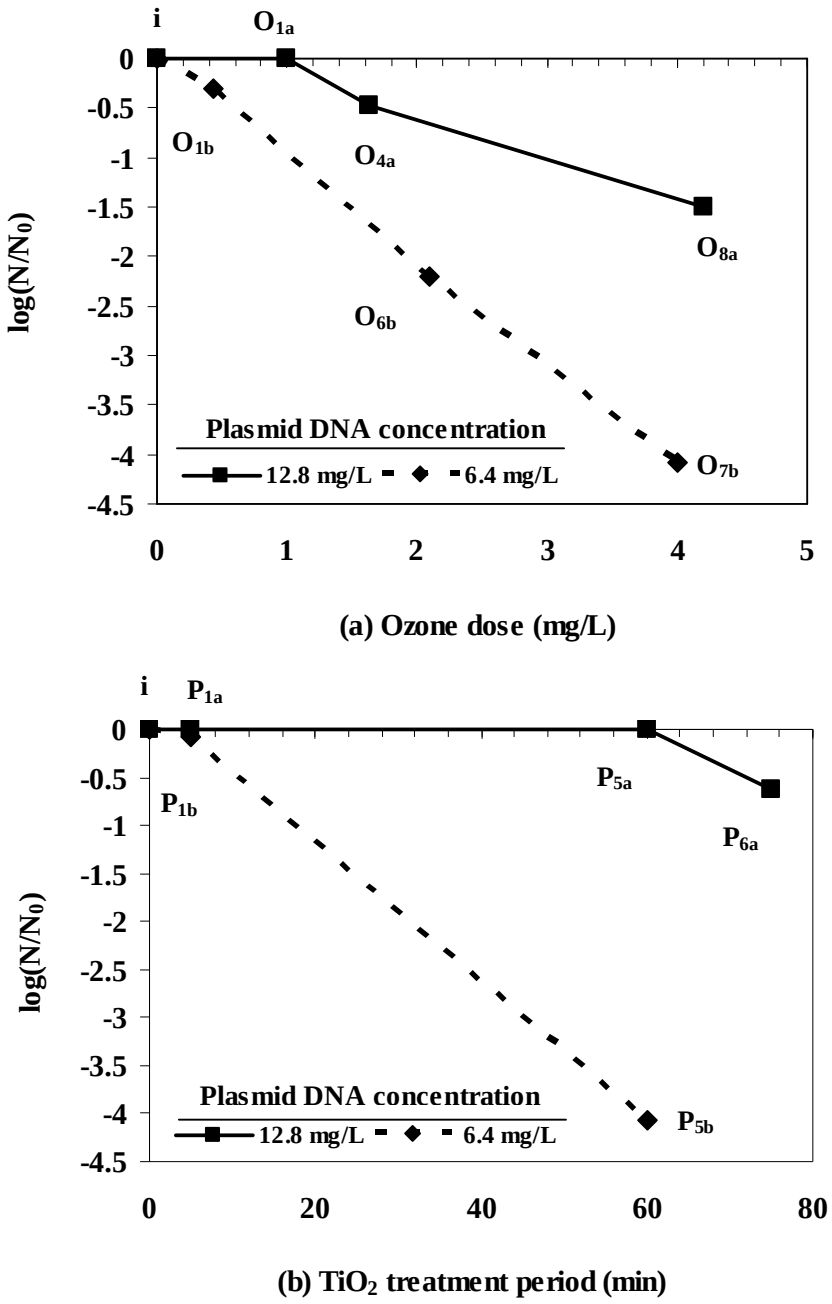


Figure 4.9. Effect of (a) ozone and (b) TiO_2-photocatalysis on the ability of plasmid DNA to transform competent XL-blue cells. Selected ozone doses are: O_{1a} – 0.4 mg/L, O_{4a} – 1.6 mg/L, O_{8a} – 4.2 mg/L for high concentration plasmid DNA – 12.8 mg/L; O_{1b} – 0.4 mg/L, O_{6b} – 2.1 mg/L, O_{7b} – 4.0 mg/L for low concentration plasmid DNA – 6.4 mg/L. Selected TiO_2 treatment periods are: P_{1a} – 5 min, P_{5a} – 60 min, P_{6a} – 75 min for high concentration plasmid DNA – 12.8 mg/L; P_{1b} – 15 min, P_{5b} – 75 min for low concentration plasmid DNA – 6.4 mg/L.

A similar finding for the effects of chlorine dioxide on transformability was reported in a previous study (Roller et al., 1980) where both plasmid DNA extracted from the treated microorganisms and treated naked plasmid DNA were investigated. Treated naked plasmid DNA was only minimally affected at a very high chlorine dosage of 20 mg/L and with longer treatment periods. Naked plasmid DNA concentration was not reported by the authors; however it is expected to be comparable with the concentration of plasmid DNA obtained from the treated microorganisms, the initial concentration of which was 2×10^8 cells/mL. This concentration is much lower than that utilized to obtain the relatively higher plasmid DNA concentrations studied in this study.

Samples, the electrophoresis images of which showed minimal change in the plasmid DNA conformation in the gels, also showed minimal effect on the ability to transform competent cells; high concentration plasmid DNA treated with ozone dose of 1 mg/L or with TiO_2 for 60 min for instance. Oxidation both with ozone and by TiO_2 photocatalysis resulted in much higher transformability decrease in the lower concentration plasmid DNA samples and no colony forming units were found on the plates of samples treated with 4 mg/L ozone dose or by TiO_2 for 75 min. In the gel images of these samples, no plasmid DNA bands were detected. Although natural transformation mechanisms and required conditions for transformation may show variations depending on the nature of the microorganism (Lorenz and Wackernagel, 1994), it has been shown that disruption of plasmid integrity leading to linear plasmid DNA conformations during in vitro ligation reactions can result in up to 2-3 fold decrease in transformability efficiencies in *E. coli* (Conley et al., 1986). Therefore linearization of plasmid DNA is expected to result in decrease in transformability. However, in transformation experiments with *E. coli* and plasmid DNA of sizes varying from 2 kb to 66 kb, Hanahan et al. (1983) have shown that the transformability efficiencies of relaxed plasmid DNA forms were approximately 75% of that of their supercoiled forms, showing that relaxed plasmid DNA can still transform competent cells effectively. The results obtained here similarly have shown that although disruption of plasmid DNA integrity did result in decrease in transformability efficiencies, complete loss of the transforming activity was only achieved with complete plasmid DNA destruction by the oxidants. Therefore, oxidant damage on plasmid DNA reported in previous studies

alone is not sufficient to conclude that the risk of resistance transfer is eliminated. As noted previously, protection of exogenous DNA by suspended material in water matrices further underlies the significance of this finding. Therefore, careful monitoring of water and application of effective oxidant doses is important. It can be proposed that ozone and TiO_2-photocatalysis can decrease and prevent ability of plasmid DNA to transform competent cells and thus are both expected to help reducing the risk of antibiotic transfer.

4.4. Conclusion

Effects of chlorination, ozonation, and TiO_2 mediated photocatalytic processes on plasmid DNA was comparatively investigated for the elimination of antibiotic resistance pollution in water. The results revealed that removal of all plasmid DNA bands from the gel image necessitated a high ozone dose and a longer photocatalytic treatment period with commercial TiO_2-P25. However, it is possible to remove lower plasmid DNA concentration with lower ozone doses and shorter photocatalysis treatment durations; but in this case mass transfer limitations should also be considered. While ozone and commercial TiO_2-P25 induced damage in the plasmid DNA structure and therefore led to decrease in competent cell transformability, no effect of chlorine and low effect of nano-fiber-TiO_2 was observed during the experiments.

5. OZONATION OF SECONDARY SEWAGE SLUDGE: ANTIBIOTIC DEGRADATION AND SLUDGE SOLUBILIZATION

5.1. Introduction

Wastewater sludge harbors valuable constituents such as organic matter and nutrients as well as a wide variety of organic and inorganic contaminants originating from human activities (Verlicchi et al., 2012; Olofsson et al., 2012; Clarke et al., 2011). Up to date, over 360 OCs have been identified in sewage sludge (Eriksson et al., 2008) and the list is expected to grow as a result of rapid developments in analytical techniques. The beneficial use of waste sludge in agriculture has gained higher importance as the waste sludge volumes generated from wastewater treatment increased considerably. In order to minimize potential negative environmental impacts, the agricultural use of waste sludge both in the EU and the US has been limited by specific regulations that mainly monitor heavy metal concentrations and pathogens (CEC, 1986; US-EPA, 1993). Due to increased environmental concerns, some countries have also adapted standards for the regulation of several OCs (Schowanek et al., 2004) based mainly on the latest EC Working Document on Sludge (CEC, 2000). Concurrently, comprehensive risk assessment studies were carried out in several EU states and in the US (Jensen et al., 2012; Eriksen et al., 2009; US-EPA, 2009; Lindberg et al., 2007; Schowanek et al., 2004). Although in the majority of these studies, it was concluded that many of the investigated OCs including brominated flame retardants, musk substances, polychlorinated biphenyls, and pharmaceuticals, were unlikely to pose a significant threat to the environment, concerns have also been emphasized regarding the limited knowledge on the ecotoxicological impacts of OCs and their metabolites, as well as potential synergistic effects in the presence of a wide span of OCs.

Among various persistent OCs, antibiotics deserve a major concern because of their possible contribution to the risk of antibiotic resistance development at subtherapeutic levels (Kim and Aga, 2007; Rooklidge, 2004). Recent reviews of occurrence data have shown that some antibiotics, specifically those belonging to TET and FQ groups tend to accumulate in sewage sludge mainly by partitioning to the solid phase (Oncu-Bilgin and

Balcioglu-Akmehmet, 2013a, Zhang et al., 2011). These antibiotics are generally detected in the sludge in the low mg/kg range concentrations; however, concentrations as high as 40.8 mg/kg and 97.5 mg/kg were also reported for CIP in some reports (US-EPA, 2009, SFT, 2007). Field experiments of sludge application to soil also revealed the long-term persistence of FQs in soil (Golet et al., 2003). Although it was pointed out that resistance development under the selective pressure of antibiotics in soil is of minimal importance, since the resistance will disappear as soon as the selective pressure is removed (Smith et al., 2009), the long-term persistence potential of some antibiotics indicates that resistance development is plausible.

The urgent concerns driven by the rapidly expanding literature on the occurrence of various persistent OCs (e.g. endocrine disrupting compounds and micro-organics such as polyaromatic hydrocarbons, pharmaceuticals and personal care products) resulted in a number of studies attempting to reduce their concentrations in sewage sludge via different chemical oxidation processes (Pham et al., 2011; Carballa et al., 2007; Carballa et al., 2006; Carrere et al., 2006; Flotron et al., 2005), which were formerly employed in order to minimize sludge production during biological wastewater treatment and to improve sludge biodegradability (Carrere et al., 2010; Bougrier et al., 2007; Wei et al., 2003). Among several treatment technologies for sludge, ozonation has gained acceptance as one of the most effective disintegration technologies (Muller, 2000) and has been utilized successfully in full-scale applications (Chu et al., 2009). Application of ozone at the recycle line of the activated sludge treatment process can reduce sludge generation up to 70%, which requires high ozone dose e.g. 50 mg/g treated volatile suspended solids (VSS) (Deleris et al., 2002). Furthermore, lower dose of ozone can improve sludge biodegradability greatly (Bougrier et al., 2007, Yeom et al., 2002). The benefits of ozonation in sludge treatment are not limited with these applications. Ozonation has also been utilized for sludge stabilization and conditioning (Park et al., 2003). Among the studies dealing with sludge treatment, the fate of antibiotics is a scarcely investigated subject. High degradation rates for sulfamethoxazole were reported by chemical and thermal pretreatment combined anaerobic digestion application (Carballa et al., 2006; 2007), however, sulfonamide antibiotics exhibit poor sorption tendency to sludge. To the best of our knowledge, the degradation of strongly sorbed TET and FQ antibiotics in sludge has not been reported in the literature.

The objective of this part of the thesis is to investigate the degradation of CIP and OTC selected from the TET and FQ group antibiotics during ozonation of secondary sewage sludge. In this context, lab-scale experiments were conducted for sludge with different TS concentrations, considering the different applications of ozone to sludge. The effect of pH on the ozonation performance was investigated both for the assessment of oxidation mechanism and the desorption of the antibiotics. Special emphasis was given for the desorption of the antibiotics from the sludge to enhance the treatment performance of ozonation. Organic matter and metal solubilization were also investigated in order to evaluate their influence on the antibiotics degradation and the performance of ozonation for secondary sludge solubilization.

5.2. Materials and Methods

5.2.1. Chemicals

Hydrochloride forms of OTC (Sigma) and CIP (Mp. Biomedicals) were utilized to spike the sludge. *para*-Chlorobenzoic acid – *p*CBA (Aldrich) was utilized as the hydroxyl radical probe and *t*-butanol – TBA (Sigma-Aldrich) was utilized as the $OH^{\bullet}$ radical scavenger. Formic acid and HPLC grade acetonitrile utilized in the HPLC analyses; magnesium sulfate and magnesium nitrate were all obtained from Sigma-Aldrich. All solutions were prepared with MQ water (Millipore, Milford, MA).

5.2.2. Preparation of Antibiotic Contaminated Sewage Sludge.

The physicochemical parameters of the sludge obtained from Pasakoy-Istanbul municipal wastewater treatment during the SC-1 are given in Table 5.1. Prior to the ozonation experiments, depending on the desired TS amount either distilled water or antibiotic containing aqueous solutions were used to dilute the sludge that was concentrated upon collection (See Section 3.1.3).

Table 5.1. Average secondary sewage sludge characteristics and total metal concentrations for the SC-1 period.

Sludge properties		Metals	
Parameter	**Value**	**Metals**	**Sludge concentrations (mg/kg DS)**
TS (g/L)	10.6±0.8	Ni	200±15
VS/TS (%)	59±4	Cr	320±10
TCOD (g/L)	10.7±1.4	Cu	220±20
SCOD (mg/L)	125±75	Zn	780±50
TKN (mg/L)	437±26	Fe	11,150±2,300
TP (mg/L)	543±51	Mn	530±20
PO_4^{3-}	67±19	Cd	BLD*
pH	6.5−7.0	Pb	10±8
Alkalinity (g/L)	1.5		

*BLD: below limit of detection. The data are the mean values obtained from n=15 samples for sludge parameters and n=3 samples for the total metal concentrations.

The degradation of antibiotics at two different concentrations was studied in sludge. While at high concentration, 20 mg/L of OTC and CIP (2–8 mg/g TS) was spiked to sludge with TS of 2.5, 5.6, and 10 g/L, at low concentration, 0.8 mg/L of these antibiotics (0.08 mg/g TS) was applied only for sludge with TS of 10 g/L. The contaminated sludge was equilibrated overnight (16±1 h) at 25±5ºC in a temperature controlled water bath at 200 rpm. Depending upon the antibiotic and TS concentration of the sludge, equilibration provided 85–95% antibiotic sorption at the natural pH of sludge. The pH adjustments were carried out by addition of 9.4 M H_2SO_4 or 12.5 M NaOH, and a period of 15 min was allowed for sludge equilibration before ozonation. The added acid and base volumes did not exceed 0.07% of the sludge volume for the pH from 4 to 9 and 0.3% for pH 11.5.

5.2.3. Ozonation of Secondary Sewage Sludge

Sludge of 1.5 L was ozonated in the cylindrical glass column reactor as described in Section 3.2.5.1 (Figure 3.4). In the experiments exploring the effect of pH, absorbed ozone doses were in the range of 0.31 – 0.45 g O_3/g initial TS within 30 min treatment period and in the experiments exploring the effect of TS the absorbed ozone doses were in the range of 0.47 – 1.17 g O_3/g initial TS within 60 min treatment period. The effects of pH value and TS content of sludge on the ozonation performance were investigated in a series of

experiments with 20 mg/L antibiotic concentration to get a better understanding of the effect of the selected process variables. At specified experimental conditions, the effect of antibiotic desorption on ozonation performance was studied at both 20 mg/L and 0.8 mg/L CIP and OTC concentrations in order to evaluate degradation of the antibiotics found in environmentally relevant concentrations. The desorption of the antibiotics was provided either by alkaline addition or magnesium salt pretreatment carried out for 60 min at pH 8. All experiments were performed at least in duplicate.

5.2.4. Analytical Methods

During ozonation, sludge samples were periodically taken from the reactor to evaluate the performance of the process. OTC and CIP were extracted from raw and ozonated sludge samples with ultrasonic solvent extraction and concentrated with SPE. Consequent analysis of the antibiotics was carried out by HPLC. Detailed description of the procedures is given in Section 3.2.6.6.

Recoverability of the antibiotics was not affected by ozonation; this was confirmed after contaminating ozonated sludge samples with the antibiotics and carrying out the described extraction and analysis procedures. Antibiotic degradation was calculated as the percent change in the antibiotic concentration after sludge treatment. Initial antibiotic concentrations were determined in each experiment in order to eliminate the probable influence of changes in the sludge characteristics on the antibiotic recoveries. In order to evaluate the desorption of CIP and OTC from sludge, soluble antibiotic concentrations in the supernatant were also analyzed after following the described extraction procedures.

Characterization of sludge samples with parameters including TS, VS, SCOD, TCOD, TKN, TP, metals, NH_4^+, and PO_4^{3-}, and SV_{60}, was performed as described in Sections 3.2.6.2 and 3.2.6.6.

Analysis of the radical probe *p*CBA was carried out with HPLC (See Section 3.2.6.7).

5.3. Results and Discussion

5.3.1. Effect of Initial pH

It is known that during ozonation dissolved components of a treated heterogeneous matrix are more vulnerable to oxidation compared to those sorbed on particulate material (De Witte et al., 2010; Carrere et al., 2006, Huber et al., 2005; Cesbron et al., 2003). In this respect, the desorption of the antibiotics could be crucial to provide efficient degradation in sludge by ozonation process. Although the sorption/desorption behavior of antibiotics on the sludge can be very complex, by taking into account the electrostatic interactions between the charged functional groups of investigated antibiotics and the negatively charged sludge particles, the desorption of CIP and OTC would be expected by increasing the pH of the sludge. Furthermore, pH can affect the degree of protonated species dissociation and their reactivity with ozone (Hoigne and Bader, 1983) as well as the ozone oxidation mechanism. Therefore, the degradation of the antibiotics over a wide pH range by varying the initial pH of sludge (TS=5.6 g/L) from 4 to 11.5 requires special attention. Before the application of ozone to sludge, the dissolved phase concentrations of the antibiotics and SCOD were determined at each investigated pH by analysis of the supernatant (Figure 5.1 (a-b)).

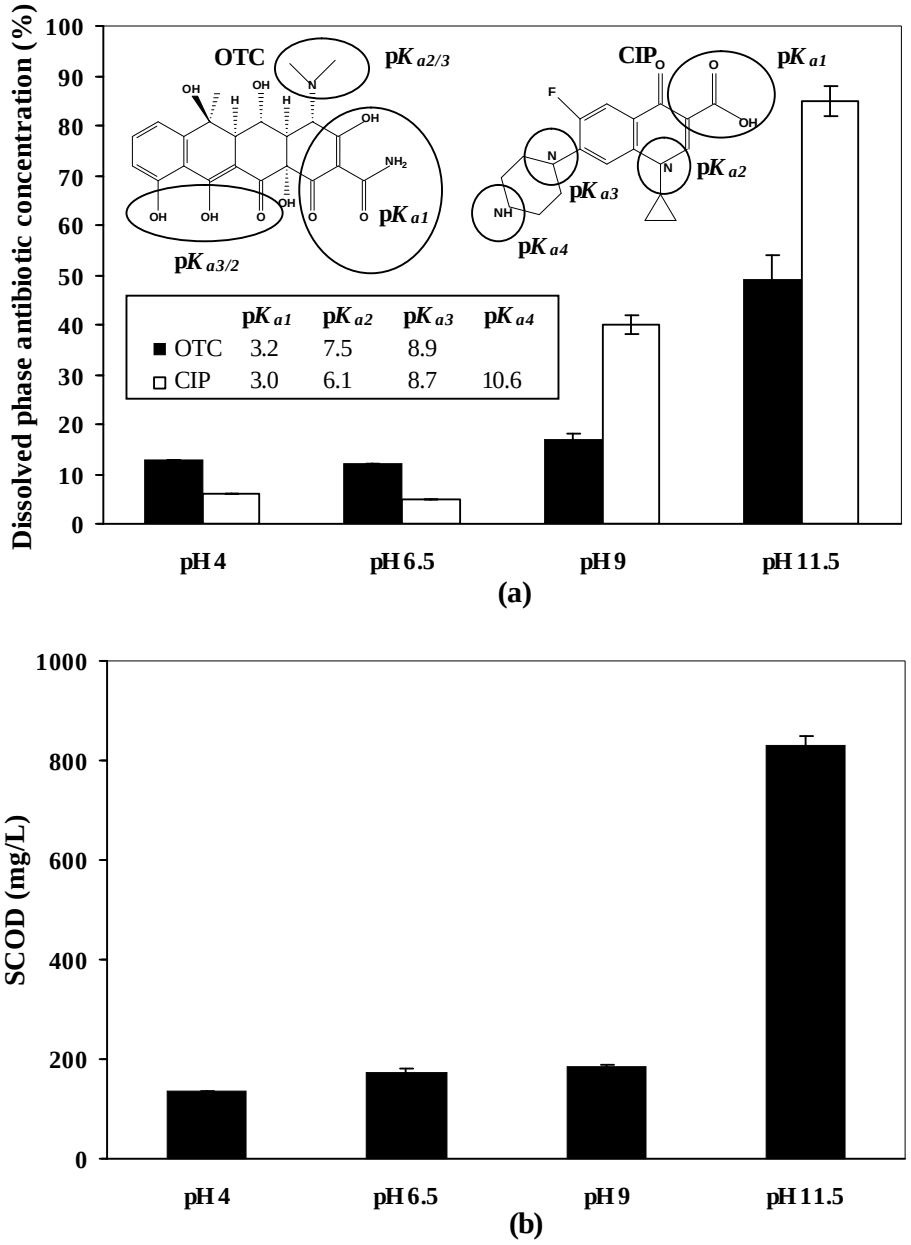


Figure 5.1. (a) Influence of pH on antibiotic desorption from sludge and pH dependent speciation of OTC and CIP (b) Influence of pH on SCOD.

As depicted in Figure 5.1 (a) and (b), the elevation of pH to 11.5 resulted in the release of organics from sludge. As well as COD solubilization, 90% CIP and 49% OTC desorption was achieved, since both antibiotics are negatively charged at pH=11.5 as deduced from the p*Ka* values (Qiang and Adams, 2004) inserted in the figure. The difference in the desorption rates of CIP and OTC could be related to structural differences

in their molecules. In fact, similar desorption behavior of FQ antibiotics was also observed in wastewater (De Witte et al., 2010).

The effect of subsequent ozonation on the degradation of CIP and OTC in sludge equilibrated at different pH values is shown in Figures 5.2 (a) and (b), respectively. The results primarily demonstrated that low ozone dose was not sufficient to effectively degrade both antibiotics. In addition, the degradation pattern of each antibiotic clearly shows that both the initial and overall ozonation efficiency was influenced by the pH of the sludge. While 48% OTC and 18% CIP degradation was obtained within 15 min treatment period at pH 6.5 (the unmodified pH value of sludge), these degradation rates improved to 76% and 84% at pH 11.5, respectively. Prolonged ozonation up to 30 min at pH 11.5 resulted in 98% CIP and 88% OTC degradation. Fast initial degradation of the antibiotics at pH 11.5 could be attributed to the higher amount of initial dissolved antibiotic obviously accomplished by the elevation of sludge's pH (Figure 5.1 (a)). About 10% of OTC degradation took place during pH equilibration due to its instability at highly alkaline pH. The effect of the pH on antibiotic degradation was significant ($p<0.05$) for the initial 20 min of ozonation and became insignificant at 30 min after desorbed antibiotic concentrations were consumed.

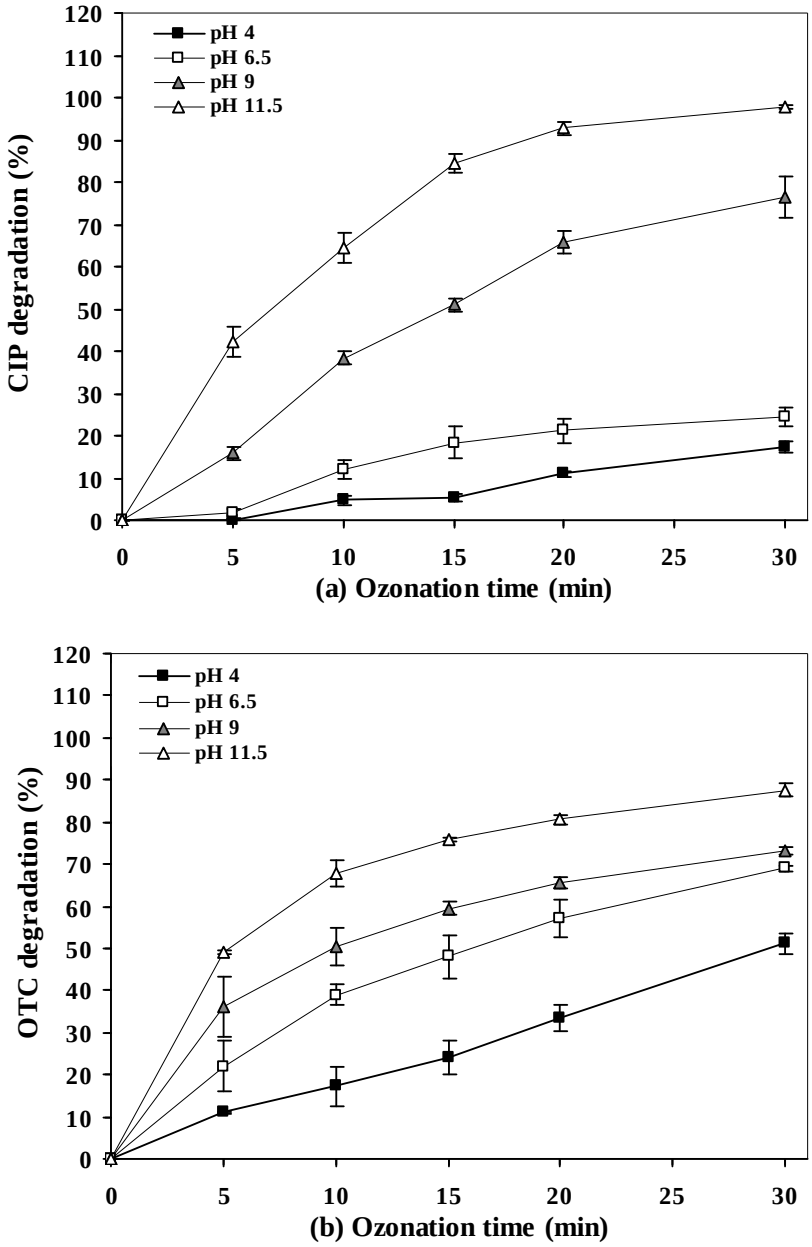


Figure 5.2. Degradation of (a) CIP and (b) OTC with ozonation at various pH values (TS=5.6 g/L, $[\text{antibiotic}]_0$ = 20 mg/L).

It is known that both OTC and CIP are highly reactive with ozone and hydroxyl radicals in liquid media and that OTC is relatively more reactive compared to CIP (second order rate constants at pH 7: $k_{O3,\ TET} = 1.9 \times 10^6$, $k_{O3,\ CIP} = 1.9–2.2 \times 10^4$, $k_{OH\bullet,\ TET} = 7.7 \times 10^9$ (Dodd et al., 2006), $k_{OH\bullet,\ CIP} = 4.1–5 \times 10^9$ (Lester et al., 2011; Dodd et al., 2006). However, as can be seen from Figure 5.2, higher degradation of CIP was achieved at pH 9

and 11.5, at which higher desorption of CIP than that of OTC was obtained. The overall degradation of OTC did not exhibit pronounced variation over a wide range of pH, which can be explained by the primary attack of ozone to the tetracycline ring system (Dodd et al., 2006). On the other hand, the reactivity of CIP increases considerably with increase in pH since the degradation of CIP is primarily governed by pH-dependent speciation (Dodd et al., 2006, De Witte et al., 2009; Buffle and Von Gunten, 2006). In this study, to assess the contribution of radical oxidation to the degradation of the antibiotics, the degradation of a hydroxyl radical probe, *p*CBA ($0.6x10^{-2}$ mM), during the ozonation of sludge was investigated. Since *p*CBA exhibited limited sorption (15%) on sludge only at pH 4 (as opposed to strong sorption of antibiotics on sludge at acidic pH), the degradation of *p*CBA was evaluated by pseudo first-order kinetics (Figure 5.3).

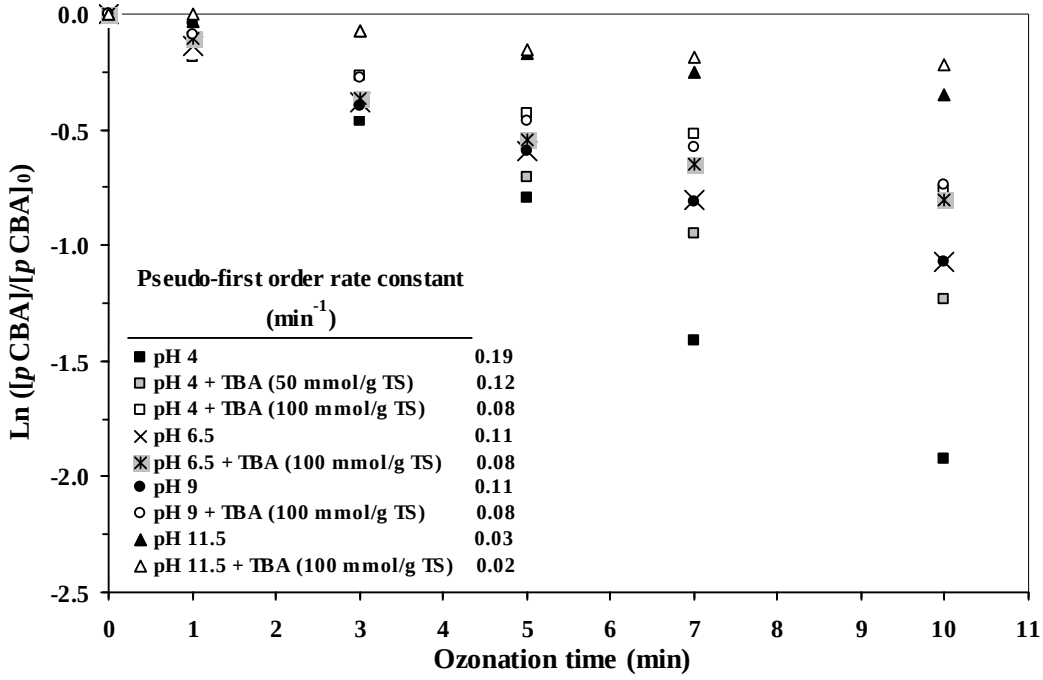


Figure 5.3. The pseudo first-order plots of *p*CBA versus time with and without TBA during ozonation at various pH values. ($[pCBA]_0=0.6\times10^{-2}$ mM, TS=5.6 g/L).

Contrary to expectations, the highest decomposition rate of *p*CBA was observed at pH 4 and the addition of TBA at two different concentrations (50 and 100 mmol/g TS) greatly reduced the decomposition of *p*CBA, suggesting the formation of reactive radicals.

However, the degradation of *p*CBA was not completely eliminated by TBA addition, which suggested that even the high dose of TBA could be insufficient for completely scavenging the radical species, probably because of the high complexity of the sludge matrix. Further increase of the TBA concentration was avoided due to possible influence on the ozone mass transfer in the sludge (De Witte et al., 2009). On the other hand, the lowest decomposition of *p*CBA was observed at pH 11.5 and the influence of TBA addition on the rate constant was minimal at this pH. The scavenging of radicals rapidly by the solubilized organic components and the alkalinity constituents (Table 5.1) of sludge (Acero and Von Gunten, 2000) could explain the lower degradation rate of *p*CBA obtained at pH 11.5. In order to clarify these results the solubilization of organic matter and metals from sludge during ozonation were also investigated; and soluble metal concentration and SCOD variations by the application of ozonation process at different pH values are represented in Figure 5.4 (a) and (b), respectively. The release of metals as a regulated pollutant group was also of interest for the evaluation of the metal load reduction in sludge.

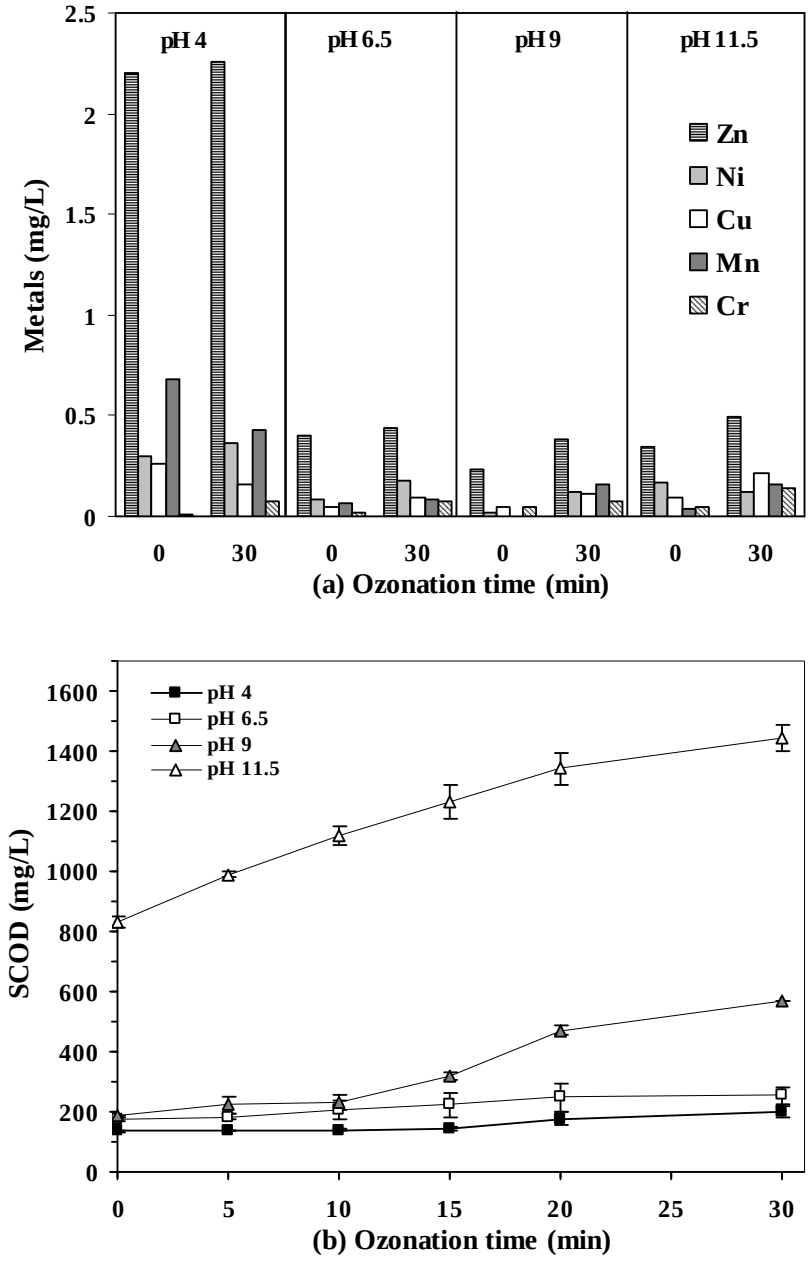


Figure 5.4. Solubilization of (a) metals and (b) SCOD with ozonation at various pH values (TS=5.6 g/L).

As shown in Figure 5.4 (a), the acidic pH resulted in the release of metals from the sludge most likely due to the dissolution of the carbonate fractions (Babel and Dacera, 2006). Solubilization of metals ranged from 20% to 48% for Zn, Ni, Mn, and Cu after equilibration at acidic pH. It should be also noted that total metal values were below the limit values that are set in the regulations for land-application of sludge (CEC, 1986).

However, higher metal solubilization at acidic pH might explain the highest degradation rate of *p*CBA, since catalytic action of dissolved metals can produce reactive radicals during ozonation. Actually, dissolved metal ions were used to accelerate solubilization of sludge in a recent study (Lee et al., 2010). Although metal solubilization and generation of reactive radicals were achieved at pH 4, the degradation of the antibiotics was poor due to their strong sorption on particulate matter at this pH. While 15% COD solubilization was achieved as a result of the alkaline addition to sludge (Figure 5.1 (b)), the overall COD solubilization attained was 33% by subsequent ozonation (Figure 5.4 (b)). The relatively high initial dissolved organic load of sludge at pH 11.5 did not eliminate the antibiotic degradation because an initial desorption of the antibiotics was already achieved. However, further solubilization of organics in the composition of the sludge by ozonation clearly caused a competition for the oxidants. At the time corresponding to higher SCOD release during ozonation (15 min), the degradation of CIP and OTC slowed down. It is also known that deprotonated species generally react readily with ozone resulting in higher ozone absorption (Buffle and Von Gunten, 2006) as observed in this study. While the absorption of ozone at pH 4 was 0.30 g/g initial TS, it was 0.45 g/g initial TS at pH 11.5 which caused higher organic matter solubilization.

5.3.2. Effect of Total Solid Concentration

As mentioned previously, ozonation of sludge is applied both to induce cryptic growth during the activated sludge process (Chu et al., 2009, Deleris et al., 2002) and to improve the biodegradability of waste sludge prior anaerobic treatment (Bourgier et al., 2007, Yeom et al., 2002). Since the TS content of sludge at these implementations can vary to a great extent, the effect of solid content on the fate of antibiotics during ozonation of sludge was investigated at three different TS values (2.5 g/L, 5.6 g/L, and 10.0 g/L) by spiking constant CIP and OTC concentrations (Figure 5.5 (a) and (b)). These experiments were carried out at pH 9. pH 11.5 was avoided due to the previously observed instability of OTC, which could hamper understanding the effect of TS on ozonation efficiency. The ozonation period was extended to 60 min in these experiments in order to clarify the degradation behavior of residual antibiotic concentrations. Depending on the TS content of sludge, the adjustment of pH to 9 resulted in different desorption rates of CIP and OTC (inserted tables in Figure 5.5 (a) and (b)).

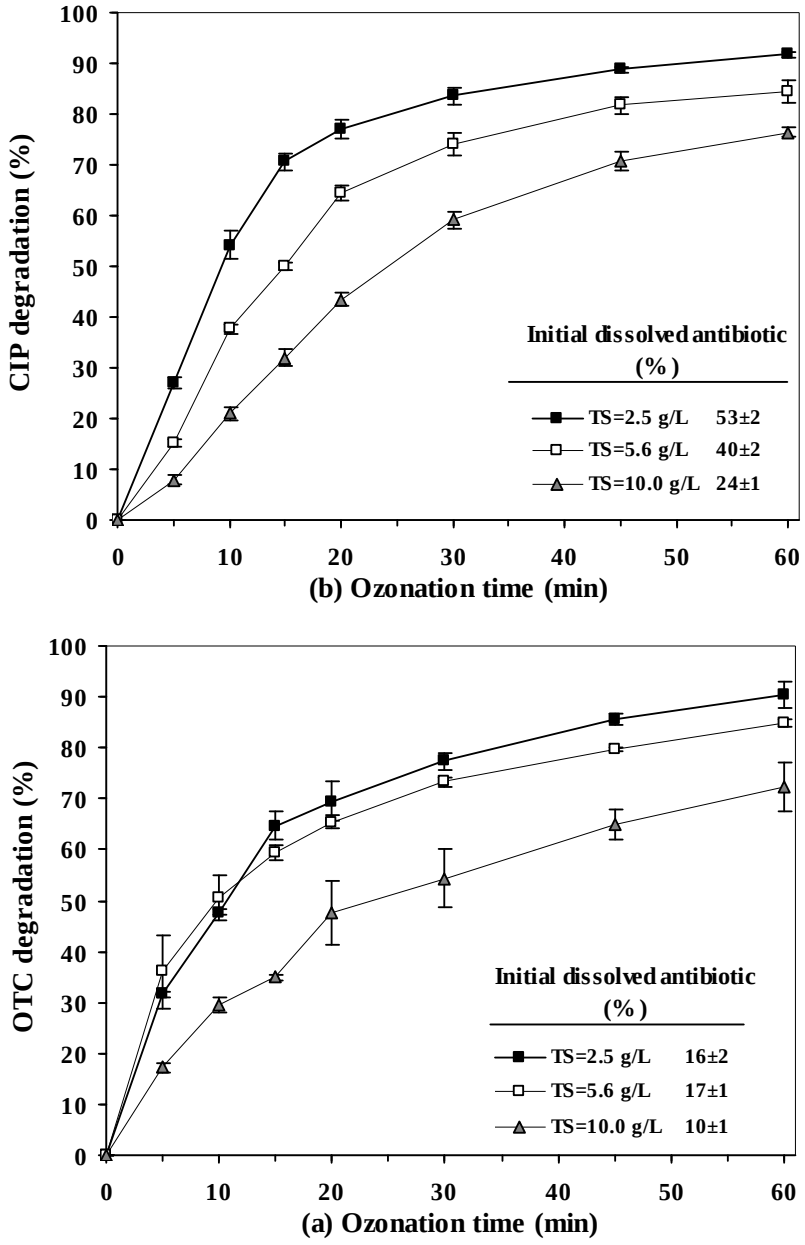


Figure 5.5. Degradation of (a) CIP and (b) OTC with ozonation at various TS values (pH 9, $[\text{antibiotic}]_0$ = 20 mg/L).

As can be seen from Figure 5.5 (a) and (b), the overall CIP and OTC degradation rate significantly (p<0.05) decreased with an increase in the TS concentration of sludge. The inefficient performance of ozonation with high TS concentration can be attributed both to the lower initial dissolved antibiotic fraction and the higher SCOD release, which is shown in Figure 5.6 (b). At high TS concentrations of sludge, the scavenging of oxidants with the

dissolved components of the sludge before encountering a dispersed particle would be expected. Accordingly, four fold increases in TS concentration of sludge resulted in 14% and 20% abatement in overall antibiotic degradation rates of CIP and OTC, respectively. However, even at the lowest concentration of TS, complete elimination of the antibiotics was not obtained within extended treatment period due to the competition of solubilized organics (Cesbron et al., 2003) that continued to be released throughout the ozonation period. Higher TS concentration of sludge resulted in higher consumption of ozone and reactive radicals (their formation were shown by the degradation of *p*CBA in Figure 5.3) and this produced higher COD and metal solubilization (Figure 5.6 (a) and (b)). As a consequence, ozonation of sludge at 2.5 g/L TS concentration resulted in 15% COD solubilization while 12% COD solubilization was achieved for sludge with TS concentration of 10 g/L.

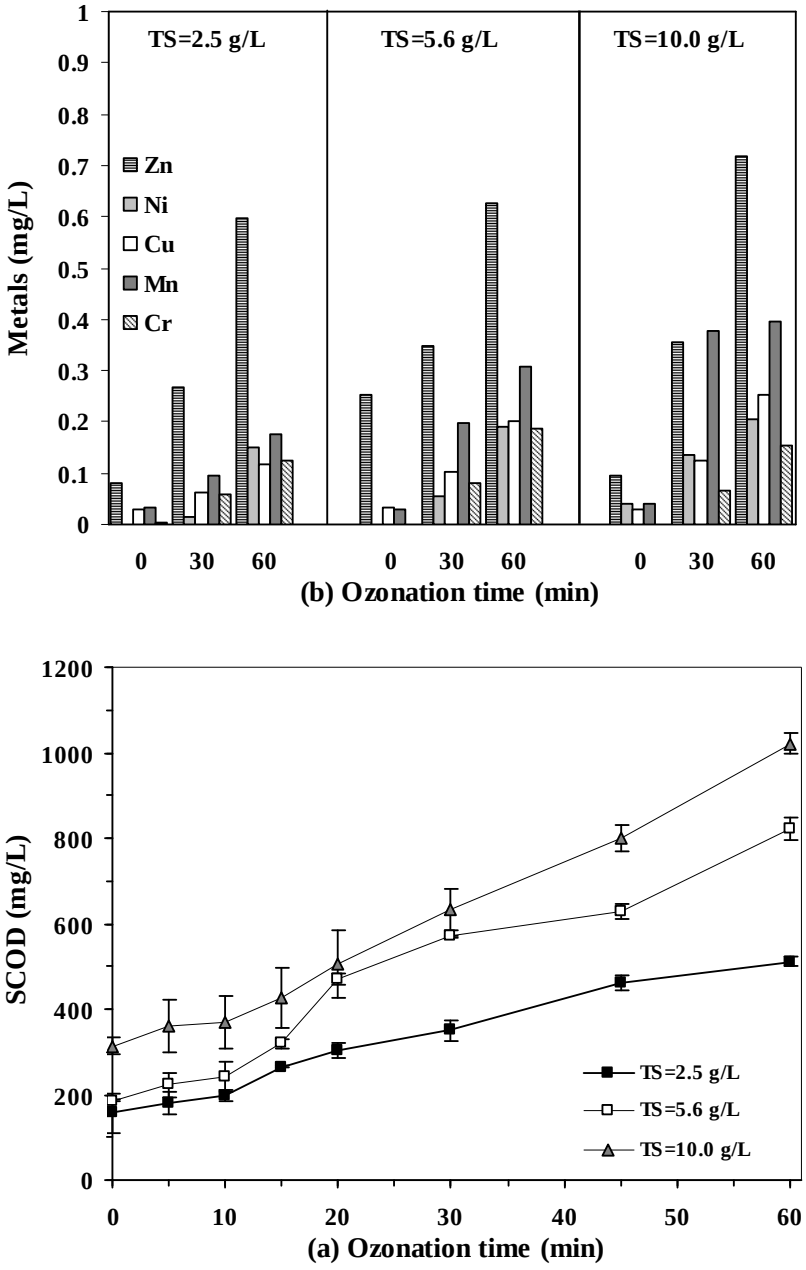


Figure 5.6. Solubilization of (a) metals and (b) SCOD with ozonation at various TS values (pH 9).

5.3.3. Effect of Antibiotic Desorption

The above mentioned results clearly indicate that efficient degradation of the contaminants in sludge during ozonation is dependent upon their desorptions. Considering this fact and higher ozone absorption at the higher TS concentration of sludge, in this part

of the study the effectiveness of two approaches for the treatment of sludge with high TS concentration (TS=10 g/L) was compared. The first approach involved the combined application of ozonation with Mg^{2+} pretreatment at pH 8, which was previously shown to provide a considerable improvement in the abatement of antibiotic contamination in manure (Uslu-Otker and Balcioglu-Akmehmet, 2009). The pretreatment of sludge spiked with 20 g/L antibiotic was performed with a Mg^{2+} dose of 0.91 g/L (75.2 meq/L) in 1 h period, which was determined by preliminary experiments (data not shown). After ozonation of pretreated sludge, 98% of the added Mg^{2+} was present in the dissolved phase of the sludge and thus can be recovered. The second approach, the effectiveness of which was already presented in this study, involved ozonation of sludge at pH 11.5 with a NaOH dose of 23.4 meq/L. The effect of the alkaline equilibration period performed prior to ozonation on the desorption of antibiotics was investigated at selected time intervals in a period of 3 h. Maximum desorption of antibiotics was achieved within 15 min and the extension of equilibration period did not increase the desorption of CIP and OTC (data not shown). The degradation rates of CIP and OTC at two different antibiotic concentrations are presented in Figure 5.7.

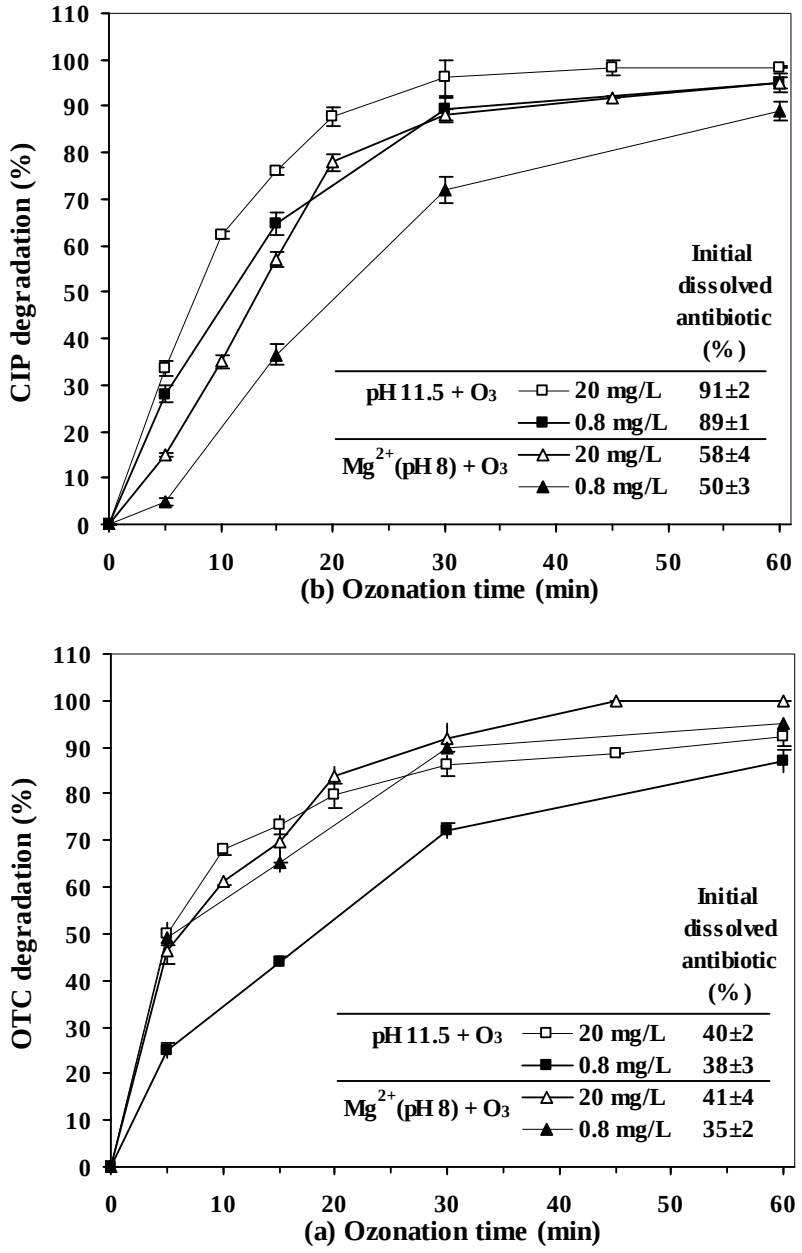


Figure 5.7. Degradation of (a) CIP and (b) OTC with Mg^{2+} pretreatment combined ozonation and ozonation at pH 11.5 (TS=10 g/L).

The complexation of tetracycline and fluoroquinolone antibiotics with magnesium (Eboka and Okeri, 2005; Tongaree et al., 2000; Ross and Riley, 1992) increased their solubility, hence 58% CIP and 41% OTC desorption were achieved at 20 mg/L antibiotic concentration (tables inserted in Figure 5.7 (a) and (b)).

Subsequent ozonation of sludge pretreated by Mg^{2+} provided almost complete OTC and 95% CIP degradation. Reducing the antibiotic concentration to 0.8 mg/L did not greatly influence antibiotic desorption (tables inserted in Figure 5.7 (a) and (b)). On the other hand, alkaline pH was remarkably more effective for the desorption of CIP at each concentration level. Despite the comparable desorption rate of CIP, ozonation efficiencies of the two different applications were lower for CIP and OTC at their low concentrations (0.8 mg/L), since the competition between the soluble compounds of sludge for oxidation depends on their concentrations as well as their reactivities (Cesbron et al., 2003). 22% COD solubilization was achieved during pH equilibration prior to ozonation and then solubilization was enhanced to 34% by the application of ozone (Figure 5.8 (b)). In case of ozonation of Mg^{2+} pretreated sludge only 9% COD solubilization was achieved at the end of the treatment period and the competition of organics did not hinder the degradation of antibiotics at the high concentration (Figure 5.8). A comparison of metal solubilization efficiencies of two approaches revealed that ozonation at alkaline pH produced higher solubilization. While solubilization of Mn, Cu, and Ni was in the range of 1–13% at the end of the magnesium salt pretreatment and ozonation combined process, the solubilization of these metals was 5–25% at the end of the alkaline ozonation process (Figure 5.8 (a)). Despite the high concentration of Fe in the sludge, solubilization during ozonation was generally $\leq$ 2% (data not shown).

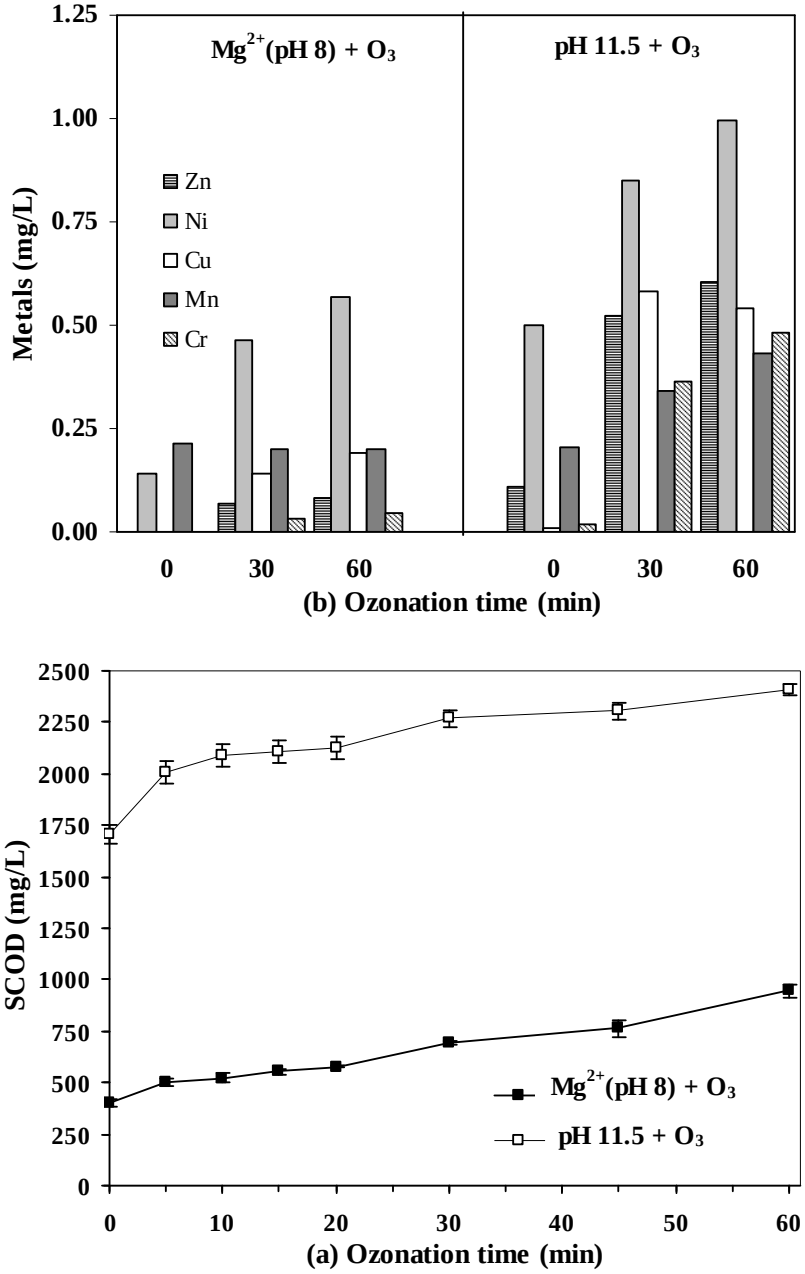


Figure 5.8. Solubilization of (a) metals and (b) SCOD with Mg^{2+}pretreatment combined ozonation and ozonation at pH 11.5 (TS=10.0 g/L).

5.3.4. Effect of Ozonation on Nutrient Solubilization

Release of NH_4^+ and PO_4^{3-} at different ozonation conditions are shown in Figure 5.8.

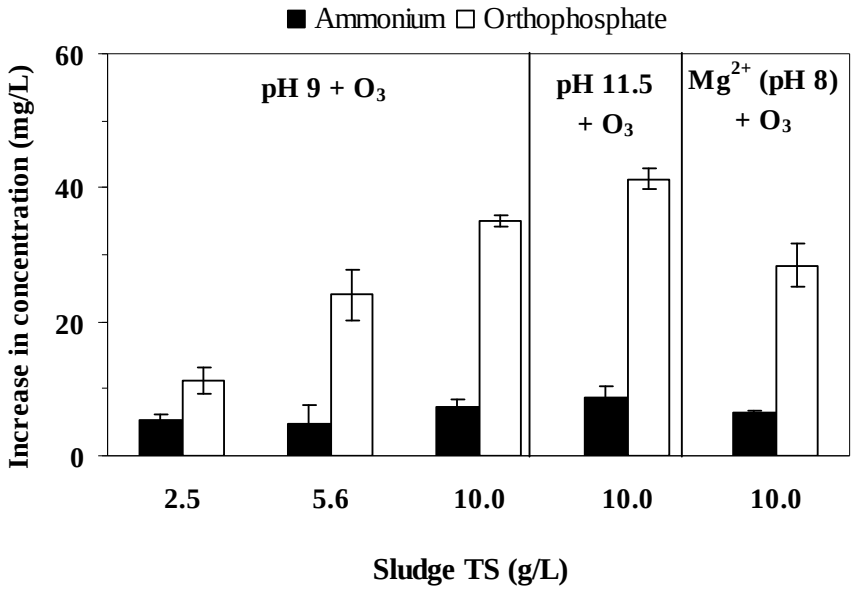


Figure 5.9. Solubilization of nutrients during treatment of secondary sludge with ozonation at different conditions.

The higher amount of organic load in the sludge at higher TS resulted in higher amounts of nutrient release. In addition, as expected, nutrient release was higher at pH 11.5 due to enhanced solubilization of the sludge and this was more obvious during equilibration period at this pH. However, the increase in nitrogen solubilization by ozonation at pH 11.5 compared to the value obtained by ozonation at pH 9 was not remarkable, probably due to loss of ammonia by volatilization. On the other hand, addition of Mg^{2+} to the sludge resulted in the precipitation of phosphate; therefore, phosphorous solubilization was lower than those obtained by ozonation at pH 10 and 9 as shown in figure. Actually, the presence of Mg^{2+}, NH_4^+, and PO_4^{3-} in the dissolved phase of the sludge presents a potential for nutrient recovery in the form of struvite (magnesium ammonium phosphate, MAP) (Le Corre et al., 2009).

5.3.5. Effect of Ozonation on Sludge Settleability

The influence of ozonation on sludge settleability, which was investigated by measuring the percentage of sludge volume settled in 60 min (SV_{60}), is illustrated in Figure 5.9.

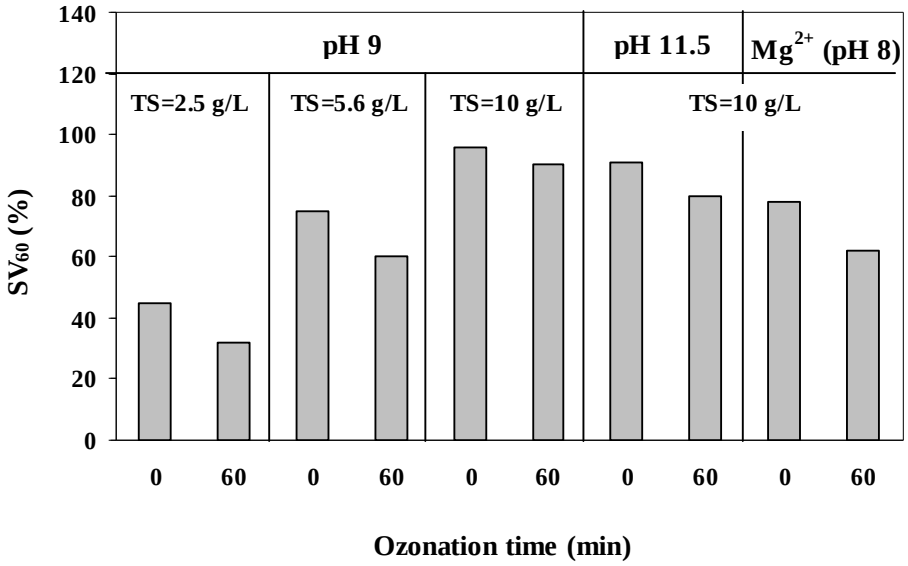


Figure 5.10. Influence of ozonation on the SV_{60} of secondary sewage sludge at different ozonation conditions.

Although ozonation reduced the SV_{60} value at each investigated TS, increasing the total solid content of the sludge had an adverse effect on the settleability of the sludge (Figure 5.9). Ozonation yielded a 13–15% decrease in the SV_{60} of sludge with TS of 2.5 g/L and 5.6 g/L, whereas the settleability of the sludge with TS of 10.0 g/L was decreased only by 6%. While ozonation at pH 11.5 slightly improved the settleability of sludge at high TS (10.0 g/L), much pronounced improvement was observed by ozonation of Mg^{2+}-pretreated sludge. This observation indicates that the beneficial effect of Mg^{2+} addition to the sludge is not limited with antibiotic desorption and metal release, but sludge settleability is also improved. This improvement can be attributed to increased floc strength by formation of Mg^{2+} bridges between the active sites on the exocellular polymers, which could have promoted particle aggregation and hence, improved settling

by flocculation (Neyens et al., 2004). Consequently, an 38% decrease in the SV_{60} of raw sludge was obtained by the application of Mg^{2+} pretreated ozonation (Figure 5.9).

5.4. Conclusion

Increasing the pH value of sludge to 11.5 improved the degradation of CIP and OTC to a great extent by providing their desorptions from the sludge. Both the dissolved fractions of the antibiotics and the differences in their reactivity with ozone could be a factor controlling their degradation rates. At alkaline pH the scavenging of radicals by the solubilized components of sludge and the high alkalinity present in sludge probably leaves molecular ozone as the sole oxidant. Although at acidic pH the low degree of organic matter solubilization could eliminate competition for the radicals generated by the catalytic decomposition of ozone with solubilized metal ions from the sludge, the degradation rates of the antibiotics were considerably low due to the lack of their efficient desorption.

Of the two approaches applied for antibiotic desorption in order to improve CIP and OTC degradation in sludge with high TS content, ozonation at pH 11.5 provided more efficient CIP degradation, while Mg^{2+} pretreatment combined ozonation provided more efficient OTC degradation. However, both approaches provided high degradation rates of both OTC and CIP, when spiked at high concentrations. When the antibiotics were spiked at low concentrations (0.08 mg/g TS), ≥95% CIP and OTC ≥85% degradation efficiencies were attained by alkaline ozonation, which in addition provided simultaneous sludge solubilization.

Ozonation was more effective in improving the dewaterability of sludge at low TS content, while with increase in sludge TS the efficiency was decreased due to the competitive influence of solubilized organics. However, application of Mg^{2+}-pretreatment to the sludge prior ozonation, considerably improved sludge settleability even at high TS. Moreover, the addition of Mg^{2+} to the sludge may constitute a potential for the recovery of the released nutrients from the dissolved phase by struvite precipitation. Enhanced sludge settleability with combined Mg^{2+}-pretreatment and ozonation of sludge can facilitate the recovery of both nutrients and metals from the sludge.

6. MICROWAVE IRRADIATION ASSISTED CHEMICAL OXIDATION OF SECONDARY SEWAGE SLUDGE: ANTIBIOTIC DEGRADATION AND SLUDGE SOLUBILIZATION

6.1. Introduction

The presence of a wide variety of emerging micro-pollutants in sewage sludge (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a; Stasinakis, 2012; Clarke and Smith, 2011) increases the concerns regarding its disposal on land. On the other hand, both the challenge of ever-growing volumes of sludge produced from biological wastewater treatment processes and the motivation for recycling the valuable constituents, has brought to the fore land-application as a means of reusing waste sludge beneficially. Although current environmental regulations require stabilization of waste sludge prior to land-application to minimize the potential risk of pathogens and heavy metals on the environment, conventional stabilization methods such as anaerobic or aerobic digestion and composting can not destruct many non-regulated organic micro-pollutants due to strong sorption to particulate material (Stasinakis, 2012).

Among various organic micro-pollutants, antibacterial substances deserve specific attention since their uncontrolled spread in the environment is suspected to contribute to increased rates of antibacterial resistance development in microorganisms (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a), thus creating a secondary pollution. Specifically, antibiotics from tetracycline and fluoroquinolone groups have been detected in sludge at mg/kg range concentrations (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a). For ciprofloxacin, a fluoroquinolone group antibiotic, sludge concentration as high as 97.5 mg/kg (SFT, 2007) as well as long-term persistence in soil (Golet et al., 2003) was reported. A recent study demonstrated that environmental concentrations of ciprofloxacin and tetracycline could exert a selective pressure and increase the prevalence of resistant bacteria in soil (Tello et al., 2012).

Currently, studies dealing with waste sludge management mainly target improving the efficiency of the biological stabilization process (Carrere et al., 2010) or the physical properties of waste sludge (Li et al., 2008), or recovering the valuable nutrients (Tyagi and Lo, 2013). For this purpose various mechanical, thermal or chemical processes have been used. However, despite the aforementioned concerns, the removal of micro-pollutants from sludge has been investigated only in a limited number of studies, while to our knowledge antibiotic degradation has not been reported elsewhere, except in our previous study. The degradation efficiency of micro-pollutants during sludge treatment can be limited by their thermal stability (McNamara et al., 2012) and more importantly by strong sorption tendencies on sludge. For example, in order to efficiently degrade antibiotics sorbed on raw sludge by ozonation, an additional pretreatment process or alteration of the sludge pH was required to provide desorption of antibiotics and enhance their contact with the oxidant (Oncu-Bilgin and Balcioglu-Akmehmet, 2013b).

Among various sludge treatment processes, MW technology has attracted much interest recently, since effective sludge disintegration, conditioning and pathogen destruction can be achieved by rapid and homogeneous heating (Wu, 2008). MW technology can be utilized as a stand-alone pretreatment process for sludge solubilization or in a combined application with chemical oxidation to further improve sludge disintegration and nutrient recovery (Tyagi and Lo, 2013). The most commonly utilized oxidant in combination with MW has been hydrogen peroxide and the synergistic enhancement of sludge solubilization in MW/H_2O_2 was demonstrated in a number of studies (Tyagi and Lo, 2013; Eskicioglu et al., 2008b; Wong et al., 2007). Besides hydrogen peroxide, persulfate alone has also been used for the enhancement of sludge dewaterability (Zhen et al., 2012a-c). However, up to our knowledge, the combined application of persulfate and MW for sludge treatment has not been investigated. Since the influence of MW on the desorption of organic pollutants (Wu et al., 2008) and the strong oxidative power of hydrogen peroxide and persulfate on degradation of organics in solid matrices are known (Uslu-Otker and Balcioglu-Akmehmet, 2009), combined benefits of MW and hydrogen peroxide or persulfate oxidants can be expected for organics degradation in sludge.

In this study the fate of sorbed antibacterial micro-pollutants in sewage sludge was investigated during MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments performed in lab-scale

experiments at different experimental conditions. For this purpose, two antibacterial micro-pollutants that are commonly detected in sludge –OTC and CIP antibiotics– were added to sludge in order to study the effects of selected operational parameters and their interactions on the efficiency of applied processes. The influences of MW/H_2O_2 and MW/$S_2O_8^{2-}$ on the release of metals as another regulated micro-pollutant group, as well as on organic matter and nutrient solubilization were also of interest. Additionally, efforts were made in this work to understand the radical mechanism contribution during combined MW treatment and chemical oxidation.

6.2. Materials and Methods

6.2.1. Chemicals

Hydrochloride forms of oxytetracycline (Sigma) and ciprofloxacin (MP. Biomedicals) were the model antibiotics used for spiking. H_2O_2 (Sigma-Aldrich) and $Na_2S_2O_8$ (Sigma-Aldrich) were used as the oxidants. Anisole (Fluka) as the radical probe and ethanol-EtOH (Sigma-Aldrich) as the radical quencher were purchased from Sigma-Aldrich. Formic acid and acetonitrile (Sigma-Aldrich, HPLC grade) were utilized in the HPLC analyses. 2,9-Dimethyl-1,10-phenanthroline (DMP) (Aldric), $CuSO_4 \cdot 5H_2O$ (Riedel-de Haën), Na_2HPO_4 (Fisher Scientific), and KH_2PO_4 (Merck) were utilized for hydrogen peroxide analysis and KI (Sigma) and $Na_2S_2O_3$ (Sigma-Aldrich) were utilized for sodium persulfate analysis. All solutions were prepared with MQ (Millipore, Milford, MA).

6.2.2. Preparation of Antibiotic Contaminated Sewage Sludge

The physicochemical parameters of the sludge obtained from Pasakoy-Istanbul municipal wastewater treatment during the SC-2 are given in Table 6.1.

Table 6.1. Average secondary sewage sludge characteristics and total metal concentrations for the SC-2.

Sludge properties		Metals	
Parameter	**Value**	**Metals**	**Sludge concentrations (mg/kg DS)**
TS (g/L)	12.2±0.3	Ni	321±8
VS/TS (%)	57±5	Cr	605±12
TCOD (g/L)	10.4±0.1	Cu	544±85
SCOD (mg/L)	105±7	Zn	909±98
TKN (mg/L)	430±7	Cd	BLD
TP (mg/L)	847±32	Pb	BLD
PO_4^{3-}	89±4	Fe	13,877±97
pH	6.5−7.0	Mn	563±67

The data are the mean values obtained from n=4 samples

When compared with sludge obtained during SC-1, it can be seen that there were changes in the concentrations of the sludge parameters. The most important difference was observed in the TP content of the sludge and in the concentrations of some heavy metals (e.g. Cr, Cu, Zn, Fe, Pb).

The TS of the sludge that was concentrated upon collection (See Section 3.1.3) was adjusted to 10.0±0.1 g/L by spiking OTC and CIP antibiotic aqueous solution as described previously (See Section 3.2.3). Taking into account the concentrations of the antibiotics already present in the sludge, in the majority of the experiments, antibiotics were spiked to achieve a 20 mg/L sludge concentration (2 mg/g TS). However, the performances of the treatment processes were also tested for sludge with an environmentally relevant antibiotic concentration of 0.8 mg/L (0.08 mg/g TS). Prior to sludge treatment experiments, the antibiotic-spiked sludge was equilibrated to provide >95% antibiotic sorption, which was verified by antibiotic analysis carried out in total and liquid phase sludge samples.

6.2.3. MW/H_2O_2 and MW/$S_2O_8^{2-}$ Treatment of Sewage Sludge

The treatment of sewage sludge was carried out in the bench scale microwave irradiation system (See Section 3.2.5.2). The sludge samples were placed in the closed vessels were treated by either adding hydrogen peroxide, H_2O_2 (30% w/w), or sodium persulfate, $Na_2S_2O_8$ (>98%), as the oxidants or without adding any oxidant. Among MW,

MW/H_2O_2, and MW/$S_2O_8^{2-}$ treatments, MW/H_2O_2 consisted of two steps. In the first step, before the dosing of hydrogen peroxide, all samples were heated at 120°C for 15 min to destruct the biological enzymes in the sludge and hence prevent the undesirable consumption of hydrogen peroxide (Wang et al., 2009). In control experiments carried out at room temperature, 30% hydrogen peroxide consumption took place in the non-preheated sludge within one min after oxidant dosing, while persulfate consumption in this sludge was only 2%. Therefore, preheating was crucial for MW/H_2O_2 but not for MW/$S_2O_8^{2-}$. Depending on the type of the treatment, 2.5 mL of the oxidant at desired concentration or deionized water was added to 25 mL of the sludge samples. MW irradiation of the sludge with a total volume of 27.5 mL for each vessel was performed at predetermined experimental conditions. No effort was spent for pH adjustment. The temperature of the sludge samples was increased at a rate of 10°C/min in the MW system. At the end of the treatment period and before carrying out the immediate subsequent analyses, the vessels were inserted in an ice bath without opening the caps and cooled down to room temperature to prevent evaporation.

6.2.4. Experimental Design

In order to explore the effects of the selected variables on the efficiencies of MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments, a 2^3 full factorial design with three independent variables (A: temperature; B: oxidant dose; C: MW holding time) was performed. It should be noted that preliminary experiments were carried out to determine the extreme values (design's corner points) of the variables. The highest MW temperature was defined as 160°C since sludge biodegradability is negatively influenced due to the Maillard reaction above temperatures of 180°C (Pinnekamp, 1989). The whole design for each of the MW/H_2O_2 and MW/$S_2O_8^{2-}$ processes consisted of 19 experimental runs carried out in a random order. The experimental range and the levels of the independent variables are given in Table 6.2.

Table 6.2. Independent variables and their levels used in the 2^3 full factorial design for sewage sludge treatment with MW/H_2O_2 and $MW/S_2O_8^{2-}$.

		MW/H_2O_2			**$MW/S_2O_8^{2-}$**		
Experimental factors	**Symbol**	**Level (-)**	**Level (0)**	**Level (+)**	**Level (-)**	**Level (0)**	**Level (+)**
Temperature (ºC)	A	120	140	160	120	140	160
Oxidant dose (g/g TS)	B	0.0	0.6	1.2	0.00	0.44	0.87
MW holding time (min)	C	5	10	15	5	10	15

As can be seen from Table 6.2, each one of the three variables received two values as indicated by the plus and the minus signs, while a central value was indicated by 0. The minus sign for the oxidant dose variable indicates the absence of it.

6.2.5. Analytical Methods

The extraction, purification, and analysis of OTC and CIP raw and treated sludge samples was carried out with ultrasonication assisted solvent extraction, SPE, and HPLC, respectively as described previously in Section 3.2.6.6. Antibiotic degradation was calculated as the percent change in antibiotic concentration after sludge treatment. Initial antibiotic concentrations were determined in each experiment in order to eliminate the probable influence of changes in sludge characteristics on the antibiotic recoveries. The degradation rates observed for the antibiotics spiked to the sludge at low concentrations were confirmed with qualitative LC-MS/MS analysis (See Section 3.2.6.6).

Characterization of raw and treated sludge samples for parameters including TS, VS, SCOD, TCOD, TKN, TP, metals, NH_4^+, and PO_4^{3-}, and CST was performed as described in Sections 3.2.6.2 and 3.2.6.6.

Analysis of the radical probe anisole was carried out with HPLC (See Section 3.2.6.7).

Both hydrogen peroxide and persulfate concentrations in the samples were determined by the iodometric method (Kolthoff and Carr, 1953). Since residual hydrogen peroxide

exerted considerable COD interference, a photometric method with copper(II)-DMP complex formation (Kosaka et al.,1998) was used to confirm the low concentrations of hydrogen peroxide (See Section 3.2.6.3). Then the COD equivalent of the residual hydrogen peroxide was subtracted from the measured COD value to eliminate its positive interference (Kang et al., 1999).

6.3. Results and Discussion

The results of antibiotic degradation and sludge solubilization of each 19 experiments for MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments are shown in Table 6.3 and Table 6.4, respectively. The statistical significances of the effects of selected independent variables on the efficiencies of the processes were evaluated and the significant factors (p<0.05) with decreasing order of effects are shown in Figure 6.1 (a)-(f) as Pareto plots. Additional results including the influence of treatment conditions on sludge pH, oxidant consumption, and solubilization of individual metals for MW/H_2O_2 and MW/$S_2O_8^{2-}$treatments, are shown in Table 6.5 and Table 6.6, respectively.

Table 6.3. 2^3 full factorial design table for the coded factors and the responses obtained for sewage sludge treatment with MW/H_2O_2.

Process parameter level				Responses					
				Antibiotic degradation (%)		Solubilization (%)			
Experiment number	A	B	C	OTC	CIP	Organic carbon (SCOD/TCOD×100)	Nitrogen (NH_4^+/TKN)	Phosphorus (PO_4^{3-}/TP)	Overall metal (Me_S/Me_T)
1	–	–	–	40.3	7.6	28.1	2.6	8.4	3.0
13	–	–	–	41.6	7.2	27.8	2.9	8.7	2.9
5	–	–	+	43.2	8.7	31.4	4.1	9.1	3.0
7	–	–	+	44.2	9.1	32.1	4.4	9.4	2.9
2	–	+	–	83.6	31.6	24.2	17.7	16.5	11.6
12	–	+	–	84.8	32.9	23.3	18.1	17.2	11.9
3	–	+	+	86.0	35.2	30.8	23.9	20.3	13.0
4	–	+	+	87.3	36.5	31.2	24.9	19.9	12.6
10	0	0	0	91.4	60.9	36.8	11.0	29.2	19.5
11	0	0	0	91.6	60.3	37.6	12.3	28.9	18.9
19	0	0	0	91.5	60.5	38.2	11.9	29.3	19.3
16	+	–	–	81.6	9.8	34.5	7.4	11.5	3.6
17	+	–	–	82.3	9.9	34.0	7.7	11.8	3.6
14	+	–	+	83.8	12.4	40.2	12.6	13.5	3.9
18	+	–	+	84.6	11.9	39.8	13.2	14.5	3.8
8	+	+	–	98.0	88.6	40.4	40.7	49.6	22.3
9	+	+	–	98.2	89.2	38.6	42.4	49.0	23.3
6	+	+	+	98.8	98.1	26.5	46.7	51.3	25.3
15	+	+	+	98.9	98.3	24.7	44.5	53.3	25.9

A: Temperature; B: Hydrogen peroxide dose; C: MW holding time

Table 6.4. 2^3 full factorial design table for the coded factors and the responses obtained for sewage sludge treatment with MW/$S_2O_8^{2-}$.

Process parameter level				Responses					
				Antibiotic degradation (%)		Solubilization (%)			
Experiment number	A	B	C	OTC	CIP	Organic carbon (SCOD/TCOD×100)	Nitrogen (NH_4^+/TKN)	Phosphorus (PO_4^{3-}/TP)	Overall metal (Me_S/Me_T)
6	–	–	–	31.2	0.3	25.0	0.5	3.1	2.5
14	–	–	–	33.5	0.2	24.4	0.4	3.0	2.4
9	–	–	+	36.7	0.7	25.8	1.9	7.2	2.8
17	–	–	+	38.9	0.8	26.4	1.5	6.9	2.6
3	–	+	–	82.3	58.3	23.8	8.1	25.6	39.1
19	–	+	–	83.3	59.8	23.8	9.7	26.2	38.7
2	–	+	+	85.9	60.6	23.9	12.1	27.5	52.0
12	–	+	+	88.0	62.1	24.1	13.2	28.2	52.2
5	0	0	0	97.4	88.2	26.3	13.5	29.3	57.0
13	0	0	0	97.1	87.0	25.7	12.9	29.3	56.5
7	0	0	0	96.4	87.3	26.1	12.1	29.4	56.1
10	+	–	–	77.4	3.3	30.0	2.2	8.1	3.0
11	+	–	–	77.6	4.8	29.6	2.5	8.3	3.0
15	+	–	+	78.3	7.0	32.8	3.1	10.6	2.5
16	+	–	+	79.1	6.9	33.2	2.9	11.0	3.0
4	+	+	–	96.2	94.2	20.4	13.8	41.2	65.7
18	+	+	–	97.0	93.9	19.9	14.1	41.9	65.4
1	+	+	+	98.5	96.6	22.1	14.9	43.9	73.6
8	+	+	+	99.6	97.3	21.5	15.5	44.2	73.4

A: Temperature; B: Persulfate dose; C: MW holding time

Table 6.5. Solubilization of individual metals, sludge pH and oxidant consumption for sewage sludge treatment with MW/H_2O_2.

Experiment number	Process parameter levels			Metal solubilization (%)						pH	Consumed H_2O_2 (%)
	A	B	C	Cr	Cu	Mn	Ni	Fe	Zn		
1	–	–	–	0.8	4.0	0.2	12.4	0.6	0.4	6.56	–
13	–	–	–	0.7	3.9	0.2	11.8	0.6	0.3	6.58	–
5	–	–	+	1.0	3.7	0.2	12.5	0.6	0.4	6.43	–
7	–	–	+	0.7	3.6	0.3	12.1	0.4	0.2	6.50	–
2	–	+	–	14.1	12.8	11.7	25.7	1.8	3.6	5.22	39.2
12	–	+	–	14.3	13.3	12.2	26.2	1.9	3.5	4.91	37.9
3	–	+	+	15.8	13.9	14.5	27.4	2.1	4.5	5.00	30.6
4	–	+	+	15.7	13.4	13.5	27.2	2.0	3.9	4.70	32.5
10	0	0	0	24.4	20.5	24.7	37.5	2.5	7.5	4.33	69.7
11	0	0	0	23.3	20.4	24.0	36.0	2.4	7.2	4.29	70.8
19	0	0	0	24.2	20.3	24.0	37.1	2.6	7.6	4.31	70.3
16	+	–	–	0.7	4.3	0.2	14.3	1.0	1.4	6.42	–
17	+	–	–	1.0	4.1	0.3	13.9	0.8	1.2	6.46	–
14	+	–	+	1.2	3.8	0.3	15.7	0.9	1.5	6.38	–
18	+	–	+	1.3	3.9	0.4	15.0	0.8	1.6	6.30	–
8	+	+	–	31.5	22.6	32.1	38.1	1.5	8.2	4.17	84.1
9	+	+	–	30.5	23.8	32.9	41.4	1.8	9.4	4.59	85.3
6	+	+	+	36.2	27.9	34.5	41.7	1.7	9.9	4.33	82.7
15	+	+	+	38.1	26.8	34.7	43.7	1.8	10.1	4.54	82.8

A: Temperature; B: Hydrogen peroxide dose; C: MW holding time

Table 6.6. Solubilization of individual metals, sludge pH and oxidant consumption for sewage sludge treatment with MW/$S_2O_8^{2-}$.

Experiment number	Process parameter levels			Metal solubilization (%)						pH	Consumed $S_2O_8^{2-}$ (%)
	A	B	C	Cr	Cu	Mn	Ni	Fe	Zn		
6	–	–	–	1.0	3.3	0.1	10.2	0.2	0.1	6.34	–
14	–	–	–	0.9	3.5	0.0	9.8	0.2	0.1	6.36	–
9	–	–	+	0.9	3.1	0.0	12.1	0.3	0.1	6.32	–
17	–	–	+	0.7	2.8	0.0	11.8	0.3	0.1	6.33	–
3	–	+	–	17.7	40.4	55.2	58.9	10.5	51.9	3.81	>99.0
19	–	+	–	16.1	40.2	52.3	63.8	11.6	48.3	3.85	>99.0
2	–	+	+	24.5	52.1	76.1	86.0	12.2	61.2	3.62	>99.0
12	–	+	+	24.0	48.9	79.9	82.1	14.6	63.6	3.41	>99.0
5	0	0	0	19.0	49.2	85.0	90.8	14.2	84.0	2.15	100
13	0	0	0	18.8	46.1	87.3	88.5	13.5	84.6	2.46	100
7	0	0	0	16.8	45.4	88.9	84.4	13.2	88.0	2.16	100
10	+	–	–	0.7	3.1	0.0	12.8	0.2	1.1	6.32	–
11	+	–	–	0.6	3.3	0.0	12.8	0.3	1.0	6.41	–
15	+	–	+	1.4	0.0	0.0	13.4	0.2	0.0	6.29	–
16	+	–	+	0.9	0.0	0.0	16.6	0.2	0.0	6.25	–
4	+	+	–	44.7	45.9	96.7	84.7	34.7	87.2	1.93	100
18	+	+	–	44.9	42.3	98.4	85.3	34.8	86.6	1.90	100
1	+	+	+	53.6	58.2	100.0	88.2	52.6	89.2	1.92	100
8	+	+	+	50.7	59.8	99.9	88.9	50.1	91.0	1.89	100

A: Temperature; B: Persulfate dose; C: MW holding time

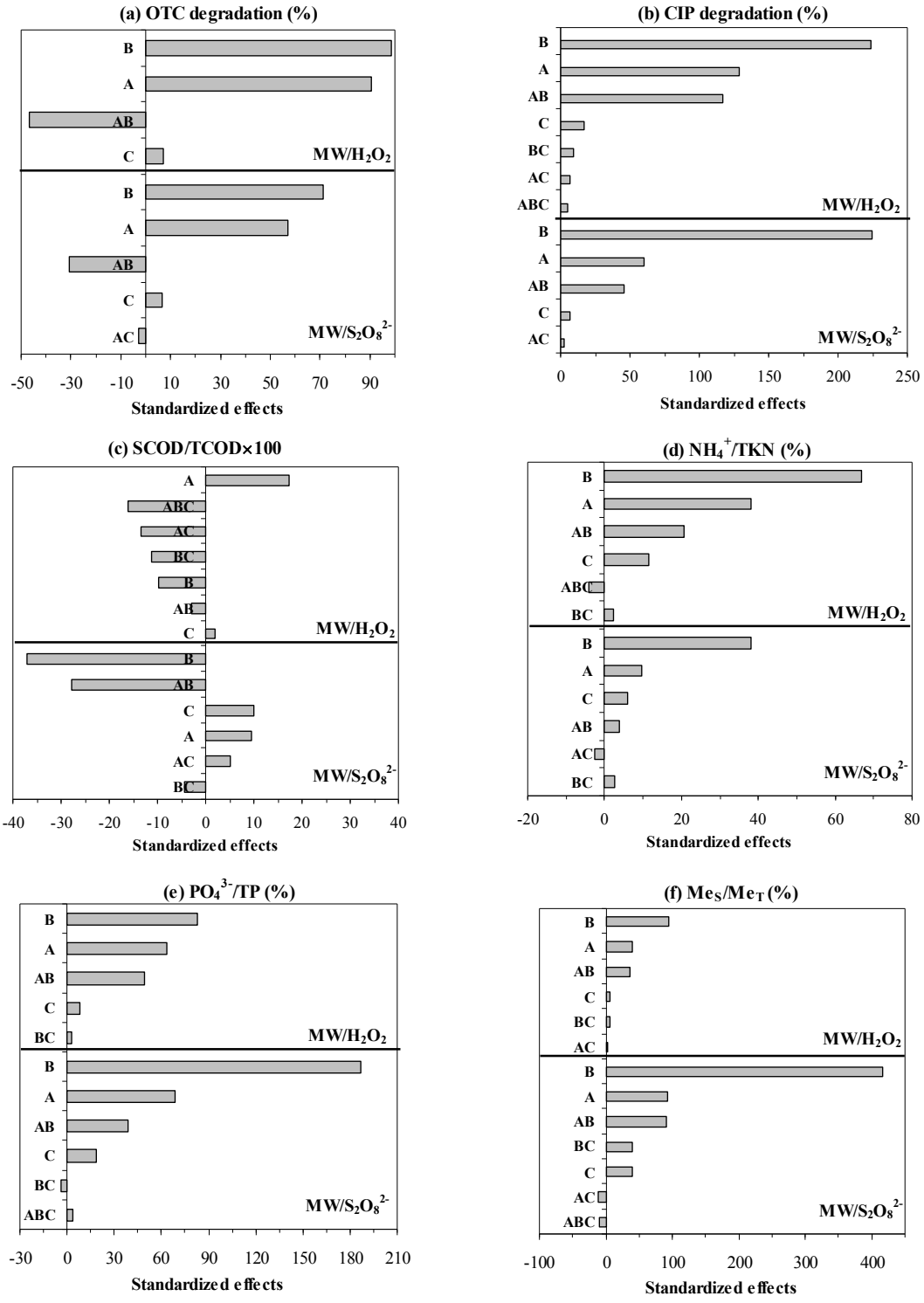


Figure 6.1. Pareto charts for (a) OTC and (b) CIP degradation, solubilization of (c) organics, (d) phosphorus, (e) nitrogen, and (f) metals during sewage sludge treatment with MW/H_2O_2 and $MW/S_2O_8^{2-}$ (A: Temperature; B: Oxidant dose; C: MW holding time).

6.3.1. Effect of MW/H_2O_2 and $MW/S_2O_8^{2-}$ Treatments on Antibiotic Degradation

The preheating stage of MW/H_2O_2 treatment resulted in a prolonged MW exposure time. Because of this, there was a difference between the results obtained for antibiotic degradation by MW treatment in the absence of hydrogen peroxide (exp. 1, 13 in Table 6.3) and persulfate (exp. 6, 14 in Table 6.4). An additional experiment that was performed to evaluate the antibiotic degradation in a single stage MW/H_2O_2 treatment (1.2 g H_2O_2/g TS; 160°C; 15 min) yielded only 81% OTC and 71% CIP degradation rates in the sludge. This poor antibiotic degradation efficiency can be explained by the undesired hydrogen peroxide consumption as stated previously. Consistently, <1% of the initially added peroxide remained in the sludge in the single-stage MW/H_2O_2 treatment while this rate was 17% with integration of the preheating step (exp. 6, 15 in Table 6.5). Furthermore, the higher availability of hydrogen peroxide in a two-stage MW/H_2O_2 treatment could also be beneficial for contaminant desorption, which is a well-known phenomenon in catalyzed hydrogen peroxide treatment systems (Watts et al., 1999).

The results presented in Tables 6.3 and 6.4 clearly indicate that the achievement of substantial antibiotic degradation depended on the dosing of the oxidants. This result was supported by the statistical analysis as seen from the Pareto plot (Figure 6.1 (a) and (b)). During the MW treatment at 160°C for 15 min, the average CIP degradation rate was improved from 12% to 98% and from 7% to 97% by the addition of 1.2 g H_2O_2 and 0.87 g $S_2O_8^{2-}$ per g of TS, respectively. On the other hand, control experiments performed in the absence of MW irradiation showed that the degradation rates of both antibiotics with these oxidants were <10% within 30 min treatment period (Figure 6.2). It can be deduced that the addition of the oxidant in MW treatment resulted in a synergistic effect on antibiotic degradation. However, the enhancement in OTC degradation with oxidant dosing was not as remarkable as for that of CIP. Even in the absence of the oxidant, 79% degradation of OTC was obtained with MW treatment at 160°C for 15 min (exp. 15, 16 in Table 6.4). Although MW temperature was the second most important factor for antibiotic degradation in both treatments, the interaction of the oxidant dose and the temperature did not show a positive influence on OTC degradation as opposed to CIP due to the instability of OTC at elevated temperatures. Thermal stabilities of antibiotics were confirmed with the experiments carried

out in deionized water under the same experimental conditions applied to the sludge (Figure 6.2).

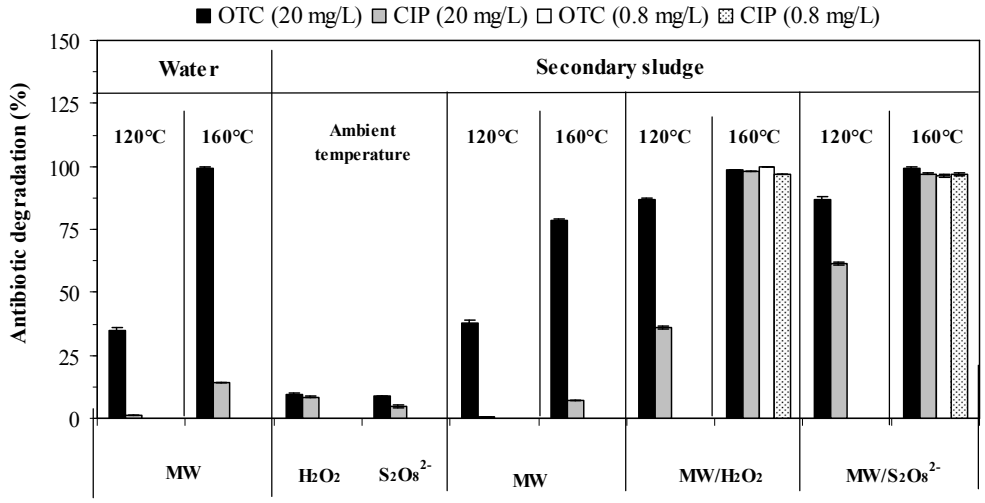


Figure 6.2. Comparison of MW, MW/H_2O_2, and MW/$S_2O_8^{2-}$ treatment efficiencies for antibiotic degradation at two different spiking concentrations in water and sewage sludge. Holding time: 15 min, oxidant dosage: 1.2 g H_2O_2/g TS and 0.87 g $S_2O_8^{2-}$/g TS, treatment time of control experiments carried out at room temperature: 30 min.

Although CIP is relatively stable at high temperatures (Figure 6.2) and almost complete persulfate consumption took place even in 5 min at 120°C (Table 6.6), the elevation of MW temperature from 120°C to 160°C enhanced the degradation of CIP by 36% in MW/$S_2O_8^{2-}$ treatment (exp. 2, 12-1, 8 in Table 6.4). This result can be attributed to the severe decline in the pH (Table 6.6), which can promote desorption of the antibiotics from the sludge due to increased electrostatic repulsions between sludge particles and the zwitterionic antibiotics (Golet et al., 2002). Hence, dissolved components of the sludge can be more vulnerable to oxidation compared to those sorbed on particulate material (Oncu-Bilgin and Balcioglu-Akmehmet, 2013b). The influence of the sludge pH on antibiotic degradation in MW treatment was investigated by additional experiments performed at an initial pH of 2 (adjusted by 1 M H_2SO_4) and at 120°C or 160°C for 15 min. The results of these experiments (Table 6.7) showed that although acidic pH caused considerable desorption of both antibiotics, particularly of CIP, (OTC=12%; CIP=58% at 120°C) and the temperature increase also exerted a slight contribution to desorption, MW treatment without

oxidant addition of did not provide efficient degradation of the antibiotics. As a result, the overall degradation rates of OTC and CIP at pH 2 at 160°C were limited to 81% and 12%, respectively, and the higher degradation rate of OTC was obviously due to its thermal instability.

Table 6.7. Degradation of antibiotics with MW treatment of sewage sludge at pH 2 with 15 min holding time.

	Antibiotic desorption (%)		**Antibiotic degradation (%)**	
Temperature (°C)	**OTC**	**CIP**	**OTC**	**CIP**
120	11.7	58.3	40.5	2.9
160	1.0	62.5	80.8	11.6

Despite being a significant factor (p<0.05) in both MW/H_2O_2 and MW/$S_2O_8^{2-}$, the effect of the holding time was not pronounced for sludge treatment in accordance with previous studies (Wong et al., 2007) (Figure 6.1 (a) and (b)). This result would be attributed to the consumption of the oxidant. On the contrary, a higher residual hydrogen peroxide concentration was observed with increased holding time in the MW/H_2O_2 treatment (Table 6.5) suggesting the production of hydrogen peroxide as a result of radical reactions. However, the remaining hydrogen peroxide in the sludge did not result in higher antibiotic degradation rates at 120°C and 140°C compared to those obtained at these temperatures in MW/$S_2O_8^{2-}$. The higher performance of MW/$S_2O_8^{2-}$ treatment can be attributed to the generation of the sulfate radical, which exhibits higher selectivity towards certain (carboxylic, anilinic and phenolic) functional groups of organics (Neta et al., 1977). Although the low effect of the holding time on antibiotic degradation may indicate a competitive influence exerted by the solubilized sludge components for the oxidants, it did not adversely affect the efficiencies of MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments. Almost the same degradation rates of the antibiotics at high and low concentrations (Figure 6.2) could be explained by their concurrent extraction with the aid of MW irradiation; hence their degradation could have been promoted in the presence of the oxidant. However, it was not possible to analyze the antibiotics in the aqueous phase due to their fast degradation which took place simultaneously with antibiotic desorption.

6.3.2. Effect of MW/H_2O_2 and $MW/S_2O_8^{2-}$ Treatments on Sludge Solubilization

As clearly seen from the results in Tables 6.3 and 6.4, although MW irradiation alone did not provide substantial antibiotic degradation, effective COD solubilization could be achieved even under mild conditions, i.e. low temperature and short reaction time. In MW treatment, the percent ratio of the soluble to total COD at 120°C with 15 min exposure time was 26% (exp. 9, 17 in Table 6.4), which increased to 32% in a two-step MW treatment with an initial preheating stage (exp. 5, 7 in Table 6.3) and a further increase of this ratio to 40% was achieved by elevation of the temperature to 160°C (exp. 14, 18 in Table 6.3). The positive influences of temperature and exposure time are also obvious in the Pareto plot (Figure 6.1 (c)). Although COD solubilization greatly depends on various factors including sludge source, TS concentration of the sludge, and MW treatment conditions, which vary considerably in different studies, the SCOD/TCOD ratios obtained in the present study were in the range of those reported in other studies (Tyagi and Lo, 2013).

Similar to previous results (Eskicioglu et al., 2008b), the addition of the oxidant to the sludge during MW treatment resulted in a decline in the SCOD/TCOD ratio due to oxidation of the solubilized organic components of the sludge as understood from the negative influence of the oxidant dose and its interactions with other factors (Figure 6.1 (c)). In addition, the oxidation of organics in MW/H_2O_2 and $MW/S_2O_8^{2-}$ treatments was also evident from the decline in both the TCOD and SCOD values (Figure 6.3). The results of TCOD decrease also underline the higher oxidative power of persulfate at 120°C compared to hydrogen peroxide, which is consistent with the results obtained for antibiotic degradation. It can be deduced that rapid sludge solubilization and oxidation took place concurrently in $MW/S_2O_8^{2-}$ while in MW/H_2O_2, substantial oxidation of the organics took place at 160°C (Figure 6.3).

An improvement in the COD solubilization with the oxidant addition was only achieved in the MW/H_2O_2 process within a short exposure time at 160°C (Table 6.3 exp. 8, 9). However, the SCOD/TCOD ratio was decreased by extending the exposure time from 5 min to 15 min, due to oxidation of solubilized organics (exp. 6, 15 in Table 6.3). This observation is also consistent with the greater increase in CIP degradation. Consequently, a

negative three-factor interaction (temperature; oxidant dose; exposure time) was revealed for the SCOD/TCOD ratio in the Pareto plot.

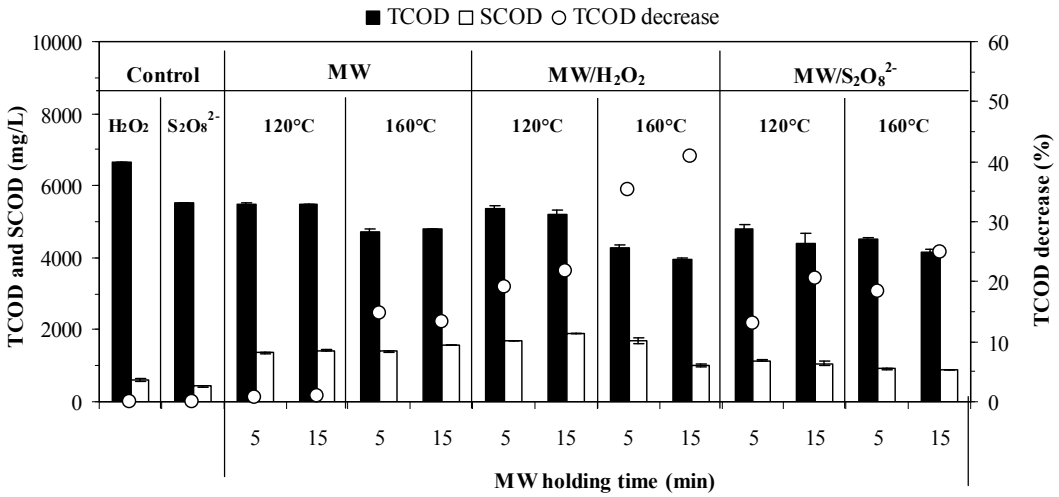


Figure 6.3. Comparison of the influences of MW, MW/H_2O_2, and MW/$S_2O_8^{2-}$ treatments on TCOD, SCOD, and decrease in TCOD. Oxidant dosage: 1.2 g H_2O_2/g TS and 0.87 g $S_2O_8^{2-}$ /g TS.

Although the MW treatment conditions which were favorable for degradation of the sorbed antibiotics led to SCOD/TCOD decline due to sludge oxidation, the nutrient solubilization from the sludge was also positively influenced by addition of the oxidant in both thermo-oxidative treatments (Figure 6.1 (d) and (e)). In accordance with a previous study (Wong et al., 2007), the effect of the oxidant dose on nutrient solubilization was greater than that of temperature. The higher release of ammonia in the presence of the oxidant can be attributed to the higher oxidation rate of the proteins released from the bacterial cells during MW irradiation whereas it is known that proteins aren't degraded with MW irradiation alone (Toreci et al., 2010). The oxidant dose exerted a higher influence on ammonia release in MW/H_2O_2 compared to that in MW/$S_2O_8^{2-}$. As can be seen from Tables 9.3 and 9.4, an average of 46% N solubilization as ammonia took place in MW/H_2O_2 treatment with 1.2 g H_2O_2/g TS dosing at 160°C, 15 min (exp. 6, 15 in Table 6.3), whereas with 0.87 g $S_2O_8^{2-}$/g TS dosing N solubilization was only 15% (exp. 1, 8 in Table 6.4). This well expected result can be attributed to the higher oxidative power of persulfate, which was

also confirmed by nitrate formation; an almost three-fold higher nitrate concentration was detected in the sludge samples treated with 0.87 g $S_2O_8^{2-}$/g TS compared to those treated with 1.2 g H_2O_2/g TS at 160°C, 15 min. Oxidation of ammonia to nitrate can decrease the potential of nitrogen loss from sludge through ammonia volatilization.

One of the most important differences between MW/H_2O_2 and MW/$S_2O_8^{2-}$ was their influence on the overall metal solubilization (e.g. exp. 6, 15 in Table 6.3 and 1, 8 in Table 6.4). While MW/H_2O_2 treatment yielded 26% metal solubilization at the extreme condition of the experimental design (1.2 g H_2O_2/g TS, 160°C, 15 min), MW/$S_2O_8^{2-}$ treatment provided 73% metal solubilization (0.87 g $S_2O_8^{2-}$/g TS 120°C, 15 min). The high metal release yield of MW/$S_2O_8^{2-}$ treatment can be attributed to the enhanced solubilization of the sludge, as well as to the influence exerted on metal speciation by the severe pH decline. This suggestion can be supported by the 32% overall metal solubilization obtained by MW treatment at pH 2 without oxidant addition. Solubilization rates of individual metals in MW/H_2O_2 and MW/$S_2O_8^{2-}$ as well as in MW treatment at pH 2 are given in Tables 9.5, 9.6 and 9.8, respectively. In sludge treatment, the reduction of concentrations of metals such as Cu which are associated mainly with organics is a common problem. While the exceptional metal solubilization in MW/$S_2O_8^{2-}$ can be related to the acidic conditions developing during the treatment, the solubilization of Cu was remarkable only at the conditions where the sludge components were oxidized, as was demonstrated by Beauchesne et al. (2007).

Table 6.8. Solubilization of metals and nutrients with MW treatment of sewage sludge at pH 2 with 15 min holding time.

	Nutrient solubilization (%)		**Metal solubilization (%)**						
Temperature (°C)	**Phosphorus (PO_4^{3-}/TP)**	**Nitrogen (NH_4^+/TKN)**	**Cr**	**Cu**	**Mn**	**Ni**	**Fe**	**Zn**	**Overall metal (Me_S/Me_T)**
120	10.0	0.8	10.2	2.0	51.5	35.7	10.8	50.9	26.9
160	34.0	1.5	12.7	2.9	63.4	39.4	19.6	54.1	32.0

6.3.3. Effect of MW/H_2O_2 and $MW/S_2O_8^{2-}$ Treatments on Sludge Dewaterability

Since sludge dewaterability is an important parameter in sludge management, the influence of MW/H_2O_2 and $MW/S_2O_8^{2-}$ on sludge dewaterability was assessed with the CST method at the extreme conditions of the processes (160°C, 15 min and oxidant doses of 1.2 g H_2O_2/g TS and 0.87 g $S_2O_8^{2-}$/g TS, respectively), where the degradation of antibiotics and solubilization of nutrient and metals were most efficient. The results, which are shown as CST per g suspended solids to better compare the two processes, revealed that both treatments improved sludge dewaterability (Figure 6.4). Although greater SS reduction was obtained in MW/H_2O_2 treatment consistently with the higher TCOD reduction, the $MW/S_2O_8^{2-}$ treatment resulted in a two-fold higher improvement in dewaterability. This difference can be due to the considerably higher amount of solubilized iron (Table 6.8), which can act as a coagulant. Detailed investigation is being currently made to further elucidate the influence of the processes on sludge dewaterability as well as to evaluate the biodegradability of sludge after treatment with MW/H_2O_2 and $MW/S_2O_8^{2-}$.

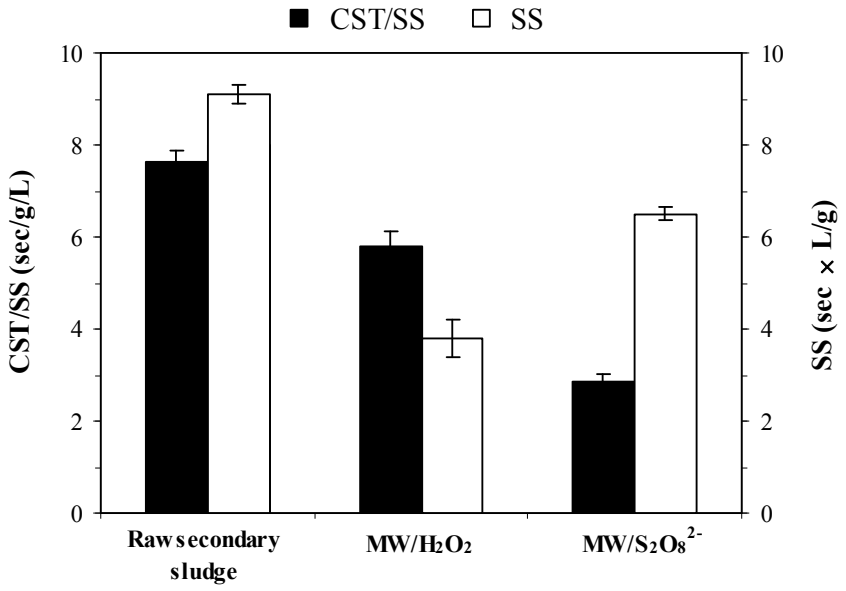


Figure 6.4. Influences of MW/H_2O_2 and $MW/S_2O_8^{2-}$ treatments on sludge dewaterability. Oxidant dosage: 1.2 g/g TS and 0.87 g/g TS, temperature: 160°C, MW holding time: 15 min.

6.3.4. Radical Mechanism Contribution in $MW/S_2O_8^{2-}$ and MW/H_2O_2 Treatments

In this part of the study, in order to explain the probable contribution of the free radicals on the efficiencies of MW/H_2O_2 and $MW/S_2O_8^{2-}$ treatments, anisole was used as a radical probe (k_{SO4}-• = 4.9 × 10^9, k_{OH}• = 7.8 × 10^9, Liang and Su, 2009) and the degradation rate of anisole at an initial concentration of 0.06 mM was investigated at 120°C and 160°C without applying holding time due to its rapid degradation (Figure 6.5).

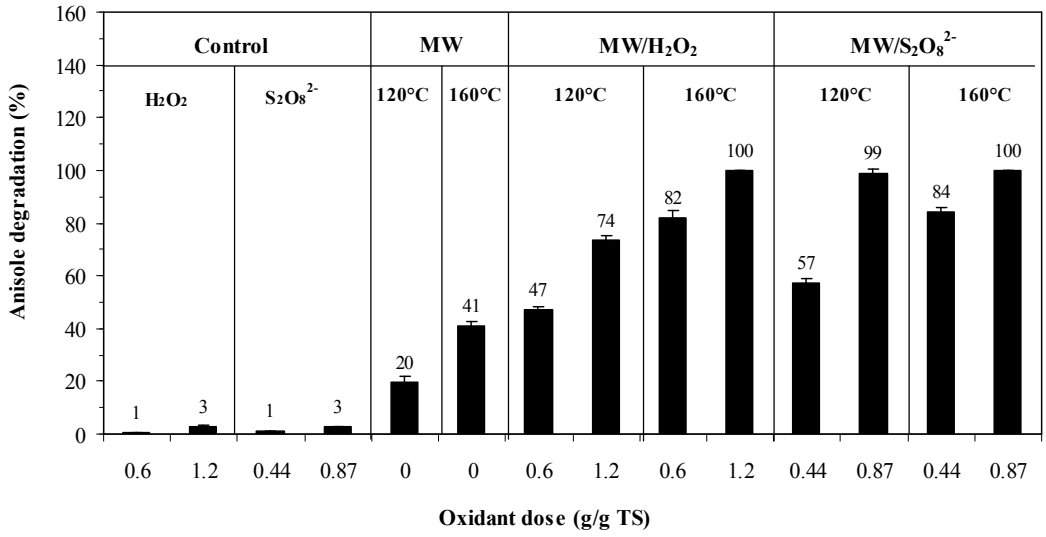


Figure 6.5. Radical probe compound degradation during sewage sludge treatment with MW, MW/H_2O_2, and $MW/S_2O_8^{2-}$. Initial anisole concentration: 0.06 mM; treatment time of control experiment carried out at room temperature: 20 min.

Compared to the results obtained in control experiments carried out either in the absence of MW irradiation or oxidant, the degradation rate of the radical probe increased in both MW/H_2O_2 and $MW/S_2O_8^{2-}$ treatments. Therefore, it can be suggested that during MW irradiation both hydrogen peroxide and persulfate can generate reactive radicals (Eqns. 6.1 and 6.2), which improved the antibiotic degradation and the oxidation of organic matter, as well as the release of nutrients and metals in the current study.

$$H_2O_2 + \text{MW irradiation} \rightarrow 2OH^{\bullet} \quad (6.1)$$

$$S_2O_8^{2-} + \text{MW irradiation} \rightarrow 2SO_4^{-\bullet} \quad (6.2)$$

In addition to MW irradiation, the metal content of the sludge (Table 6.1), especially the metals released during sludge treatment (Tables 6.3 and 6.4) may contribute to the activation of oxidants to produce reactive radicals in both MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments via Eqns. 2.15 and 2.25 (Siegrist et al., 2011).

Metal activation could have been especially important in MW/$S_2O_8^{2-}$ treatment. Severe pH decline took place particularly at 160°C (Table 6.5). These acidic conditions could have further increased the activation of persulfate to produce the sulfate radicals as was shown in Eqn. 2.26 (Peyton, 1993), causing even a higher sulfate radical concentration, which in turn could have resulted in higher radical probe compound and antibiotic degradation rates:

Since the hydrogen peroxide residuals observed in the treated sludge (Table 6.5) could be an indicator for the formation of hydroxyl radicals in MW/H_2O_2 treatment, hydrogen peroxide formation in water dosed with 356 mM H_2O_2 (corresponding to the 1.2 g/g TS dosage in sludge) and treated by MW irradiation was studied in additional experiments, which confirmed this suggestion (Figure 6.6). In these experiments hydrogen peroxide was formed at all conditions and the elevation of MW temperature from 120°C to 160°C at 15 min increased the residual hydrogen peroxide concentration from 400 mM to 492 mM. Since sludge components constitute considerable oxidant demand, hydrogen peroxide was formed at higher concentrations in water compared to those observed in the sludge. Consistently, the degradation rate of anisole in MW/H_2O_2 treatment at 120°C was lower than that obtained in MW/$S_2O_8^{2-}$ treatment. Therefore, it can be concluded that at this temperature hydrogen peroxide activation to produce hydroxyl radicals in the sludge could not be efficient probably due to the lack of sufficiently acidic conditions (Table 6.5) and also due to the competitive influence of sludge constituents solubilized during the preliminary heating stage (Table 6.3). It should be also noted that the differences in anisole degradation rates in MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments at 120°C were not as high as for those observed for CIP, since anisole was mainly present in the dissolved phase and was more available for oxidation. On the other hand, at 160°C, both MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments provided almost the same anisole and antibiotic degradation rates, although the oxidation mechanisms of the sulfate and hydroxyl radical and their rates of reaction with the inorganic anions produced in the treated sludge are considerably different (Neta et al., 1977).

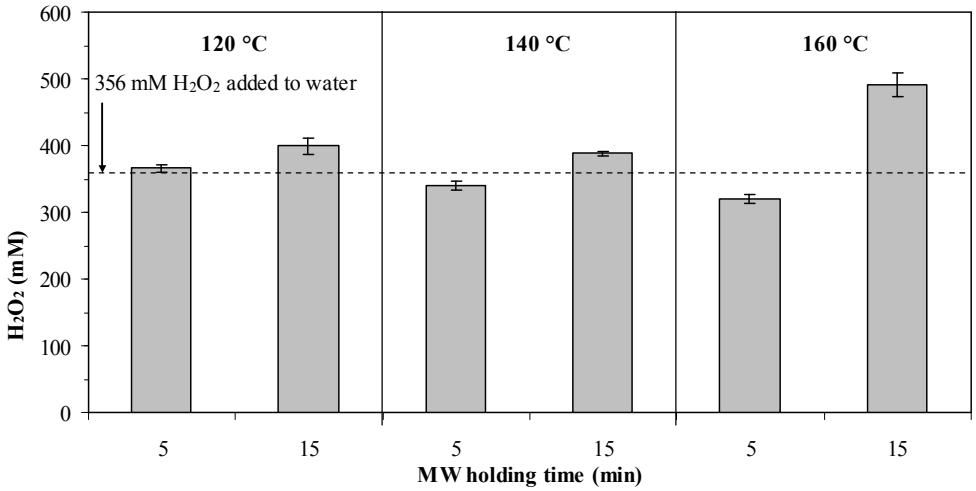


Figure 6.6. Hydrogen peroxide concentration variation in water during MW/H_2O_2 treatment (initial H_2O_2 dosage: 356 mM).

6.4. Conclusion

The efficiency of a two-stage MW/H_2O_2 treatment was compared with a novel single-stage $MW/S_2O_8^{2-}$ treatment for simultaneous sludge solubilization and antibiotic degradation. Thermo-oxidative sludge treatment with either MW/H_2O_2 or $MW/S_2O_8^{2-}$ was shown to effectively degrade sorbed antibiotics constituting a problem for the beneficial reuse of sludge on land. Degradation yields as high as ≥97% were obtained even when the antibiotics were spiked at low concentrations. Therefore either H_2O_2 or $S_2O_8^{2-}$ oxidation assisted with MW can eliminate the need of a pretreatment step to desorb micro-pollutants from the sludge.

Although comparable antibiotic degradation rates were achieved in MW/H_2O_2 and $MW/S_2O_8^{2-}$ treatments at 160°C within 15 min, consumption of hydrogen peroxide by the sludge components required the application of MW/H_2O_2 as a two-stage treatment to enhance antibiotic degradation. As a result, while the nonproductive consumption of the oxidant was prevented by the preheating stage, the actual treatment time was prolonged to 30 min in a two-stage MW/H_2O_2 treatment.

It was demonstrated that MW/$S_2O_8^{2-}$ treatment can concurrently provide the desorption of the antibiotics with the aid of the generated acidic conditions, and their rapid degradation due to the high reactivity of the sulfate radical. MW/$S_2O_8^{2-}$ treatment applied at the extreme conditions of the experimental design did not only provide antibiotic desorption but also 48% higher release of overall metals. Furthermore two-fold higher sludge dewaterability was obtained and the oxidation of ammonia was observed in this treatment.

The experimental design methodology that was used in the study revealed that while the oxidant dosage had a negative influence on COD solubilization, it was the most crucial factor for the degradation of the antibiotics and the solubilization of nutrients and metals in both MW/H_2O_2 and MW/$S_2O_8^{2-}$ treatments. The contribution of reactive radicals for the degradation of thermally stable micro-pollutants, as well as for nutrient and metal solubilization were confirmed for both treatments.

Of the two treatments, since MW/$S_2O_8^{2-}$ can be applied as a single stage process, while MW/H_2O_2 requires a pre-heating stage to prevent unproductive consumption of the oxidizing species, MW/$S_2O_8^{2-}$ can be more promising for sludge treatment. However, further optimization and evaluation of MW/$S_2O_8^{2-}$ for the production of sludge suitable for land-application should be carried out.

7. Fe^{2+} AND CONVENTIONAL HEATING ACTIVATED PERSULFATE TREATMENT OF SECONDARY SEWAGE SLUDGE: ANTIBIOTIC DEGRADATION AND SLUDGE SOLUBILIZATION

7.1. Introduction

Management of waste sewage sludge is a multidimensional challenge owing to the fact that sludge comprises of both valuable constituents as well as of problematic organic and inorganic contaminants. Among different sludge management options, land-application is still the most desired alternative, since it offers the beneficial reuse of valuable sludge constituents. On the other hand, in order to prevent potential harmful impacts on the environment, waste sludge has to be stabilized prior land-application to reach specific limits of pollutants defined by the regulations. However, contamination of the sludge is not limited to regulated pollutants; occurrence of various non-regulated pollutants in the sludge has been proved recently (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a; Stasinakis, 2012; Clarke and Smith, 2011). Owing to intensive global consumption of antimicrobial products, antimicrobial contaminants such as antibiotics and antibiotic resistance carriers (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a) constitute an important problem for the land-application of biosolids and have to be controlled along with other regulated pollutants. Some antibiotics such as TET and FQ groups tend to accumulate in sewage sludge mainly by partitioning to the solid phase and are therefore less vulnerable to biodegradation (Oncu-Bilgin and Balcioglu-Akmehmet, 2013a; Zhang et al., 2011). Recent studies demonstrate the significance of antimicrobial contamination originating from sewage treatment plants. Incomplete removal of antimicrobial agents during wastewater treatment and selection of resistant bacteria among other populations during tertiary wastewater treatment (LaPara et al., 2011) is believed to contribute to the dissemination of antibiotic resistant pathogens.

While sludge treatment methods including chemical oxidation have generally been developed to reduce sludge production and accelerate anaerobic digestion (Carrere et al., 2010), fewer number of studies have explored the role of these for micro-pollutant degradation and to the best of our knowledge no literature is available regarding antibiotic degradation except our previous study (Oncu-Bilgin and Balcioglu-Akmehmet, 2013b). The superiority of chemical

oxidation technologies such as ozone and photocatalytic oxidation over conventional chlorination in degrading resistance carriers in water was shown (Oncu-Bilgin et al., 2011) and powerful oxidation technologies can be also applied to remove recalcitrant antibiotics from sludge. A promising chemical oxidant is persulfate, which can generate sulfate radicals upon different activation aids such as heat and transition metal catalysts (Siegrist et al., 2011; Tsitonaki et al., 2010). Sulfate radicals have higher selectivity than hydroxyl radicals and thus can provide better removal of target organics from matrixes with high organic content (Uslu-Otker and Balcioglu-Akmehmet, 2009; Peyton, 1993).

Utilization of iron is one of the most commonly applied persulfate activation aids. As both ferrous (Fe^{2+}) and ferric forms (Fe^{3+}) of iron are well known coagulants and can precipitate orthophosphate in the sludge (Zhang et al., 2010; Amuda and Amoo, 2007), addition of iron to the sludge during persulfate treatment can provide the preservation of phosphorus in the solid phase. This can be an important benefit since, during chemical treatment processes generally the nutrients are released to the dissolved phase as a result of sludge solubilization and while the recovery of the released nutrients from the dissolved phase is possible, some treatment processes can result in biosolids that are not rich enough in nutrients and are thus of low fertilizer value (Shanableh and Ginige, 1999). Another benefit with persulfate treatment can be provided by the acidic conditions that are typical to the process when the pH is not controlled; these can facilitate the solubilization of metals that are another regulated pollutant group in biosolids. Moreover, treatment of sludge with Fe^{2+} activated persulfate can result in considerable enhancement in the dewatering properties of sludge (Zhen et al., 2012a, 2012b, 2012c) thus facilitating both the separation of the solubilized metals and decreasing the costs associated with sludge drying.

Considering the above mentioned facts, the potential of persulfate activated with Fe^{2+} for the degradation of two antibiotics from the TET and FQ groups – OTC and CIP – in artificially contaminated sewage sludge was investigated along with organic matter, nutrient and metal solubilization at different experimental conditions. In addition, heat was also utilized as activation means of persulfate along with Fe^{2+}.

7.2. Materials and Methods

7.2.1. Chemicals

Hydrochloride forms of OTC (Sigma) and CIP (MP. Biomedicals) were utilized for the spiking of the secondary sludge samples. $Na_2S_2O_8$ as oxidant, $FeSO_47H_2O$ as the Fe^{2+} source were purchased from Sigma-Aldrich. $Fe_2(SO_4)_3xH_2O$ was used as the Fe^{3+} source for the antibiotic complexation and desorption control experiments. KI (Sigma) and $NaHCO_3$ (Sigma-Aldrich) were utilized for oxidant analysis. Ferrozine (Fluka), ammonium acetate (Sigma), and hydroxylamine hydrochloride (Sigma-Aldrich) were used for determination of iron forms. Formic acid and acetonitrile (Sigma-Aldrich) were utilized in the HPLC analyses. All solutions were prepared with MQ water (Millipore, Milford, MA).

7.2.2. Preparation of Antibiotic Contaminated Sewage Sludge

The secondary sewage sludge, the physicochemical properties of which for the course of the study (SC-3) are presented in Table 7.1, was contaminated with CIP and OTC antibiotics as described previously (See Section 3.2.3).

Table 7.1. Average secondary sewage sludge characteristics and total metal concentrations for the SC-3 period.

Sludge properties		**Metals**	
Parameter	**Value**	**Metals**	**Sludge concentrations (mg/kg DS)**
TS (g/L)	12.7±2.9	Ni	385±11
VS/TS (%)	57±5	Cr	329±13
TCOD (g/L)	11.2±1.9	Cu	509±20
SCOD (mg/L)	83±46	Zn	848±19
TKN (mg/L)	495±84	Fe	11,987±3,137
TP (mg/L)	653±16	Mn	495±185
PO_4^{3-}	61±6	Cd	BLD
pH	6.5−7.0	Pb	BLD

The data are the mean values obtained from n=4 samples

When compared with sludge obtained during the SC-1 and SC-2, as previously the most important difference in sludge properties was observed in the TP content and in the concentrations of some heavy metals (e.g. Cr and Fe).

Antibiotics were spiked to the sludge and the TS was adjusted 10.0±0.1 g/L, which was used throughout the study (See Section 3.2.3). Two antibiotic concentrations of 20 mg/L and 0.8 mg/L were spiked to the sludge as was described previously (Sections 5.2.2 and 6.2.2).

7.2.3. Fe^{2+}/Heat/$S_2O_8^{2-}$ Treatment of Sewage Sludge

Treatment of sewage sludge with Fe^{2+} and conventional heating activated persulfate was performed in bench scale experiments within a low temperature range of 40–75°C closed TFE tubes as described in Section 3.2.5.3. Sludge samples of 25 mL were placed in the TFE tubes, then determined amounts of Fe^{2+} as and persulfate were added consecutively and the samples were mixed for 60 s on a vortex (Scientific Industries, Vortex Genie 2; VIRTIS Sentry 5L). No effort was spend for pH control. A total of 27.5 mL sludge samples (TS=10 g/L) containing determined amounts of Fe^{2+} and oxidant ($S_2O_8^{2-}$) in the TFE tubes were inserted in a temperature controlled water bath at 200 rpm for a reaction period of 120 min, which was determined by preliminary experiments. At the end of the treatment process, before the subsequent analyses were carried out, the vessels were immediately inserted in an ice bath to quench the reaction (Liang et al., 2003).

7.2.4. Experimental design

In order to explore the effects of the selected variables on the efficiency of Fe^{2+}/heat/$S_2O_8^{2-}$ for sludge treatment, a 2^3 full factorial design with three independent variables (A: temperature; B: oxidant dose; C: $Fe^{2+}/S_2O_8^{2-}$ molar ratio) was performed. The Fe^{2+} concentration was varied accordingly to provide the desired $Fe^{2+}/S_2O_8^{2-}$ molar ratio for a given oxidant dosage. The Fe^{2+} dosages utilized in the study were in the range of 4.0−28.4 mM. It should be noted that preliminary experiments were carried out to determine the extreme values (design's corner points) of the variables. The experimental range and the levels of the independent variables are given in Table 7.2.

As can be seen from Table 7.2, each one of the three variables received two values as indicated by the plus and the minus signs, while a central value was indicated by 0. The minus sign for the oxidant dose variable indicates the absence of it.

Table 7.2. Independent variables and their levels used in the 2^3 full factorial design for sewage sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

Experimental factors	Symbol	Level (-)	Level (0)	Level (+)
Temperature (ºC)	A	40.0	57.5	75.0
Oxidant dose (g/g TS)	B	0.15	0.29	0.44
$Fe^{2+}/S_2O_8^{2-}$ (molar ratio)	C	0.50	0.87	1.25

7.2.5. Analytical methods

Extraction, purification, and analysis of OTC and CIP in raw and treated sludge samples was carried out with ultrasound assisted solvent extraction, SPE, and HPLC, respectively as described previously in Section 3.2.6.6. Antibiotic degradation was calculated as the percent change in antibiotic concentration after sludge treatment. Initial antibiotic concentrations were determined in each experiment in order to eliminate the probable influence of changes in sludge characteristics on the antibiotic recoveries. The degradation rates observed for the antibiotics spiked to the sludge at low concentrations were confirmed with qualitative LC-MS/MS analysis (See Section 3.2.6.6).

Characterization of raw and treated sludge samples for SCOD, TCOD, nutrients, and metals was performed as described previously (Section 3.2.6.2 and Section 3.2.6.6). Persulfate concentrations in the samples were determined spectrophotometrically by the modified iodometric method of Liang et al. (2008) as described in Section 3.2.6.3. The concentration of Fe^{2+} and total iron were measured in some samples with a modified ferrozine method (See Section 3.2.6.4).

7.3. Results and Discussion

The experimental design results of antibiotic degradation and sludge solubilization of each 19 experiments for Fe^{2+}/heat/$S_2O_8^{2-}$ treatment are shown in Table 7.3. Additional results including the influence of treatment conditions on sludge pH and persulfate consumption are given in Table 7.4. The statistical significances of the effects of the selected independent variables on the efficiencies of the processes were evaluated and the significant factors (p<0.05) with decreasing order of effects are shown in Figure 7.1 (a)-(f) as Pareto charts.

Table 7.3. 2^3 full factorial design table for the coded factors and the responses obtained for sewage sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

Process parameter levels				Responses					
				Antibiotic degradation (%)			Solubilization (%)		
Experiment number	A	B	C	OTC	CIP	PO_4^{3-} precipitated (%)*	Organic carbon (SCOD/TCOD×100)	Nitrogen (NH_4^+/TKN)	Overall metal (Me_S/Me_T)
8	–	–	–	27.5	6.7	86.3	9.3	0.6	31.1
12	–	–	–	25.4	5.9	85.6	9.4	0.6	31.5
6	–	–	+	33.6	19.1	97.8	8.9	1.0	46.5
15	–	–	+	34.3	17.5	98.3	9.8	1.2	45.9
1	–	+	–	70.4	57.4	94.2	12.5	2.4	47.9
14	–	+	–	68.2	55.4	94.5	13.9	2.6	47.4
4	–	+	+	79.0	77.9	79.9	11.7	6.3	60.7
5	–	+	+	81.2	75.8	78.7	10.1	6.8	59.9
7	0	0	0	92.3	96.1	94.7	11.8	2.7	48.8
10	0	0	0	94.1	95.2	92.9	13.2	2.7	50.3
17	0	0	0	96.0	96.5	92.7	14.2	2.7	49.6
16	+	–	–	68.1	31.3	73.5	18.5	1.9	40.5
19	+	–	–	70.0	33.3	73.7	17.7	1.8	41.8
3	+	–	+	85.2	42.8	96.0	13.2	2.0	52.1
9	+	–	+	88.1	41.6	96.5	12.6	2.2	52.8
11	+	+	–	99.9	96.1	92.1	24.7	6.1	53.2
18	+	+	–	100**	97.2	90.9	23.4	6.0	54.9
2	+	+	+	100**	100**	73.5	22.1	9.1	65.6
13	+	+	+	100**	100**	73.2	20.4	8.8	65.1

A: Temperature; B: Persulfate dose; C: $Fe^{2+}/S_2O_8^{2-}$ molar ratio

* Percentage of precipitation based on initial PO_4^{3-} present in the sludge

** BLD

Table 7.4. Solubilization of individual metals, iron precipitation, sludge pH, and oxidant consumption for sewage sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

	Process parameter levels			Metal solubilization (%)						pH		
Experiment number	A	B	C	Cr	Cu	Mn	Ni	Zn	Fe precipitated (%)*	pH_i	pH_f	Consumed $S_2O_8^{2-}$ (%)
8	–	–	–	0.9	31.3	26.2	50.0	47.4	98.6	5.1	5.0	50.2
12	–	–	–	0.5	32.9	26.9	49.1	48.3	98.3	5.0	4.8	49.0
6	–	–	+	20.4	40.6	56.1	54.8	60.4	86.9	3.4	3.0	96.4
15	–	–	+	21.1	41.7	56.8	57.2	52.9	86.3	3.5	3.1	97.5
1	–	+	–	23.0	47.7	46.0	65.7	57.3	19.5	3.2	2.6	45.7
14	–	+	–	24.3	47.8	46.9	60.2	57.7	21.9	3.0	2.4	46.2
4	–	+	+	49.5	48.9	77.3	72.0	55.8	47.3	2.7	2.5	69.1
5	–	+	+	46.3	48.9	77.2	71.7	55.4	49.0	2.7	2.3	68.3
7	0	0	0	28.9	48.4	47.3	63.0	56.7	63.2	3.0	2.5	96.2
10	0	0	0	30.6	46.2	48.0	71.4	55.5	51.3	3.0	2.4	96.3
17	0	0	0	32.8	49.1	45.2	66.9	53.8	59.3	3.1	2.4	96.0
16	+	–	–	7.8	34.3	42.0	61.4	56.8	51.6	4.9	4.4	93.5
19	+	–	–	10.4	34.1	45.3	60.5	58.7	53.8	4.6	4.5	93.8
3	+	–	+	26.9	45.6	71.6	59.0	57.6	59.6	3.3	3.2	98.3
9	+	–	+	27.7	46.9	70.9	59.9	58.3	60.1	3.3	3.0	98.6
11	+	+	–	39.6	49.9	59.2	56.3	61.2	29.1	3.1	2.2	92.7
18	+	+	–	39.3	48.9	58.5	66.3	61.6	28.1	3.3	2.2	92.1
2	+	+	+	62.4	51.1	84.2	73.5	56.7	39.9	2.8	2.1	99.6
13	+	+	+	59.0	51.0	84.6	73.5	57.2	42.2	3.0	2.0	99.8

**Percentage of precipitation based on added Fe

A: Temperature; B: Persulfate dose; C: $Fe^{2+}/S_2O_8^{2-}$ molar ratio; pH_i: initial pH after iron and oxidant addition; pH_f: final pH after sludge treatment

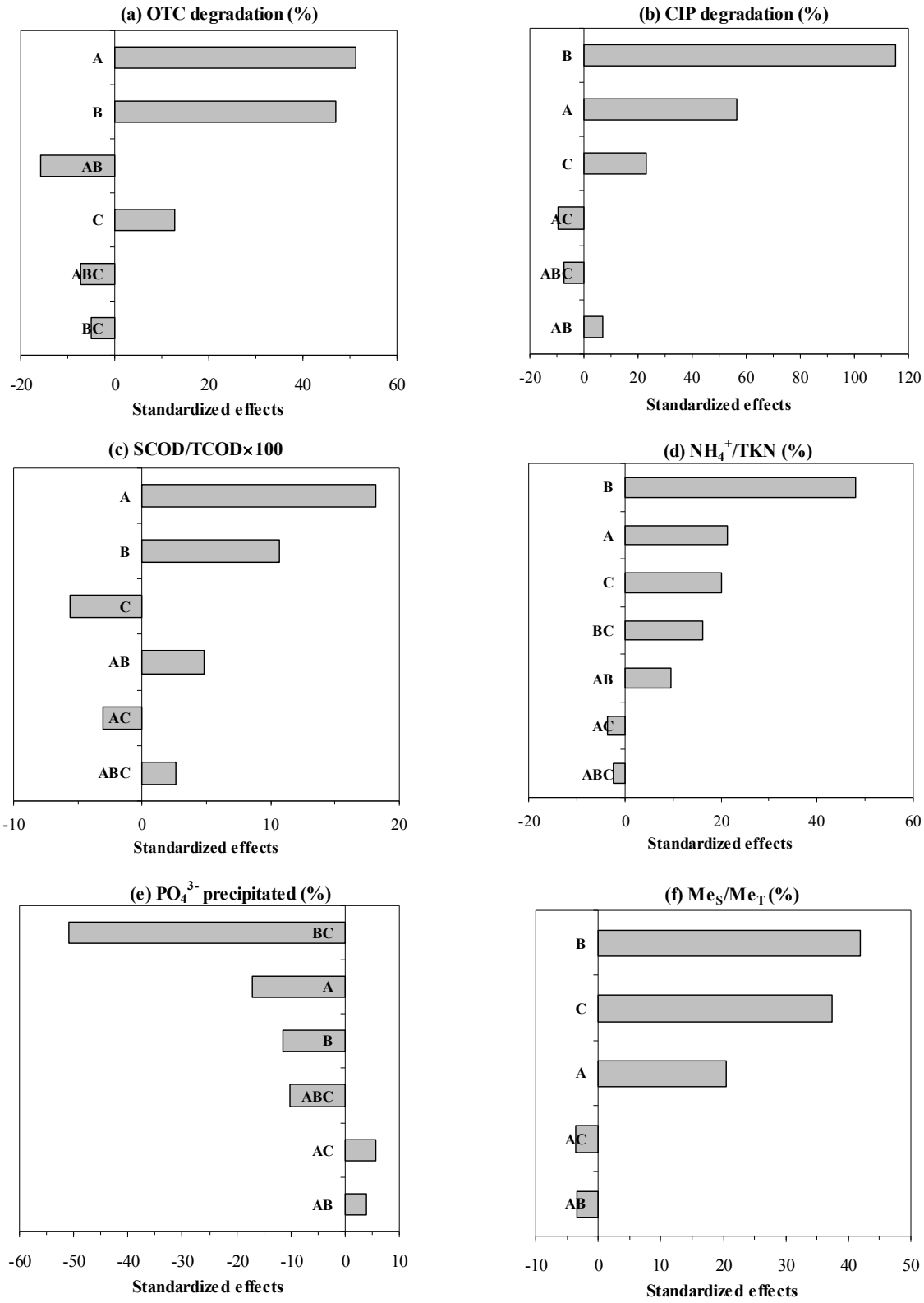


Figure 7.1. Pareto charts for (a) OTC and (b) CIP degradation, solubilization behavior of (c) organics, (d) phosphorus, (e) nitrogen, and (f) metals during sewage sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$ (A: Temperature; B: Persulfate dose; C: Fe^{2+}/$S_2O_8^{2-}$ molar ratio).

7.3.1. Effect of Fe^{2+}/heat/$S_2O_8^{2-}$ Treatment on Antibiotic Degradation

Degradation of OTC and CIP in control experiments and at selected conditions from the experimental design and at constant persulfate dosage of 0.44 g/g TS are displayed in Figure 7.2. In addition, degradation rates of the antibiotics for two different spiking concentrations (20 mg/L and 0.8 mg/L, respectively) are compared at 75°C.

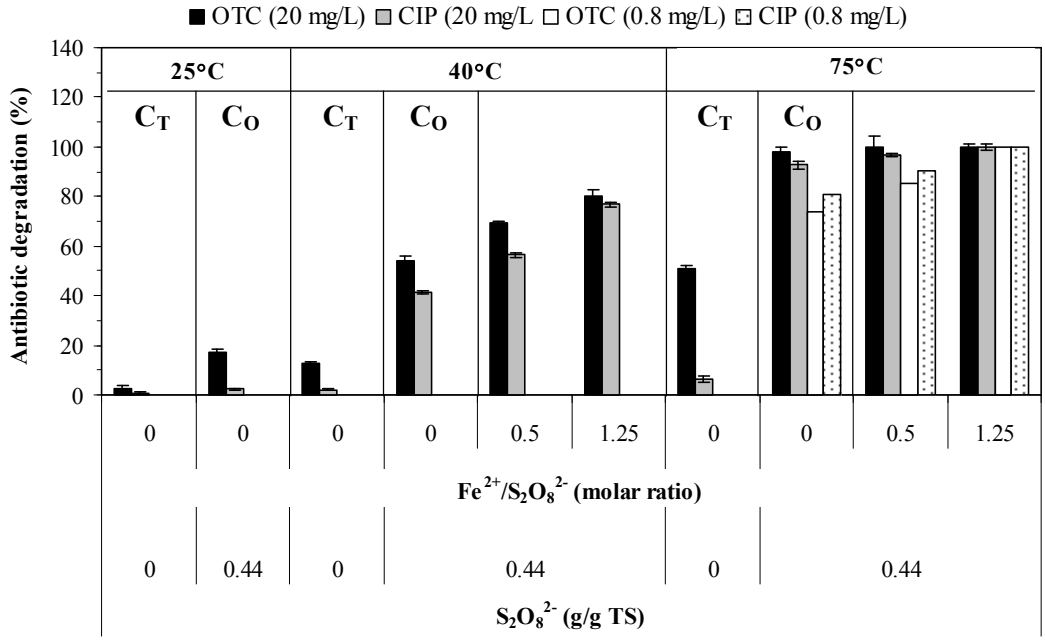


Figure 7.2. Degradation of OTC and CIP at two different spiking concentrations during treatment of sewage sludge with Fe^{2+}/heat/$S_2O_8^{2-}$ (C_T: temperature control; C_O: oxidant control).

Negligible degradation of OTC and CIP took place in the control experiment carried out at 25 °C with 0.44 g $S_2O_8^{2-}$/g TS (Figure 7.2), which shows that persulfate was not considerably activated at room temperature. As can be seen from Table 7.3 and Figure 7.2, the degradation of the antibiotics increased with temperature, oxidant dose and Fe/$S_2O_8^{2-}$ ratio. These results indicate that higher degree of persulfate activation took place with temperature and Fe^{2+} dosage according to Eqns. 2.24 and 2.25, respectively.

Pareto charts demonstrated that the most important factors for both OTC and CIP degradation were the oxidant dose and the temperature (Figure 7.1). While OTC degradation mainly depended on temperature, CIP degradation depended mainly on oxidant dose (Figure 7.1). As mentioned in the Section 6, this result can obviously be attributed to the thermal instability of OTC as opposed to the relatively high thermal stability of CIP. For instance, OTC degradation improved from 13% to 51% only with temperature increase from 40°C to 75°C in the absence of persulfate (Figure 7.2). On the other hand, CIP degradation at 75°C improved from 32% to 97% when the persulfate dosage was increased from 0.15 g/g TS to 0.44 g/g TS ($Fe/S_2O_8^{2-}$ = 0.5) (exp. 16, 19-11, 18 in Table 7.3). As in the case of persulfate oxidation assisted with MW, due to the thermal instability of OTC, the enhancement in its degradation with oxidant dosing at high temperature was not as remarkable as for that of CIP, which resulted in a negative interaction of the temperature and oxidant dose (Figure 7.1). Complete degradation of both antibiotics was obtained at the most extreme conditions of the design (75°C, 0.44 g/g TS, $Fe/S_2O_8^{2-}$ = 1.25).

The $Fe/S_2O_8^{2-}$ ratio was the third most important factor for CIP degradation and the fourth most important factor for OTC degradation (Figure 7.1). The positive influence of $Fe/S_2O_8^{2-}$ ratio indicates enhanced persulfate activation at higher iron dosage. Possible enhancement in persulfate activation by iron addition was also demonstrated by the higher persulfate utilization rate at higher $Fe^{2+}/S_2O_8^{2-}$ dose especially at 40°C (Figure 7.3 and exp. 8, 12-6, 15 in Table 7.4).

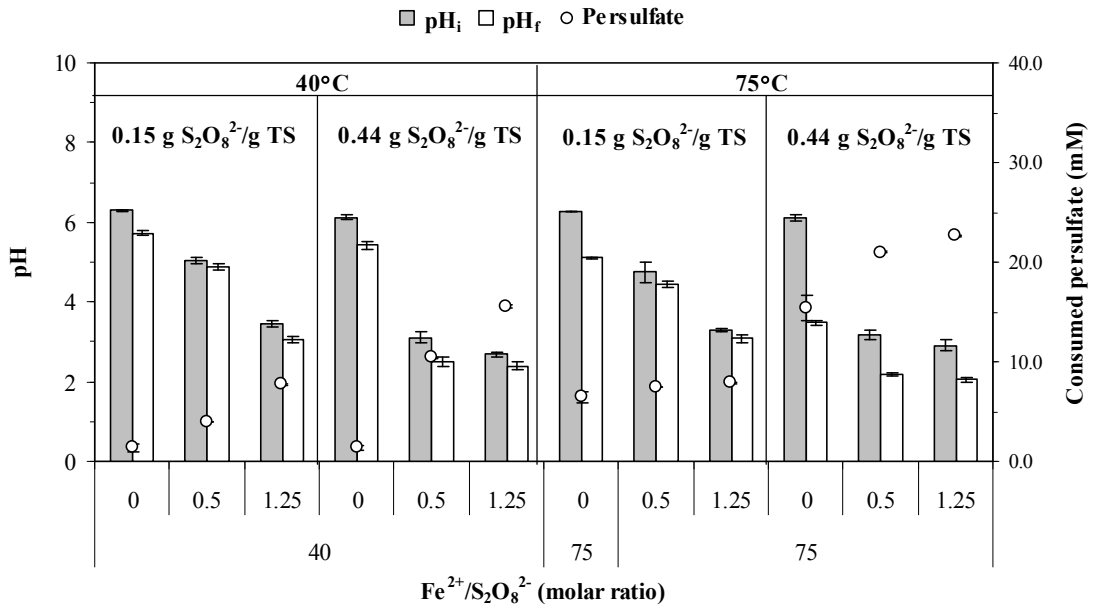


Figure 7.3. Persulfate consumption and pH change as a result of sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

By the addition of Fe^{2+} to the sludge pH exhibited a rapid decline and a higher decline in the pH value of the sludge was obtained by higher $Fe^{2+}/S_2O_8^{2-}$ dose application. These observations support the well-known fast activation of persulfate with iron, which requires lower activation energy than that of heat activation (Kolthoff and Miller, 1951; Gupta and Gupta, 1981). The acidic conditions developed by the addition of iron activator can also provide increased rate of sulfate radical generation via Eqn. 2.26.

As can be seen from Table 7.3, increase in the Fe^{2+} dosage enhanced CIP degradation to a higher degree at 40°C (e.g. exp. 1, 14- 4, 5) than that at 75°C (e.g. exp. 11, 18-2, 13); this resulted in a negative interaction between the temperature and the $Fe^{2+}/S_2O_8^{2-}$ ratio for CIP. This can be since at 75°C, the temperature should be the major activator for persulfate, which resulted in lower influence to be observed for the iron dosage. This result was also supported by the higher increase in CIP degradation with addition of 0.44 g $S_2O_8^{2-}$/g TS in the absence of iron at 75°C compared to 40°C (difference between CIP degradation at C_O and C_T at 40°C and at 75°C in Figure 7.1). The interaction of temperature and $Fe^{2+}/S_2O_8^{2-}$ ratio was not significant for OTC (p<0.05).

Regarding contaminant degradation, different results were reported for the combined activation of persulfate with heat and Fe^{2+} in the literature. For example, while Romero et al. (2010) reported enhancement in diuron degradation with persulfate in water by addition of Fe^{2+} at 50°C (initial pH 4-5 and not controlled during oxidation), Oh et al. (2009) reported that the activation of persulfate in a system utilizing Fe^{2+}/heat/persulfate to treat polyvinyl alcohol in water was mainly heat dominated even at moderate temperatures (e.g. 40°C, pH was not reported). In the present study, the activation of persulfate was mainly heat dominated at 75°C.

Fe^{2+} could not be identified in the supernatant of the sludge due to fast oxidation to Fe^{3+} via Eqn. 2.25 as was also observed by Liang et al. (2004). Nevertheless, it was suggested that activation of persulfate by Fe^{3+} was probable by the following chain reactions (Eqns. 10.1-10.2) that involve the reaction of Fe^{3+} with organic radicals ($R^{\bullet}$), which leads to regeneration of Fe^{2+} (Liang et al., 2009):

$$SO_4^{-\bullet} + RH \rightarrow R^{\bullet} + H^{+} \quad (7.1)$$

$$R^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + \text{products} \quad (7.2)$$

However, Liang et al. (2009) reported that the influence of Fe^{3+} on persulfate activation was negligible in the absence of a chelating agent that could keep iron in the soluble phase, since ferric iron precipitates to iron hydroxide $Fe(OH)_3$ or hydrous ferric oxide $Fe_2O_3nH_2O$ especially above pH 3.

The most important positive contribution of the iron dose on antibiotic degradation in the current study can be its influence on antibiotic desorption. As opposed to CHP (catalyzed hydrogen peroxide), persulfate may not oxidize strongly sorbed compounds very efficiently (Teel et al., 2009). On the other hand, it is known that iron can influence antibiotic desorption by complexation. Complexation of ferric iron both with CIP and OTC to produce soluble complexes is well-known (Kara et al., 1991; Machado et al., 1995). In order to explore this possibility, control experiments were carried out, in which the desorption of the antibiotics by formation of soluble complexes with ferric iron was investigated. For this purpose Fe^{3+} at three different concentrations was added to the sludge, which was then equilibrated at 200 rpm for 30 min at different temperatures. Then the

influence of equilibration period was investigated at the optimum conditions obtained for 30 min. The highest Fe^{3+} concentration used in these experiments corresponded to the highest amount of Fe^{2+} used in the study. The results of these experiments are shown in Figure 7.4.

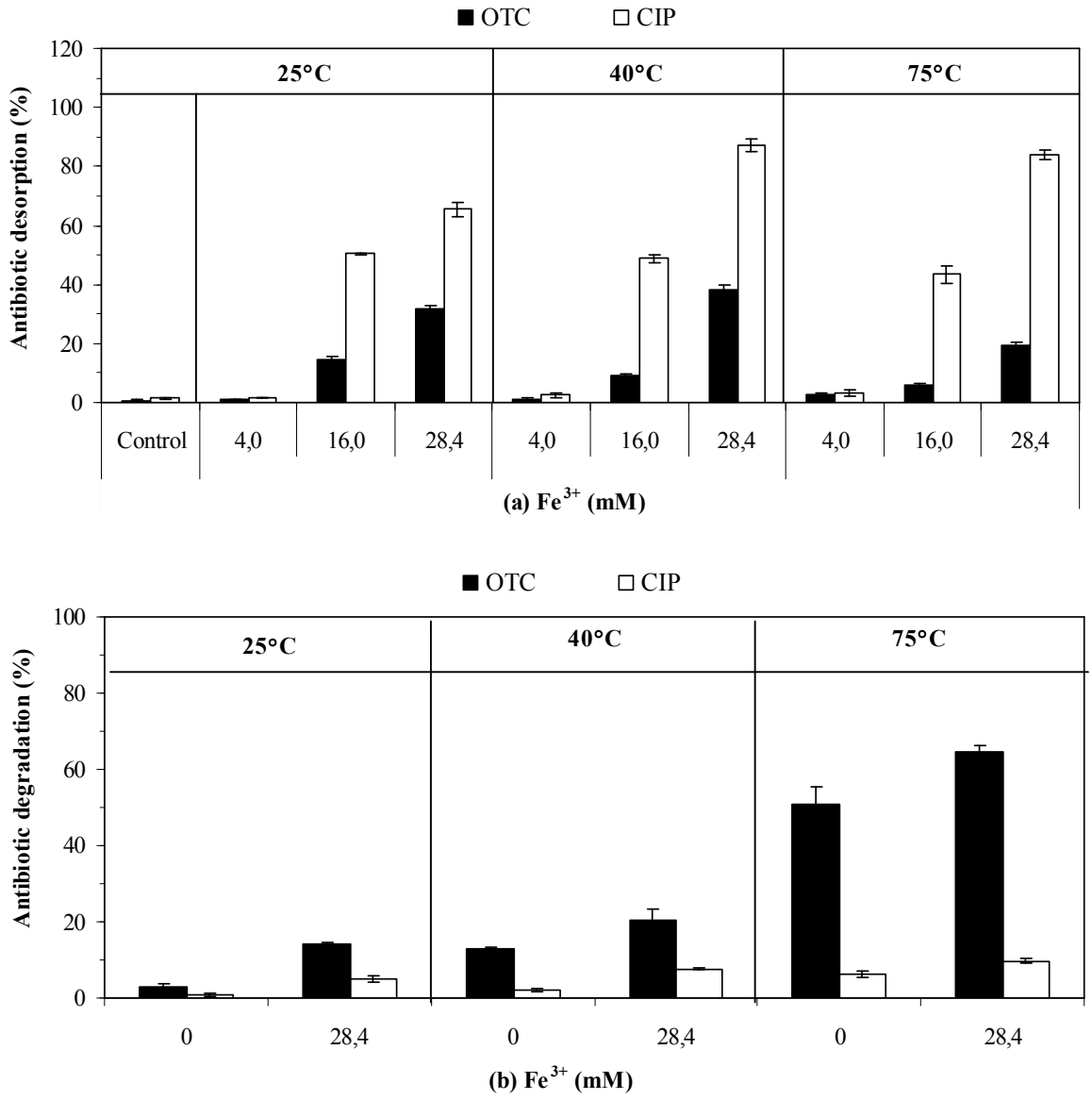


Figure 7.4. Influence of ferric iron on (a) antibiotic desorption and (b) on antibiotic degradation at different temperatures in equilibration time of 30 min.

As can be seen from the figure, Fe^{3+} resulted in antibiotic desorption, which was more pronounced for CIP (Figure 7.4 (a)). Degradation of OTC especially with temperature increase could have hampered evaluating reliably the influence of Fe^{3+} on it's desorption.

On the other hand, 84% of the CIP concentration in the sludge was desorbed to the dissolved phase in 30 min at 75°C by the addition of 28.4 mM Fe^{3+}. Prolonging the equilibration period to one hour as well as to two hours did not improve antibiotic desorption rates considerably (data not shown) indicating that the antibiotics desorbed in a relatively short time and were then available for oxidation with persulfate. The highest Fe^{3+} dose resulted in the highest rate of antibiotic desorption.

Lower degradation rates were observed for antibiotics spiked at low concentrations during treatment of sludge with Fe^{2+}/heat/$S_2O_8^{2-}$ at a lower Fe^{2+}/$S_2O_8^{2-}$ dose (85% and 91% for OTC and CIP, respectively, Figure 7.2) and much lower degradation rates were observed when iron was not added (74% and 81% for OTC and CIP, respectively). On the other hand, when the Fe^{2+}/$S_2O_8^{2-}$ dose was increased to 1.25, complete degradation of the antibiotics spiked at low concentrations was observed. This can be attributed to the greater competitive influence exerted by solubilized organics for the oxidant when antibiotics are present at lower concentrations and when their sufficient desorption is not provided due to insufficient amount of iron. While concurrent antibiotic desorption and degradation took place in MW/$S_2O_8^{2-}$ because of the specific characteristics of the MW process, in the Fe^{2+}/$S_2O_8^{2-}$ process, antibiotic desorption during oxidation was provided by complexation with iron and by pH decline (Table 7.5 and Figure 7.3.). While both the iron and persulfate dosages utilized in the study for sludge treatment were higher than those utilized previously by Zhen et al. (2012a, b, c), it is obvious that these concentrations are necessary to achieve complete antibiotic degradation especially when the antibiotics are present at low concentrations in the sludge.

The oxidative influence of Fe^{3+} produced during persulfate oxidation process would also have a positive contribution on antibiotic degradation, as described by Liang et al. (2009), who suggested the reduction of organics with ferric iron by Eqn. 7.3. Fe^{3+} has reducing potential of 0.77 V and thus can contribute to higher oxidizing conditions in the medium.

$$RH + Fe^{3+} \rightarrow Fe^{2+} + RH^{+} \qquad (7.3)$$

While it is known that Fe^{3+} can especially oxidize quinone intermediates that are produced during radical oxidation reactions of aromatics (Chen and Pignatello, 1997), this reaction is very slow as suggested by the findings of Anipsitakis and Dyonisiou (2004) who reported 7.5% degradation of 2,4-dichlorophenol by Fe^{3+} at pH 3 within 30 min of reaction period. In the present study, it is obvious that the most important contribution of Fe^{2+} dosing on antibiotic degradation was the desorptive influence of Fe^{3+} by its complexation with the antibiotics.

7.3.2. Effect of Fe^{2+}/heat/$S_2O_8^{2-}$ Treatment on Sludge Solubilization

As can be seen from the results in Table 7.3 and the Pareto chart displayed in Figure 7.1 (c), an increase in both temperature and oxidant dose enhanced organic carbon solubilization, which indicated increased cell lysis. The highest SCOD/TCOD ratio (24%) was obtained at 75°C with a persulfate dose of 0.44 g/g TS and a $Fe^{2+}/S_2O_8^{2-}$ ratio of 0.5 (exp. 11, 18 in Table 7.3). The positive interaction of temperature and oxidant dose indicated their synergistic influence on organic carbon solubilization.

While the addition of iron during persulfate treatment at both 40°C and 75°C resulted in increase in the SCOD/TCOD ratio compared to that obtained both in the absence of iron or in the absence of both persulfate and iron in control experiments (Figure 7.5), lower organic carbon solubilization was obtained with a $Fe^{2+}/S_2O_8^{2-}$ dosage of 1.25, compared to that obtained with 0.5 (e.g. exp. 11, 18-2, 13 in Table 7.3).

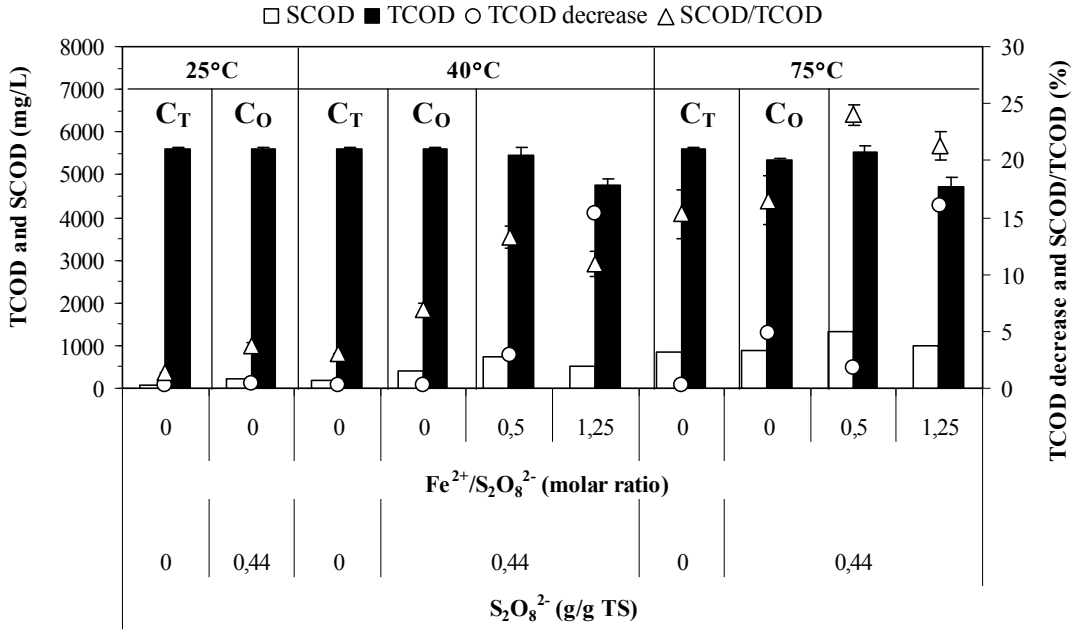


Figure 7.5. Influence of Fe^{2+}/heat/$S_2O_8^{2-}$ treatment on soluble and total organic content of sludge.

This negative influence of increased $Fe^{2+}/S_2O_8^{2-}$ dosage was also obvious in the Pareto chart (Figure 7.1 (c)). In addition, a negative interaction of the temperature and the $Fe^{2+}/S_2O_8^{2-}$ dose suggested a decrease in organic carbon solubilization. This result can be partially due to increased sludge oxidation compared to solubilization via enhanced persulfate activation at higher iron dosage, which is also consistent with the declines observed in the TCOD and SCOD values (Figure 7.5). This result is also consistent with the higher antibiotic degradation rates at higher $Fe^{2+}/S_2O_8^{2-}$ dosage and indicates that enhanced antibiotic degradation cannot be attributed solely to desorption, but also to enhanced persulfate activation. Since higher dissolved iron concentrations were provided at a higher $Fe^{2+}/S_2O_8^{2-}$ ratio, precipitation of SCOD with iron is another possibility for the decline in solubilization. Both ferrous and ferric forms of iron are used as flocculants to precipitate COD and orthophosphate; and precipitation can take place in a wide range of pH (Zhang et al., 2010; Amuda and Amoo, 2009). Comparison of the COD values obtained in temperature control experiments (Figure 7.5) and control experiments performed with 28.4 mM Fe^{3+} (Table 7.5) at 40°C and 75°C demonstrated that some COD precipitation could have taken place in the present study during sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

Table 7.5 Influence of Fe^{3+} (28.4 mM) on organic carbon solubilization and PO_4^{3-} precipitation within equilibration time of 30 min.

Temperature (°C)	SCOD (mg/L)	TCOD (g/L)	Organic carbon solubilization (SCOD/TCOD×100)	PO_4^{3-} precipitation (%)*
40	128.4	5.6	2.3	98.8
75	499.4	5.6	8.9	91.5

* Percentage of precipitation based on initial PO_4^{3-} in dissolved phase

As a result of higher oxidation rate of proteins released from bacterial cells, higher degree of nitrogen solubilization took place at higher oxidant dose (e.g. 6, 15-4, 5 in Table 7.3), in accordance with previous studies (Wong et al., 2007) and with the results obtained for MW/$S_2O_8^{2-}$. Thus the highest influence on nitrogen solubilization was exerted by the oxidant dose (Figure 7.1 (d)). The temperature and the $Fe^{2+}/S_2O_8^{2-}$ ratio also exerted a positive influence on ammonia solubilization.

As anticipated, the addition of iron resulted in precipitation of the orthophosphate that was initially present in the liquid phase of the sludge (Zhang et al., 2010); therefore, the influence of process parameters on phosphorus fate was expressed as percentage of precipitated orthophosphate. Since both temperature and oxidant dose enhanced sludge solubilization, these factors and their interaction exerted a negative influence on phosphate precipitation (Figure 7.1 (e)). While the $Fe^{2+}/S_2O_8^{2-}$ ratio on phosphate precipitation was not significant (p<0.05), it is more appropriate to analyze the relationship of precipitated orthophosphate at different initial Fe^{2+} concentrations, since the added iron amounts varied depending on the persulfate dosage. This relationship is displayed in Figure 7.6.

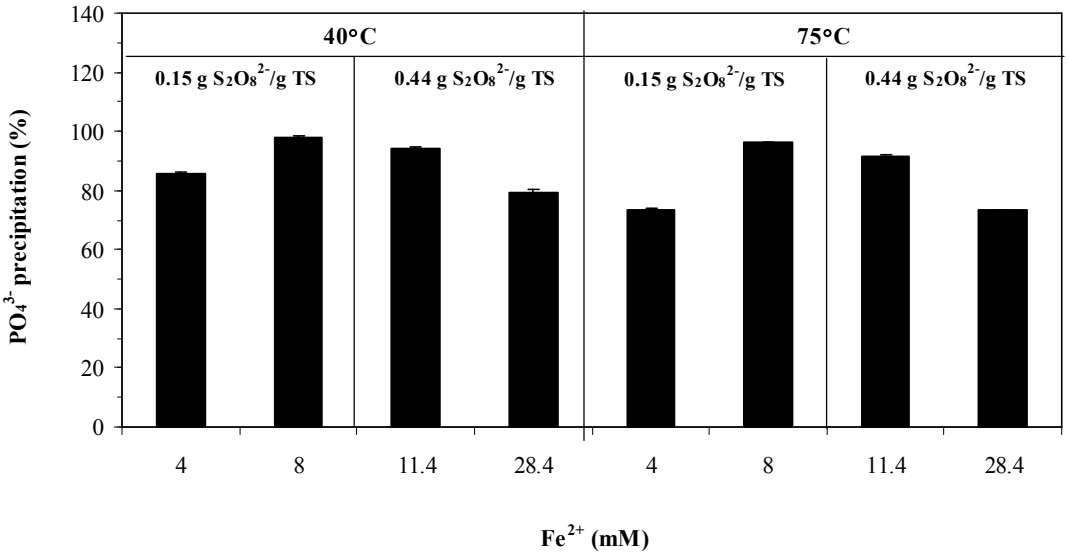


Figure 7.6. Precipitation of PO_4^{3-} based on initial concentration at different iron concentrations utilized during sludge treatment with Fe^{2+}/heat/$S_2O_8^{2-}$.

As can be seen from the figure, higher amount of PO_4^{3-} precipitated with increased Fe^{2+} dose when the persulfate dosage was 0.15 g/g TS. The precipitation of PO_4^{3-} with $Fe^{3+}$was also confirmed in the control experiments performed with 28.4 mM Fe^{3+} as shown in Table 7.5. However when the persulfate dosage was fixed at 0.44 g/g TS, increase in the Fe^{2+} dose resulted in higher phosphorus solubilization, which decreased the overall amount of precipitated PO_4^{3-}. This indicates that precipitation of PO_4^{3-} was overcome by solubilization at these conditions. As a result, a positive interaction of the oxidant dosage and the $Fe^{2+}/S_2O_8^{2-}$ ratio was observed in the Pareto chart (Figure 7.1 (e)). At higher persulfate dosage and with increase in the $Fe^{2+}/S_2O_8^{2-}$ ratio, greater decline in the pH with higher activation degree of persulfate could have also promoted the solubilization of phosphorus.

It should be noted that the amount of iron that will be spent for PO_4^{3-} precipitation in the sludge will depend on the initial PO_4^{3-} content present in the dissolved phase of the sludge, which will in turn influence the oxidation efficiency. For example, if the initial PO_4^{3-} content is low, excess iron can act as a scavenger according to Eq. 7.4 and can reduce the process efficiency (Oh et al., 2009).

$$Fe^{2+} + SO_4^{-\bullet} \rightarrow Fe^{3+} + SO_4^{2-} \qquad k = 4.6 \times 10^9\ M^{-1}s^{-1} \qquad (7.4)$$

The highest metal release (65%) was obtained with Fe^{2+}/heat/$S_2O_8^{2-}$ treatment of sludge at 75°C and with 0.44 g $S_2O_8^{2-}$/g TS and Fe^{2+}/$S_2O_8^{2-}$ ratio of 1.25 (exp. 2, 13 in Table 7.3). This observation can be attributed to the enhanced solubilization of the sludge, as well as to the influence exerted on metal speciation by the severe pH decline (Table 7.4). Solubilized metals can also promote persulfate activation in a similar manner with Fe^{2+}. Solubilization rates of individual metals are also given in Table 7.4. Precipitation of iron took place after treatment as expected and the detected liquid phase iron concentrations were lower than the added concentrations. However, due to the complexity of the matrix and changes in sludge characteristics at different treatment conditions, the precipitated iron concentrations could not be statistically correlated with the precipitated PO_4^{3-} concentrations.

7.4. Conclusion

Complete degradation of CIP and OTC antimicrobial micro-pollutants in secondary sewage sludge was achieved along sludge solubilization with Fe^{2+}/heat activated persulfate treatment. All of the studied process parameters exerted significant and important effects on both antibiotic degradation and sludge solubilization. Especially the dosing of iron provided considerable antibiotic desorption and thus was crucial for the complete elimination of the antibiotics that were spiked to the sludge at low concentrations. Addition of iron also provided precipitation of phosphorus and thus can enhance the fertilizing value of the treated sludge. However, specific attention should be paid to optimize the process depending on the initial phosphorus concentration in the sludge, which may influence the efficiency of the process.

8. SUMMARY AND CONCLUSIVE REMARKS

The effectiveness of conventional water treatment technologies for the prevention of antimicrobial pollution dissemination has been questioned due to considerable increase in the prevalence and proliferation of antimicrobial resistance. Antibiotic resistance carrier elements are increasingly found in the effluents of treatment plants and can inevitably reach natural waters and contaminate drinking water sources. Therefore, preventive efforts must focus on monitoring both drinking water quality and effluent quality of treatment plants.

On the basis of this objective, chemical oxidation processes were evaluated in the thesis for their effectiveness to destruct antimicrobial pollution in water as well as in waste sewage sludge, which can be considered a significant point source. In the first part of the thesis, the effectiveness of ozone and heterogeneous photocatalytic oxidation on the reduction of antibiotic resistance transfer risk through a resistance carrier bacterial plasmid DNA was evaluated and compared with conventional chlorine oxidation. Two different catalysts, a commercial TiO_2-P25 and a nano-fiber-TiO_2 photocatalyst that was prepared in the laboratory were utilized for heterogeneous photocatalytic oxidation of the water. In the second part of the thesis, destruction of CIP and OTC, model antibiotics commonly detected in waste sewage sludge was investigated during chemical treatment of sludge with ozonation, hydrogen peroxide oxidation assisted with microwave irradiation (MW/H_2O_2), persulfate oxidation assisted with microwave irradiation ($MW/S_2O_8^{2-}$), and persulfate oxidation activated with ferrous iron and conventional heating. The major outcomes of the thesis can be listed as follows:

- Both ozonation and photocatalytic treatment with TiO_2-P25 induced considerable structural damage on the resistance carrier plasmid DNA. Increase in conformational changes of plasmid DNA structure either with increased ozone dose or with photocatalytic treatment period resulted in higher inhibition in the transformation efficiency of competent cells, which indicated decline in the antibiotic resistance transforming ability of the plasmid DNA.

- Ozone and TiO_2-P25 were more effective in damaging the plasmid DNA having a low initial concentration of 6.4 mg/L compared to a high initial concentration of 12.8 mg/L. Although, complete destruction of the plasmid DNA with initial concentration of 6.4 mg/L and complete prevention of antibiotic resistance transfer necessitated an ozone dose of 4 mg/L or a long photocatalytic treatment period of 75 min, the considerable improvement in the degradation of the plasmid DNA at lower initial concentration indicates that ozone doses commonly used for water treatment and heterogeneous photocatalysis with TiO_2-P25 can be effective to destruct plasmid DNA that is found at environmental concentrations.

- While the nano-fiber-TiO_2 induced slight modifications in the structure of the plasmid DNA and thus further improvement in material properties is required to enhance its photocatalytic efficiency, it had excellent uniform fiber structure and the surface area was similar to that of the commercial TiO_2-P25. Therefore, the material can be promising for supported photocatalytic system applications and can facilitate catalyst removal after water treatment.

- Chlorination, which is still one of the most widely applied technologies, was ineffective even at a dose higher than those utilized commonly for water treatment, indicating that chlorine cannot be the technology of choice to prevent antimicrobial contamination in drinking water. It is suggested that more effective oxidation processes such as ozonation and heterogeneous photocatalysis can be much appropriate to control antibiotic resistance contamination in water.

- The fate of CIP and OTC during the treatment of sludge with chemical oxidation presented the dependency of the degradation efficiency on two major factors: i) the degree of antibiotic desorption and ii) the degree of competitive influence exerted by solubilized sludge components. When the desorption of the antibiotics was provided prior chemical oxidation or concurrently with sludge oxidation, the solubilized organics did not considerably influence the degradation efficiency and almost complete degradation of the antibiotics was achieved. However, the degradation rate of the antibiotics at low concentrations slowed down considerably when the desorption efficiency was not sufficient and when the sludge contained high amounts of competing solubilized constituents.

- Among the studied processes, it was shown that ozonation is more suitable for the treatment of sludge with a low TS content (2.5 g/L), since at high TS (10 g/L) ozone gas transfer within the sludge was inefficient and the competitive influence of the solubilized sludge organics exerted considerable negative influence on the process efficiency. Therefore, further process modification had to be applied to desorb the target contaminants and to improve their degradation.

- The desorption of antibiotics in the ozonation process was achieved either with Mg^{2+} pretreatment (0.09 g Mg^{2+}/g TS) or by increasing the pH to 11.5. While increasing the pH improved CIP desorption (90%) to a greater extent compared to Mg^{2+} pretreatment, the desorption rates of OTC both with Mg^{2+} pretreatment and at pH 11.5 were similar (~38%). As a result, considerable degradation of CIP (>95%) was obtained by ozonation at pH 11.5, while the higher amount of organics that solubilized (33%) at pH 11.5 compared to Mg^{2+} pretreatment exerted higher competitive influence on OTC degradation. While the organics as well as the antibiotics were desorbed by the pH adjustment, Mg^{2+} pretreatment did not cause a remarkable increase in the SCOD. Ozonation at pH 11.5 provided higher degree of organic matter solubilization and ~two-fold higher metal solubilization, while Mg^{2+} pretreatment and subsequent ozonation provided ~two-fold higher improvement in the sludge settleability.

- Thermo-oxidative treatment of sludge with MW/H_2O_2 and MW/$S_2O_8^{2-}$ processes could effectively degrade the antibiotics in the sludge having high TS content (10 g/L) owing to the desorption ability of MW. In addition, antibiotic desorption was promoted by pH decline during treatment with MW/$S_2O_8^{2-}$ and most probably in the presence of high hydrogen peroxide concentrations during treatment with MW/H_2O_2. It was revealed that oxidant dosing in MW treatment is necessary to synergistically improve micro-pollutant degradation, as well as nutrients and metals solubilization in both MW/H_2O_2 and MW/$S_2O_8^{2-}$ processes by the production of reactive radicals.

- >97% antibiotic degradation was obtained at both low and high antibiotic concentrations along with 22-26% carbon solubilization at 160°C within 15 min holding time in both MW/H_2O_2 and MW/$S_2O_8^{2-}$ processes, with the oxidant dosages of 1.2 g H_2O_2/g TS and 0.87 g $S_2O_8^{2-}$/g TS, respectively. However, while MW/$S_2O_8^{2-}$ can be

applied as a single stage process, MW/H_2O_2 requires a pre-heating stage to prevent the inefficient consumption of the oxidizing species and thus the overall treatment time is prolonged. In addition, under appropriate treatment conditions, MW/$S_2O_8^{2-}$ provided 48% higher overall metal solubilization, two-fold higher improvement in sludge dewaterability and oxidation of solubilized ammonia leading to 3-fold higher concentrations of nitrate. Oxidation of ammonia to nitrate can decrease the potential of nitrogen loss from sludge through ammonia volatilization.

- The efficiency of persulfate oxidation process activated by conventional heating depended on the addition of iron, which provided both concurrent selective antibiotic desorption via complexation and the generation of reactive radicals. The addition of iron was crucial especially at low antibiotic concentrations, which were affected by the competitive influence of solubilized organic to a greater extent.

- Compared to MW/$S_2O_8^{2-}$, the Fe^{2+}/heat/$S_2O_8^{2-}$ process required longer treatment period, but almost complete degradation of the antibiotics was achieved at 75°C within 120 min, with an oxidant dose of 0.44 g $S_2O_8^{2-}$/ g TS, and with a Fe^{2+}/heat/$S_2O_8^{2-}$ molar ratio of 1.25. On the other hand, as a result of much rapid persulfate consumption in the MW process due to the high temperature, residual antibiotics were left in the MW/$S_2O_8^{2-}$ process. Besides the high antibiotic degradation rate, the Fe^{2+}/heat/$S_2O_8^{2-}$ process resulted in organic carbon solubilization of 21% and nitrogen solubilization of 9%, which were comparable to those obtained with the MW/$S_2O_8^{2-}$ process. Metal solubilization was 65%, which was 10% lower than that obtained with the MW/$S_2O_8^{2-}$ process. Additional benefit of ferrous iron addition during persulfate oxidation can be enhanced sludge dewaterability due to iron's coagulation action. Hence, the 73% precipitation of the initial orthophosphate concentration that took place at appropriate conditions in the Fe^{2+}/heat/$S_2O_8^{2-}$ process can increase the fertilizer value of the sludge.

- Of the studied processes, Mg^{2+} pretreatment combined ozonation, oxidation with MW/$S_2O_8^{2-}$, and oxidation with Fe^{2+}/heat/$S_2O_8^{2-}$ can provide enhanced dewaterability and thus can facilitate the separation of solubilized metals and nutrients from the sludge.

- The high resistance of CIP to heat and ozone resulted in lower degradation rates compared to OTC. Thermo-oxidative sludge treatment by the addition of persulfate provided high antibiotic destruction rate, due to the more selective nature of the sulfate radicals compared to hydroxyl radicals. Therefore, persulfate oxidation can be more suitable for matrices with high organic matter content that can exert considerable competitive influence.

- In the thesis, it was shown that chemical oxidants are promising for the effective elimination of antimicrobial contaminants in both water and waste sewage sludge. Much powerful oxidative radicals are required to properly control antimicrobial pollution in water and sludge.

- Further optimization and evaluation of the processes either for direct sludge stabilization and conditioning or for sludge pretreatment can be promising.

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