FATE OF MICROPOLLUTANTS DURING THE ADVANCED TREATMENT OF SEWAGE SLUDGE: DEGRADATION OF TRICLOSAN, CIPROFLOXACIN, AND OXYTETRACYCLINE

by

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ABSTRACT

Antimicrobials are emerging organic contaminants that enter wastewater network as consequence of human activities. In contrast to other emerging organic contaminants, antimicrobials have the potential to cause a secondary pollution. In order to eliminate the environmental risk of both antimicrobial and antimicrobial resistance bacteria pollution the treatment of these contaminants in sewage sludge could be necessary. Considering these facts, the fate of a model antimicrobial Triclosan (TCS) in waste secondary sewage sludge was investigated during the application of heat and ferrous iron activated persulfate process. An experimental design methodology was applied for this thermochemical process to evaluate the combined effects of selected parameters. The degradation rate of TCS was >99.9% with 22.7 mM dose of persulfate and 0.5 molar ratios of $Fe^{2+}/S_2O_8^{2-}$ at 75 °C in 90 min treatment period. While increasing dose of persulfate and temperature have positive influence on TCS degradation higher dose of Fe^{2+} reduced the treatment efficiency of the process. However, the addition of iron to persulfate process shortened the treatment period required for effective antimicrobial degradation, enhanced the solubilisation of sludge constituents, and caused a reduction in Adsorbable orgnic halogens (AOX) values of sludge by providing both sulphate and hydroxyl radicals in the sludge. The beneficial effects of iron addition in persulfate process were not limited with these it also provided the recovery of phosphorous in the solid phase of the sludge and slight improvement in sludge dewaterability. Activation of persulfate with microwave (MW) irradiation resulted in lower extractable protein in the sludge than that obtained by Heat/$Fe^{2+}/S_2O_8^{2-}$ treatment.

ÖZET

Antimikrobiyaller atıksu ortamlarında insan aktiviteleri sonucu organik kirleticiler olarak ortaya çıkmaktadırlar. Açığa çıkan diğer organik kirleticilerin aksine antimikrobiyallerin ikincil kirliliğe sebebiyet verme potansiyelleri vardır. Antimikrobiyal ve antimikrobiyallere dirençli bakteriyellerin çevresel risklerini azaltabilmek için bu kirleticilerin evsel çamur ortamında arıtılması önemlidir. Bu gerçekler göz önüne alındığında, antimikrobiyal Triklosan'ın (TCS) Isı/Demir/Persülfat süreci ile ikincil evsel çamur sistemlerinden giderimi araştırılmıştır. Dizayn edilmiş deneysel yöntem uygulanarak seçilmiş parametrelerin kombinasyonlarının bu termokimyasal süreç üzerindeki etkileri değerlendirilmiştir. Triclosan'ın, 75 °C'de 90 dakika arıtma süresi, 22.7 Mm persülfat dozu ve $Fe^{2+}/S_2O_8^{2-}$ =0.5 uygulandığında 99.9% 'dan dan fazla giderim verimi elde edilmiştir. Persülfat dozu ve sıcaklık artışı TCS giderimini artırırken Fe^{2+} dozunun artması arıtma verimini düşürmüştür. Ancak, persülfat sürecine demir ilavesi antimikrobiyal giderimi için gerekli süreyi azaltmış, çamur çözünürlüğünü arttırmış ve çamurda sülfat ve hidroksil radikallerinin oluşması sonucu AOX değerlerini düşürmüştür. Ayrıca, persülfat sürecine demir ilavesinin olumlu etkileri arasında çamurun katı fazından fosfor kazanımı ve çamur susuzlaştırılmasının arttırılması gibi etkiler de gözlenmiştir. Mikrodalga destekli persülfat aktivasyonu uygulaması sonucu, çamurda bulunan ekstre edilebilen proteinin Isı/$Fe^{2+}/S_2O_8^{2-}$ arıtımı ile elde edilen çamurdakinden daha düşük olduğu bulunmuştur.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**	**Unit**
A^2/O	Anaerobic-Anoxic-Oxic	-
AOPs	Advanced Oxidation Processes	-
AOX	Adsorbable Organic Halogens	mg/L
APHA	American Public Health Association	-
CIP	Ciprofloxacin	-
COD	Chemical Oxygen Demand	mg/L
CST	Cappilary Suction Time	S
DS	Dissolved Solids	g/L
EPA	Environmental Protection Agency	-
EPS	Extracellular Polymeric Substances	mg/ g VSS
Fe^{+2}	Ferrous Iron	-
Fe^{+3}	Ferric Iron	-
H_2O_2	Hydrogen Peroxide	-
HLB	Hydrophilic Lipophilic Balanced	-
HPLC	High-Performance Liquid Chromatography	-
HR	Hydroxyl Radical	-
ICP	Inductively Coupled Plasma	-
IPA	Isopropyl Alcohol	-
ISO	International Organization for Standardization	-
KHP	Potassium Hydrogen Phthalate	-
LB-EPS	Loosely Bound- Extracellular Polymeric Substances	mg/ g VSS
MLSS	Mixed liquor suspended solids	mg/L
MLVSS	Mixed liquor Volatile suspended solids	mg/L
MW	Microwave	-
NB	Nitrobenzene	-
NH_3-N	Amonium Nitrogen	mg/L
NO_3^--N	Nitrate Nitrogen	mg/L

OCs	Organic Compounds	-
OTC	Oxytetracycline	-
PAH	Polycyclic Aromatic Hydrocarbons	-
PBS	Phosphate Buffer Solution	-
PO_4^{3-}	Phosphate	mg/L
SCOD	Soluble Chemical Oxygen Demand	mg/L
S-EPS	Soluble- Extracellular Polymeric Substances	mg/ g VSS
SPE	Solid Phase Extraction	-
SRA	Sulfate Radical	-
TBA	Tert-Butyl Alcohol	-
TB-EPS	Tightly Bound- Extracellular Polymeric Substances	mg/ g VSS
TCOD	Total Chemical Oxygen Demand	g/L
TCS	Triclosan	-
PTFE	Polytetrafluoroethylene	-
TKN	Total Kjeldahl Nitrogen	mg/L
TOC	Total Organic Carbon	mg/L
TP	Total Phosphorus	mg/L
TS	Total Solids	g/L
US	United States	-
VLB-EPS	Very Loosely Bound Extracellular Polymeric Substances	mg/ g VSS
VS	Volatile Solids	g/L
VSS	Volatile Suspended Solids	g/L
ZPC	Zero Point Charge	-

1. INTRODUCTION

Sewage sludge which is produced from various treatment operations and processes applied to urban wastewater, was previously disposed by direct land application. However, this disposal method is currently controlled by strict regulations to protect the environment (Regulation on Application of Sewage Sludge to Soil, 2010). Since during the treatment of wastewater, the sludge tends to concentrate heavy metals and poorly biodegradable trace organic compounds as well as potentially pathogenic organisms (viruses, bacteria etc) present in wastewaters, serious deterioration of environmental quality is well-expected consequences of dumping of raw sludge into receiving systems. Besides undesirable quality of waste sludge, the quantity of it has increased along with population and implementation of stringent wastewater regulations. Although innovative wastewater treatment processes significantly reduce sludge production, expected annual sewage sludge will be 13 million tons dry matter up to 2020 only in Europe (Leonard, 2011). Therefore, acceptable management of high amount of urban sludge is a current challenging issue and sludge treatment is an important part of integrated wastewater management.

Among the principal sludge disposal alternatives the application of incineration to sewage sludge is increasing in some countries although this alternative can be unfavourable due to its adverse effects on the environment and economic costs (Milieu Ltd., WRc and RPA, 2010; Fytili and Zabaniotou, 2008). Landfilling option is considered an unsustainable option due to increasing competition for landfill space, higher costs, and more stringent environmental standards. On the other hand, recycling of biosolid to agricultural land is regarded as the most environmentally sustainable approach to manage the urban sludge (CEC, 1986). This disposal alternative is encouraged worldwide as long as its management complies with sanitary and environmental requirements since beneficial use of the sludge as fertilizer or soil amendment is possible due to its valuable nutrient content.

In order to eliminate the risks on the environment, the current sewage sludge regulations for land application addresses both pathogen reduction and the potential for accumulation of persistent organic pollutants and metals in soils (Regulation on Application of Sewage Sludge to Soil, 2010). Different stabilization processes (anaerobic digestion, composting, chemical stabilization by using lime or ozone) can be used for land application of sewage sludge to meet regulatory requirements. To reduce the pathogens of sewage sludge anaerobic digestion and composting can be used as conventional processes all over the world. However, recent studies clearly showed the reactivation of pathogenic microorganisms during subsequent dewatering operation of anaerobically stabilized sludge (Higgins et al., 2007; Erkan and Sanin, 2013). It was also reported that this situation is more pronounced for thermophilically digested sludge that is known to be safer.

On the other hand, tremendous number of studies indicates the presence of emerging contaminants including pharmaceuticals in biosolids and the inefficiency of biological stabilization processes for the degradation of these contaminants (e.g. Clarke and Smith, 2011; Smith, 2009; Harrison et al., 2006). In a recent environmental risk assessment study, potential risks of pharmaceutical compounds in digested sludge and compost were explained by their high concentrations or their high toxicity (Martins et al., 2012). Although the ecotoxicological risk significantly decreased after the application of digested sludge or compost to the soils (Martins et al., 2012) during this period antimicrobials can exert another risk by promoting the development of resistance in microorganisms (Oncu and Balcioglu, 2013a). Therefore, antimicrobials as emerging biosolid contaminants deserve a special attention by producing a secondary pollutant in the environment.

Concerns about the nonregulated pollutants in sewage sludge promote the search for alternatives in sludge management. Although advanced oxidation processes gain importance in sludge treatment for sludge solubilization (Carrera et al., 2010) and some of chemical oxidation processes are already developed as commercial sludge treatment process their beneficial effects on the degradation of xenobiotic compounds have been scarcely investigated (Oncu and Balcioglu, 2013b; Mohapatra et al., 2010). Among the studied processes by Oncu and Balcioglu (2013b), MW mediated persulfate oxidation produced promising results for tetracycline and flourquinolone antimicrobials degradation in secondary sludge in reasonable treatment time.

Considering above mentioned facts, this study focused on the treatment of secondary sewage sludge by persulfate which was activated by the addition of ferrous iron and conventional heating operation. A hydrophobic antimicrobial, Triclosan (TCS) was selected as main target pollutant, which is not previously investigated in the literature although its load in the sludge was well known (Stasinakis, 2012). The degradation of Oxytetracycline (OTC) and Ciprofloxacin (CIP) as well as TCS was investigated during the application of persulfate oxidation to the sludge. In addition, the efficiency of persulfate oxidation for sludge treatment was compared with those of MW assisted persulfate and peroxide oxidations at the determined experimental conditions. The variation in solubilisation, dewaterability, and organic constituents of the sludge were used to evaluate the process efficiency.

2. THEORETICAL BACKGROUND

2.1. Emerging Organic Contaminants in Sewage Sludge

Treatment processes for wastewater are able to reduce organic contaminants (OCs). On the other hand, many lipophilic OCs are transfered to sewage sludge during the treatment of wastewater and eventually the sludge of wastewater can contain various organic contaminants (Clarke and Smith 2011; Stevens-Garmon et al., 2011; Ottmar et al., 2010). In a recent review (Clarke and Smith 2011), it was reported that over 360 organic compounds have been identified in sewage sludge. The expansion of the contaminant list is well expected due to faster developments in analytical techniques. Clarke and Smith (2011) and Stasinakis (2012) compiled various studies to classify frequently detected sludge organic contaminants, which are Perfluorinated chemicals (PFOS, PFOA), Polychlorinated alkanes (PCAs), Polychlorinated naphthalenes (PCNs), Organotins (OTs), Polybrominated diphenyl ethers (PBDE), Triclosan (TCS), Triclocarban (TCC), Benzothiazoles, Antibiotics and pharmaceuticals, synthetic musks, Bisphenol A, Quaternary ammonium compounds (QACs), Steroids, Phthalate acid esters (PAEs), Polydimethylsiloxanes (PDMSs). Among these triclosan, triclocarban, and antibiotics are antibacterial compounds and quaternary ammonium compounds are biocides. Figure 2.1 demonstrates the maximum detected concentration of some organic contaminants in sewage sludge. Compare to other contaminants, polybrominated diphenyl ethers are found at relatively high concentration (>15 g/kg dry solid). While the polydimethylsiloxanes and quaternary ammonium compounds concentrations are about 5 g/kg DS in biosolids (Figure 2.1), antibiotics are detected at about 0.01 g/kg DS. Classification of antibiotics and their maximum detected values are shown in Figure 2.2.

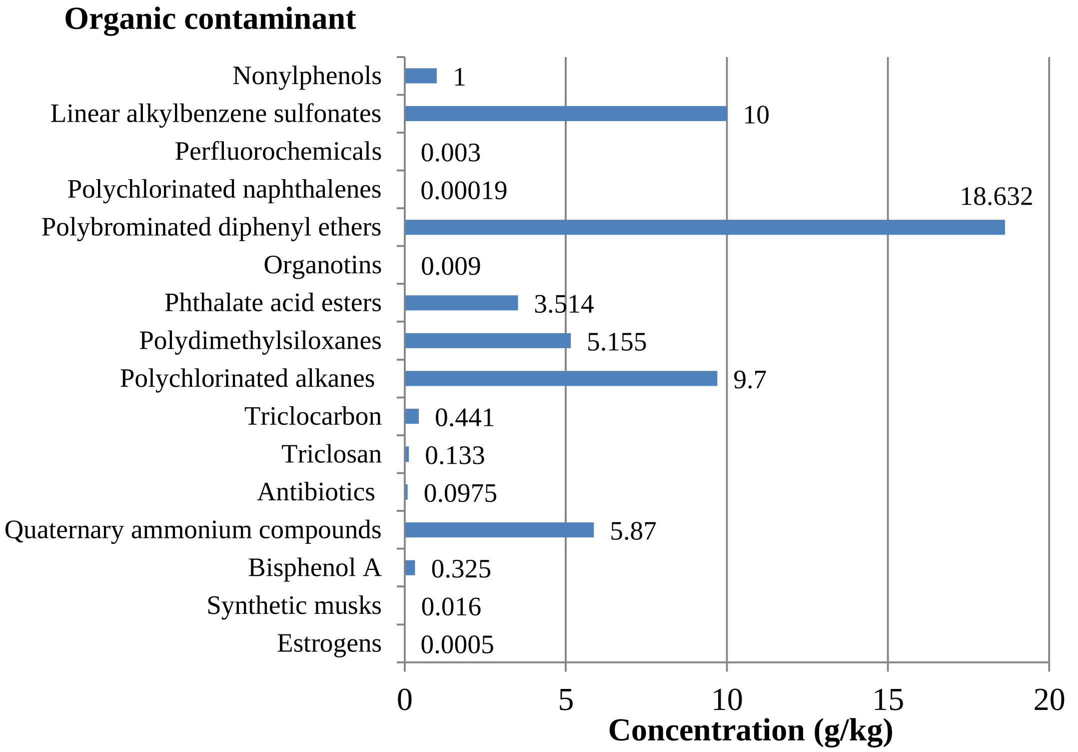


Figure 2.1. Organic contaminants in sewage sludge.

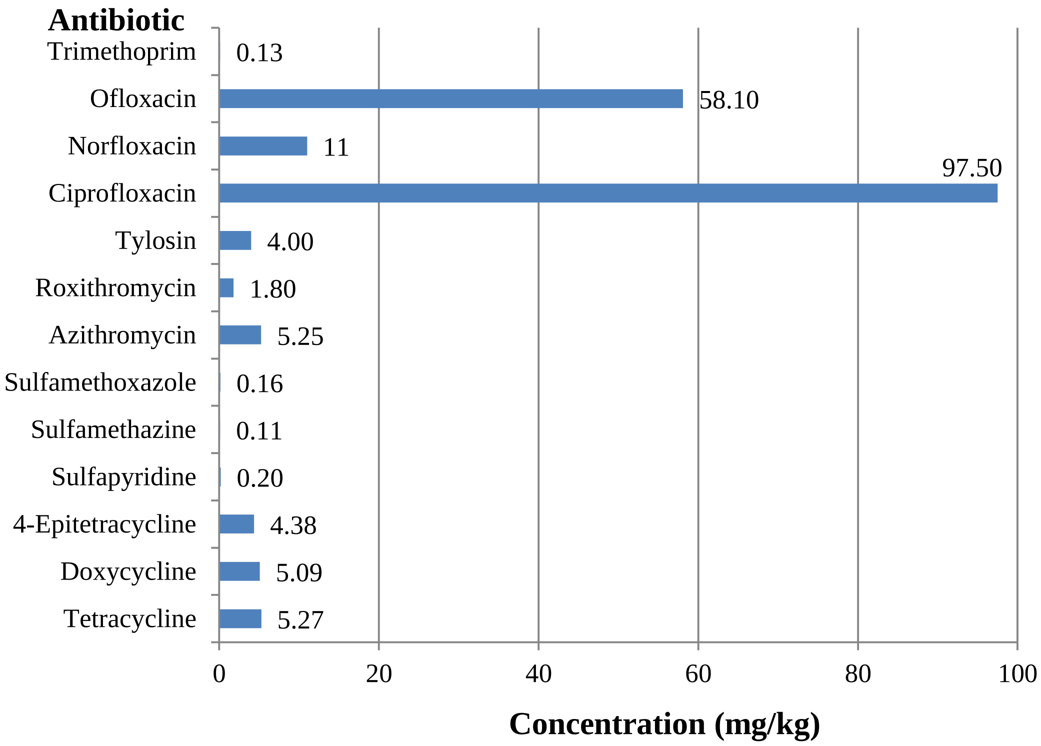


Figure 2.2. Antibiotic concentrations in sewage sludge.

Although the concentrations of antibiotics in the sludge are not high, their effects on environment can be significant. Previous studies have found that releasing both antibacterials and biocides into the environment contributes to the development of resistance (Kopmann et al., 2009; Oncu and Balcioglu, 2013b). However, the risk of these emerging contaminants is not limited to promotion of resistance development. The presence of antibiotics in the environment also provokes toxic effects to some microorganisms (Schmitt et al., 2004; Brown and Balkwill, 2009; Daughton and Ternes, 1999; Flaherty and Dodson, 2005; Isidori et al., 2005) and adverse health effects on human health (Thiele-Bruhn and Beck, 2005; Pomati et al., 2006). Another concern related to these emerging contaminants (including antibacterials and resistance microrganisms) is the potential entry of these into groundwater or uptaking by crops (Richardson, 2012). Although controversial results have been published about the level of risk of these emerging contaminants (Clarke and Smith, 2011) they cannot be disregarded. Therefore, it can be concluded that land applications of biosolids can constitute a major source of these contaminants entering the environment.

2.2. Fate of Emerging Pollutants in Sludge Treatment

Anaereobic digestion, which is commonly used stabilization method for sludge in Europe (Werle and Wilk, 2010) can provide reduction of sludge volume, while energy-rich biogas and nutrient-rich final product are obtained. However, the occurrence of various emerging pollutants in digested sludge indicates the inefficiency of this sludge stabilization process for these compounds (Martin et al., 2012). Since the application of anaerobic digestion is limited by long retention time and low process efficiency, various sludge pretreatment techniques have been applied to sludge in order to enhance its anaerobic digestion. These processes are thermal, mechanical, chemical with oxidants (mainly ozonation) or acids or alkalis (Carrère et al., 2010), biological and combination of these processes (Kim et al., 2010). Although pre-treatment processes increase the overall cost of operation, they can provide some beneficial effects on digested sludge quality (Carballa et al., 2008). The risks due to the residuals of heavy metals and organic micropollutants can be reduced by the pre-treatment. The abatement of micropollutant concentration and solubilization of heavy metals can be well expected results of chemical oxidation of sludge.

Advanced oxidation processes (AOPs) utilizing free radicals as a primary oxidant, have been given growing attention in sludge treatment due to short treatment time and high treatment efficiency. Although ozone has been widely used in sludge solubilization the efficiency of the process is limited with gas transfer rate. While the application of hydrogen peroxide has been subjected on various investigations (Neyens et al., 2003; Dewil et al., 2007; Lo et al,. 2008; Erden and Filibeli, 2010; He and Chao-Hai, 2010; Pham et al., 2010; Abe et al., 2011) the use of persulfate (Balcioglu et al., 2012; Zhen et al. 2012 a, b, c) and MW assisted persulfate for sludge treatment has been scarcely investigated. In addition, the fate of micro organic pollutants has been rarely investigated during the chemical oxidation of sludge (Mohapatra et al., 2014; Carlsson et al., 2012; Carrera et al., 2010).

2.3. Theory of Persulfate Oxidation

Persulfate oxidation is more widely investigated for the treatment of contaminated soil due the high stability and solubility of persulfate although its application to the sludge is not a common practice. It is well known that persulfate anion ($S_2O_8^{2-}$) and hydrogen peroxide can produce reactive sulfate ($SO_4^{\bullet-}$) and hydroxyl ($HO^{\bullet}$) radicals, respectively. The radical generation from these oxidants can be achieved through the application of (i) heat or (ii) transition metal catalysts or (iii) UV radiation and eventually a series of radical chain reactions can generally provide the oxidation of reduced species in a treated matrix.

$$S_2O_8^{2-} + \text{heat/UV} \rightarrow 2SO_4^{\bullet-} \tag{2.1}$$

$$H_2O_2 + \text{UV} \rightarrow 2HO^{\bullet} \tag{2.2}$$

$$S_2O_8^{2-} + Me^{n+} \rightarrow SO_4^{\bullet-} + SO_4^{2-} + Me^{n+2} \tag{2.3}$$

$$H_2O_2 + \text{Metals}^{\,n+} \rightarrow 2OH^{\bullet} + \text{Metals}^{\,n+2} \tag{2.4}$$

These radicals have high oxidation power and oxidation potentials of sulfate and hydroxyl radicals are compared with those of some oxidants in Table 2.1. Both sulfate and hydroxyl radicals have a short lifespan, however, oxidation mechanism with persulfate and peroxide can be significantly different. Sulfate radical is more electrophilic than hydroxyl radical and it preferably removes electrons from an organic molecule to produce an organic radical cation (Forsey, 2004), whereas the hydroxyl radicals add to C=C bonds or abstract hydrogen from the C-H bond (Neta et al., 1977).

Table 2.1. Standard redox potentials of selected oxidants.

Oxidant	**Redox Potential (Volts)**
Flourine	3.03
Hydroxyl radical	2.70
Sulfate radical	2.60
Atomic oxygen	2.42
Ozone	2.07
Persulfate anion	2.01
Hydrogen peroxide	1.78
Permanganate	1.68
Chlorine dioxide	1.57
Hypochlorus acid	1.49
Chlorine	1.36
Bromine	1.09
Iodine	0.54

Oxidation reaction by hydrogen peroxide and persulfate depends upon pH, type and dose of activator. It has been demonstrated that at alkaline pH (i.e., pH >8.5) persulfate can produce hydroxyl radicals by interconversion of sulfate radical to $HO^{\bullet}$:

$$SO_4^{\bullet -} + OH^- \rightarrow HO^{\bullet} + SO_4^{2-} \qquad (k=6.5x10^7\ M^{-1}\ s^{-1}) \tag{2.5}$$

It has been suggested that under highly acidic conditions (pH<2) persulfate may decompose without generating sulfate radicals. Hence a decrease in the efficiency of the process in terms of the target contaminants degradation could be observed (Tsitonaki et al., 2010). In addition, it has also been shown that sulfate radical can react with water at a wide pH range to produce $HO^{\bullet}$:

$$SO_4^{\bullet -} + H_2O \rightarrow HO^{\bullet} + SO_4^{2-} + H^+ \qquad (k=2x10^{-3}\ s^{-1}) \tag{2.6}$$

However, it was reported that the rate constant of above reaction 2.6 is considerably low (Norman et al., 1970). Therefore, in persulfate oxidation hydroxyl radicals have minor importance except at alkaline pH. It is also known that pH is an important parameter for hydrogen peroxide oxidation since the stability of oxidant decreases by increasing pH. Besides, metal ions that catalyze the production of radicals from hydrogen peroxide become unavailable for the oxidant due to reduced solubility of metals at alkaline pH. Consequently, hydrogen peroxide exhibits higher performance at acidic pH.

In metal activation, the ratio of metal^{n+}/oxidant (H_2O_2 or $S_2O_8^{2-}$) is another important parameter that influences the efficiency of these oxidation processes since excess metals or oxidants can scavenge the reactive radicals (Buxton et al., 1988) e.g:

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + OH^{-} \quad (k=3\text{-}4x10^{8}\ M^{-1}\ s^{-1}) \tag{2.7}$$

$$SO_4^{\bullet-} + Fe^{2+} \rightarrow Fe^{3+} + SO_4^{2-} \quad (k=4.6x10^{9}\ M^{-1}\ s^{-1}\ pH=3\text{-}5) \tag{2.8}$$

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO^{\bullet}_2 \quad (k=3.3x10^{7}\ M^{-1}\ s^{-1}) \tag{2.9}$$

$$SO_4^{\bullet-} + S_2O_8^{2-} \rightarrow S_2O_8^{\bullet-} + SO_4^{2-} \quad (k=6.1x10^{5}\ M^{-1}\ s^{-1}) \tag{2.10}$$

Besides the above mentioned scavenging reactions, treated matrix components can be responsible for the deterioration of reaction efficiency. In case of sludge treatment released inorganic anions can give reaction with sulfate and hydroxyl radicals hence reduce their attack on target pollutants. In Table 2.2, the reaction rate constants of some inorganic anions with these radicals are compared.

Table 2.2. Rate constants of sulfate and hydroxyl radicals with some inorganic ions**.**

Anions	**$SO_4^{\bullet-}$**	**$HO^{\bullet}$**
Cl^{-}	$2.7x10^{8}$–$6.1x10^{8}$ [a, b]	$4.3x10^{9}$ [b]
HCO_3-	$1.6x10^{6}$-$9.1x10^{6}$ [c, d]	$8.5x10^{6}$ [b]
CO_3^{2-}	4.1-6.1 $x10^{6}$[c, e]	$3.9x10^{8}$ [b]

a: Huie and Clifton, 1990 b: Buxton et al., 1988 c: Zuo et al., 1999 d: Dogliotti and Hayon, 1967
e: Padmaja et al., 1993

As shown in Table 2.2, the rate constants of sulfate radical towards most commonly encountered anions are smaller compared to those of hydroxyl radical. Therefore, higher performance of persulfate on the degradation of specific pollutant during the in sludge treatment would be expected.

2.4. Theory of Microwave Irradiation

Recent studies focused on the application of microwave (MW) irradiation for the treatment of sewage sludge (Jones et al., 2002; Appleton et al., 2005; Eskicioglu et al., 2007; Beszedes et al., 2008; Wu, 2008; Tyagi et al., 2011; Tyagi et al., 2013). This process is used as a thermal treatment of sludge as well as the activation of oxidant during the thermo-chemical treatment. Microwaves are a form of electromagnetic radiation with an oscillating frequency between 0.3 and 300 GHz, (Figure 2.3). However, in most applications the radiation between 3 and 30 GHz is utilized (Appleton et al., 2005) to avoid interference with telecommunications and cellular phone frequencies. Under the oscillating electrical field of MW irradiation, the rotation of dipole molecules in the treated matrices resulted in heating. Actually, temporary dipole movement of non-polar molecules or atoms, which are the absorbers of MW irradiation, causes a friction inside the medium and this energy is dissipated subsequently as heat. The wavelength of MW irradiation is full electromagnetic spectrum is shown in Figure 2.3. This figure also shows schematic representation of two different effects of MW irradiation.

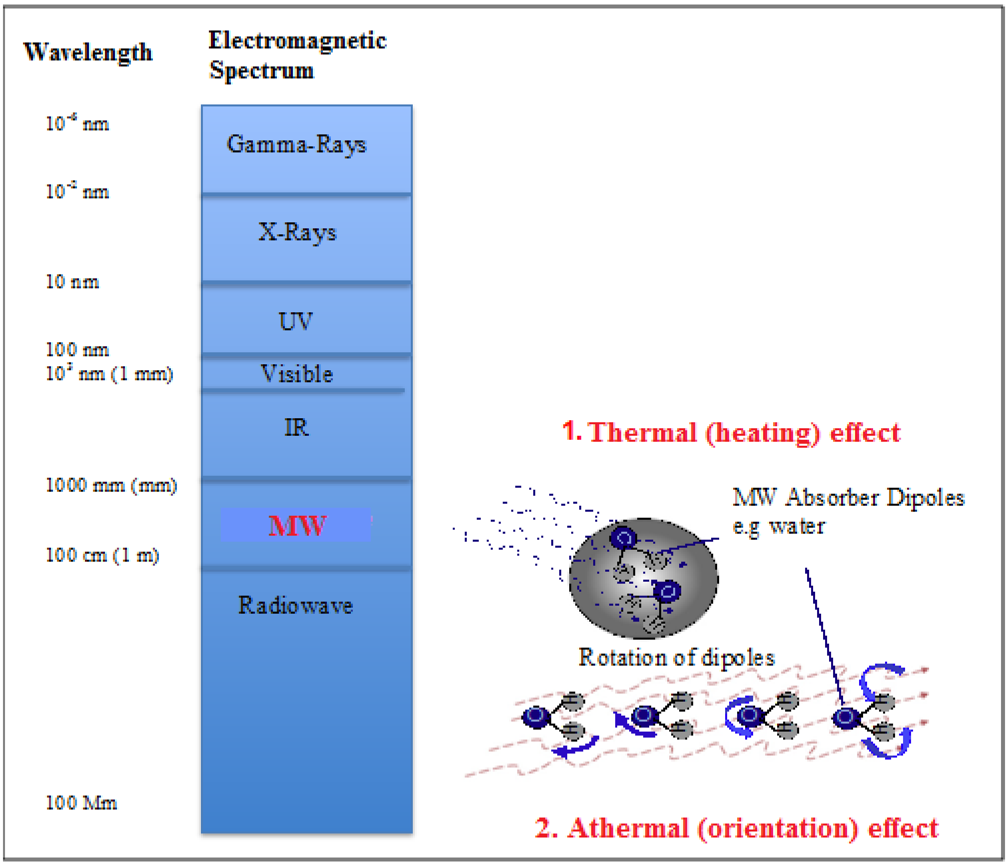


Figure 2.3. MW irradiation and its effects.

Heating with MW energy is considerably rapid compare to conventional heating. In conventional method, heat is transferred to the surface of the material by conduction, convection or radiation and into the interior of the material by thermal conduction (Figure 2.4). Therefore, the rate of conventional heating is limited by diffusion. Conventional heating is not only slow, but also non-uniform with the surfaces, edges and corners being much hotter than the inside of the treated matrices. In contrast to conventional heating, microwave can penetrate into the treated matrices and can generate hotspots, which enhance diffusion (Salvi et al., 2011). Consequently, fast heating throughout the volume of the treated matrices can be achieved. Conventional and MW heating are comparably shown for a sample in Figure 2.4.

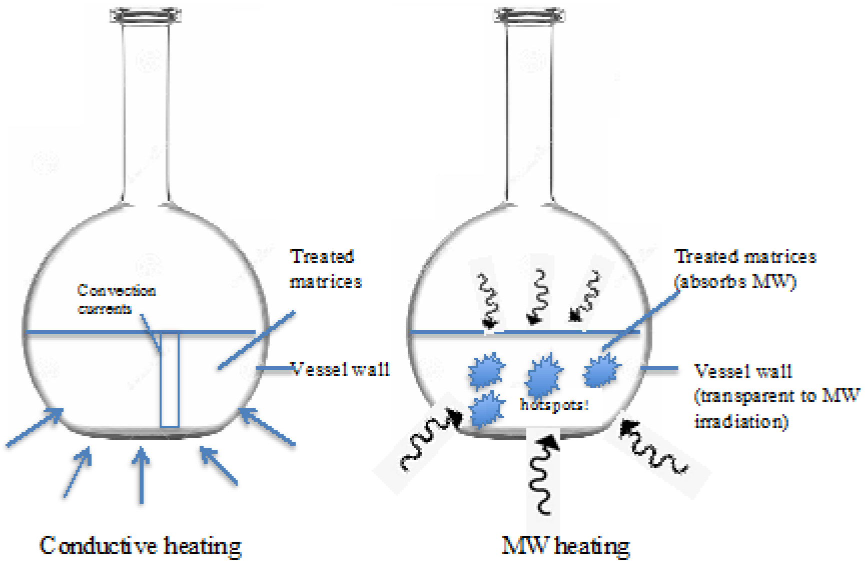


Figure 2.4. Comparison of conventional and MW heating (adapted from Eskicioglu et al., 2006).

Higher heating rate, selective heating, and better process control are the major advantages of MW heating. Heating effect of microwave energy may provide the overcoming of activation energy barrier and accelerate the reaction rate of many chemical reactions. On the other hand, in some studies (e.g Bergqvist et al., 1994: Eskicioglu et al., 2007) it was suggested that besides the thermal effect, microwave irradiation also induces an athermal (non-thermal) effect due to dipole orientation of polarized parts of macromolecules, which results in possible breakage of hydrogen bonds. Therefore, MW irradiation has been proved to enhance many reactions during the remediation of environmental matrices (Jones et al., 2002; Wu, 2008). Although thermal and athermal effects of MW irradiation are not always distinguishable MW treatment has ability to breakage of the extracellular polymeric substance (polysaccharide, proteins) and divalent cation network of sludge flocs; hence resulted in solubilization of waste activated sludge (Tyagi et al., 2011, 2013).

In numerous recent studies MW irradiation was used to enhance the efficiency of hydrogen peroxide, (Wong et al, 2006; Wong et al., 2007; Kenge et al., 2008; Yin et al, 2008; Eskicioglu et al., 2008; Kenge et al., 2009 a, b; Wang et al., 2009; Lo et al., 2010;

Yu et al., 2010 a, b, c; Xiao et al., 2012: Oncu and Balcioglu, 2013b), persulfate (Oncu and Balcioglu, 2013b) and ozone (Yin et al., 2008) for the treatment of sludge. MW assisted chemical oxidation process is known as advanced oxidation and synergistic effect of MW irradiation and chemical oxidant on sludge disintegration has been reported (Oncu and Balcioglu, 2013b). Persulfate and hydrogen peroxide can be activated by MW irradiation to generate reactive radicals and the radical formation has been proved in sludge treatment (Oncu and Balcioglu, 2013b). It is also known that MW irradiation can efficiently release heavy metals from the sludge that exert an additional effect on the formation of reactive radicals from the oxidants.

2.5. Treatment of Sewage Sludge by Persulfate and Peroxide Processes

A review of selected literature covering applications of persulfate, peroxide, and MW assisted peroxide for sludge treatment is given in Table 2.3.

Table 2.3. Persulfate and peroxide treatment of sludge.

Reference	**Oxidant dose and other treatment conditions**	**Results**
		Persulfate oxidation
Zhen et al. (2012a)	$S_2O_8^{2-}$ = 1.2 mmol/ g-VSS Fe(II)=1.5 mmol/g-VSS pH=1.5 - 10.0 TS=16 g/L	89% capillary suction time (CST) reduction within 1 min.
Zhen et al. (2012b)	$S_2O_8^{2-}$ = 1.2 mmol/ g-VSS Fe(II)= 1.5 mmol/g-VSS TS= 17 g/L for sewage sludge TS=26 g/L for industrial sludge TS=42 g/L for special sludge	80-86% CST reduction within 1 min.
Zhen et al. (2012c)	$S_2O_8^{2-}$ =1.2 mmol/ g-VSS Fe (II)=1.5 mmol/g-VSS Temperature= 20,40,60, 80 °C TS=26 g/L	95% CST reduction within 5 min.
		Hydrogen peroxide oxidation
Neyens et al. (2003)	H_2O_2=5 g /kg TS, Fe^{2+}= 1.67 g /kg TS pH= 3 DS=6%	50% CST reduction in the presence of polyelectrolyte. 20% DS increase in sludge cake.
Lu et al. (2003)	H_2O_2=3000 mg/l Fe^{2+}or Fe^{3+} =6000 mg/l TS=8.3 g/L	90% reduction of specific resistance to filtration. 75% moisture in sludge cake.
Valo et al. (2004)	H_2O_2= 150 mmol/L Fe^{2+}= 5 mmol/ L TS=17 g/L 90 °C	13% total COD reduction. Enhancement of biogas production in subsequent biological digestion.
Flotron et al. (2005)	H_2O_2= 4.9 M Fe^{2+}= 0.01 M no pH arrangement DS=20%	38% to nearly 100% PAH degradation.

Table 2.3. Persulfate and peroxide treatment of sludge (cont.).

Rivero et al. (2006)	H_2O_2= 2.0 g/g VSS	15.2% increase in solid removal.
Dewil et al. (2007)	H_2O_2 =50 g/kg DS Fe^{2+}/ H_2O_2 =0.07(w/w) pH =3 DS=3%	Significant decrease of Cd, Cu, Ni and Zn metals in sludge cake by destroying EPS but not for Pb and Hg.
Beauchesne et al. (2007)	H_2O_2= 0-31 g/kgTS [Fe^{2+}]/ [H_2O_2]= 0-5.7 pH=2.5	95% CST reduction. High solublization of Cd, Cu, Mn, and Zn whereas poor solubilization of Cr, Ni and Pb along with 4-25% COD solubilization.
Zheng et al. (2007)	H_2O_2= 92.5 g /kg TS Fe^{2+}/ H_2O_2=0.83 pH= 2.08 TS=18 or 30 g/L	30% total degradation of PAH species in the presence of nonionic surfactant.
Lo et al. (2008)	H_2O_2=300 g /kg TS [Fe^{2+}]/ [H_2O_2] =0.02 pH= 6.5 TS=10 g/L	9% COD solubilization by MW heating. Reduced solid reduction by the addition of Fe^{2+}.
Kaynak and Filibeli. (2008)	H_2O_2 =60 g /kg DS 0.067 g Fe^{2+}/ g H_2O_2 pH= 3	Decrease in CST did not have a positive effect on sludge dewatering.
Erden and Filibeli. (2010)	H_2O_2=60 g/kg DS, 4.02g Fe^{2+}/kg DS pH=3 TS=13.5 g/L	28.2% reduction in DS by Fenton pretreatment followed by thermophilic anaerobic digestion.
He and Chao-Hai (2010)	H_2O_2= 8000 mg/L, Fe^{2+}=200 mg/L pH~7 SS=113 mg/L	66% MLSS reduction accompaning by COD decrease.
Pham et al. (2010)	H_2O_2= 0.01 mL /g SS [H_2O_2] /[Fe^{2+}]= 150/25g/L TS pH= 3 TS=10-40 g/L	High solubilization (70%) and biodegradation (74%).

Table 2.3. Persulfate and peroxide treatment of sludge (cont).

	MW assisted peroxide oxidation	
Abe et al. (2011)	$[H_2O_2]/[Fe^{2+}]$= =15 /0.1 g/L pH =3-8 Temp=50-105 ºC TS=14 g/L	50-55%.VSS degradation at acidic pH. Higher VSS degradation by increasing temperature.
Liao et al. (2007)	71 mL/L H_2O_2 (30% by wt) T= 20 ºC/min, TT= 5min	Over 96% of TCOD was dissolved into the solution.
Kenge et al. (2008)	1 mL H_2O_2 (30%)/1%TS T= 80 ºC, TT= 5 min	25% increase in SCOD.
Lo et al. (2008)	3.3 g H_2O_2/g TS, $FeSO_4/H_2O_2$= 0.4 (w/w)	18% increase in COD solubilisation.
Eskicioglu et al. (2008)	1 g H_2O_2 (30%v/v)/g TS T= 120 ºC, TT= 10 min	COD solubilisation increased from 3% to 24%.
Yin et al. (2008)	0.5-4.7 g H_2O_2/g TS T= 80-120 ºC, TT= 1.5-5 min	Maximum sludge disintegration achieved at the highest TS with addition of H_2O_2.
Yu et al. (2010b)	0.06-0.31 g H_2O_2/g TS T= 55 - 70 °C, TT= 5 min	Better sludge solubilisation at higher temperature.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Source of Waste Secondary Sludge

Secondary sewage sludge samples were collected from Paşaköy Municipal Advanced Biological Wastewater Treatment Plant, Istanbul/Turkey. The plant has a wastewater treatment capacity of 500,000 m^3/d and serves about 2,500,000-population equivalent (average flow of 100,000 m^3/d). The treatment plant consists of inlet pumping station, fine screens, aerated grit chamber, distribution chamber, anaerobic tanks, aeration tanks, final clarifiers, dissolved air flotation unit, aerated sludge storage tanks, sludge dewatering unit, and return of dewatering supernatant to the distribution chamber. The A^2/O process (anaerobic unit followed by anoxic and then aerobic units) is applied for the removal of nutrients from wastewater.

Sampling location was selected as the return sludge pumping station of the treatment plant. Samples were immediately transported to the laboratory with plastic containers. The sludge was stored at 4 ˚C for 20 hours to concentrate it. By decanting the supernatant of settled sludge, the solid content was adjusted to 25±5 g/L. Due to rapid change in the composition of the sludge, maximum storage time at 4 ˚C was not longer than one week.

3.1.2. Chemicals and Model Micropollutants

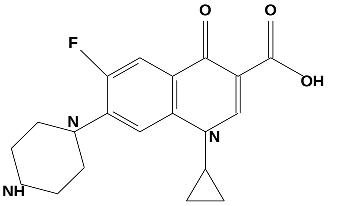
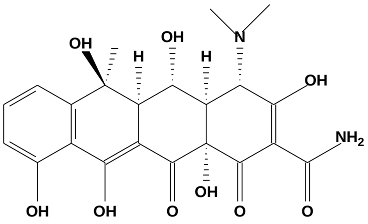
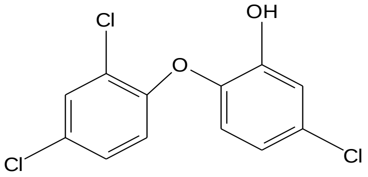
Both of chemicals and model micropollutants that were used to perform experiments are listed in Table 3.1.

Table 3.1. List of chemicals used in experiments.

Chemical	Formula	Experimental Use	Supplier
Oxytetracycline-HCl	$C_{22}H_{24}N_2O_9.HCl$	Model antibiotic	Sigma
Ciprofloxacin-HCl	$C_{17}H_{18}FN_3O_3.HCl$	Model antimicrobial	Mp.Biomedicals
Triclosan	$C_{12}H_7Cl_3O_2$	Model antimicrobial	Sigma
Anisole	$CH_3OC_6H_5$	Radical probe	Fluka
Nitrobenzene	$C_6H_5NO_2$	Radical probe	Fluka
Tertiary-butanol	$C_4H_{10}O$	Radical scavenger	Sigma-Aldrich
Isopropyl alcohol	C_3H_8O	Radical scavenger	Sigma-Aldrich
Sodium persulfate	$Na_2S_2O_8$	Sludge treatment	Sigma-Aldrich
Hydrogen peroxide (34.5–36.5 %)	H_2O_2	Sludge treatment	Sigma-Aldrich
Ferrous sulfate	$FeSO_4.7H_2O$	Persulfate activator	Sigma-Aldrich
Ammonia solution (2.5%)	NH_3	Solid phase extraction	Merck
Magnesium nitrate	$Mg(NO_3)_{2.}6H_2O$	Sample preparation	Sigma-Aldrich
Magnesium sulfate	$MgSO_4.7H_2O$	Sludge pretreatment	Sigma-Aldrich
Formic acid (HPLC grade)	HCOOH	HPLC analysis	Sigma-Aldrich
Methanol (HPLC grade)	CH_3OH	HPLC analysis	Sigma-Aldrich
Potassium iodide	KI	Persulfate analysis	Sigma
Phos Ver3 Kit		Ortho-phosphate analysis	Hach
Nessler reagent		Ammonia and TKN analysis	Hach
Nitra Ver 6 Kit		Nitrate analysis	Hach
Potassium dichromate	$K_2Cr_2O_7$	COD analysis	Sigma-Aldrich
Sulfuric acid (95-98%)	H_2SO_4	COD analysis	Aldrich
Silver sulfate	Ag_2SO_4	COD analysis	Sigma-Aldrich
Mercury(II) sulfate	$HgSO_4$	COD analysis	Fluka
Hydrochloric acid	HCl	AOX analysis	Merck
Sulfuric acid (98%)	H_2SO_4	AOX analysis	Merck
Copper oxide	Cu_2O	AOX analysis	Merck
Nitric acid (65%)	HNO_3	AOX analysis	Riedel-de-Haen
Sodium perchloridemonohydrate	$NaClO_4.H_2O$	AOX analysis	Merck
Aminodosulfuric acid	H_2NSO_3H	AOX analysis	Merck
Sodium nitrate	$NaNO_3$	AOX analysis	Merck
Potassium chloride	KCl	EPS analysis	Sigma
Sodium chloride	NaCl	EPS analysis	Riedel-de-Haen
Sodium dihydrogen phosphate	$NaH_2PO_4.(H_2O)$	EPS analysis	Sigma
Sodium phosphate dodecahydrate	$Na_3PO_4.12(H_2O)$	EPS analysis	Sigma
Dowex marathon C sodium form		EPS analysis	Sigma
Anthron	$C_{14}H_{10}O$	EPS analysis	Sigma
Bovine serume albumin		EPS analysis	Sigma
Folin reagent	$C_{10}H_5NaO_5SNa_2$	EPS analysis	Sigma
Sodium tartrat	Tartrat. $2(H_2O)$	EPS analysis	Sigma
Copper sulfate	$CuSO_4.5(H_2O)$	EPS analysis	Sigma
Sodium hydroxide	NaOH	EPS analysis	Sigma
Sodium carbonate	Na_2CO_3	EPS analysis	Horasan
Glucose	$C_6H_{12}0_6$	EPS analysis	Sigma

The molecular formulas and some physicochemical properties of antimicrobials selected as micropollutants are given in Table 3.2.

Table 3.2. Structure and properties of Ciprofloxacin, Oxytetracycline, and Triclosan.

Name	**Chemical structure**	**Molecular weight (g/mole)**	**Log K_{ow}**	**pK_a**
Ciprofloxacin		331.346	0.3[a]	3.0, 6.1, 8.7, 10.6[b]
Oxytetracycline		460.4	1.6[c]	3.2, 7.5, 8.9[b]
Triclosan		289.54	4.8[d]	8.1[e]

a: Chenxi et al., 2008, b: Qiang and Adams, 2004, c: Berthod et al., 2014, d: Aranami and Readman, 2007, e: Jakel, 1990

3.2. Methods

3.2.1. Contamination of Secondary Sludge with Antimicrobials

First, the concentrations of the antimicrobials already present in the sludge were analyzed. Then, concentrated sludge was diluted by using either distilled water or an aqueous solution of CIP, OTC, and TCS to adjust the TS concentration to 10 g/L and micropollutant concentration to 100 mg/kg TS. After spiking the sludge with model micropollutants, the equilibration was performed in a temperature controlled water bath at 200 rpm and 25±5 ºC for 1.5 h to provide >95% sorption of them.

3.2.2 Treatment of Secondary Sludge

In this study, two different oxidants, hydrogen peroxide, H_2O_2 (34.5–36.5%) or sodium persulfate, $Na_2S_2O_8$, were used for treatment of waste sludge. To produce sulfate radical, ferrous iron, conventional heating, and microwave heating were used for the activation of persulfate. The sludge samples in polytetrafluoroethylene (PTFE) tubes, which have capacity of 50 mL, were subjected to Heat/$S_2O_8^{2-}$ and Fe^{2+}/Heat/$S_2O_8^{2-}$ treatments in a temperature controlled water bath (Julabo, SW22). Pre-determined amounts of Fe^{2+} and $S_2O_8^{2-}$ from stock solutions were successively added to 25 mL of the sludge preheated to the desired temperature in order to initiate chemical reaction. By the addition of these solutions, the total volume of treated sludge was 27.5 mL. In addition, peroxide oxidation was performed with same procedure in persulfate oxidation process.

Microwave treatment of waste sludge was performed by using bench scale microwave irradiation system (Berghof, Speed wave MWS-3, 2.54 GHz), which had the capacity to accommodate up to 12 TFM vessels (each with a volume of 60 mL) and could be operated at a maximum temperature, power, and pressure of 230 °C, 1450 W, and 4000 kPa, respectively. After the addition of H_2O_2 or $Na_2S_2O_8$, to 25 mL of the sludge sample, the samples in closed vessels were treated according to pre-determined experimental conditions. The temperature of the sludge samples was increased at a rate of 10 °C/min to desired value in MW system. MW/H_2O_2 treatment consisted of a preheating stage. Before dosing hydrogen peroxide, all of the samples were heated at 120 °C for 15 min in order to destruct the biological enzymes in the sludge, whereby undesirable consumption of hydrogen peroxide would be prevented (Wang et al., 2009). At the end of the treatment period and before carrying out subsequent analyses, the vessels were cooled to room temperature without opening the caps to avoid evaporation. In all treatment experiments, no effort was made to adjust the pH of the sludge. However, the pH of raw and treated samples was measured. In all treatment systems, control experiments were performed in the absence of oxidants.

3.2.3. Experimental Design

2^3 full factorial experimental design was used to identify significant influences of selected factors and their interactions on TCS degradation and sludge solubilisation with the minimum number of experiments (Minitab Inc.). Three independent variables of design were temperature (A), oxidant dose (B), and $Fe^{2+}/S_2O_8^{2-}$ molar ratio (C). Preliminary experiments were carried out to determine the extreme values (corner points) of selected variables. The levels of each factor along with their codes and values are listed in Table 3.3. As shown in table each one of three variables received two values as indicated by the plus and the minus signs, while a central value was indicated by 0. The Fe^{2+} concentration was varied accordingly to provide the desired $Fe^{2+}/S_2O_8^{2-}$ molar ratio for a given oxidant dose.

Table 3.3. Independent variables and their levels used in the 2^3 full factorial design for sewage sludge treatment.

Experimental factors	Symbol	Level (-)	Level (0)	Level (+)
Temperature (ºC)	A	40.0	57.5	75.0
$S_2O_8^{2-}$ dose (mM)	B	8.0 (0.15 g/g TS)	15.4 (0.29 g/g TS)	22.7 (0.44 g/g TS)
$Fe^{2+}/S_2O_8^{2-}$ (molar ratio)	C	0.50	0.87	1.25

Statistical analysis of the results was performed with a software (Minitab 17).

3.2.4. Evaluation of Radical Mechanism for $Fe^{2+}/Heat/S_2O_8^{2-}$ Treatment

To evaluate the formation of reactive radicals in the sludge during Fe^{2+}/heat/$S_2O_8^{2-}$ treatment, two radical probe compounds, the hydroxyl radical (HR) probe nitrobenzene (NB) (Neta et al., 1977; Zepp et al., 1987), and the combined sulfate radical (SRA) and HR probe anisole (AN) (Zepp et al., 1987; O'Neill et al., 1975) were spiked into the sludge at concentrations of 0.06 mM. As radical scavengers, tert-butyl alcohol (TBA) and isopropyl alcohol (IPA) at 100 millimole/g TS were used to quench HR and HR/SRA, respectively (Buxton et al., 1988; Clifton and Huie, 1989). After centrifugation and filtration of the sludge samples, the probe compounds were analyzed by HPLC (acetonitrile/water 60:40 v/v; flow rate: 0.7 mL/min; detection with a diode array detector: 254 nm for AN, 275 nm for NB).

3.2.5. Micropollutant Extraction and Analysis

Micropollutant extraction from both the liquid and the solid portions was performed after centrifugation of the sludge samples (27.5 mL) at 5000 g for 20 min. The extraction and analysis procedure for the OTC and CIP antibiotics were based on published methods with slight modifications (Blackwell et al., 2004; Turiel et al., 2006). For this purpose, the sludge samples were mixed with an aqueous solution of $Mg(NO_3)_2$ (1 M, pH 8.1) for CIP and with an extraction buffer consisting of methanol/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 in a vortex for 30s. Subsequent extraction of the samples by ultrasonication (Sonorex super RK 510–640 W, Morfelden-Walldorf) for 30 min was followed by centrifugation at 5000 g for 10 min. Thereafter, the supernatant was withdrawn and the residue was re-extracted two times as described above. The combined supernatant was filtered through a 0.45 μm membrane syringe filter (regenerated cellulose, Sartorius).

For OTC analysis, the filtered samples were purified and concentrated by solid phase extraction, for which SAX (6 mL/500 mg, Phenomenex) and HLB cartridges (6 mL/200 mg, Waters, Milford) were utilized in tandem, and the elution of OTC was accomplished with methanol (4 mL).

TCS was analyzed according to Langdon et al. (2012) with some modifications. Briefly, a methanol/acetone (50/50, v/v) mixture was used for TCS extraction. The extracts were passed through 0.45 μm filters (regenerated cellulose, Sartorius, Germany) after centrifugation (5000 g, 10 min) and purified with Oasis HLB cartridges (6 mL/200 mg, Waters, Milford, MA, USA). Elution of TCS from the cartridge was attained with a dichloromethane/acetone (50:50) solution. The eluate was evaporated to complete dryness under a gentle stream of nitrogen gas, and it was then reconstituted in methanol (1 mL) and finally quantified by HPLC. Quantification of the micropollutants was carried out with HPLC by separation on a C18 column (YMC-Pack ODS-AQ; 3 μm, 50×4.0 mm). Elution was applied with acetonitrile and Milli-Q water, both containing 0.1% formic acid. CIP was detected with a fluorescence detector at excitation and emission wavelengths of 280 and 450 nm, respectively, while TCS were detected with a diode array detector at 360 nm and at 234 nm, respectively. All procedure for each micropollutant analysis is summarized in Figure 3.1. HPLC chromatograms of OTC, CIP, and TCS in sludge samples are represented in Appendix A.

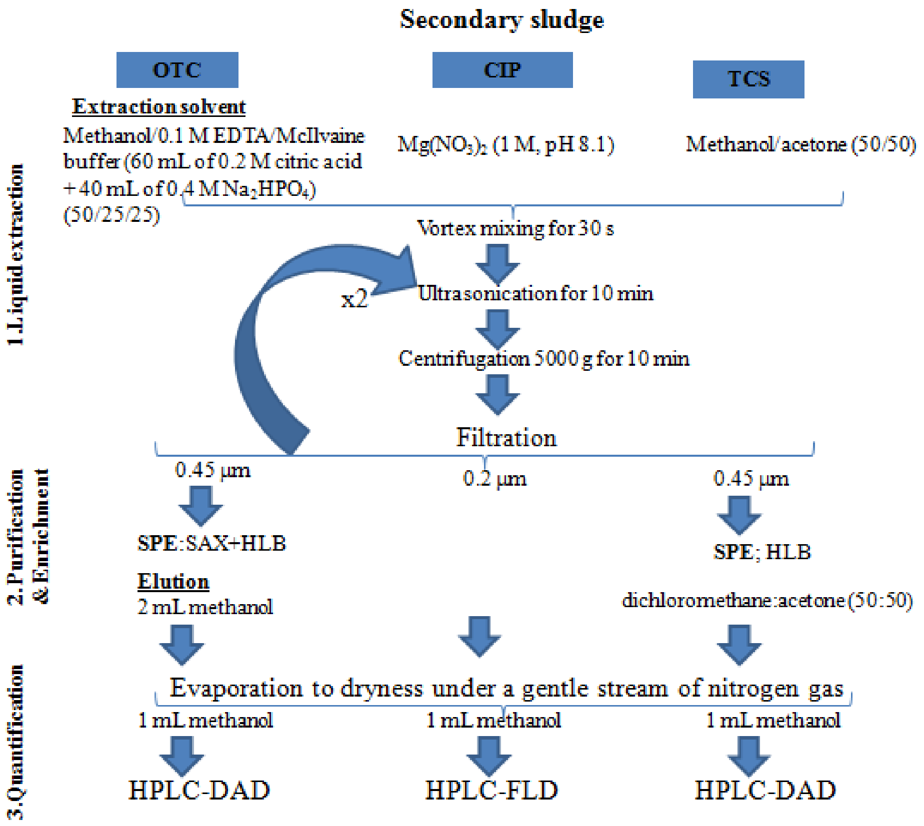


Figure 3.1. Schematic representation of OTC, CIP and TCS analysis.

Degradation was calculated as the percent change in micropollutant concentration after sludge treatment. For each experiment before treatment of the sludge, the initial concentration of target pollutant was determined to eliminate the probable influence of changes in sludge characteristics on the recoveries. In order to assess the performance of analysis method recovery study was performed and the recovery ratio of CIP, OTC and TCS from the sludge was calculated by using the following equation:

$$\text{Recovery (\%)} = \frac{\text{CIP,OTC,or TCS conc.quantified by HPLC analysis (mg/kgTS) x100}}{\text{spiking level (mg/kgTS)}} \qquad (3.1)$$

3.2.6. Adsorbable Organic Halogens Analysis

Adsorbable organic halogen contents of solid and liquid portions of sludge samples were separately estimated based on the procedure described BEHR manual and ISO 9565-2004 standard, respectively. This measurement includes three steps:

(i) Adsorption of the organochlorine compounds onto the activated carbon,
(ii) Mineralization of organically bound halogen through combustion,
(iii) Determination of chloride concentration by micro coulometric titration.

The instruments used AOX analysis is shown in Figure 3.2.

The solid samples were obtained by centrifugation of the sludge at 6500 rpm for 15 min. After drying obtained solid sample at 105 °C for overnight, it was grounded. Subsequently, 20 mg of it was placed in a 25 ml stoppered erlenmeyer flask and mixed with 20 mg activated charcoal (VM0000002). They were dispersed in 25 ml of the nitrate solution in order to eliminate the adsorption of inorganic halide on the activated carbon.

As an adsorbent of 50 mg of activated charcoal was use for 40 mL of liquid samples and the identical procedure applied to solid samples was followed. The suspensions of both liquid and solid samples were shaken in mechanical agitator at 200 rpm for one hour. Samples were filtered through a polycarbonate membrane at 0.4 bar pressure of nitrogen for the separation of activated carbon from aqueous phase. The carbon cake on the filter was washed with 25 ml of a diluted nitrate solution and then the filter was transferred to the quartz boat placed in a combustion tube. Combustion of the sample in oxygen stream (12 L/h) at 950 °C was performed by using a program which provides gradual increase in the temperature (200 °C for 2 min); 100 °C/min; 500 °C for 1 min); 950 °C for 6 min)). A mixture of free halogen and hydrogen halides released from the combustion of organochlorine compounds was bubbled through a vessel containing concentrated sulfuric acid, which removes the water vapor. Finally, titration of the hydrogen halide with a microcoulometer by silver provides AOX result. After measuring and entering the current values of the blank, the interpretation of results was done using installed software in µg/l or mg/kg for liquid and solid samples, respectively.

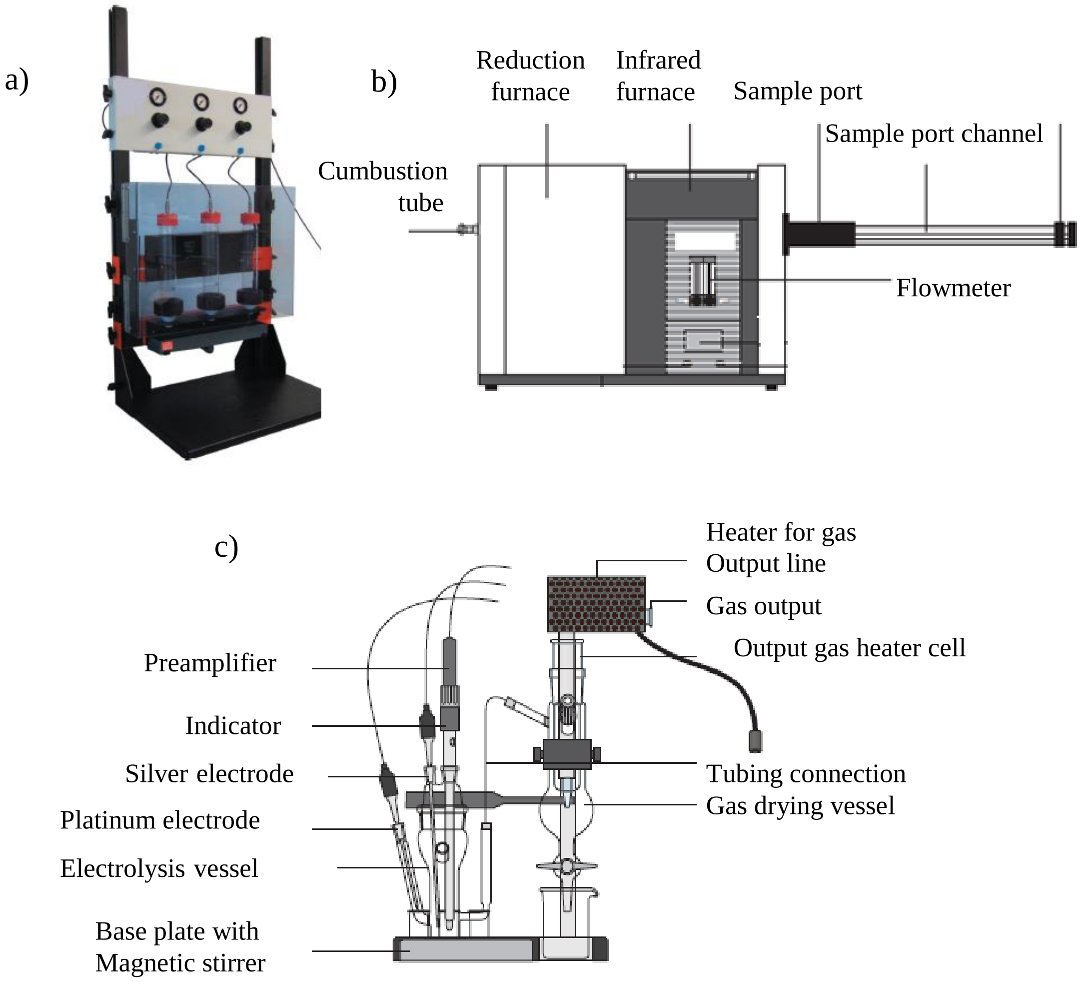


Figure 3.2. a) Filtration, b) combustion, and c) microcoulometric titration modules for AOX analysis.

3.2.7. Capillary Suction Time Measurement

Dewatering properties of raw and treated sludge samples were evaluated by CST measurement using a CST apparatus (Triton Electronics, Type 304M) following standard procedure (APHA, 2005). According to manufacturer instruction 2 mL of the sludge sample was poured in the test cell reservoir (1.8 cm diameter) as shown in Figure 3.3.

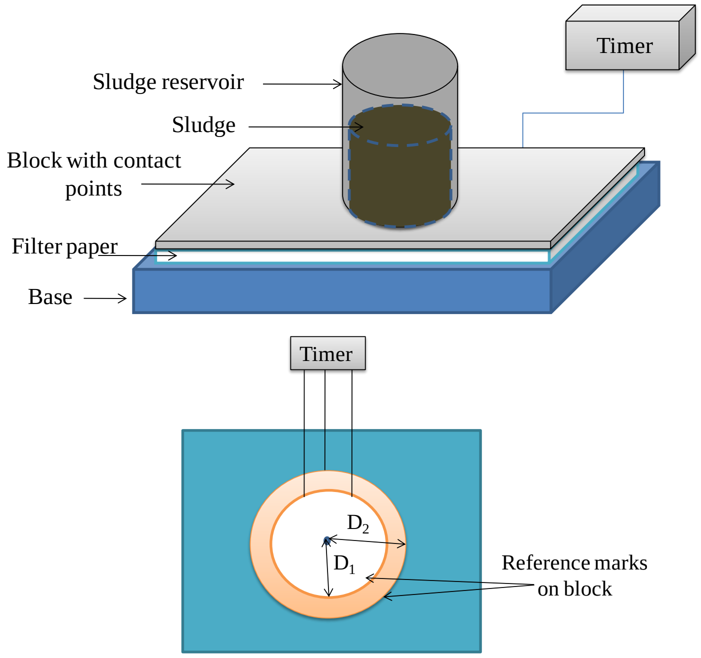


Figure 3.3. Schematic representation of CST measurement.

CST apparatus measures how quickly the sludge wet filter paper (Triton Electronics, 7x9 cm). After contacting the sludge with filter paper, water from the sludge starts to wet the paper due to capillary suction phenomena and the water proceeds radially. The time required for water to proceed from D1 to D2, which is called CST, is measured using a conductivity sensor. Duplicate CST measurements were performed and average values were reported.

3.2.8. Extraction and quantification of extracellular polymeric substances (EPS)

In order to classify EPS, a proper volume of the sludge having 500 mg MLVSS was subjected to extraction with phosphate buffer saline (PBS) according to the procedure of Geyik and Çeçen (in press). Proteins and polysaccharides in EPS were quantified in the extracts of raw and treated sludge. The procedure to determine Very Loosely Bound-EPS (VLB-EPS), Loosely Bound-EPS (LB-EPS) and Tightly Bound-EPS (TB-EPS), soluble EPS (S-EPS) was summarized in Figure 3.4.

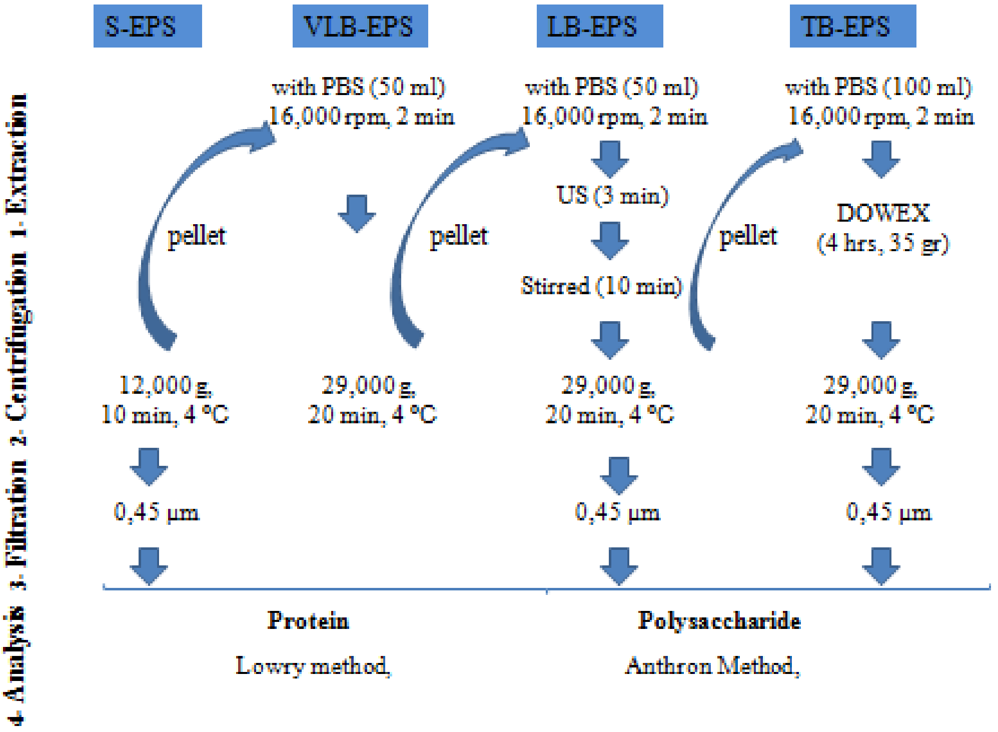


Figure 3.4. Schematic representation of EPS extraction.

After centrifugation of the sludge at 12,000 g (Beckman Coulter, Allegra 64R High speed Refrigerated Centrifuge) for 10 min S-EPS was determined in the filterate (0.45 µm filter, Sartorius). The remaining pellet was washed with deionized water with vortex. Sludge pellet was separated by centrifugation at 16,000g for 10 min. Protein and carbohydrate analyses were performed in the washing solution. Pellet was extracted three times.

The first extraction was carried out by phosphate buffer solution (PBS) (2 mM $Na_3PO_4.12H_2O$, 4 mM NaH_2PO_4. $2H_2O$, 9 mM NaCl, 1 mM KCl) in a vortex (nüve, NM 110) to determine Very Loosely Bound EPS (VLB-EPS). Then, sludge was homogenized for 2 min at 16,000 rpm by using the Heidolp Silent Crusher Homogenizer. It was centrifuged for 20 min at 29,000g at 4 °C. The supernatant was filtered through 0.45 µm filter paper.

The second extraction was performed to determine loosely bound EPS (LB-EPS). Pellet was treated by 50 ml PBS similar to previous extraction step. Additionally, ultrasonic extraction (Bandelin Sonorex ultrasonic water bath at 50W for 3 min) was applied to the pellet. Following the stirring at 800 rpm for 10 min extract was separated by centrifugation at 29,000g for 20 min. Before the analysis of protein and carbohydrate the supernatant was filtered through 0.45 µm syringe filter.

The last extraction was performed to determine tightly bound EPS (TB-EPS).This fraction of EPS was extracted by using Dowex® Marathon™ C sodium form (Sigma-Aldrich). Homogenized suspension was contacted with 35 g washed, drained and filtered DOWEX resin. The sample was stirred for 4 hours at 800 rpm and at 4 °C under dark conditions. The sample was then centrifuged, filtered and stored as introduced in previous step. All extractions were performed in duplicate.

Proteins and carbohydrates in the filtrates of each extraction step were analyzed colorimetrically according to Lowry (Lowry et al., 1951) and Anthron methods (Frølund et al., 1996), respectively. While bovine serum albumin was used as a standard for protein analysis glucose was the standard of carbohydrates. Calibration curves for protein and carbohydrate analysis are given in Appendix B.

3.2.9. Other Analyses

3.2.9.1. *Solids Analysis.* Solid concentration of secondary sludge was determined in accordance with Standard Methods (APHA/ 2005) by using the methods from 2540B to 2540E. In order to determine suspended solids content, sludge samples were filtered through 0.45 μm Millipore filter paper which was dried at 105 ˚C for 24 hour in drying oven (Fn 500). Volatile or fixed solids content was determined by the ignition at 550 ˚C for one hour.

3.2.9.2. *Chemical Oxygen Demand (COD) Analysis.* COD measurements were conducted in accordance with Standard Methods (APHA/ 2005) by closed reflux colorimetric method. For determination of TCOD, prior to COD analysis sludge samples were digested with NaOH (1 M) by the aid of mixing at 150 rpm for 24 hours (Tiehm et al., 2001). Samples and standards that were prepared by using standard solutions of KHP with known COD values (100-500 ppm) were digested at 150 ˚C with high range dichromate COD reagents for 2 hours. Absorbance of the samples and standards were measured at 600 nm, and COD values of samples were calculated by using the calibration curve that was plotted according to standards' absorbance values. Solubilization degree of organic matter was calculated by using the equation 3.2:

$$\text{Solubilization degree}= \frac{\text{SCOD*100}}{\text{TCOD}} \tag{3.2}$$

where,
SCOD= soluble COD, (mg/L)
TCOD= total COD, (mg/L)

3.2.9.3. *Total Kjeldhal Nitrogen (TKN) and Ammonia Analysis (NH_3-N).* After the digestion of sludge samples by using digestion apparatus (Hach Digesdahl) TKN determination was performed in accordance with Nessler Method (Method 8075) suggested by Hach (Hach, 1997). The volumes of sample and digestion reagents were selected depending on solid content and expected TKN value of each sample. Ammonia Analysis of sludge samples was performed in accordance with Nessler Method (Method

8038) suggested by Hach (Hach, 1997). Quantitative analysis of both TKN and NH_3-N were carried by Hach spectrophotometer (Hach DR/2010).

3.2.9.4. Total and Soluble Phosphorous Analysis. The digestions of samples were carried out with the same method used for TKN analysis. Soluble and total phosphate determinations were performed with Ascorbic Acid Method (Method 8048) suggested by Hach (Hach, 1997). Following the dilution, phosphorous content of samples was measured by Hach spectrophotometer (Hach DR/2010) by using Hach PhosVer 3 test kits.

3.2.9.5. pH Measurements. WTW pH 330 pH meter was used for pH measurements.

3.2.9.6. Metal Analysis. Total and soluble metal concentrations in raw and treated sludge samples were determined by ICP analysis (Perkin-Elmer Optima 2100 DV). Total concentration of metals was measured after the digestion of sludge samples in accordance to TS EN ISO 11885 and soluble metals were determined in filtered samples. Solubilization of metals from the sludge was calculated by using following equation:

$$\text{Metal Release} = \frac{\mathrm{sMe_f} - \mathrm{sMe_i}}{\mathrm{TMe_i}} \tag{3.3}$$

Where,
TMe_i= initial total Metal Concentration, (mg/L)
sMe_i= initial soluble Metal Concentration, (mg/L)
sMe_f= final soluble Metal Concentration, (mg/L)

4. RESULTS

4.1. Characterization of Waste Sewage Sludge

Raw waste sludge was characterized by different parameters listed in Table 4.1 in order to evaluate solubilisation efficiency of applied oxidation processes. This table shows average values of parameters for the sludge samples collected in different sampling campaigns are shown.

Table 4.1. Characteristics of waste sewage sludge (mean ± standard deviation, n=6 samples).

Parameter	**Value**	**Metals**	**Value (mg/kg TS)**
TS (g/L)	16.8±0.1	Cr	330.9±157.4
VS/TS	0.63±2.7	Mn	476.5±104.0
TCOD (g/L)	10.6±6.8	Fe	13956.9±1142.1
SCOD (mg/L)	38.9±7.6	Ni	278.2±25.3
TKN (mg/L)	549.4±108.7	Cu	414.9±91.2
TP (mg/L)	954.5±98.3	Zn	1046.4±146.3
PO_4^{3-} (mg/L)	122.5±42.5	Cd	BLD[a]
NO_3^--N (mg/L)	2.1±1.5	Pb	BLD[a]
NH_3-N (mg/L)	1.1±0.3		
pH	6.3−6.9		

BLD[a]: below limit of detection

As can be seen from Table 4.1 SCOD value of raw sludge is only 0.37% of the TCOD value, which was used to estimate both soluble and particulate organic matter in the sludge. On the other hand, soluble phosphate constitutes about 13% of the total phosphorus due to the applied advanced biological phosphorus removal process in the treatment plant. The average VS/TS ratio and TS value are typical for raw sewage sludge (Metcalf and Eddy, 1991).

Although regulations and guides for wastewater sludge that is considered for land application includes limit values of some persistent organics antimicrobials are not found in the pollutant list. However, OTC, CIP, and, TCS were analyzed in the collected sludge samples in this study. While OTC and TCS concentrations were found below the detection limit of applied analysis method, CIP concentration was 8±2 mg/kg. The limits of detection of OTC, CIP, and TCS in the sludge were 2, 4, and 3 μg/g DS solid, respectively.

4.2. Preliminary Experiments for the Degradation of OTC, CIP, and TCS by Persulfate Treatment

Preliminary experiments were performed to investigate the degradation of OTC, CIP, and TCS as a function of time during treatment of sludge by persulfate oxidation. Two different concentrations of persulfate ($S_2O_8^{2-}$= 11.3 mM and 22.7 mM) were used in the experiments carried out at 75 °C within 120 min. Besides, the effect of ferrous iron addition on the efficiency of oxidation process was studied at $Fe^{2+}/S_2O_8^{2-}$ ratio of 1.25 (22.7 mM $S_2O_8^{2-}$, 28.4 mM Fe^{2+}). The average values of the results obtained by the application of Heat/$S_2O_8^{2-}$ and Fe^{2+}/Heat/$S_2O_8^{2-}$ processes are shown as a function of treatment time in Figure 4.1 (a-c). In addition, a control experiment was performed at pH = 2.5 and 75°C (Heat/pH 2.5) without addition of the oxidant in order to access the influence of acidification of sludge on the fate of target micropollutants.

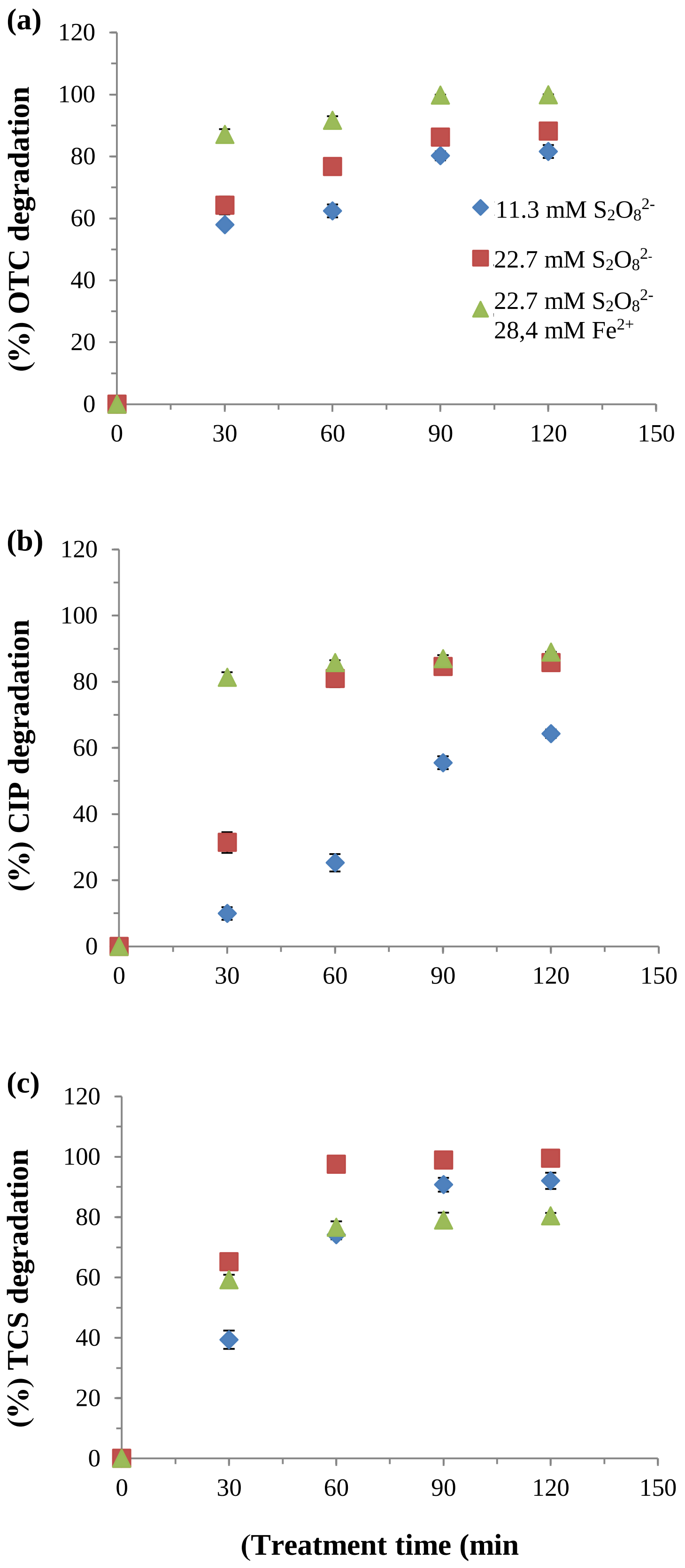


Figure 4.1. Effect of treatment time on the degradation of OTC, CIP, and TCS.

The results of control experiments in the absence of oxidant at pH=2.5 revealed that all micropollutants remained within the sludge without decomposition in the treatment period. On the other hand, increasing dose of persulfate did not only enhance initial degradation rate but also total removal rate of micropollutants within 120 min. As can be seen from the Figure 4.1 remarkable degradation rates of OTC, CIP, and TCS were achieved within 90 min and further increase of treatment time did not caused pronounced enhancement in degradation rates. While the application of persulfate treatment with 11.3 mM oxidant dose to the sludge for 30 min treatment time resulted in 58%, 10%, and 39% degradation rates of OTC, CIP, and TCS, respectively increasing the dose of persulfate to 22.7 mM provided more than 20% increase in the degradation rates of CIP and TCS. However, this enhancement was only 6% for OTC. The addition of ferrous iron during the treatment caused a further increase in the degradation rates of OTC, CIP, and TCS to 87%, 81%, and 59% within 30 min. On the other hand, overall degradation rate of TCS did not exhibit an improvement by the addition of ferrous iron to persulfate process.

During the application of persulfate treatment to the sludge the consumption of the oxidant and pH were also monitored (Figure 4.2). In accordance to the results of micropollutant degradation rates, noticeable change in the pH value of the sludge and the consumption of oxidant were observed within 90 min of the process. The addition of ferrous iron to persulfate treatment resulted in a high (68%) initial consumption of oxidant and concomitantly drastic change in the pH value. On the other hand, without addition of ferrous iron the oxidant at both high and low dose remained in the sludge even at the end of the treatment period. Nevertheless, degradation rates of micropollutants declined by the treatment time probably due to the competition of solubilized components of the sludge for the oxidant.

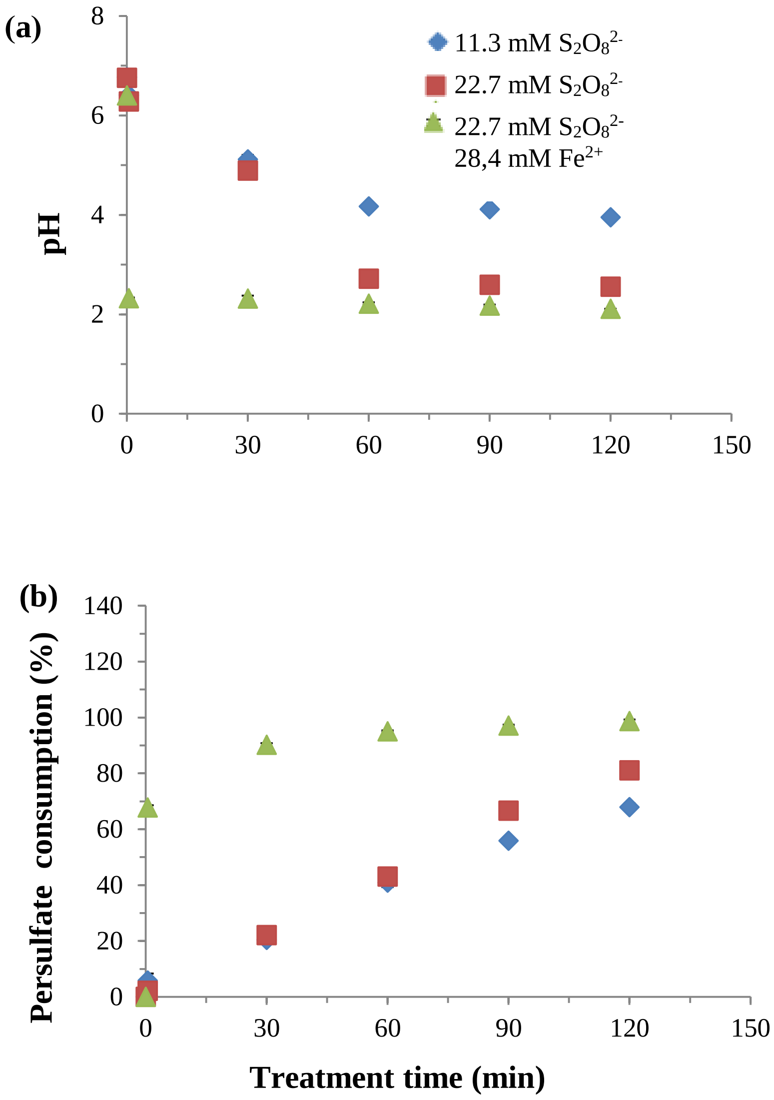


Figure 4.2. Effect of treatment time on (a) the pH of waste sludge and (b) persulfate consumption.

4.3. Effect of Treatment Time on Sludge Solubilization

Besides the micropollutant degradation the solubilisation of organic carbon, phosphorus, and metals were studied as a function of treatment time. In Figure 4.3 (a) and (b), the variations of SCOD and TCOD are displayed separately while Figure 4.3 (c) shows the solubilisation of organic carbon in terms of SCOD/TCOD as function of treatment time. This figure also contains the results of control experiment performed with acidified sludge in the absence of oxidant.

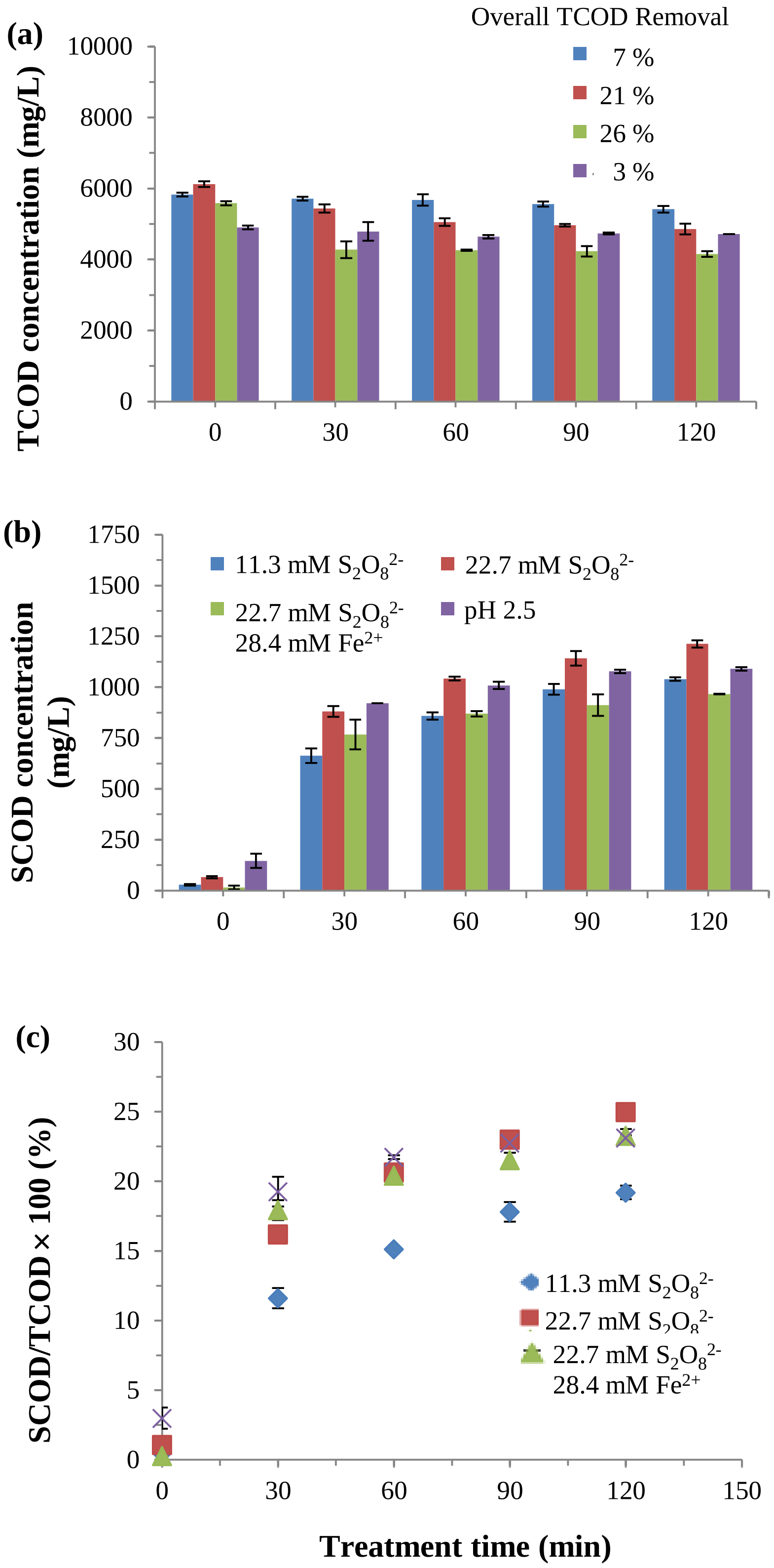


Figure 4.3. Effect of treatment time on the solubilisation of organic carbon.

As opposed to the results of micropollutants, acidification of the sludge to a pH value of 2.5 at 75 °C caused a variation in the overall organic content of the sludge and these conditions led to a SCOD/TCOD ratio of 23% (in 120 min) that was comparable to the value obtained with Fe^{2+}/Heat/$S_2O_8^{2-}$. As can be seen from Figure 4.3, increasing treatment time induced continuous SCOD release throughout treatment performed at each experimental condition. However, remarkable increase in SCOD was achieved up to 30 min and then it was slowed down. While higher dose of persulfate caused the higher SCOD release in the sludge, Fe^{2+}/Heat/$S_2O_8^{2-}$ process provided simultaneous degradation of solubilised COD and deteriorated the organic carbon solubilisation. Hence, highest rate of overall organic carbon solubilisation took place with Heat/$S_2O_8^{2-}$ at a $S_2O_8^{2-}$ dose of 22.7 mM (25% in 120 min) (Figure 4.3 (c)) and overall TCOD mineralization in the sludge with Heat/$S_2O_8^{-2}$ (22.4 mM) and Fe^{2+}/$S_2O_8^{2-}$ processes were 21% and 26%, respectively.

The results of these experiments demonstrated that during treatment of sludge either with Heat/$S_2O_8^{2-}$ and Fe^{2+}/Heat/$S_2O_8^{2-}$ substantial increase in the solubilised organic carbon content, induced a competitive effect for the oxidizing species and this resulted in eventual decline in micropollutant degradation as shown in Figure 4.1.

The effects of treatment time on the solubilisation of phospahate and metals were also investigated during the treatment of sludge by Heat/$S_2O_8^{2-}$ and Fe^{2+}/Heat/$S_2O_8^{2-}$. While the results of these experiments as well as control experiment are demonstrated in Figure 4.4 and 4.5, respectively the solubilisation of individual metals is listed in Table 4.2. It should be noted that the solubilisation rate of iron was not included in the overall metal solubilisation rate for the Fe^{2+}/Heat/$S_2O_8^{2-}$.

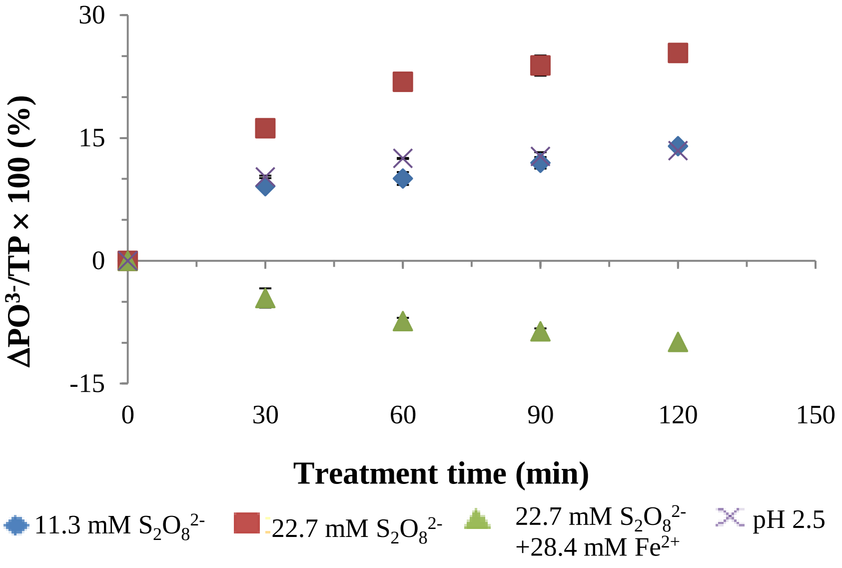


Figure 4.4. Effect of treatment time on the solubilisation of phosphorus.

While Heat/pH 2.5 resulted in considerable phosphorus solubilisation (13% at 120 min) that was comparable to that obtained with Heat/$S_2O_8^{2-}$ at a low $S_2O_8^{2-}$ dose of 11.4 mM (12% at 120 min), the highest rate of phosphorus solubilisation was obtained with Heat/$S_2O_8^{2-}$ at the higher $S_2O_8^{2-}$ dose of 22.7 mM (25% at 120 min) (Figure 4.4 (a)). As expected, the addition of Fe^{2+} in Fe^{2+}/Heat/$S_2O_8^{2-}$ resulted in PO_4^{3-} precipitation, and its rate increased with treatment time.

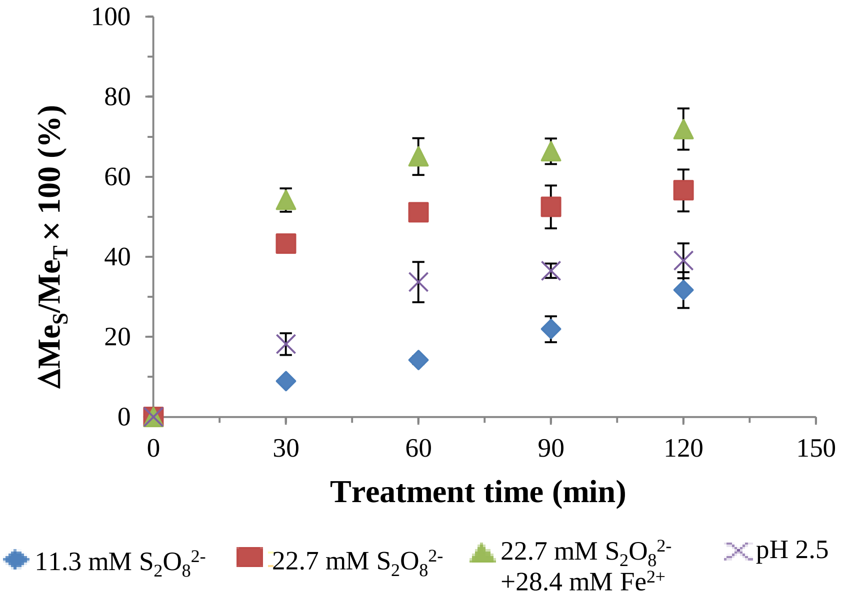


Figure 4.5. Effect of treatment time on the solubilisation rate of metals.

Table 4.2. Solubilization of individual metals for sewage sludge treatment with Heat/$S_2O_8^{2-}$, Heat/ Fe^{2+}/$S_2O_8^{2-}$, and Heat/pH 2.5.

Metal	Process	Solubilization (%)			
		Treatment time (min)			
		30	60	90	120
Cr	Heat /$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	47.1	56.5	53.5	60.0
	Fe^{2+}/ Heat /$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	70.9	62.8	68.1	73.1
	Heat /pH 2.5	1.5	1.6	2.9	4.9
Cu	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	44.3	45.9	53.2	54.8
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	46.0	55.0	57.6	57.9
	Heat/pH 2.5	14.7	32.1	36.5	39.1
Mn	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	82.1	79.3	80.6	81.1
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	82.8	86.3	92.2	95.3
	Heat/pH 2.5	35.5	68.8	72.0	75.0
Ni	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	17.1	63.5	67.7	74.0
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	36.6	68.1	69.8	74.9
	Heat/pH 2.5	29.8	57.8	61.6	69.2
Zn	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	42.9	48.3	51.5	59.2
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	58.6	69.6	67.0	72.4
	Heat/pH 2.5	27.8	35.6	35.9	37.5
Fe	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	2.2	2.3	2.1	2.2
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	-	-	-	-
	Heat/pH 2.5	0.0	0.5	2.0	2.7
Overall[a]	Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM)	46.7	58.7	61.3	65.8
	Fe^{2+}/Heat/$S_2O_8^{2-}$ ($S_2O_8^{2-}$=22.7 mM, Fe^{2+}=28.4 mM)	59.0	68.4	70.9	74.7
	Heat/pH 2.5	21.9	39.2	41.8	45.1

The highest rate of overall metal solubilisation (72%) was obtained with the Fe^{2+}/Heat/$S_2O_8^{2-}$ possibly due to the higher disintegration rate of the sludge with this process (Figure 4.5). Nevertheless high solubilisation rate of the overall metal was also achieved with Heat/$S_2O_8^{2-}$ at a high dose of $S_2O_8^{2-}$ (57%), while at a dose of 11.4 mM the solubilisation rate was lower (32% in 120 min) than that obtained with Heat/pH 2.5 (39% in 120 min). This result can be attributed both to the higher pH value (Figure 4.2. a) and the considerably low sludge disintegration rate attained during sludge treatment with a lower $S_2O_8^{2-}$ dose. Despite the high rate of overall metal solubilisation, Heat/pH 2.5 could not substantially solubilise some metals such as Cr and Cu, which are associated considerably with the organic phase of the sludge (Babel and Daceda, 2006) and thus their solubilisation require the oxidative disintegration of the sludge.

4.4. Degradation of TCS by Persulfate Treatment

The effects of persulfate process parameters on the degradation rates of OTC and CIP were investigated in a previous study by using full factorial design (Oncu, 2013). In this study, the same experimental design was applied to the sludge spiked with TCS. Considering the results of preliminary experiments, in each experiment the sludge was treated for 90 min. The results 19 experiments for TCS degradation are presented in Table 4.3 together with experimental conditions.

Table 4.3. 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			
Experiment number	A Temperature (–: 40°C; +: 75°C)	B $S_2O_8^{2-}$ dose (–: 8.0 mM; +: 22.7 mM	C $Fe^{2+}/S_2O_8^{2-}$ molar ratio (–: 0.5; +: 1.25)	TCS (%)
8	–	–	–	30.9
12	–	–	–	34.6
6	–	–	+	2.1
15	–	–	+	4.3
1	–	+	–	83.0
14	–	+	–	83.3
4	–	+	+	19.6
5	–	+	+	20.1
7	0	0	0	47.7
10	0	0	0	46.4
17	0	0	0	45.5
16	+	–	–	84.1
19	+	–	–	87.0
3	+	–	+	15.5
9	+	–	+	14.3
11	+	+	–	99.9
18	+	+	–	100.0[a]
2	+	+	+	80.1
13	+	+	+	79.7

The statistical significances of temperature, $S_2O_8^{2-}$ dose, and $Fe^{2+}/S_2O_8^{2-}$ molar ratio effects on micropollutant degradation were evaluated and they were shown in Figure 4.6 with Pareto chart. This chart displays the estimated effects of these studied factors and their interactions that are significant with a level of confidence greater than 95% in descending order.

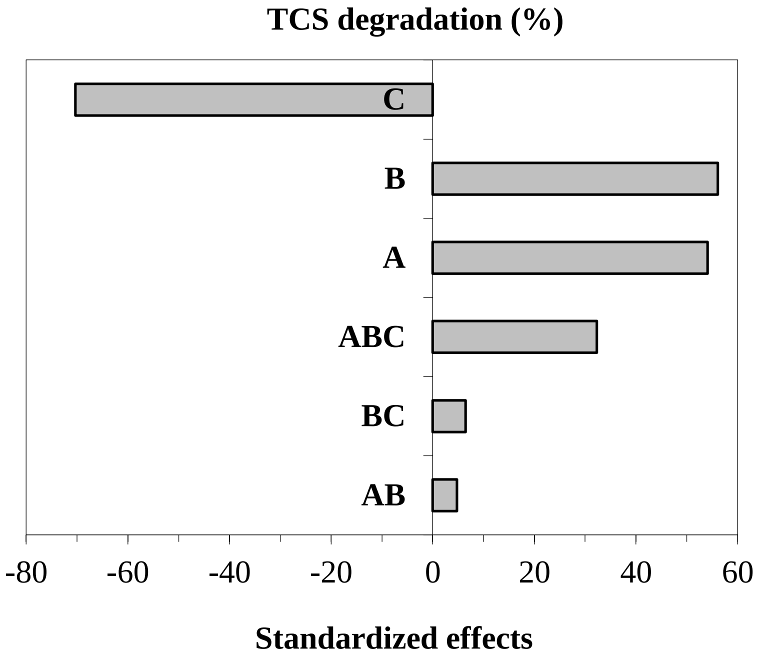


Figure 4.6. Pareto chart for TCS degradation.

From the results obtained in the experimental design, it can be deduced that both temperature and $S_2O_8^{2-}$ dose increased TCS degradation rate (e.g. exp. 8, 12-1, 14 and 16, 19 in Table 4.3). The molar ratio of $Fe^{2+}/S_2O_8^{2-}$ on the other hand exerted a negative influence on the degradation of TCS (Figure 4.5). By increasing the $Fe^{2+}/S_2O_8^{2-}$ molar ratio from 0.5 to 1.25 the rate of of TCS degradation declined by 63% in the presence of $S_2O_8^{2-}$=22.7 mM at 40 °C (exp. 1, 14 and 4, 5 in Table 4.3). It is known that, iron can pose conditioning effect on sludge particles besides promotion of radical generation during the applied oxidation process. Hence, this conditioning effect can intercept the contact of the oxidant with TCS. On the other hand, in a previous study, the addition of iron enhanced the degradation rates of zwitterionic antibiotics by providing their desorptions (Oncu, 2013). However, the formation of metal complexes has not been reported for TCS. Furthermore, desorption of TCS was not expected at acidic pH values owing to its high pK_a value and its hydrophobicity (Table 3.2). In order to support this suggestion, a series of control experiments was performed in the absence of oxidant to clarify the effects of pH, temperature and the addition of ferric iron on the fate of TCS in the sludge. While the effect of temperature on the fate of TCS was investigated at the natural pH of the sludge an acid control experiment at pH=2.5 was investigated only at 75 °C considering the prevailing of the acidic conditions during the treatment. Two different concentrations of Fe^{3+} (11.3 mM and 28.4 mM, respectively) corresponding to the low and high Fe^{2+} dose used in the experimental design were added to the sludge, which was then equilibrated at 200 rpm at different temperatures. All these control experiments are shown out for a 30 min treatment period in Figure 4.7 as prolonged treatment did not influence the results. In these experiments, to differentiate dissolved and sorbed fractions of TCS separate analyses were performed. However, TCS was not detected in the dissolved phase in each experiment therefore the total concentration of TCS in Figure 4.7 implies the sorbed concentrations of it.

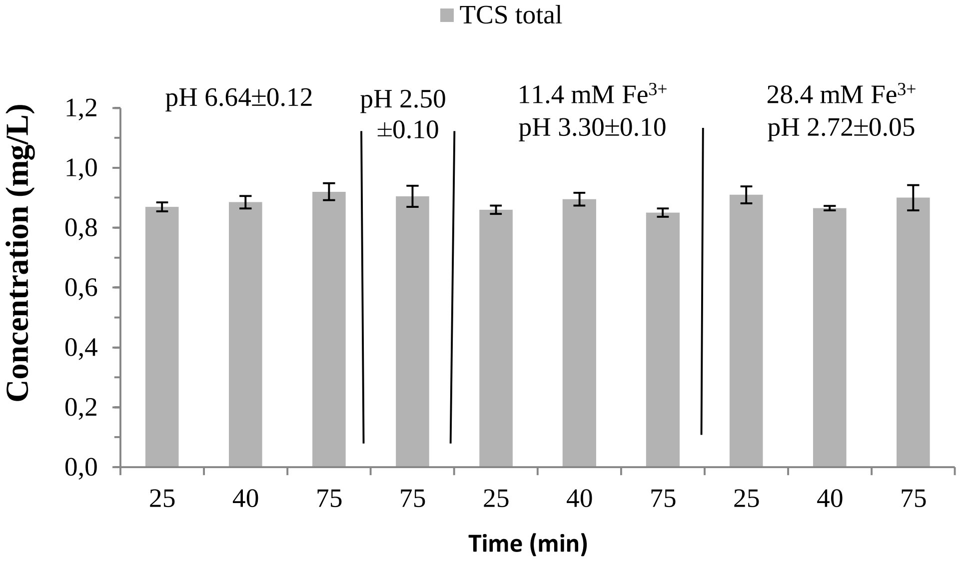


Figure 4.7. Influence of temperature, pH, and Fe^{3+} dose on the dissolved and total concentrations of the TCS.

In spite of the negative influence of $Fe^{2+}/S_2O_8^{2-}$ on the degradation of TCS, the three-way interaction of the factors exerted a major positive influence (Figure 4.6), probably as a result of the efficient utilization of persulfate by simultaneous increase in factor values. Same effects of these interactions were reported on the solubilisation of sludge organic carbon in a previous study (Oncu, 2013). In Figure 4.8, the results of TCS degradation obtained at the corner points of the experimental design are presented together with previous sludge solubilisation data (Oncu, 2013) as a cube plot.

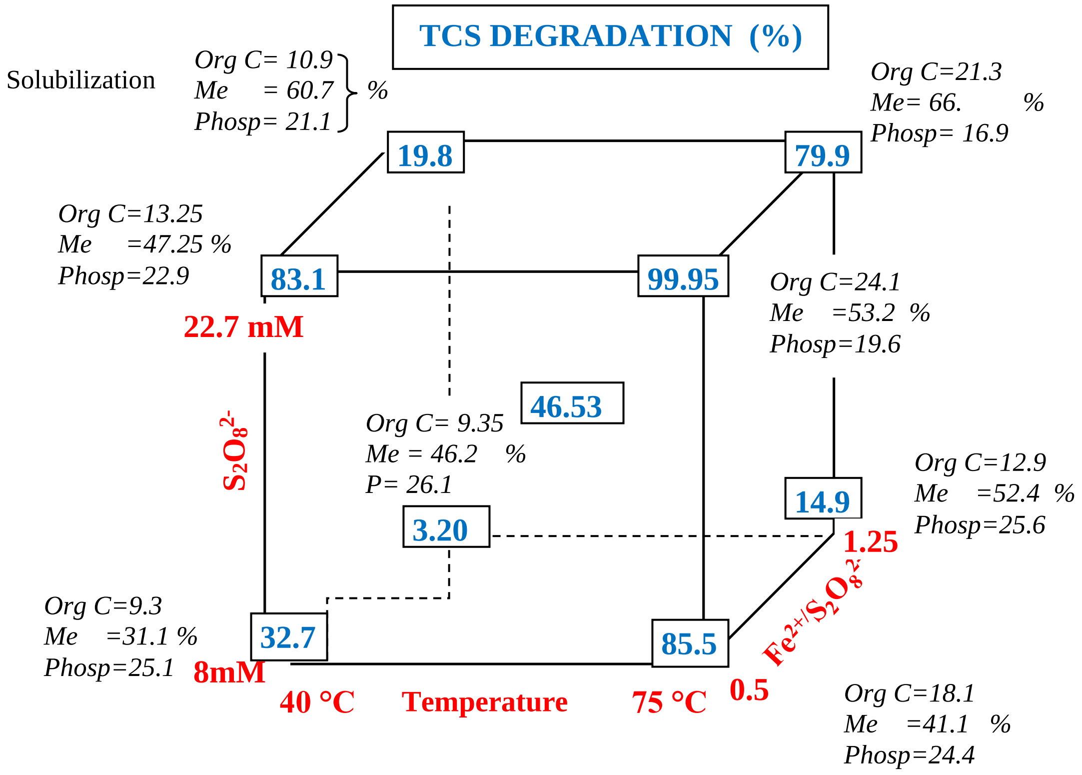


Figure 4.8. Cube plot for different factors affecting persulfate process performance in terms of TCS degradation and sludge solubilization.

As summarised in the cube plot (Figure 4.8), the highest degradation rate of TCS was achieved with 22.7 mM dose of persulfate and 0.5 molar ratios of $Fe^{2+}/S_2O_8^{2-}$ at 75 °C. Under the same experimental conditions, the highest organic carbon solubilisation rate (24.1%) was also obtained whereas this does not correspond to the highest phosphorus and metal solubilisation rates. At the high dose of persulfate, the reduced phosphorus solubilisation rate by increasing iron dose clearly indicates the precipitation of iron phosphate.

4.5. Effects of Radical Mechanisms in Persulfate Treatment of Sludge

In order to explain the probable contribution of free radicals during persulfate treatment of sludge, experiments were performed with two different radical probe compounds and radical scavengers, the degradation rates of which at two different iron and oxidant doses at both 40 and 75 °C are given in Figure 4.9 for a treatment period of 15 min. NB was used as a HR probe, whereas AN was used to detect both the HR and the SRA. The literature values of the rate constants of probe and scavengers with the radicals are inserted in Figure 4.9. The rate of degradation of AN was remarkably higher than that of NB within a short treatment period; therefore, its degradation is depicted only in the presence of the TBA and IPA radical scavengers.

Although the degradation of NB was only 4 and 19% with $S_2O_8^{2-}$ =22.7 mM at 75 °C in the absence and presence of iron, respectively, the degradation of AN at 75 °C was 91% in the absence of iron, and almost complete degradation (>99%) was achieved by the addition of iron (Figure 4.9a, b). A high temperature was not responsible for the degradation of the radical probe compounds, which was proven by control experiments performed in the absence of oxidant at 75 °C. From these results it can be deduced that persulfate could be activated by heat and iron in the sludge according to Eqn. 4.1 and 4.2 (Peyton, 1993; House, 1962):

$$S_2O_8^{2-} + \text{heat} \rightarrow 2SO_4^{-\bullet} \qquad k{=}< 10^{-7}\ s^{-1}\ (25\ °C);\ 10^{-4.5}\ s^{-1}\ (77\ °C) \qquad (4.1)$$

$$S_2O_8^{2-} + Me^{n+}\ (Fe2+) \rightarrow Me^{(n+1)} + (Fe^{3+}) + SO_4^{2-} + SO_4^{-\bullet} \qquad k{=}27\ M^{-1}s^{-1}\ (Fe^{2+}) \qquad (4.2)$$

This activation of persulfate is clearly necessary for the efficient degradation of the antimicrobials, which exhibited negligible degradation rates (<10%) with a $S_2O_8^{2-}$ dose of 22.7 mM in the absence of heat and iron as deduced from the results of control experiment presented in Table 4.4.

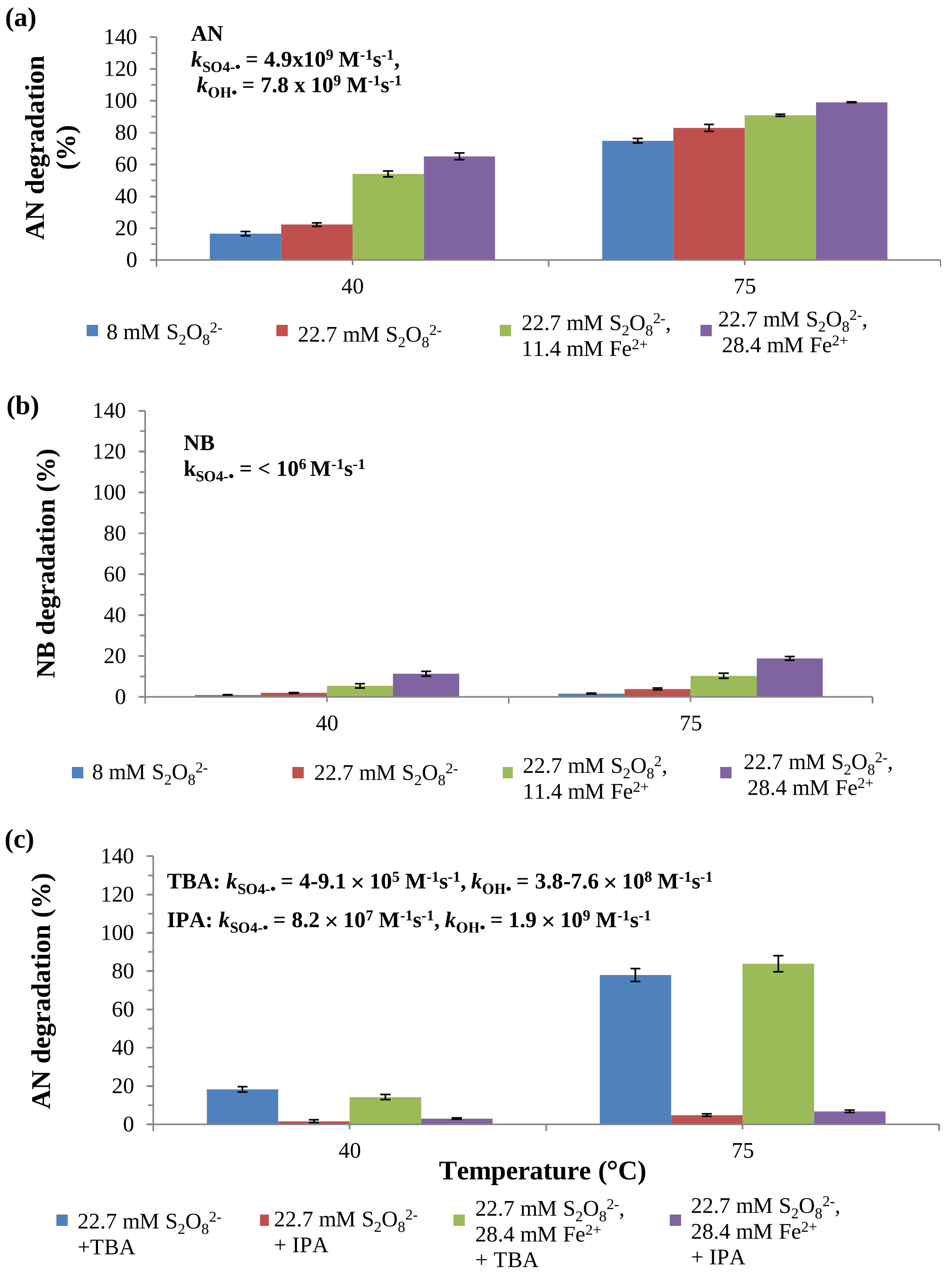


Figure 4.9. Degradation of (a) AN and (b) NB with heat/$S_2O_8^{2-}$ and Fe^{2+}/heat/$S_2O_8^{2-}$, and (c) AN with heat/$S_2O_8^{2-}$ and Fe^{2+}/heat/$S_2O_8{}^{2-}$ in the presence of radical scavengers; Treatment period=15 min.

Table 4.4. Micropollutant degradation in control experiments of persulfate treatment at ambient conditions and at 75°C in the absence of iron.

Temperature (°C)	**Oxidant dose (mM)**	**Fe^{2+}/ $S_2O_8^{2-}$ (mol/mol)**	**Micropollutant degradation (%)**		
			OTC	**CIP**	**TCS**
24 ±3	22.725	-	9.5±3.2	6±1.6	<1
75	22.725	-	93.1±2.0	79.8±3.6	99.5±0.3

The obtained results indicated a negligible contribution of the HR to the treatment of the sludge. The limited contribution of the HR was supported by the addition of a HR scavenger, which resulted in a decrease in the rate of degradation of AN by only 15%, whereas the addition of IPA clearly ceased its degradation (Figure 4.9c). These results are consistent with previous studies, in which similarly the predominance of the SRA at acidic pH both at ambient and high temperatures was reported (Liang and Su, 2009; Liang et al., 2007; Yan et al., 2011).

The contribution of iron to the extent of radical formation depended on the applied temperature. Although its influence on the degradation of AN was not pronounced at 75 °C, the situation was reversed at 40 °C owing to the relatively low activation rate of persulfate by heat at this temperature (Eqn. 4.1) (Peyton, 1993). The rate of degradation of AN was 83% with $S_2O_8^{2-}$ =22.7 mM if only heat was applied for persulfate activation at 75 °C, whereas it was 65% if the major activation route of persulfate at a dose of $S_2O_8^{2-}$ =22.7 mM was by iron at a $Fe^{2+}/S_2O_8^{2-}$ ratio of 1.25 at 40 °C. Although the oxidation of Fe^{2+} to Fe^{3+} according to Eq. 4.2 and subsequent precipitation as ferric hydroxide would limit the sustained activation of persulfate (Liang et al., 2009), the considerably low pH values attained during treatment resulted in the presence of soluble iron (Fe_S) (Oncu, 2013). Hence, Fe^{2+} regeneration according to Eqs. 4.3 and 4.4, given below (Liang et al., 2009), and enhanced metal solubilisation from the sludge at acidic pH values (Oncu, 2013) can provide further activation of persulfate:

$$R^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + \text{products} \quad (4.3)$$

$$S_2O_8^{2-} + H^{+} \rightarrow HS_2O_8^{2-} \rightarrow SO_4^{-\bullet} + SO_4^{2-} + H^{+} \quad (4.4)$$

However, as mentioned previously, Fe^{2+} could not be identified in the supernatant of the sludge owing to an almost immediate decrease in its concentration to below the detection limit as a result of its fast oxidation to Fe^{3+}. Liang et al. (2007) similarly reported that in an iron-activated persulfate treatment system, Fe^{2+} was almost immediately oxidized to Fe^{3+} in the absence of a chelating agent. Generation of the HR in the process can take place by Eqs. 4.5, 4.6, and 4.7 (House, 1962):

$$S_2O_8^{2-} + 2H_2O \rightarrow 2HSO_4^- + H_2O_2 \qquad k = 10^{-7}\ s^{-1}\ (25\ °C);\ 5.2\ x10^{-5}\ s^{-1}\ (70\ °C) \tag{4.5}$$

$$H_2O_2 + Me^{n+} \rightarrow Me^{(n+1)\ +} + OH^- + HO^{\bullet} \tag{4.6}$$

$$SO_4^{-\bullet} + H_2O \rightarrow HSO_4^- + OH^{\bullet} \qquad k = 2x10^3\ M^{-1}s^{-1} \tag{4.7}$$

Specifically, the increase in the degradation of NB with the addition of Fe^{2+} suggests that the generation of HR can be promoted in an iron-activated persulfate system, as reported by Ji et al. (2014) and Anipsitakis and Dionysiou (2004). However, the contribution of these reactions cannot be important because of their low second-order rate constants.

4.6. Comparison of Advanced Chemical Oxidation Processes Efficiency

A recent study by Oncu (2013) showed that MW process provides the degradation of micropollutants and solubilisation of sewage sludge. In this study, MW irradiation was used to activate two oxidants (persulfate and hydrogen peroxide) under the experimental conditions determined in a previous PhD thesis (Oncu, 2013) to investigate the effects of this activation on the specific components of the sludge. The concentrations of adsorbable organic halogens, proteins and carbohydrates in the sludge treated by MW assisted chemical oxidation were compared with those of treated by persulfate process activated by both heat and iron. During these treatments dewaterability of the sludge was also determined.

4.6.1. AOX Values of Treated Sludge

AOX values in solid and liquid portions of sludge samples treated by chemical oxidation processes under the conditions, at which the higher amount of antimicrobial degradation was achieved, were separately determined. While persulfate treatment was applied to the sludge for 90 min, MW assisted persulfate, and MW assisted peroxide treatments were performed for 15 min. In Table 4.5., the results are summarized together with the results of control experiments carried out in the absence of oxidants.

Table 4.5. AOX values in solid and liquid portions of sludge samples.

	Solid (mg/L)	**Liquid (µg/L)**
Untreated sludge	175.15±6.34	21.57±5.57
140 °C	212.53	72.76
160 °C	250.35	110.51
$S_2O_8^{2-}$ (0.44 g/g TS) MW 140 °C	186.70	83.05
$S_2O_8^{2-}$ (0.87 g/g TS) MW 140 °C	205.06	158.66
$S_2O_8^{2-}$ (0.44 g/g TS) MW 160 °C	222.09	54.67
$S_2O_8^{2-}$ (0.87 g/g TS) MW 160 °C	222.71	134.12
H_2O_2 (0.6 g/g TS) MW 140 °C	300.87	45.52
H_2O_2 (1.2 g/g TS) MW 140 °C	275.95	47.18
H_2O_2 (0.6 g/g TS) MW 160 °C	259.105	63.85
H_20_2 (1.2 g/g TS) MW 160 °C	208.82	38.56
$S_2O_8^{2-}$ (0.44 g/g TS) 75 °C	252.00	363.42
$Fe^{2+}/S_2O_8^{2-}$ (0.44 g/g TS) = 1.2, 75°C	168.50	111.44

While AOX values of solid and liquid portions of raw sludge were 175.15±6.34 mg/kg and 21.57±5.57 µg/L, respectively higher values were determined in MW treated samples in the absence of oxidants. Increasing MW temperature from 140 to 160 °C caused a remarkable increase AOX value especially for liquid phase. These results could indicate the formation of halogenated organics due to the reactions between organics and halogens during the sludge treatment. Recent studies reported that the formation of AOX could occur in presence of high chlorine concentrations (Baycan et al., 2005; Baycan et al., 2007; Aken et al., 2013).

Although the addition of persulfate at a dose of 0.44 g/g TS to the sludge during MW treatment reduced the AOX formation increasing the oxidant dose to 0.87 g/g TS resulted in higher liquid phase AOX values than that of the sludge treated MW alone. In contrast to the results obtained by MW assisted persulfate process, liquid phase AOX value of the sludge treated by high dose of H_2O_2 at higher MW temperature was comparable low indicating the nonselective nature of hydroxyl radicals generated in the sludge during the treatment. However, it should be taken into account that the application of low dose of hydrogen peroxide at 140 °C MW temperature to the sludge caused the elevation of solid phase AOX value to 300 mg/L which is about 60% higher than that of raw sludge. This could be the result of preheating stage of MW/H_2O_2 process since longer exposure of the sludge to MW irradiation increased AOX formation possibility. However, an increase in both temperature and oxidant dose in MW/H_2O_2 process could provide the degradation of generated AOX exerting compounds.

Similar to MW assisted oxidation process, persulfate and peroxide treatment with conventional heating increased the AOX values of the sludge. As a result of persulfate treatment of the sludge at 75 °C, the increase in AOX value especially in liquid phase was noticeably high. On the other hand, the addition of iron to the sludge during this treatment has a positive influence on AOX values in both liquid and solid phases.

4.6.2. Dewaterability of Treated Sludge

In order to reduce the volume of sludge and the cost of transportation sludge dewatering is one of the basic steps in sludge treatment (Neyens and Baeyens, 2003). As mentioned in Table 2.3 chemical and thermal treatment greatly influence sludge dewaterability, which is generally characterized by CST. In this study, after the application of advanced chemical oxidation processes, the dewaterability of treated sludge was evaluated by CST and obtained results are compared with that of raw sludge in Table 4.6. This table also includes the results of control experiments for MW treatment performed in the absence of oxidant.

Table 4.6. CST values of raw and treated sewage sludge.

	CST (s)
Untreated sludge	44.45
MW 140 °C	High*
$S_2O_8^{2-}$ (0.44 g/g TS)/MW 140 °C	15.15
$S_2O_8^{2-}$ (0.87 g/g TS)/MW 140 °C	17.75
$S_2O_8^{2-}$ (0.44 g/g TS)/MW 160 °C	16.15
$S_2O_8^{2-}$ (0.87 g/g TS)/MW 160 °C	16.45
H_2O_2 (0.6 g/g TS)/MW 140 °C	53.75
H_2O_2 (1.2 g/g TS)/MW 140 °C	20.75
MW 160 °C	High*
H_2O_2 (0.6 g/g TS)/MW 160 °C	19.90
H_2O_2 (1.2 g/g TS)/MW 160 °C	19.55
$S_2O_8^{2-}$ (0.44 g/g TS) 75°C	22.20
$Fe^{2+}/S_2O_8^{2-}$= 1.2 with 0.44 g/g TS oxidant at 75 °C	16.05

*Higher than 1000 s

As can be seen from table, the results of control experiment carried out with 15 min contact of MW irradiation were not evaluated due to exceeding the measurement limit of CST. In fact, deterioration of sludge dewaterability depends upon the contact time and energy of MW irradiation (Tyagi et al., 2013; Yu et al., 2009; Wojciechowska et al., 2005). In case of longer exposure of MW irradiation, the lack enhancement in sludge dewaterability was attributed to the destruction of microorganisms’ cells and the release of intercellular macromolecules (Yu et al., 2009). In addition, formation of fine particles by the disintegration of sludge could be another reason for this result (Yu et al., 2009).

Dependent upon the type and dose of oxidant added to the sludge during MW treatment dewaterability exhibited different trend. Combined application of MW irradiation and chemical oxidation except H_2O_2 (0.6 g/g TS)/MW 140 °C process substantially decreased CST value of the sludge (Table 4.6). MW assisted peroxide process with 0.6 g/g TS dose at 140 °C caused a higher CST value for treated sludge than that of raw sludge. In contrast to the results obtained by MW assisted peroxide, MW assisted persulfate process yielded improvement in the dewaterability of sludge regardless of MW temperature and oxidant dose. However, by increasing the MW temperature from 140 °C to 160 °C a slight enhancement in dewaterability was achieved at the high dose of oxidant. Although combined application of persulfate and MW 52rradiation to sludge has not been investigated in the literature similar beneficial effect of hydrogen peroxide addition on the sludge dewaterability during MW irradiation was observed in previous studies (Eskicioglu et al, 2008; Yu, 2007) and this is attributed to the destruction of EPS (Eskicioglu et al., 2008; Yu, 2007). A slight difference in the effect of persulfate and peroxide at high oxidant dose and at high temperature applied in present study on the sludge dewaterability could also be explained by pH attained during the treatment. It was suggested that a reduction in the pH value below Zero point charge (ZPC) of sludge could provide the minimization of repulsive force effect on the particles that are stable due to their negative charge in the sludge liquor (Neyens et al., 2003). While the pH value of the sludge treated with $S_2O_8^{2-}$ (0.87 g/g TS)/MW 160 °C was below 2 it was 4.5 in the sludge treated with H_2O_2 (1.2 g/g TS)/MW 160 °C process (Oncu, 2013; Oncu and Balcioglu, 2013b). It should also be taken into account that pretreatment in MW/H_2O_2 process caused a longer exposure time of MW irradiation and resulted in higher organic matter solubilisation than the MW/$S_2O_8^{2-}$ process (Oncu, 2013; Oncu and Balcioglu, 2013b) that may cause slight deterioration of sludge dewaterability.

Application of conventionally heated persulfate oxidation resulted in lower dewaterability enhancement (50%) than that obtained by MW heated persulfate oxidation performed even at the low dose of the oxidant. However, the addition of ferrous iron to persulfate reduced the CST value from 44 s to 16 s implying 64% dewaterability enhancement. However, in the study of Zhen et al. (2012 a,b,c) 80–86% CST reduction efficiency was achieved by the application of Fe (II)-activated persulfate (1.2 mmol/gVSS) to the sludge. The reason of the higher CST reduction value than that obtained in this study can be attributed to the use of higher dose of oxidant (3.78 mmol/gVSS) that probably provided higher disintegration of the sludge.

4.6.3. Organic Constituents of Raw and Treated Sludge

Thermal, chemical treatment or combination of them, thermo-chemical treatment, can change polymeric substances of the sludge, which is composed proteins, polysaccharides, nucleic acids, lipids etc. Recent studies reported that proteins and polysaccharides are dominant components of EPS (Neyens et al., 2004; Dongsu et al., 2015). It is also known that the concentration and the characteristics of polymeric substances play a predominant role in sludge dewaterability (Yu et al., 2009) and settleability (Sheng et al., 2010; Liao et al., 2001). Hence, recent studies focused on the fractionation of polymeric substances which have double-layered structure: tightly bound EPS (TB-EPS) that surround the cells and loosely bound EPS (LB-EPS), which diffuse from the tightly bound EPS (Zhen et al., 2012b; Li and Yang, 2007). It was suggested that the amount of LB-EPS affects the performance of sludge–water separation more than the amount of TB-EPS (Li and Yang, 2007). Considering forementioned literature investigations, protein and polysaccharide contents of treated sludge by selected advanced chemical oxidation processes were evaluated and soluble portions (S- Protein and S-Polysaccarides) are compared in Figure 4.10 while extractable portions are shown in Table 4.7. Figure 4.10 also demonstrates the total organic carbon (TOC) content of the sludge samples. On the other hand, figure and table did not include the results of MW/H_2O_2 process since the residual peroxide in the sludge after treatment interfered the analysis of polymeric substances.

Table 4.7. Extractable protein and polysaccharide content of sludge.

	Polysaccharide (%)		**Protein (%)**	
	Loosely bound	**Tightly bound**	**Loosely bound**	**Tightly bound**
Untreated sludge	8.60	11.77	23.00	49.76
$S_2O_8^{2-}$ (0.44 g/g TS) 75 °C	3.35	2.99	4.94	4.02
$Fe^{2+}/S_2O_8^{2-}$ (0.44 g/g TS) = 1.2 75 °C	1.96	3.54	1.97	3.30
MW 140 °C	2.80	3.39	5.36	5.68
$S_2O_8^{2-}$(0.87 g/g TS) MW 140 °C	0.99	2.47	1.03	2.81

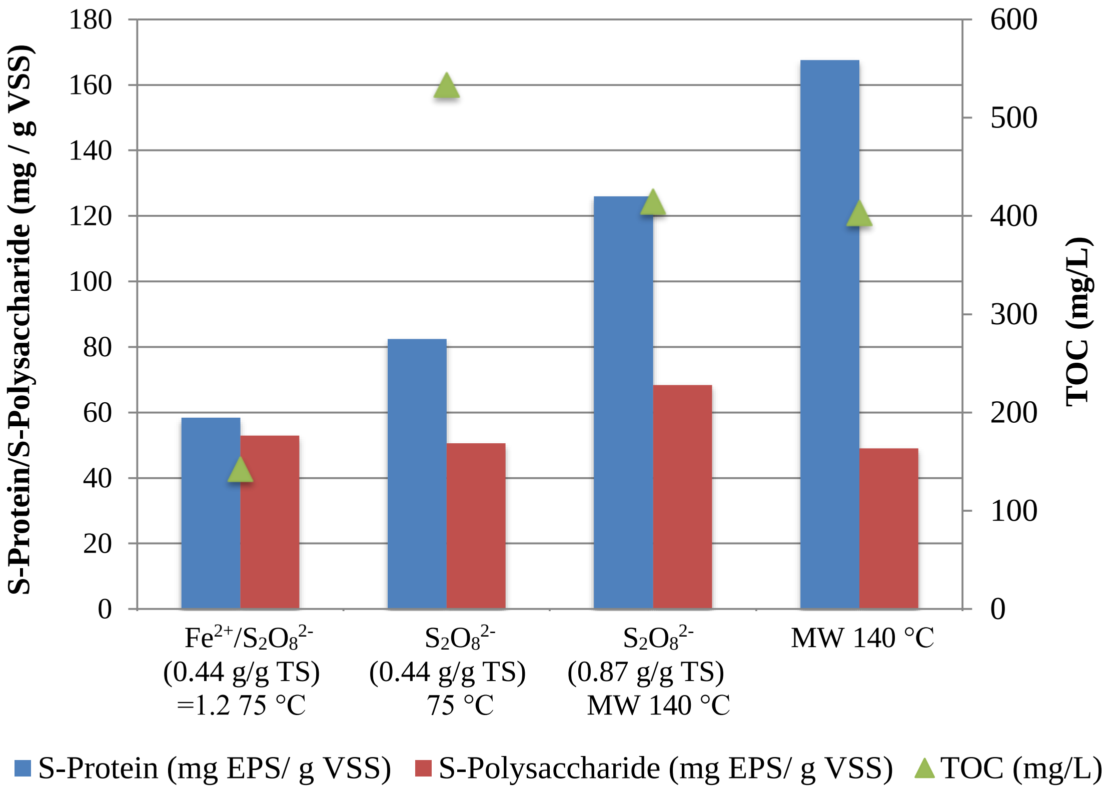


Figure 4.10. Soluble organic constituents of treated sludge.

By the application of selected treatments to the sludge extractable protein and polysaccharide decreased while soluble protein and polysaccharide increased (Figure 4.10 and Table 4.7). These results clearly proved the cell lysis and destruction of protein and polysaccharides. In accordance to the previous studies (Eskicioglu et al, 2006; Shao et al., 2009; Yuan et al., 2011) proteins were found in higher concentrations than polysaccharide in soluble phase of the sludge.

It can be seen in Figure 4.10, compare to MW irradiation the combined application of it with persulfate substantially decreased soluble proteins while it caused an increase in the concentrations of soluble polysaccharides. Consequently, TOC in soluble phase of the sludge was almost invariable. However, the addition of persulfate to the sludge during MW irradiation decreased the each fraction of extractable proteins and polysaccharides (Table 4.7). Similarly, the addition of iron to heat activated persulfate process enhanced the reduction of extractable polymeric substances and also reduced the amount of soluble proteins in the sludge. In accordance to previous findings of this thesis, these results obviously show the strong oxidizing conditions in sludge treatment with Heat/Fe^{2+}/$S_2O_8^{2-}$ and MW/$S_2O_8^{2-}$ processes. Remarkable difference in both soluble concentrations of proteins and TOC values of the sludge treated by these processes could be attributed to longer treatment period of Fe^{2+}/$S_2O_8^{2-}$ process, which enabled the destruction of released components of the sludge. On the other hand, higher extractable protein content of the sludge treated by Heat/Fe^{2+}/$S_2O_8^{2-}$ suggested simultaneous precipitation of solubilised proteins. It was suggested that reduction of protein amount improves the sludge dewaterability of sludge due to its high water-holding capacity (Cetin and Erdincler, 2004). This can explain the higher efficiency of Heat/Fe^{2+}/$S_2O_8^{2-}$ process for dewatering of the sludge.

5. CONCLUSIONS

In this study, the degradation of antimicrobials in waste sewage sludge was investigated by the application of persulfate oxidation. Solubilisation of sludge in terms of organics, metals and nutrients was also investigated to clarify the multi functional role of persulfate process in the sludge treatment. Additionally, the efficiency of persulfate oxidation was compared with those of MW assisted persulfate and peroxide oxidation processes for dewaterability of waste sludge.

Conclusions of this study were explained below.

1. The average concentration of CIP in the waste sludge samples were found as 8±2 mg/kg and the concentrations of OTC and TCS were below the detection limit of applied chromatographic analysis method while the amounts of organic matter, metal and nutrient contents in waste sludge used in this study have typical values for a sewage sludge.

2. During the treatment of waste sludge at strong acidic condition and 75 °C in the absence of oxidant the solubilization of sludge components was obtained but the degradation of OTC, CIP, and TCS antimicrobials spiked to the sludge was not achieved within 120 min.

3. A remarkable change in both the degradation of antimicrobials and the solubilization of organic carbon and metals was achieved at 75 °C within 90 min of persulfate treatment. Further increase of treatment time did not cause a pronounced enhancement in antimicrobial degradation due to competition of solubilized sludge components for oxidant.

4. The addition of iron to persulfate process shortened the treatment period required for effective antimicrobial degradation and enhanced simultaneous solubilization and mineralization of organic constituents of the sludge.

5. Similar to OTC and CIP degradations investigated in a previous study TCS degradation was positively affected by increasing both the temperature and the oxidant dose of persulfate process whereas as opposed to those of other antimicrobials TCS degradation was negatively influenced by increasing molar ratio of $Fe^{2+}/S_2O_8^{2-}$. In spite of this, the three-way interaction of selected process parameter exerted a major positive influence probably due to the efficient utilization of persulfate. Almost complete degradation of TCS was achieved with 22.7 mM $S_2O_8^{2-}$ dose and $Fe^{2+}/S_2O_8^{2-}$ molar ratio of 0.5 at 75 °C.

6. The formation of the selective sulfate radical in persulfate process explained the high rate of degradation of the antimicrobials, even in the presence of a high organic content of the sludge. The concurrent use of iron and heat for persulfate activation enhanced the rate of degradation of the antimicrobials, promoted solubilization of the metal, and also resulted in precipitation of a major portion of sludge phosphorus.

7. Sulphate radical generated from the activation of persulfate was mainly responsible for the degradation of antimicrobials although hydroxyl radicals could also be generated in the presence of both iron and heat. The contribution of iron to the radical formation depended upon the applied temperature to the sludge. The concurrent use of iron and heat for persulfate activation served multi-purposes: enhanced degradation of the antimicrobials, promoted solubilization of the metals and the precipitation of a major portion of sludge phosphorus.

8. Regardless of treatment type, AOX content of the sludge both in liquid and solid phase exhibited an increase indicating the reactions of halogens with the organic under the treatment conditions. Nonselective hydroxyl radicals mainly generated from the activation of peroxide caused lower AOX values in liquid phase of the sludge compared to that treated by sulfate radical formed by the activation persulfate. During MW assisted persulfate treatment accumulation of AOX was attributed to selective nature of sulfate radical. The activation of persulfate by heat and ferrous iron instead of MW irradiation resulted in not only lower AOX values but also lower metals values than the regulated limits for the sludge that is considered for land application.

9. Although the advanced chemical oxidation processes at the high oxidant dose which was necessary for efficient antimicrobial degradation deteriorated sludge dewaterability slight reduction in CST of the sludge was obtained with each of the applied processes. Type of persulfate activation did not cause a significant change on sludge dewaterability.

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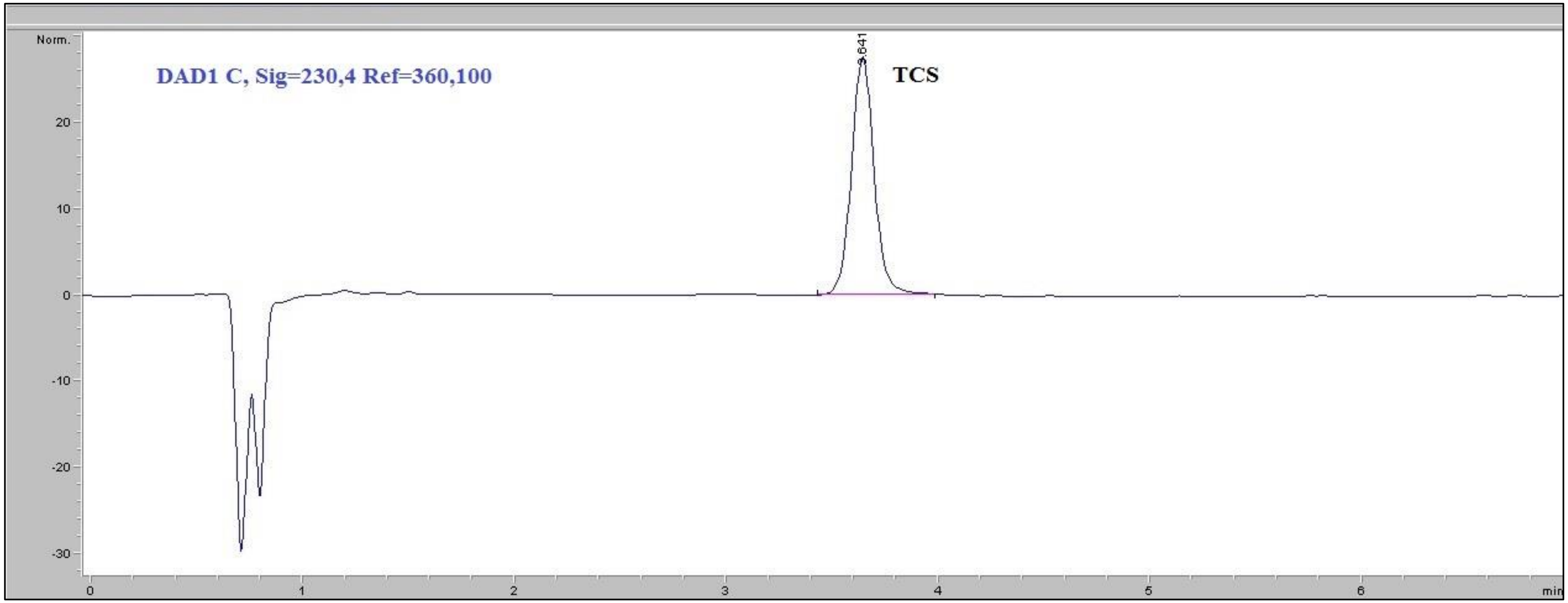
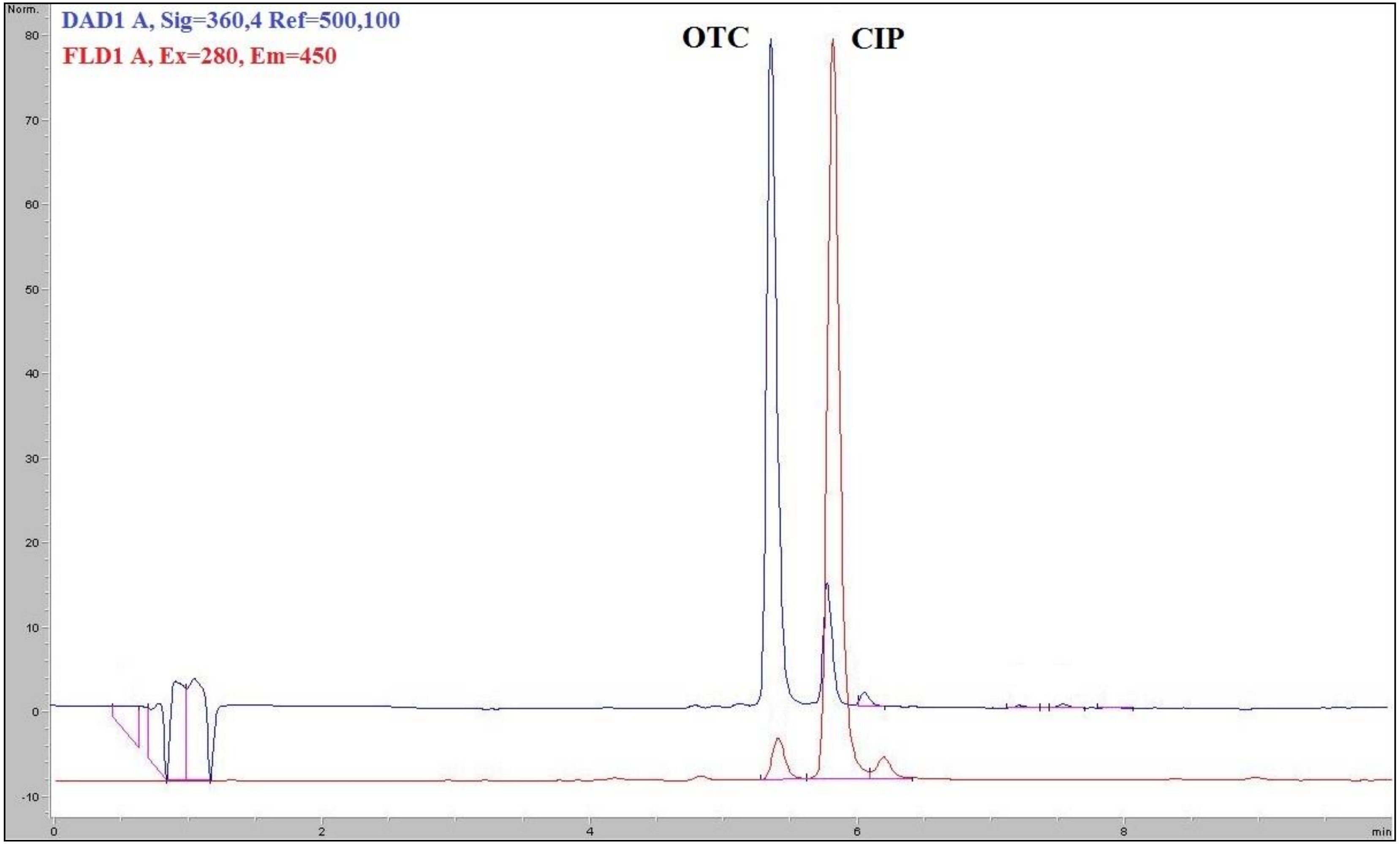
APPENDIX A

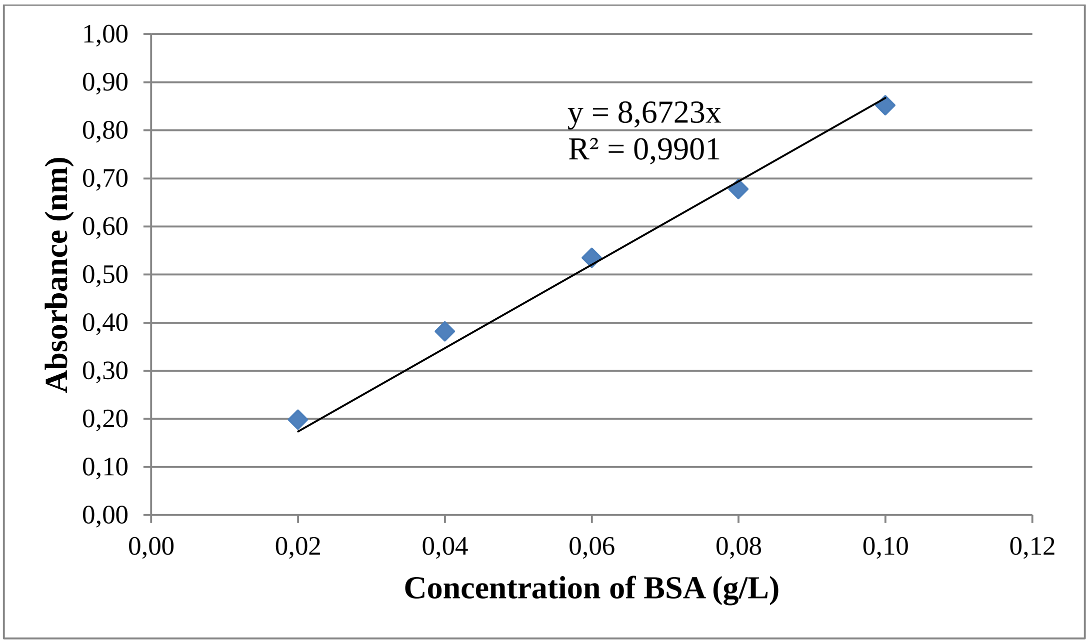


A1. HPLC chromatogram of TCS

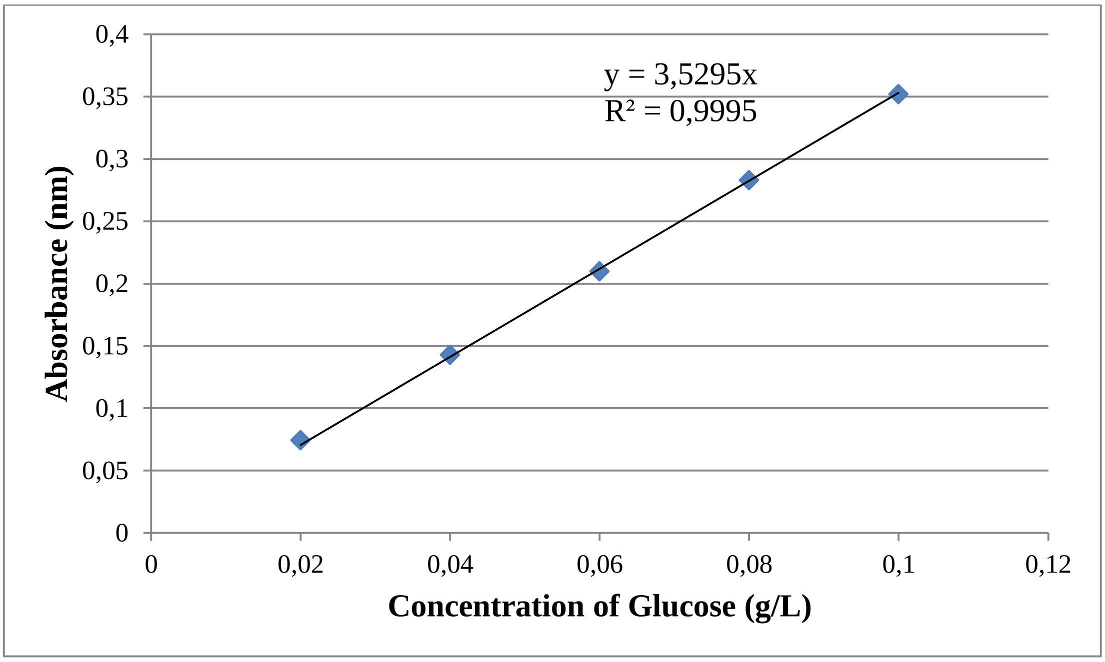


A2. HPLC chromatograms of OTC and CIP

APPENDIX B



B1. Calibration Curve for Protein Analysis



B2. Calibration Curve for Polysaccharide Analysis