

DEGRADATION OF OXYTETRACYCLINE ANTIBIOTIC FROM WASTE  
ACTIVATED SLUDGE BY FENTON OXIDATION PROCESS

by

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## ABSTRACT

Antibiotics are emerging organic contaminants that enter wastewater network as consequence of human activities, and transferred to sewage sludge by sorption during wastewater treatment. The treatment of antibiotics in waste activated sludge (WAS) deserves special attention since antibiotics have potential to cause the spreading of antibiotic resistant bacterial pathogens in contrast to other emerging organic contaminants. Although Fenton oxidation has been considered as a promising pre-treatment method by providing sludge disintegration and degradation of recalcitrant compounds, there is a lack for the determination of antibiotics' fate during Fenton oxidation of WAS.

The efficiencies of Fenton and Fenton-like oxidation for oxytetracycline (OTC) antibiotic, which exhibits high sorption capacity, were evaluated in synthetically contaminated WAS at different experimental conditions. While increased  $\text{H}_2\text{O}_2$  dosage and  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio affected process efficiency positively, higher total solid content of WAS drastically reduced the OTC degradation. Fenton and Fenton-like oxidations provided 94 % and 87% OTC degradation with 150 g/kg TS  $\text{H}_2\text{O}_2$  and 1.2 Fe/ $\text{H}_2\text{O}_2$  molar ratio in 120 minutes treatment time, respectively. Metal and nutrient release and enhanced physical properties were additional results of the Fenton oxidation.

## ÖZET

Antibiyotikler beşeri faaliyetler sonucu atık su ağına giren ve emilim yoluyla atık su çamuruna transfer olan organik kirleticilerdir. Diğer inatçı organik kirleticilerin aksine antibiyotiğe dirençli bakteriyel patojenlerin yayılmasına neden olabilmeleri dolayısıyla atık su çamurundaki antibiyotiklerin arıtılması önem arz etmektedir. Fenton oksidasyonu çamur ayrışması ve inatçı moleküllerin çamurdan uzaklaştırılması konularında ümit veren bir ön arıtma yöntemidir. Ancak antibiyotiklerin atık su çamurunun Fenton oksidasyonu ile arıtılması esnasındaki akıbeti ile ilgili yeterli veri yoktur.

Bu çalışma ile Fenton ve Fenton-Benzeri oksidasyonlarının yüksek emilim kapasiteli oksitetrasiklin antibiyotiğinin giderilmesindeki verimleri sentetik olarak kirletilen çamur üzerinde değişik şartlarda incelenmiştir. Hidrojen peroksit dozunun ve  $Fe^{+2}:H_2O_2$  molar oranının arttırılması proses verimini olumlu yönde etkilerken çamurdaki toplam katı madde miktarı antibiyotik giderimini yüksek oranda düşürmüştür. Fenton ve Fenton-Benzeri oksidasyonları 150 g/kg TKM ve 1.2 Fe:  $H_2O_2$  molar oranda 120 dakikalık arıtma süresi içinde, sırasıyla %94 ve %87 oranında antibiyotik giderimi sağlamıştır. Metal ve nutrientlerin çözünmesi ve çamurun bazı fiziksel özelliklerinin iyileşmesi de Fenton oksidasyonu ile elde edilen diğer sonuçlardır.

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## LIST OF SYMBOLS/ABBREVIATIONS

| <b>Symbol</b>                 | <b>Explanation</b>                         | <b>Unit</b> |
|-------------------------------|--|-------------|
| AD                            | Anaerobic Digestion                        | -           |
| AOP                           | Advanced Oxidation Processes               | -           |
| COD                           | Chemical Oxygen Demand                     | mg/L        |
| CST                           | Capillary Suction Time                     | S           |
| DOC                           | Dissolved Organic Carbon                   | mg/L        |
| EOC                           | Emerging Organic Compounds                 | -           |
| EPA                           | Environmental Protection Agency            | -           |
| Fe <sup>+2</sup>              | Ferrous Iron                               | -           |
| Fe <sup>+3</sup>              | Ferric Iron                                | -           |
| H <sub>2</sub> O <sub>2</sub> | Hydrogen Peroxide                          | -           |
| LOD                           | Limit Of Detection                         | mg/g        |
| NaN <sub>3</sub>              | Sodium Azide                               | -           |
| OTC                           | Oxytetracycline                            | -           |
| PAH                           | Polycyclic Aromatic Hydrocarbons           | -           |
| PPCP                          | Pharmaceuticals And Personal Care Products | -           |
| SCOD                          | Soluble Chemical Oxygen Demand             | mg/L        |
| SSV                           | Settled Sludge Volume                      | -           |
| TCOD                          | Total Chemical Oxygen Demand               | mg/L        |
| TKN                           | Total Kjeldahl Nitrogen                    | mg/L        |
| TS                            | Total Solids                               | g/L         |
| US                            | United States                              | -           |
| VS                            | Volatile Solids                            | g/L         |
| VSS                           | Volatile Suspended Solids                  | g/L         |
| WAS                           | Waste Activated Sludge                     | -           |
| WWTP                          | Waste Water Treatment Plant                | -           |

## 1. INTRODUCTION

The growing population and intensified agricultural and industrial activities are leading to increasing consumption of various organic compounds. These compounds enter into wastewater network as consequence of all these human activities. Depending upon their physicochemical properties some of these compounds are transferred to sludge by means of sorption during primary and secondary treatment (Byrns 2001; Diaz-Cruz et al., 2009; Clarke and Smith, 2011). Among these emerging organic contaminants accumulating in the sludge, antibiotics deserve special attention due to their potential for toxicity to microorganisms, enzyme inhibition, and development of antibiotics resistance even they are discharged in low concentrations (Halling-Sørensen et al., 2000; Lindberg et al., 2007; Smith et al., 2009). Therefore, antibiotic pollution can have potential to exert adverse effects on the biological sludge treatment processes as well as on the environment.

Land application, which is a recommended ultimate sludge disposal method by EU, requires the treatment of sludge produced during the biological treatment of municipal wastewater in order to reduce its pathogen and heavy metal concentrations according to current regulations. Frequent occurrence of antibiotics in waste activated sludge (WAS) (Öncü and Balcıoğlu, 2011) and their adverse effects (Halling-Sørensen et al., 2000; Giger et al., 2003; Thiele-Bruhn and Beck, 2005; Turkdogan and Yetinmezsoy, 2009) clearly indicate a need for treatment of them before land application. Although EU Working Documents (1986/278/EEC) and EPA Guides (Guide 503, 1993) consider some of emerging organic contaminants during sludge management, antibiotics have not yet been regulated. Revision of existing regulations to minimize their possible adverse effects of antibiotics requires an extensive investigation for the fate of them in the sludge treatment processes as well as development of efficient treatment methods.

Stabilization of WAS is commonly performed by anaerobic digestion due to its high biogas yield potential but this process is not efficient to remove antibiotics (Radjenovic et al., 2009). Moreover, anaerobic digestion efficiency is limited by biological hydrolysis (Vavilin et al., 1996; 2008; Tiehm et al., 2001; Appels et al., 2008). Therefore, pre-

treatment methods are investigated in order to improve the performance of anaerobic digestion by destructing the cell walls of microorganism and solubilizing the organics as well as the degradation of recalcitrant contaminants. Although there are many studies investigated the occurrence of tetracycline, fluoroquinolone, and sulfonamide antibiotics in WAS, their removal from the sludge by pre-treatment processes is scarcely questioned (Carballa et al., 2007; Cengiz et al.; 2010; Acar et al., 2011).

Recent studies indicated Fenton oxidation as a promising pre-treatment method by having an ability to provide microbial cell wall decomposition, solubilization, and mineralization of sludge and degradation of recalcitrant contaminants (Neyens and Baeyens, 2003). Both ozone and Fenton oxidations are advanced oxidation processes that are effective on the degradation of recalcitrant compounds in WAS. However, Fenton oxidation can ensure some advantages such as simpler application than ozone oxidation, enhancement of metal release due to acidic pH and ability to supply coagulation by added iron catalyst.

Considering the lack of antibiotic fate in Fenton oxidation for the sludge, the efficiency Fenton oxidation is evaluated to destruct a tetracycline antibiotic sorbed on WAS. Synthetically contaminated WAS samples were treated at different  $\text{H}_2\text{O}_2$  dosage,  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio, and temperature conditions in this thesis. In order to assess the effects of matrix components on treatment performance, additional experiments were carried out with different total solid content of WAS. The efficiency of process was evaluated by organic matter, metal, and nutrient solubilization as well as antibiotic degradation.

## **2. THEORETICAL BACKGROUND**

### **2.1. Emerging Organic Contaminants in Waste Activated Sludge**

In recent years, the presence of various organic contaminants that are used in daily life for domestic, industrial, and agricultural purposes has been getting more attention. These compounds are commonly referred as emerging organic contaminants (EOCs) including pharmaceuticals and personal care products, pesticides, industrial compounds/by-products, and food additives. EOCs refer not only newly developed compounds but also compounds newly discovered in different environmental matrices by the developments in analytical techniques (Diaz-Cruz et al., 2009; Clarke and Smith, 2011; Stevens-Garmon et al., 2011).

Wastewaters are the main sources of EOCs. During the primary and secondary treatment of municipal wastewater many of EOCs tend to be transferred to sewage sludge and then accumulate on the sludge reaching to a concentration level of mg/kg DS (Table 2.1) due to their lipophilic properties (Byrns 2001; Diaz-Cruz et al., 2009; Clarke and Smith, 2011). A successful treatment of wastewater by biological processes can result in a sludge production of about 20-40 kg dry matter per population equivalent per year (Carballa et al., 2009). Annual quantity of sludge produced in the world will increase in the future due to not only increasing population but also contaminants by increasing numbers of wastewater treatment plants. Therefore, management of the waste sludge becomes more important. The progressive implementation of current regulations prescribe numerical limits for heavy metals and pathogenic microorganisms for WAS whereas most of EOCs have not been considered for regulation in many countries (EU, 2000).

Recent reviews have covered the occurrence of EOCs in sludge produced from wastewater treatment (Diaz-Cruz et al., 2009; Clarke and Smith, 2011). In accordance with their findings, Table 2.1 lists maximum and minimum concentrations of these pollutants in the sludge. The concentrations depend on their initial concentrations in the wastewater, physicochemical properties, and the degree of destruction during treatment (Clarke and Smith, 2011).

Table 2.1. Occurrence data of organic emerging contaminants in sludge

| <b>Contaminant</b>     | <b>Range (<math>\mu\text{g}/\text{kg DM}</math>)</b> |
|------------------------|--|
| Estrogens              | 1-406  |
| Illicit drugs          | 4-3,000  |
| UV-filters             | 10-19,000  |
| Flame retardants       | 0.2-59,000   |
| Perfluorated compounds | 0.4-3,370  |
| Surfactants            | 1-1,500x10 <sup>3</sup>                              |
| Synthetic Musks        | 120-13,900   |
| Bisphenol A            | 4-1,363  |
| Antibiotics            | 3-15,600   |

EOCs are considered to present a risk to human health and the environment when biosolids are used for land application which is a recommended ultimate disposal method by international authorities (CEC, 1991; US EPA, 1993; EC, 2010). The occurrence of EOCs in the soil amended with manure and sludge clearly reveals a risk for the plants that can uptake these pollutants (Kümmerer, 2004). Therefore, higher quality standards for waste sludge can be required in case of land disposal.

### **2.1.1. Antibiotics in Waste Activated Sludge as Emerging Pollutants**

Antibiotics are pharmaceuticals that are commonly used in human and veterinary medicine to prevent or treat bacterial infections. Degradation of antibiotics during municipal wastewater treatment is rarely complete, thereby creating a pathway for entry of these compounds into aquatic and terrestrial environments via wastewater reclamation (Ternes, 1998; Daughton and Ternes, 1999; Hirsch et al., 1999; Halling-Sørensen et al., 2000) and land application of biosolids (Ternes et al., 2005).

Antibiotics and their presence in the environment deserve major concern due to their proven adverse effects such as toxicity to green algae and microorganisms (Halling-

Sørensen et al., 2000), and enzyme inhibition (Levine et al., 1997). But most importantly, unlike to other emerging contaminants, antibiotics cause development of resistance in microorganisms when exist in the environment even at low concentrations (Halling-Sørensen et al., 2000).

Elimination of antibiotics in a complex system of wastewater treatment plant (WWTP) will depend on various parameters e.g. type of treatment process, presence of some other pollutants, and properties of compound (Byrns 2001). In a biological treatment process, the elimination of antibiotics is largely influenced by sorption processes rather than biodegradation (Kim et al., 2005). Previous studies proved that especially fluoroquinolones and tetracyclines are mainly removed from wastewater by sorption on the sludge (Giger et al., 2003; Golet et al., 2003; Kim et al., 2005). Both hydrophobic and electrostatic interactions of pharmaceuticals with particulate matter can be responsible for their adsorption (Trovo et al., 2008; Barnabe et al., 2009) as well as composition of the sludge (Carballa et al., 2006). Table 2.2 compiled the concentrations of various antibiotics found in sewage sludge samples.

Table 2.2. Occurrence data of antibiotics in sludge

| <b>Antibiotic Group</b> |                     | <b>Concentration<br/>(mg/kg DM)</b> | <b>Reference</b>           |
|-------------------------|---------------------|-------------------------------------|----------------------------|
| <b>Fluoroquinolone</b>  | Ciprofloxacin       | 0.05-4.8                            | Harrison et al., 2006      |
|                         |                     | <0.8                                | Diaz Cruz et al., 2009     |
|                         |                     | <10.8                               | McClellan and Halden, 2010 |
|                         |                     | 2.2±0.4                             | Golet et al., 2003         |
|                         |                     | 1.4-2.4                             | Giger et al., 2003         |
|                         | Norfloxacin         | 0.01-4.2                            | Harrison et al., 2006      |
|                         |                     | <0.42                               | McClellan and Halden 2010  |
|                         |                     | 2.1±0.1                             | Golet et al., 2003         |
|                         |                     | 1.4-2.4                             | Giger et al., 2003         |
|                         | Ofloxacin           | <0.01-2                             | Harrison et al., 2006      |
|                         |                     | <0.55                               | Diaz Cruz et al., 2009     |
|                         |                     | 5.4 ±1.9                            | McClellan and Halden, 2010 |
| <b>Tetracycline</b>     | Doxycycline         | <1.2-1.5                            | Harrison et al., 2006      |
|                         | Tetracycline        | 1-2                                 | McClellan and Halden, 2010 |
|                         | Minocycline         | 1-2                                 | McClellan and Halden, 2010 |
|                         | 4 4-epitetracycline | 1-2                                 | McClellan and Halden, 2010 |
| <b>Triclosan</b>        | Triclosan           | ND-15.6                             | Harrison et al., 2006      |
| <b>Sulfanomide</b>      | Sulfapyridine       | 0.028±0.003                         | Göbel et al., 2005         |
|                         | Sulfamethoxazole    | 0.068 ± 0.02                        | Göbel et al., 2005         |
| <b>Trimethoprim</b>     | Trimethoprim        | 0.041 ± 0.015                       | Göbel et al., 2005         |
| <b>Macrolide</b>        | Azithromycin        | 0.064 ± 0.03                        | Göbel et al., 2005         |
|                         | Clarithromycin      | 0.067 ±0.028                        | Göbel et al., 2005         |

Although they are generally discharged in very low concentrations, the total concentration of antibiotics may be toxicologically significant (Lindberg et al., 2007).

Studies performed on antibiotic toxicity indicate that amino glycosides, macrolides, quinolones, sulphonamides, and tetracyclines pose high environmental risk by exhibiting toxic effects to cyanobacteria and green algae at one g/L and few mg/L concentrations, respectively (Halling-Sørensen et al., 2000; Giger et al., 2003; Turkdogan and Yetinmezsoy, 2009) It is proved that sulfapyridine and oxytetracycline antibiotics at environmentally relevant concentrations inhibit microbial activity by 10% in top soils and continuous exposure of tetracycline to soil result in increased levels of tetracycline-resistant bacteria (Thiele-Bruhn and Beck, 2005). High mobility of some antibiotics can create a risk for aquatic environment while immobile antibiotics in soil fertilized with manure or biosolid create a risk for plant uptake and soil microorganisms in addition to resistance development (Kümmerer, 2004). As in some countries, the occurrence of various antibiotics has been detected in the soil amended with manure in Turkey (Cengiz et al., 2010; Karcı and Balcıoğlu, 2009). On the other hand, high sorption capacity of sludge and soil amended with manure reduce the availability of them for biodegradation and hence their accumulation is apparent (Clarke and Smith, 2011).

## **2.2. Fate of Emerging Pollutants in Sludge Treatment**

For a conventional wastewater treatment plant, primary and secondary sludge or both are main produced solid products. Primary sludge is produced through the mechanical wastewater treatment process and consists of particulate wastewater contaminations including a high portion of organic matters (faeces, vegetables, fruits, textiles, paper etc). On the other hand, secondary sludge is produced throughout the biological process and the mechanism is simply conversion of soluble organics into biomass, in other words particulate matter. Secondary sludge is separated from liquid phase in the secondary clarifier and the dense solids are called activated sludge.

Martin et al. (2010) observed in their study that 13 of 16 pharmaceuticals were still detectable in primary and secondary sludge samples. They observed that in primary sludge highest concentrations were of ibuprofen and salicylic acid (4100 µg/ kg DM and 350 µg/kg DM, respectively), whereas antibiotics and beta blockers were observed at lower concentrations. In secondary sludge, carmazepine showed highest concentration (266 /kg DM) and 12 pharmaceuticals increased in concentration from primary to secondary sludge.

Electrostatic interactions and difference in organic fractions in primary and secondary sludge caused different concentrations of different compounds in them.

Ternes et al. (2005) claimed that different  $K_d$  values of pharmaceuticals and characteristics of sludge affect sorption behaviors. For example, Golet et al. (2003) observed that higher organic content of secondary sludge resulted in higher adsorption ( $K_d$  of 20 L/g SS) of ciprofloxacin antibiotic when compared to primary sludge ( $K_d$  of 2 L/g SS).

### **2.2.1. Efficiency of Conventional Sludge Stabilization Processes for Emerging Pollutants**

The expansion of wastewater treatment plants and increasing restricted regulations lead to increased production of sludge with requirement of sludge treatment and disposal (Liu et al., 2011). Not only quantities but also highly concentrated pollution content of sludge makes its handling important. Hence sludge treatment and disposal processes constitute a wide portion of wastewater treatment cost. Although formerly generated sludge during wastewater treatment was wasted by direct land filling, today various sustainable sludge management targets are being followed and advanced. Treated sludge is used for agricultural purposes as fertilizer or soil conditioner as well as energy production purposes with its high methane gas potential. Sludge produced by wastewater treatment needs further treatment aiming mainly reduction of water and pathogen content. Sludge volume minimization and pollution prevention are other purposes included in sludge management applications. Main sludge stabilization processes are aerobic and anaerobic digestion; composting and lime stabilization.

Recent studies revealed that conventional stabilization methods are not efficient enough for treatment of recalcitrant pollutants alone because of their strong sorption capacities on sludge and slow rates of biodegradation (Wilson et al., 1997; Trovo et al., 2008).

2.2.1.1. Aerobic Digestion. The digestion process is one of the most important sludge management practices used for sludge stabilization in wastewater treatment plants. Aerobic

digestion of excess biological sludge occurs in the presence of oxygen and under endogenous conditions (Ros, 1993). Aerobic heterotrophic microorganisms degrade and utilize organic material by turning them into metabolic energy for cell synthesis and maintenance. Compared to anaerobic digestion, the process is expensive due to the high energy requirement associated with continued aeration (Bernard and Gray, 2000). Hence, aerobic digestion is more suitable for low capacity treatment plants where anaerobic digestion is no longer economic. In case of auto thermal thermophilic aerobic digestion, generated sludge has high quality like humus and can be called as Class A biosolid (US EPA, 1990).

Recalcitrant compounds are not completely degraded during aerobic digestion. Banat et al. (2000), investigated degradation of nonylphenol recalcitrant organic pollutant by aerobic digestion of sewage sludge. They observed up to 60% degradation of the compound within seven days, however further extension of the retention time to 10 days did not increase degradation more than 66%. Pham et al. (2011) investigated the degradation of bis(2-ethylhexyl)phthalate by aerobic digestion with and without pre-treatment steps. They observed 21% degradation of the target contaminant at the end of aerobic digestion period, however pre-treatment resulted in enhanced degradation efficiency. On the other hand, some antibiotics such as sulfonamides, fluoroquinolones, macrolides, and trimethoprim have been found in the digested sludge (Golet et al., 2002; Göbel et al., 2005; Kinney et al., 2006; Yang and Lin, 2009; Nieto et al., 2010). Incomplete degradation of antibiotics clearly indicates the requirement of pre-treatment (Song et al., 2010).

2.2.1.2. Anaerobic Digestion. Treatment of sewage sludge via anaerobic digestion has been performed widely due to its ability to produce biogas. It is suggested as most viable treatment method for sewage sludge (Rudd et al., 2003). The process is less stable and more complex to manage when compared to aerobic digestion (Tomei et al., 2011). Complexity of sludge characteristics makes hydrolysis rate-limiting step of sludge aerobic and anaerobic digestion leading to slow process (Vavilin et al., 1996; Tiehm et al., 2001; Appels et al., 2008). Consequently, longer sludge retention times are required for digestion of waste activated sludge.

A wide variety of substances such as heavy metals, ammonia, sulphide and organic chemicals are known to be inhibitory for anaerobic digestion when they are in substantial concentrations in the sludge. They may cause proton imbalance, methane production suppression, toxicity to bacteria by slowing down or destructing their growth (Chen et al., 2008). Additionally, heavy metals and organic pollutants in the sludge pose threat on environment and public health when land application or land filling is applied to the treated sludge (Cao and Pawlowski, 2012). These inadequacies emphasize necessity of pre-treatment of WAS prior to anaerobic digestion.

Previous studies reveal that anaerobic digestion is not efficient for emerging pollutants degradation by itself. Radjenovic et al. (2009) studied the degradation of pharmaceuticals including antibiotics. Most of pharmaceuticals present in primary sludge were found in detectable concentrations after anaerobic digestion of the sludge. On the other hand, Carballa et al. (2007) achieved the degradation of 12 PPCPs (Pharmaceuticals and Personal Care Products) at varying (between 0-99%) values during anaerobic digestion of sewage sludge. They suggested that the degradation of PPCPs is highly dependent on their physicochemical properties. In another study, anaerobic digestion of pre-treated sludge provided limited degradation of polycyclic aromatic hydrocarbons (PAH) (Bernal-Martinez et al., 2009). Anaerobic digestion also displayed low efficiency for estrogens despite of their low sorption capacity. Therefore, high estrogens were found in treated sludge (Muller et al., 2010). In order to eliminate the above mentioned imperfections of conventional digestion methods several pre-treatment methods were developed (Chang et al., 2011).

2.1.1.3. Composting. Composting is an aerobic process that involves mixing the wastewater solids with sources of carbon such as sawdust, straw or wood chips by making sure that the aeration helps accelerated aerobic degradation in order to produce compost. In the presence of oxygen, bacteria digest both the wastewater solids and the added carbon source, and a large amount of heat is produced (US, EPA).

Composting reduces sludge volume, greenhouse gas emissions, and also produces a useful soil improving compost (Himanen and Hannenen, 2011). The major drawback of

sewage sludge composting is the high heavy metal concentrations in the end-product (Fang and Wong, 1999).

Limited studies investigated antibiotic degradation in sludge during composting. Lillenberg et al. (2010) investigated the composting for degradation of antibiotics in sewage sludge. In this study, concentrations of fluoroquinolone antibiotic residues in compost were significantly lower (0-20 µg/kg DM) compared to untreated sewage sludge (2.6-111 µg/kg DEM). Besides the antibiotics, abatement of PAHs in sludge was investigated by Hua et al. (2008). After 50 days of composting, 50% of total 16 PAHs were degraded. However, in these studies degradation was incomplete despite of long composting period.

In addition to sludge, the composting of manure including veterinary antibiotics has been studied in some recent studies. Arıkan et al. (2007) investigated the effect of composting on OTC and OTC resistant bacteria in manure and concluded that degradation of OTC antibiotic was primarily related to abiotic processes which are temperature depended such as adsorption and degradation. They did not observe the toxic effect of this antibiotic on heterotrophic bacteria population. In this study the population of OTC resistant bacteria was reduced 10 times within 35 days where OTC concentration was decreased well below the detection limit. Recently, Kim et al. (2012) also applied composting to manure including antibiotics and they observed 96, 99 and 95% reduction in extractable tetracyclines, sulfonamides, and macrolides concentrations, respectively.

2.2.1.4. Lime Stabilization. Lime stabilization is another process where sludge is mixed with lime to raise the pH to alkali range (pH >12) in order to stop the growth of pathogens. For stabilization purpose, lime in the form of lime hydrated (calcium hydroxide) or quicklime (calcium oxide) are commonly used. These chemicals react with water and produce  $\text{Ca}(\text{OH})_2$  and heat (exothermic reaction) in isolated and closed reactors. The heat generation and the alkali pH are the decisive disinfecting factors reducing pathogens and offensive odour. Hydrated lime is also used as flocculant in sludge dehydration. On the other hand, there is not any study investigating the degradation of emerging organic pollutants with lime stabilization.

## **2.3. Sludge Pre-treatment Methods**

In order to enhance efficiency of sludge stabilization processes, pre-treatment is applied to sewage sludge in some cases. Mechanical, thermal, chemical, and seldom biological forces are applicable for the disintegration and cell disruption (Audrey et al., 2011). Sludge pre-treatment includes stressing of sewage sludge through external forces and leading to release of cell components and decrease in particle size extensively. Thereby, an improvement and acceleration of sludge stabilization, provision of internal hydrogen sources for the denitrification process, better sedimentation of the sludge, and reduced foaming problems are achieved (Muller, 2000).

### **2.3.1. Mechanical Pre-Treatment**

Mechanical methods are mainly applied to sludge in order to release intercellular organic material by cell disruption.

2.3.1.1. Stirred Ball Mill. Stirred Ball Mill is an efficient technique that destructs cell walls and disintegrate the microorganisms with a subsequent release of intercellular components (Elliott and Mahmood, 2007; Wett et al., 2010). This process enhanced biogas yield but its performance was not higher than that of thermo-pressure-hydrolysis process (Wett et al., 2010).

2.3.1.2. High pressure Homogenizer. Pressure impulses produced as a result of cavity collapse are responsible for cell disruption in high pressure homogenizer. By imploding the cavitation bubbles temperature attains several hundred degrees Celsius that induce local pressure peaks (Muller, 2000; Weemaes and Verstraete, 1998).

2.3.1.3. Mechanical Jet Technique. High pressurized sludge exits from an outlet that suppresses the pressure after exiting sludge particles hit a plate and rupture leading to cell lysis (Muller, 2000).

2.3.1.4. High Performance Pulse Technique. Electrical pulses with a frequent pulse durations (<10 milliseconds) are applied to compose shock waves resulting in cell

disintegration. Frequent pulses with high frequency are applied due to the high inertia of organic matter (Muller, 2000).

### **2.3.2. Thermal Pre-Treatment**

Thermal pre-treatment allows significant reduction of excess sludge production. Heat treatment, in the moderate temperature range from 60 to 180 °C can be applied to WAS to destruct the cell wall. However, independent of the temperature, heat application results in solubilization and hydrolysis of particulate matter. Audrey et al. (2011) reported that thermal pre-treatment below 100°C leads to solubilization of intercellular structure by cell destruction; whereas significant floc destruction is not achieved. Improvement of substrate availability for anaerobic digestion and increased methane production rate were advantages of thermal pre-treatment.

McNamara et al. (2012) investigated the effect of thermal heat treatment on degradation of nonylphenols and it was found that the high temperature and pressure conditions used for thermal pre-treatment were not sufficient to degrade nonylphenols in sludge similarly to bisphenol A and triclosan.

Thermal treatment is generally applied to sludge by combining the action of temperature and acid/alkali treatment. However, there is not any study for the application of this combined process for the antibiotics destruction in WAS.

### **2.3.3. Alkali-Acid Pre-Treatment**

Alkaline pre-treatment is a commonly examined method whose mechanism is to induce swelling of particulate organics at high pH, making the cellular substances more susceptible to enzymatic reaction (Baccay and Hashimoto, 1984; Na et al., 2011). When properly executed alkaline treatment can provide a well-disinfected sludge that neither attracts vectors nor emits unpleasant odors.

The efficiency of alkali treatment for organic matter solubilization depends upon the applied alkaline dose. The efficiency of this treatment was investigated for PPCPs and it

was found that the degradation efficiency varied between 20 and 80% depending upon the type of the contaminant (Carballa et al., 2006; Eskicioglu et al., 2009; Chang et al., 2011; Na et al., 2011).

In recent years, alkaline pre treatment has been combined with other disintegration methods such as thermal, ultrasonic, and microwave treatments to achieve higher disintegration degrees. Liu et al. (2008) observed that among all combinations the ultrasonic-alkaline combination yielded the best performance by the solubilization of 38% of total solids content. Kim et al. (2010) applied alkali-US combined pre-treatment to WAS and achieved significantly increased methane yield in anaerobic digestion with low intensity alkaline (pH 9)/ultrasonic (7500 kJ/kg TS) treatment. However, 20% increased soluble-N concentration resulted in additional load for nitrogen degradation.

Acid pre-treatment involves disintegration of sludge particles by the addition of acids and the recommended pH is about two. In recent years, acid hydrolysis of sludge has been studied showing that the effect of pH, temperature, and reaction time are of primary importance. The potential of acid hydrolysis to enhance the dewaterability of sewage sludge was also observed (Neyens et al., 2003). Devlin et al. (2011) investigated the effect of acid pre-treatment on anaerobic digestion performance and dewatering. Pre-treatment at pH 2 resulted in increased soluble carbohydrate, protein and COD in WAS. Similar to alkaline treatment, by the application of acid pre-treatment methane yield and rate of gas production was increased.

#### **2.3.4. Ozone Oxidation Pre-Treatment**

The effect of ozone oxidation on the characteristics of waste activated sludge was investigated by several studies (Yasui and Shibata, 1994; Goel et al., 2003; Weemaes et al., 2000; Elliott and Mahmood, 2007; Bernal-Martinez et al., 2007; Carballa et al., 2007).

Similar to the other pre-treatment methods ozone has ability to rupture the cell walls and hence it enhances the sludge solubilization by producing smaller weight compounds (Bablon et al., 1991). Mineralization of the released cellular components may also be achieved by the ozonation (Elliott and Mahmood, 2007).

The degradation of polycyclic aromatic hydrocarbons in sewage sludge by ozonation was investigated to enhance the subsequent biological treatment efficiency (Bernal-Martinez et al., 2007).

There is only one study related with the performance of pre-ozonation on the degradation of pharmaceuticals. Carballa et al. (2007) observed that ozone pre-treatment enhanced degradation of carmazepin antibiotic up to 60% while no elimination was achieved with anaerobic digestion by itself. On the other hand, ozonation did not enhance degradation efficiency of other PPCPs due to their high sorption characteristics.

Patureau et al. (2008) applied ozone as post treatment following the anaerobic digestion. They observed that ozone treatment yielded two times higher degradation efficiency for nonylphenol ethoxylates when compared to single digestion operation.

### **2.3.5. Ultrasonic Pre-Treatment**

Similar to ozonation, recent studies related with sludge solubilization have been focusing on ultrasonic treatment which is considered as a powerful method. This treatment has full-scale applications for the purpose of excess sludge pre-treatment and enhancing dewaterability before aerobic/anaerobic digestion (Neyens et al., 2003; Elliot and Mahmood, 2007; Nickel and Neis, 2007). The process has been also used for cell rupturing and release of intracellular materials by means oxidants leading to break down of recalcitrant compounds (Adewuri, 2001). Ultrasonic pre-treatment requires high energy leading to the need of combined applications with other methods (Zhang et al., 2008; Kim et al., 2010).

Bougrier et al. (2006) compared the effects of ultrasound, thermal treatment, and ozone on sludge solubilization and anaerobic biodegradability. For ultrasonic treatment they observed that particle size and viscosity were reduced leading to an enhanced filterability in addition to higher biodegradability.

Pham et al. (2011) observed that ultrasonic pre-treatment increased the degradation of bis(2-ethylhexyl)phthalate in the subsequent aerobic digestion from 21 to 40% suggesting

that pre-treatment of sludge by ultrasonication is effective in improvement of pollutant degradation.

#### **2.4. Fenton Oxidation Pre-Treatment of WAS**

Fenton technology is an advanced oxidation process (AOP) that can oxidize organic compounds efficiently. Mixture of  $\text{H}_2\text{O}_2$  oxidant and ferrous iron ( $\text{Fe}^{+2}$ ) is called as Fenton's reagent; reaction between  $\text{Fe}^{+2}$  catalyst and  $\text{H}_2\text{O}_2$  generates hydroxyl radicals ( $\text{OH}\cdot$ ).

Fenton oxidation was applied to various matrices to degrade the recalcitrant pollutants (wastewater, soil, sediment, and sludge) (Lu et al., 2003; Flotron et al 2005; Tekin et al., 2006). Depending upon the type and concentration of the contaminant, the degradation efficiency may differ. However, the application of excess hydrogen peroxide provided efficient degradation of contaminants (Flotron et al., 2005).

In recent years, there has been various studies on Fenton oxidation of WAS for different purposes. Literature about the application of Fenton oxidation on WAS are listed in Table 2.3.

Table 2.3. Fenton Oxidation Applications on WAS

| Study                      | Operational Conditions  | Observations  |
|----------------------------|---|---|
| Neyens et al. (2003)       | 5 g H <sub>2</sub> O <sub>2</sub> /kg TS, 1.67 g Fe <sup>+2</sup> /kg TS at pH 3.   | 30% improved dewaterability. Decreased CTS to 40 s.   |
| Flotron et al.( 2005)      | 4.9 M H <sub>2</sub> O <sub>2</sub> and 0.01 M Fe <sup>+2</sup> no pH arrangement.  | High PAH degradation from sludge samples. High influence of matrix characteristics on Fenton oxidation of PAHs.   |
| Dewil et al. (2007)        | 0.07 g Fe <sup>+2</sup> / g H <sub>2</sub> O <sub>2</sub> and 50 g H <sub>2</sub> O <sub>2</sub> /kg DS at pH 3.  | Significant release of Cd, Cu, Ni and Zn metals. 26.6% reduction in VS in of biological sludge by Fenton Process.   |
| Beauchesne et al. (2007)   | 0-31 g/kg TS H <sub>2</sub> O <sub>2</sub> and 0-5.7 [Fe <sup>+2</sup> ]/[H <sub>2</sub> O <sub>2</sub> ] ratio at pH 2.5.                                      | 4-25% COD solubilization. 30-80% of metal release.  |
| Zheng et al. (2007)        | 92.5 g H <sub>2</sub> O <sub>2</sub> /g TS, 12 mL FeSO <sub>4</sub> at pH 2,08.   | 30% total degradation of PAH species by METIX-AC treatment.   |
| Lo et al. (2008)           | 0.02 [Fe <sup>+2</sup> ]/ [H <sub>2</sub> O <sub>2</sub> ] and 300 g H <sub>2</sub> O <sub>2</sub> /kg TS at pH 6.5   | 9% COD solubilization. Increased orthophosphate (88.1mg /L) and soluble ammonia 22.7 (mg /L) concentrations.  |
| Kaynak and Filibeli (2008) | 0.067 g Fe <sup>+2</sup> / g H <sub>2</sub> O <sub>2</sub> and 60 g H <sub>2</sub> O <sub>2</sub> /kg DS at pH 3.   | 1.53 times greater VS reduction(25.4%) in the mesophilic reactor fed with Fenton processed sludge in 20 days.   |
| Erden and Filibeli (2010)  | 60 g H <sub>2</sub> O <sub>2</sub> /kg DS, 4.02g Fe <sup>+2</sup> /kg DS and pH 3.  | Higher solid reduction and higher methane production for each experiment. Decrease of biosolids' resistance to dewatering in terms of capillary suction time (CST). |
| He and Chao-Hai (2010)     | 8000 mg/L H <sub>2</sub> O <sub>2</sub> , 200 mg/L Fe <sup>+2</sup> at acidic pH.   | Increased SCOD from 38 to 2213 mg/L in 60 minute and decreased COD to 1800mg/L by oxidation of dissolved matter at the end of two hours reaction period.            |
| Pham et al. (2010)         | 0.01 mL H <sub>2</sub> O <sub>2</sub> /g SS, 150 [H <sub>2</sub> O <sub>2</sub> ] <sub>0</sub> /[Fe <sup>+2</sup> ] <sub>0</sub> , 25g/L TS, at pH 3 and 25 °C. | High solubilization (70%) and biodegradation (74%)  |
| Abe et al., 2011           | 0.1 g/L Fe <sup>+2</sup> and 15 g/l H <sub>2</sub> O <sub>2</sub> at different pH and temperatures.   | VSS degradation of 50-55%. Higher VSS degradation at 105 °C than 50 °C.   |

### **3. MATERIALS AND METHODS**

#### **3.1. Materials**

##### **3.1.1. Source of Waste Activated Sludge**

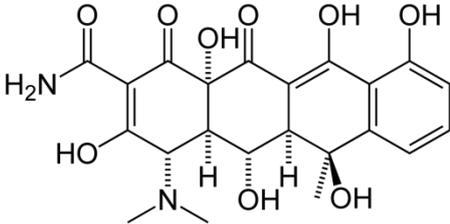
WAS samples were collected from Paşaköy municipal advanced biological wastewater treatment plant, Istanbul (from October 2010 to July 2011). The plant has a wastewater treatment capacity of 500,000 m<sup>3</sup>/day and serves about 2,500,000-population equivalent. The treatment plant consists of inlet 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, return of dewatering supernatant to the distribution chamber. The A2/O process (anaerobic unit followed by anoxic and then aerobic units) is applied to degrade 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. Following sampling, sludge was settled for three or four hours at 4°C and supernatant was discarded. Hence, the total solids content of the sludge was concentrated from 7-12 g/L to 20 g/L. Concentrated sludge sample was stored at +4 °C for a maximum storage time of about one week. For some of the oxidation experiments total solids content of stock sludge was diluted with deionized water.

##### **3.1.2. Chemicals**

A tetracycline group antibiotic, oxytetracycline (OTC, Sigma) was selected as the model pollutant. Chemical structure and some properties of OTC are listed in Table 3.1.

Table 3.1. Structure and properties of oxytetracycline antibiotic

|  |  |
|--|--|
| <b>Chemical Structure</b>                            |  |
| <b>Molecular Weight (g/mol)</b>                      | 496.891  |
| <b>Solubility (g/L)</b>                              | 6.9  |
| <b>Sorption Coefficient, <math>K_d</math> (mg/L)</b> | 3020   |

All the chemicals used for treatment and analysis purposes in this study were reagent grade.

## 3.2. Methods

### 3.2.1. Procedure of Sludge OTC Spiking

Prior to spiking, 5 g/L sodium azide ( $\text{NaN}_3$ , Merck) was added to sludge in order to inhibit possible biodegradation of the antibiotic. A stock solution of OTC with 100 mg/L concentration was prepared and then, OTC solution added to the sludge sample to attain 5 mg/L concentration in it. Spiked sludge samples were equilibrated at 150 rpm in a temperature controlled shaker (Julabo SW22) over night in order to ensure its sorption on sludge particles.

### 3.2.2. Experimental Procedure for Fenton and Fenton-like Oxidations

The pH value of 300 mL sludge samples was adjusted to 3 by the addition of 1 N  $\text{H}_2\text{SO}_4$  (Sigma-Aldrich). Then ferrous or ferric iron taken from the stock solution of iron(II)sulphate  $\text{FeSO}_4$  (Reidel de Haen) or iron(III)chloride (Sigma-Aldrich) were added to acidified samples. Finally, oxidation reaction was initiated by adding the desired dose of  $\text{H}_2\text{O}_2$  (Reidel de Haen, 30% w/w) from the stock solution (30% w/w).

Sludge samples were mixed at 200 rpm during Fenton oxidation. Fenton oxidation was performed at constant temperature. Dosage of Fenton reagent, temperature, and the TS content of the sludge were the investigated parameters of Fenton oxidation. The treatment time chosen based on the results of screening experiment was 120 minutes. Preparation of OTC solution, spiking of sludge with antibiotic and treatment of sludge with Fenton oxidation were performed in dark in order to prevent photo-degradation of OTC.

### 3.2.3. Analytical Methods

In order to evaluate antibiotic degradation in sludge by the application of Fenton oxidation, sludge samples (particulate and dissolved portions together) were subjected to analysis. On the other hand, dissolved COD, dissolved metals, and dissolved nutrients were analyzed in treated sludge samples that were filtered through 0.45  $\mu\text{m}$  Millipore filter papers following centrifugation (by Eppendorf Centrifuge 5804) at 4500 rpm for 5 minutes. Besides, total COD, total metals and nutrients were determined after digestion of the sludge samples. All measurements were performed in duplicate except COD analysis which was carried out in triplicate.

3.2.3.1. Extraction of Oxytetracycline. Extraction of OTC in sludge samples was performed in accordance with the USEPA method (821-R-08-002, Method 1694) by following the main steps:

- a) extraction on a vortex (Nüve NM 110) for 30 s by the addition of 2.5 mL of extraction buffer (0.1 M EDTA: McIlvaine buffer (60 ml of 0.2 M citric acid + 40 ml of 0.4 M  $\text{Na}_2\text{HPO}_4$ ), 1:1) and 2.5 mL of MeOH,
- b) ultrasonic extraction (Sonorex super RK 510, Morfelden-Walldorf, Germany) for 10 minutes,
- c) centrifugation at 4500 rpm for 5 minutes (Eppendorf 5804).

This procedure (from a to c) was repeated for 3 times and supernatants of each extraction were collected into 500 ml erlenmeyer. After that, the whole solution was diluted to 400 ml with Milli-Q water in order to reduce methanol content below 2%, and the pH of solution was adjusted to 2.9 by the addition of  $\text{H}_3\text{PO}_4$  (Riedel de Haen).

Solid phase extraction (SPE) and following HPLC analysis were carried out according to a method previously used for manure study (Uslu, 2009). Diluted extracts were passed through SPE cartridges which were SAX (strong anion exchange, 500 mg sorbent, 6 ml capacity; Phenomenex, Torrence, CA) and HLB (200 mg sorbent, 6 ml capacity; Waters). They were set up in tandem and conditioned by methanol and McIlvaine buffer. After passing the entire sample at a loading rate of about 5 mL/min, SAX cartridge was degraded and the HLB cartridge was washed with 5 mL of McIlvaine buffer, 5 mL of 0.1M NaOAc, 5 mL of Milli-Q water, and 2 mL of 20% MeOH. HLB cartridge was dried for 10 minutes to minimize moisture in it and then eluted with 2 ml methanol for the analysis in HPLC system (Agilent Technologies 1100 series, Santa Clara, CA). All chemicals used for antibiotic extraction purposes were purchased from Merck.

3.2.3.2. HPLC Analysis. Consequent analysis of OTC was performed by HPLC using an analytical column (Eclipse XDB C18 equipped with ODS guard column). Gradient elution was carried out with acetonitrile (solvent A, Sigma Aldrich) and water (Milli-Q) containing 0.1% formic acid (solvent B). Elution started with A:B:10:90 rising linearly to 40:60 from 0 to 5 minutes and then returning to initial composition from 5 to 8 minutes. Detection of OTC was achieved at 356 nm with a retention time of 5.9 minutes. A calibration curve was prepared by using OTC standards which were obtained by making dilution of 100 ppm stock solution. HPLC chromatograms of OTC in untreated and Fenton treated sludge samples are represented in Appendix A. Schematic diagram of OTC extraction, SPE steps and HPLC analysis are represented in Figure 3.1.

In order to assess the performance of analysis method recovery study was performed and the recovery ratio of OTC from the sludge was calculated by using the following equation:

$$\text{Recovery (\%)} = \frac{\text{OTC conc. quantified by HPLC analysis (mg/kgTS)}}{\text{spiking level (mg/kgTS)}} \times 100 \quad (3.1)$$

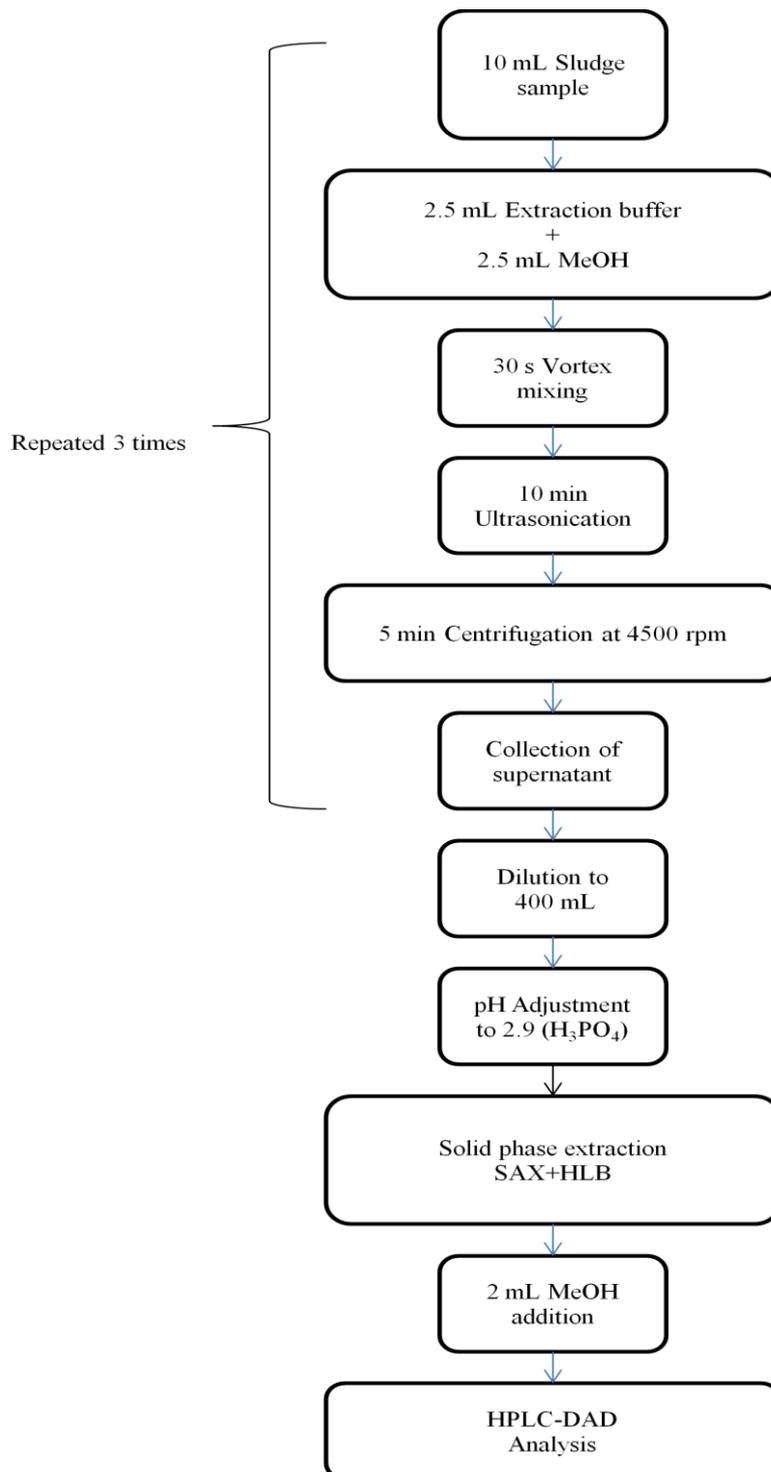


Figure 3.1. Schematic diagram of antibiotic extraction and SPE steps.

**3.2.3.3. Solids Analysis.** Solid concentration of WAS was determined in accordance with Standard Methods (APHA/AWWA/WPCF, 1995) by using the methods from 2540B to 2540E. In order to determine suspended solids content, sludge samples were filtered through 0.45  $\mu\text{m}$  Millipore filter papers which were dried at 105  $^{\circ}\text{C}$  for 24 hour in drying

oven (Fn 500). Volatile or fixed solids contents were determined by the ignition at 550 °C for one hour.

3.2.3.4. Chemical Oxygen Demand (COD) Analysis. COD measurements were conducted in accordance with Standard Methods (APHA/AWWA/WPCF, 1995) by closed reflux colorimetric method. Samples and standards that were prepared by using standard solutions of KHP with known COD values (100-1000 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.

In order to eliminate H<sub>2</sub>O<sub>2</sub> interference for COD analysis manganese oxide (MnO, Merck) was added to sludge samples and they were kept for 30 minutes to destruct remaining H<sub>2</sub>O<sub>2</sub>. Solubilization degree of organic matter was calculated by using the equation 3.2.

$$\text{Solubilization degree} = (\text{SCOD}_f - \text{SCOD}_i) / \text{tCOD}_i \quad (3.2)$$

where,

TCOD<sub>i</sub> = initial total COD, (mg/L)

SCOD<sub>i</sub> = initial soluble COD, (mg/L)

SCOD<sub>f</sub> = final soluble COD, (mg/L)

3.2.3.5. Dissolved Organic Carbon (DOC) Analysis. DOC determination was performed by a TOC (Shimadzu TOC-V CSH Analyzer) analyzer operating in the non-purgeable organic carbon (NPOC) mode. The instrument was calibrated by standard solutions of KHP (10-1000 ppm). After dilution, already filtered samples were acidified to pH 1-2 with HCl and purged for 1.5 minutes prior to injection with instrument grade air to ensure that all carbonate, bicarbonate and carbonic acid are degraded as carbon dioxide from the solution.

3.2.3.6. Total Kjeldhal Nitrogen (TKN) and Ammonia Analysis. After the digestion of samples by using digestion apparatus (Hach Digesdahl digestion apparatus) 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. Quantitative analysis of TKN was carried by Hach spectrophotometer (Hach DR/2010). TKN value was calculated by equation 3.3.

$$\text{TKN (mg/L)} = (\text{A} \times 75) / (\text{B} \times \text{C}) \quad (3.3)$$

where,

A= reading from instrument, (mg/L)

B= sample volume used for digestion, (mL)

C= digested sample volume used for analysis, (mL)

3.2.3.7. Total and Soluble Phosphorous Analysis. Samples were digested in accordance with Nessler Method by digestion apparatus (Hach Digesdahl). Soluble and total phosphate determination was 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, 10 mL. Phosphorous concentration was calculated by using equation 3.4.

$$\text{Total P (mg/L)} = (\text{A} \times 2500) / (\text{B} \times \text{C}) \quad (3.4)$$

where,

A= reading from instrument, (mg/L)

B= sample volume used for digestion, (mL)

C= digested sample volume used for analysis, (mL)

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

3.2.3.9. Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Analysis. H<sub>2</sub>O<sub>2</sub> concentration was determined in accordance with iodometric method (Gordon et al., 1992). Samples were titrated with sodium thiosulfate solution (0.1 N) in the presence of potassium iodide (0.1 N) and acid mixture (prepared by dissolving 0.18 g of ammonium molybdate in 750 mL water and

slowly adding 300 mL of H<sub>2</sub>SO<sub>4</sub>). End point of titration was determined by starch indicator.

3.2.3.10. Metal Analysis. Total and soluble metal concentrations in raw and treated sludge samples were measured by ICP analysis (Perkin-Elmer Optima 2100 DV). Total concentration of metals was measured after application of microwave digestion (Berghof Speed Wave Microwave) in accordance with Standard Methods (APHA/AWWA/WPCF, 1995) and soluble metals were determined in filtered samples. Metal release was calculated by using following equation;

$$\% \text{ Metal Release} = (sMe_f - sMe_i) / tMe_i \quad (3.5)$$

where,

tMe<sub>i</sub> = initial total Metal Concentration, (mg/L)

sMe<sub>i</sub> = initial soluble Metal Concentration, (mg/L)

sMe<sub>f</sub> = final soluble Metal Concentration, (mg/L)

3.2.3.11. Settled Sludge Volume (SSV) Measurement. SSV measurement was conducted in accordance with standard methods (APHA/AWWA/WPCF, 1995). Raw or treated sludge of 1 L was kept in graduated cylinder for 60 minutes and volume of settled sludge was recorded in definite time intervals.

3.2.3.12. Viscosity Measurement. Viscosity of 500 mL sludge samples were measured by using Brookfield Viscometer with an operating speed of 100 rpm.

3.2.3.13. Capillary Suction Time (CST) Measurement. Filterability of raw and treated sludge were compared with CST measurement by Venture Innovation CST measurement instrument. The capillary suction apparatus consisted of a sludge column contained in the sample cylinder which is centered in the middle in the two of concentric electrodes at diameter D1 and D2 resting on a Whatman-17 filter paper. A timing device is started when the waterfront reaches the inner electrode at D1 and is stopped when the water reaches the outer electrode at D2. The time elapsed is the CST. For each sample CST value was measured at six times and an average CST was calculated.

3.2.3.14. Particle Size Distribution Measurement. Particle size distribution was measured by using Malvern Mastersizer 2000 device (with the wet dispersion unit of Hydro2000MU). During the measurement stirrer and pump speed were kept at 600 rpm, which is the minimum pump/stirrer speed available, to minimize the damage of sludge particles. Diluted sludge sample in the dispersion tank was introduced into the measuring cell. Particle sizes of sludge samples were measured four times and average values were calculated.

## 4. RESULTS AND DISCUSSION

### 4.1. Characterization of Waste Activated Sludge

To evaluate the applied Fenton oxidation performance and to determine the occurrence of the selected antibiotic, the chemical characteristics of waste activated sludge (WAS) samples was investigated. In Table 4.1 each parameter for various samples is presented as a range.

Table 4.1. Chemical characteristics of raw waste activated sewage sludge

| <b>Parameter</b>              | <b>Range</b> | <b>N*</b> |
|-------------------------------|--------------|-----------|
| <b>TS (mg/L)</b>              | 7,000-12,100 | 15        |
| <b>VS (mg/L)</b>              | 4,200-8,500  | 15        |
| <b>VS/TS</b>                  | 0.6-0.7      | 15        |
| <b>TSS (mg/L)</b>             | 6,100-10,000 | 15        |
| <b>VSS (mg/L)</b>             | 2,900-6,500  | 15        |
| <b>TCOD (mg/L)</b>            | 7,200-11,000 | 15        |
| <b>SCOD (mg/L)</b>            | 50-160       | 15        |
| <b>BOD<sub>5</sub> (mg/L)</b> | 91           | 1         |
| <b>DOC (mg/L)</b>             | 7.5-9.1      | 4         |
| <b>Ph</b>                     | 5.9-6.7      | 15        |
| <b>TKN (mg/L)</b>             | 376-408      | 2         |
| <b>TP (mg/L)</b>              | 57-68        | 2         |
| <b>Soluble P (mg/L)</b>       | 9.7-11.5     | 2         |

N\*=number of the samples

As it is seen from the table, WAS has high total COD (TCOD) value which was used to estimate the organic content of the sludge and about 1% of TCOD is in dissolved portion. Average VS/TS ratio was 0.65 which is typical value for untreated activated sludge (Metcalf and Eddy, 2002). In Paşaköy wastewater advanced treatment plant, advanced biological phosphorous degradation process is applied to wastewater. As

consequence, high phosphorous concentration with low soluble portion (9.7-11.5 mg/L) of WAS was determined.

Before the application of Fenton oxidation process, total concentrations of six heavy metals were determined in the sludge to evaluate their solubilizations since the heavy metal content of WAS is a regulated parameter for its ultimate disposal and it can also cause upset in the anaerobic digestion of the sludge. Table 4.2 compiled the concentrations of these metals and their permissive levels regulated by US EPA (40 CFR Part 503), EU (Directive 86/278/EEC), and Turkish authorities (Regulation on Application of Sewage Sludges to Soil) when sewage sludge is applied to soil.

Table 4.2. Concentrations of metals in the sludge and their permissive levels

| Metal     | Permissive Levels |                  |                        | Sludge used<br>in this study |
|-----------|-------------------|------------------|------------------------|------------------------------|
|           | EU Directives     | US EPA<br>Guides | Turkish<br>Regulations |                              |
|           | mg/kg DS          |                  |                        |                              |
| <b>Cr</b> | -                 | 3,000            | 1,200                  | <b>293</b>                   |
| <b>Ni</b> | 300-400           | 420              | 400                    | <b>149</b>                   |
| <b>Cu</b> | 1,000-1,750       | 4,300            | 1,750                  | <b>203</b>                   |
| <b>Zn</b> | 2,500-4,000       | 7,500            | 4,000                  | <b>721</b>                   |
| <b>Cd</b> | 20-40             | 85               | 40                     | <b>ND</b>                    |
| <b>Pb</b> | 750-1200          | 840              | 1,200                  | <b>ND</b>                    |
| <b>As</b> | -                 | 75               | -                      | <b>*</b>                     |
| <b>Hg</b> | 16-25             | 57               | 10                     | <b>*</b>                     |
| <b>Mo</b> | -                 | 75               | -                      | <b>*</b>                     |
| <b>Se</b> | -                 | 100              | -                      | <b>*</b>                     |

ND: under the detection limit; \*: Not measured

As Table 4.2 demonstrates, heavy metal contents of the raw sludge are below the limit values that are set by relevant directives and regulations. In addition to heavy metals presented in the table, iron and manganese contents of WAS are 5.6 g/kg TS and 0.45 g/kg TS, respectively.

The occurrence of OTC, which was used as model pollutant to evaluate the performance of Fenton oxidation, was determined in the WAS samples. Although the presence of antibiotics in sludge is of particular interest due to widespread occurrence of them, regulations and guides that were prepared by EU commission or U.S Environmental Agency do not set any limit value for this pollutant group. As result of analyses in 13 different WAS samples, OTC concentration was found below the detection limit of applied analysis method in which LOD and recovery values were 0.01 mg/g and 86%, respectively.

#### **4.2. Effect of Fenton Oxidation on Antibiotic Degradation and Solubilization**

Fenton oxidation was applied to synthetically contaminated waste activated sludge (40 mg OTC/kg TS) at ambient temperature by varying  $\text{H}_2\text{O}_2$  dosage,  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio, process temperature, and solid content of the sludge in order to investigate its performance. Relatively high spiking level of OTC was preferred in order to understand effect of the investigated parameters more accurately. While the primary objective of this study is to degrade OTC from the sludge, the solubilization of organics, metals, and nutrients were also investigated in order to clarify the fate of antibiotic during Fenton oxidation.

The kinetics of OTC degradation and COD solubilization were primarily screened. For this purpose, WAS with 20 g/L TS (2% w/w) was treated by 150 g  $\text{H}_2\text{O}_2$ /kg TS (0.27% w/w) and  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio of 0.4 at pH 3 as a function of treatment time. Fenton oxidation was performed at pH 3 because it is known to be most suitable pH value for the formation of radicals (Pignatello et al., 1992). Considering the results of previous studies (Zheng et al., 2007; Neyens et al., 2003; Tokumura et al., 2009) overall treatment period was selected as 240 minutes. OTC degradation and COD solubilization are represented as a function of treatment time in Figure 4.1. The results given for antibiotic involve both soluble and adsorbed concentrations whereas COD values indicate those found in supernatant of the sludge (SCOD).

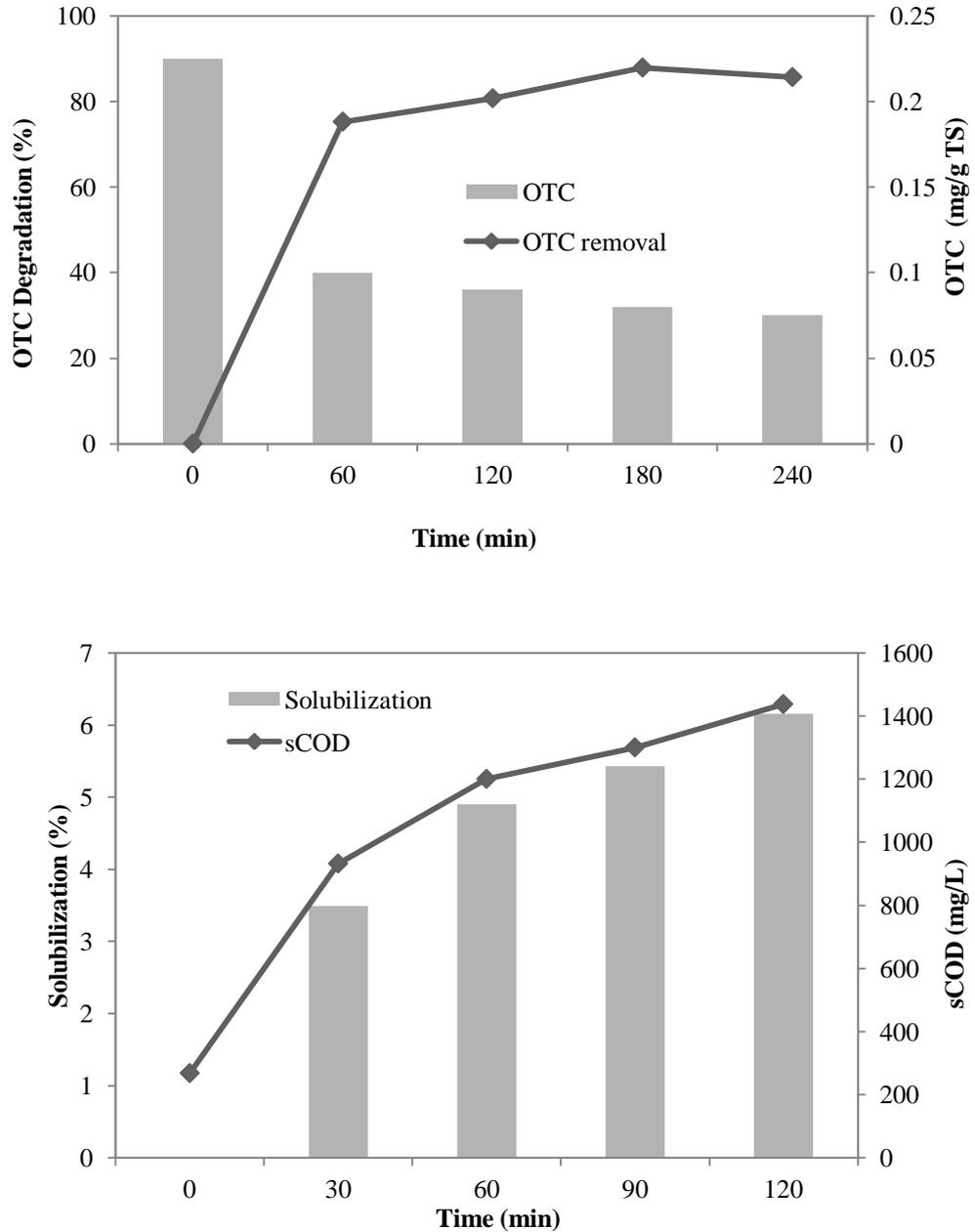


Figure 4.1. Change in OTC degradation and COD solubilization with time. Experimental conditions:  $\text{H}_2\text{O}_2=3000$  mg/L;  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]=0.4$ ; TS = 20 g/L;  $\text{pH}_i=3$ ;  $T=25$  °C.

OTC was drastically degraded by the application of Fenton's reagent and most of the antibiotic degradation (75%) occurred within 60 minutes at which 94% of initially added  $\text{H}_2\text{O}_2$  was rapidly utilized (Figure 4.1). Subsequently, OTC degradation was slowed down due to almost complete utilization of the oxidant. Hence, the extension of reaction time to 240 minutes enhanced OTC degradation only up to 85%.

As shown in Figure 4.1, although COD solubilization achieved at the end of 60 minutes is more pronounced (4.9%), soluble COD steadily increased thereafter and reached to a value of 6.2% within two hours. Continuous release of COD could be attributed to the acidity of the medium (Devlin et al., 2011). Based on the obtained results, the reaction time for Fenton oxidation process was determined to be two hours for further experiments.

In order to indicate the contribution of  $\text{OH}\cdot$  radical for both OTC degradation and COD solubilization,  $\text{H}_2\text{O}_2$  at dosage of 150 g/kg TS was applied to sludge without addition of iron at pH 3. Separate experiments were also carried out without addition of both iron and hydrogen peroxide to evaluate the effect of acidification of the sludge solubilization. Finally, Fenton oxidation process was performed without initial pH adjustment and all results are presented in Table 4.3.

Table 4.3. OTC degradation and COD solubilization at different experimental conditions

| $\text{H}_2\text{O}_2$<br>(g/kg TS) | $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$ | pH  | Antibiotic<br>Degradation (%) | Solubilization<br>(%) |
|-------------------------------------|---|-----|-------------------------------|-----------------------|
| -                                   | -   | 3.0 | nc                            | 0.70                  |
| 150                                 | -   | 3.0 | 19.0                          | 0.95                  |
| 150                                 | 0.4                                       | 3.0 | 85.0                          | 6.20                  |
| 150                                 | 0.4                                       | 6.5 | 48.4                          | 4.10                  |

nc: not considered

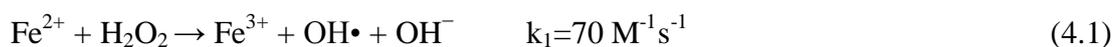
As shown in Table 4.3, the treatment efficiency of Fenton oxidation strongly depends on the experimental conditions. Although  $\text{H}_2\text{O}_2$  is a strong oxidant (standard potential 1.80 and 0.87 V at pH 0 and 14, respectively) (Neyens and Baeyens, 2003), treatment by only 150 g  $\text{H}_2\text{O}_2$ /kg TS did not provide high antibiotic degradation. Within 120 minutes treatment period 19% OTC degradation and 0.95% COD solubilization were achieved (Table 4.3) without addition of the catalyst. This limited degradation of OTC could be attributed to the oxidizing ability of peroxide by itself (Neyens et al., 2003). The catalyst addition resulted in improved treatment efficiency proving the activation of  $\text{H}_2\text{O}_2$  to produce hydroxyl radicals (oxidation potential of 2.8 V) that are mainly responsible for

OTC degradation and sludge solubilization. Hence, 85% antibiotic degradation and 6.20% COD solubilization were achieved with  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio of 0.4 at 150 g  $\text{H}_2\text{O}_2/\text{kg TS}$ .

At pH 6.5 the efficiency of Fenton oxidation process for both COD solubilization and antibiotic degradation was lower than that obtained at acidic pH. Since both of  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{+2}$  are not stable above the acidic pH, low pH leads to a higher performance for Fenton oxidation process in accordance with previous studies (Zhang et al., 2005; Bautista et al., 2008).

#### 4.2.1. Effect of $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$ Ratio on Fenton Oxidation Process Efficiency

The Fenton reaction (reaction 4.1) produces hydroxyl radicals in acidic medium by iron-catalyzed decomposition of  $\text{H}_2\text{O}_2$  (Rigg et al., 1954);



Although hydroxyl radicals can be generated readily in aqueous solution, the addition of Fenton reagent to the sludge may be insufficient to produce  $\text{OH}\cdot$  radicals because of the complex matrix of the sludge. Moreover, the degradation of target pollutant can be inefficient due to the presence of radical scavengers in the sludge. The reactions occurred simultaneously in the sludge upset the stoichiometry of the  $\text{Fe}^{+2}\text{-H}_2\text{O}_2$  reaction. Therefore,  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio and  $\text{H}_2\text{O}_2$  were investigated as being important process parameters that affect the efficiency.

In previous studies, the applied  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio to sewage sludge for disintegration and dewatering purposes exhibited a wide variation (Neyens et al., 2003; Flotron et al., 2005; Beauchesne et al., 2007; Dewil et al., 2005; Zheng et al., 2007; Lo et al., 2008; Tokumura et al., 2009; Pham et al., 2011). However, the most commonly used  $\text{H}_2\text{O}_2$  dosages in sludge disintegration studies were in the range of 60-300 g / kg TS. Considering the previous studies series of experiments with the sludge having 20 g/L TS concentration was carried out by varying the  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio between 0.02 and 0.8 while keeping the  $\text{H}_2\text{O}_2$  dosage constant at three different values the obtained results are presented in terms of antibiotic degradation and COD solubilization in Figure 4.2, respectively.

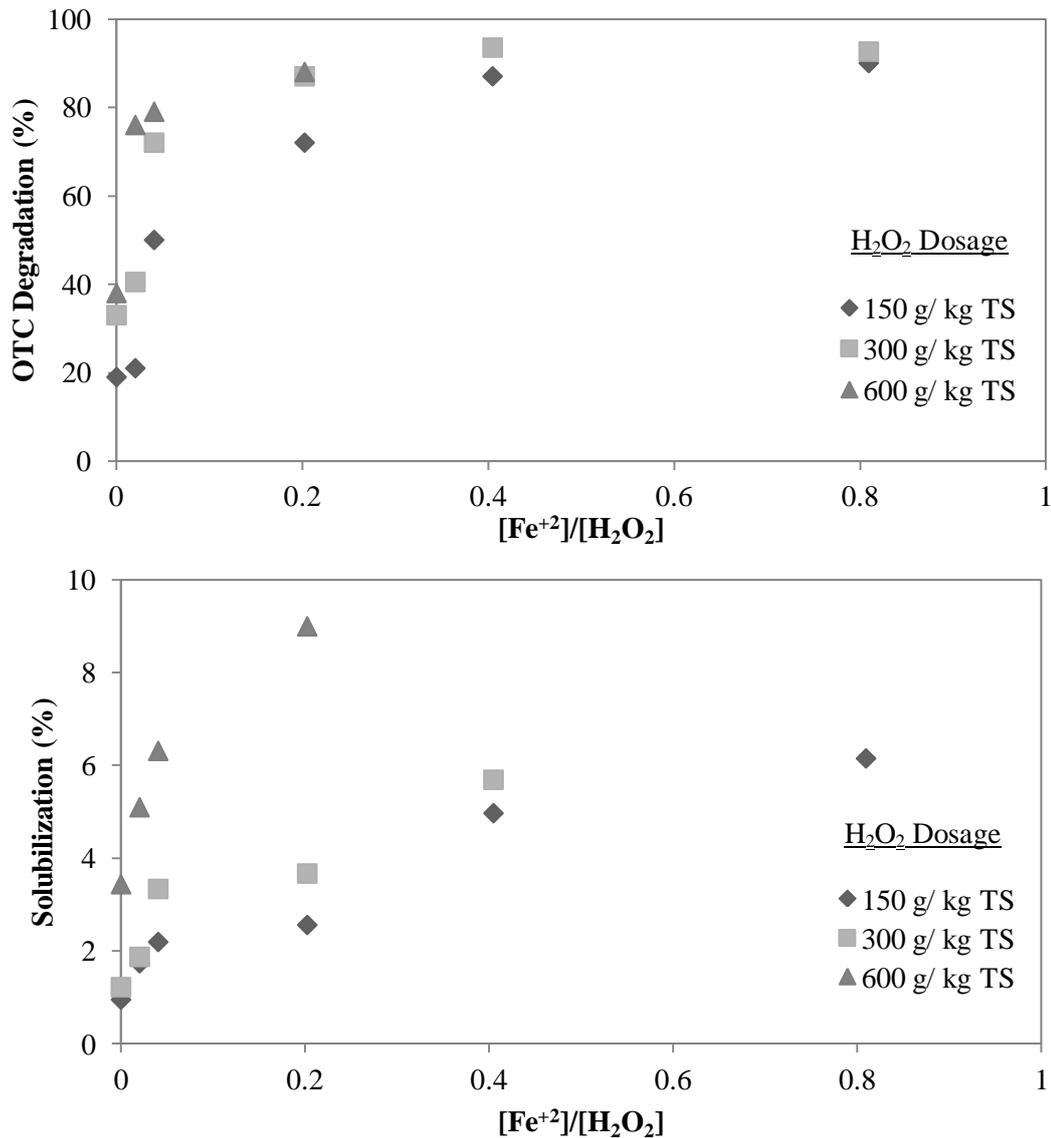
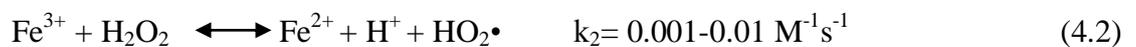


Figure 4.2. Effect of  $[\text{Fe}^{2+}]/[\text{H}_2\text{O}_2]$  ratio on OTC degradation and COD solubilization in WAS. Experimental conditions: TS= 20 g/L;  $\text{pH}_i= 3$ ;  $T= 25\text{ }^\circ\text{C}$ ; reaction period=120 min.

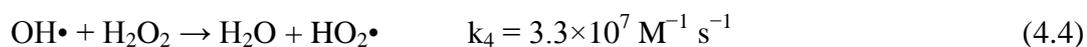
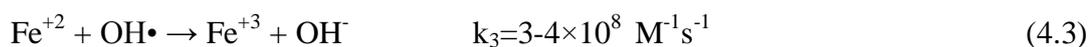
As illustrated in Figure 4.2, 21% of OTC degradation and 1.7% of COD solubilization were achieved at 150 g  $\text{H}_2\text{O}_2/\text{kg TS}$  with the  $[\text{Fe}^{2+}]/[\text{H}_2\text{O}_2]$  ratio of 0.02. These results are slightly higher than those obtained in the absence of  $\text{Fe}^{2+}$  catalysts (Table 4.3). Considering the indigenous metal content of the sludge, an additional control experiment was conducted in the presence of 1 M of *t*-butanol to indicate the contribution of hydroxyl radicals for the process efficiency and 18% of OTC degradation was obtained under these experimental conditions. Although a slight contribution of  $\text{Fe}^{2+}$  addition to the peroxidation performance of the sludge was observed at the lower peroxide dosage a pronounced improvement was achieved for both antibiotic degradation and COD solubilization by the

addition of  $\text{Fe}^{2+}$  at the highest dosage of hydrogen peroxide (600 g  $\text{H}_2\text{O}_2$ / kg TS). These results indicated that the higher dosage of hydrogen peroxide is necessary for hydroxyl radical generation in the sludge. Moreover, hydrogen peroxide at high dosage can provide desorption of contaminants as suggested by Watts et al. (1994). Previous studies (Neyens and Baeyens, 2003; Flotron et al., 2005) reported that the presence of iron minerals in sewage sludge could enable the Fenton oxidation to proceed without any iron addition at high concentration of hydrogen peroxide. However, the results of this study revealed that indigenous iron content of the sludge (5.6 g/L) was not sufficient to proceed the Fenton reaction hence  $\text{OH}\cdot$  generation since limited antibiotic degradation was achieved by  $\text{H}_2\text{O}_2$  oxidation only.

By increasing  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio from 0.02 to 0.4 drastic increase in antibiotic degradation rate was achieved for each peroxide dose (150, 300, and 600 g/ kg TS) and it attained 87% at 150 g  $\text{H}_2\text{O}_2$ / kg TS (Figure 4.2). Increasing sludge treatment performance of Fenton oxidation by increasing ferrous iron dosage can be attributed to enhanced  $\text{OH}\cdot$  radical formation similar to previous studies (Lu et al., 2003; Neyens et al., 2003; Büyükkamacı, 2004; Bach et al., 2010). However, further increase in  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio to 1.2 did not remarkably improve the results and only 7% additional increase was achieved at 150 g/ kg TS  $\text{H}_2\text{O}_2$  dosage (data not shown). At high  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio the consumption of hydrogen peroxide by ferrous iron (reaction 4.1) can eliminate the reaction between ferric iron and hydrogen peroxide (reaction 4.2), hence the reduction of oxidation reaction can be resulted (Walling and Goosen, 1973; Neyens and Baeyens, 2003):



Both rapid consumption of hydrogen peroxide and the lack of ferrous iron formation by the reduction of ferric iron (reaction 4.2) could be the reason of the obtained result at higher  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio. Furthermore, some previous studies (Chen et al., 1997; Joseph et al., 2000; Tony et al., 2008) revealed that high  $\text{Fe}^{+2}$  concentrations (>0.1 mM) could cause the scavenging of  $\text{OH}\cdot$  radical since the reaction between  $\text{OH}\cdot$  and the ferrous ion is ten times faster than that between  $\text{OH}\cdot$  and hydrogen peroxide (reaction 4.4) (Buxton and Greenstock, 1988).



In order to evaluate the fate of oxidant, the consumption of  $\text{H}_2\text{O}_2$  was determined at different  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratios during Fenton oxidation (Figure 4.3).

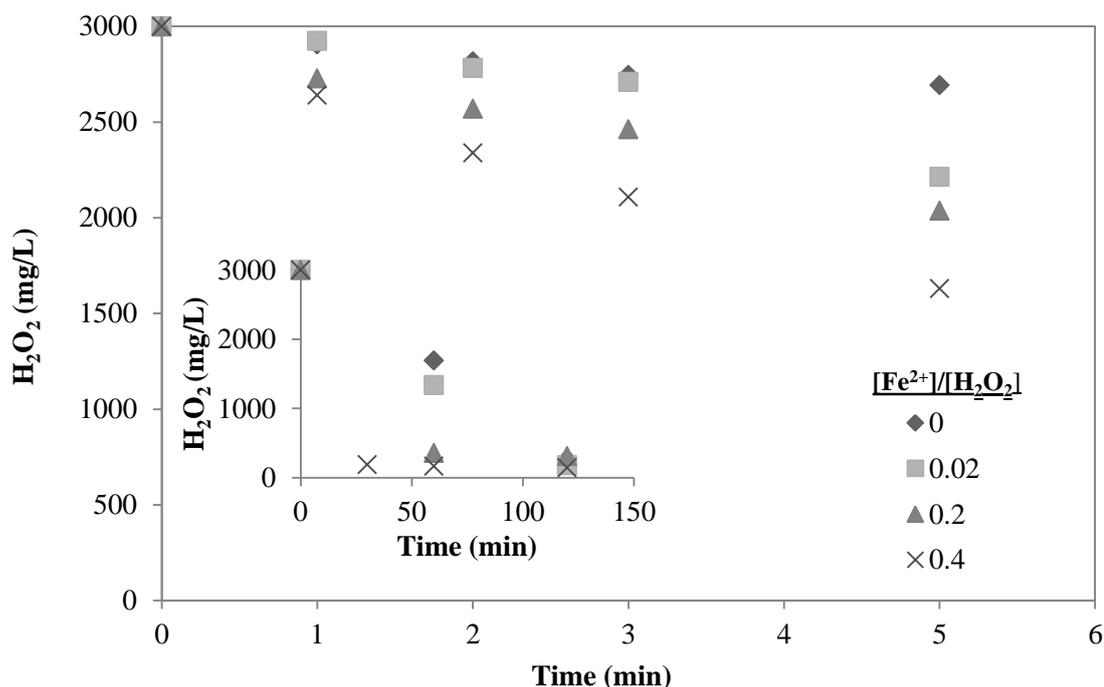


Figure 4.3.  $\text{H}_2\text{O}_2$  utilization rate at different  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratios.

Experimental conditions:  $\text{H}_2\text{O}_2 = 3000 \text{ mg/L}$ ;  $\text{TS} = 20 \text{ g/L}$ ;  $\text{pH}_i = 3$ ;  $T = 25 \text{ }^\circ\text{C}$

Increasing  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio to 0.4 increased initial  $\text{H}_2\text{O}_2$  consumption rate. However, total consumption of it was approximately same (94%) at the end of 120 minutes as shown in Figure 4.3. Therefore, the increase in OTC degradation by increasing  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio could be attributed to the increased production of  $\text{OH}\cdot$  radical by increasing  $\text{H}_2\text{O}_2$  consumption rates as mentioned before.

By taking into account rapid consumption of  $\text{H}_2\text{O}_2$  and slowing down OTC degradation (Figure 4.1), the effect of intermittent Fenton's reagent addition was investigated in order to enhance the treatment efficiency. Three intermittent dosing was

applied during the treatment process and the results obtained by 150 g/ kg TS total added H<sub>2</sub>O<sub>2</sub> dosage are summarized in Table 4.4.

Table 4.4. Effect of intermitted Fenton reagent addition to the performance

|                                      | Overall OTC     | Overall COD        |
|--------------------------------------|-----------------|--------------------|
|                                      | degradation (%) | solubilization (%) |
| Direct addition of Fenton Reagent    | 72              | 2.6                |
| Intermitted dosing of Fenton Reagent | 70              | 3.1                |

As shown in the table, intermittent dosing did not enhance antibiotic degradation. In contrary, it caused a slight decrease that supported the suggestion for desorption of strongly sorbed contaminant in the presence of high hydrogen peroxide. However, it enhanced COD solubilization.

#### 4.2.2. Effect of Fenton-like Oxidation on Antibiotic Degradation

Previous studies indicated that Fenton-like process that uses ferric salts as source of iron salt has also been applied to sewage sludge for conditioning, dewatering, and organic pollutant degradation purposes (Tony et al., 2008; Zheng et al., 2007; Lu et al., 2003; Tokumura et al., 2006). In this study, the effect of Fe<sup>+3</sup> catalyst instead of Fe<sup>+2</sup> was also investigated by taking into account that OTC molecules are able to form complex with dissolved Fe<sup>+3</sup> ions (Tanis et al., 2008). It would be expected to be an enhanced oxidation due to desorption of OTC. For this purpose, Fenton oxidation experiments were carried out at 150 g H<sub>2</sub>O<sub>2</sub>/kg TS with [Fe<sup>+3</sup>]/ [H<sub>2</sub>O<sub>2</sub>] ratio of 1.2. In a separate experiment, the added Fe<sup>+3</sup> was allowed to mix for 30 minutes prior to the addition of H<sub>2</sub>O<sub>2</sub>. This mixing period allowed time for the OTC desorption from the sludge and then the treatment was continued by the addition of H<sub>2</sub>O<sub>2</sub> for 120 minutes. In Figure 4.4 antibiotic degradation efficiency of these processes are presented as a function of treatment time.

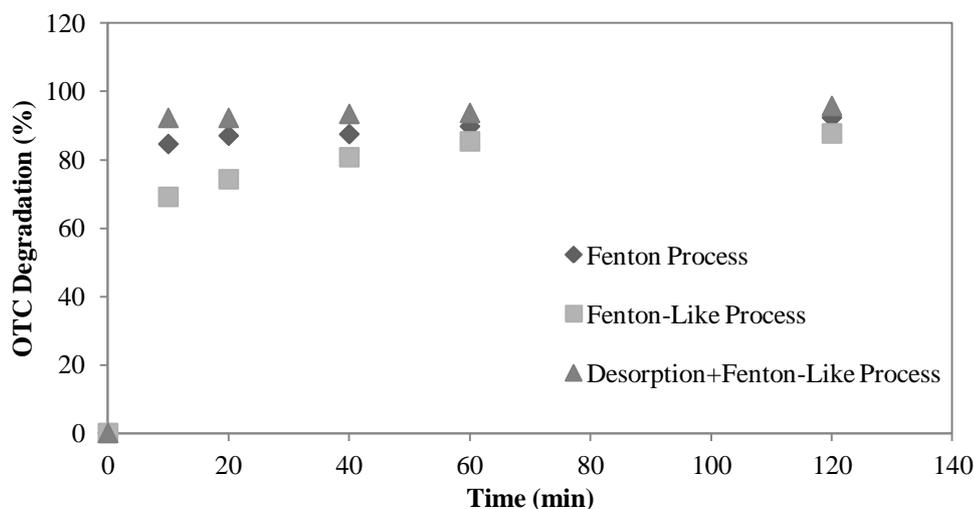


Figure 4.4. Comparison of Fenton and Fenton-like processes for antibiotic degradation.

Experimental conditions:  $\text{H}_2\text{O}_2=150$  g/ kg TS; TS= 20 g/L;  $\text{pH}_i=3$ ;  $T= 25$  °C

From Figure 4.4, it can be deduced that the complex formation of OTC with  $\text{Fe}^{+3}$  ions enhanced the efficiency of Fenton-like process and OTC degradation attained 92% within 10 minutes of treatment period. This result clearly revealed that the oxidation reaction with dissolved antibiotic improved the treatment performance while the extension of reaction period did not provide the complete removal of antibiotic (desorption+Fenton-like) probably due to the strongly sorbed OTC on the sludge. On the other hand, Fenton-like process without desorption period did not positively affect overall OTC degradation compared to Fenton process. On the contrary, higher antibiotic removal was achieved by Fenton process since  $\text{OH}\cdot$  has higher oxidation potential than that of hydroperoxyl radical, which is generated by reaction 4.2 (Wang, 2008; Malik and Saha, 2003; Fu et al., 2009).

#### 4.2.3. Effect of $\text{H}_2\text{O}_2$ Dosage on Fenton Oxidation Process Efficiency

As illustrated in Figure 4.2, over a wide  $\text{H}_2\text{O}_2$  dosage range (150-600 g/kg TS) the efficiency of Fenton process could be high even with the low dose of  $\text{H}_2\text{O}_2$  depending on  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio (Figure 4.2). Therefore, experiments were performed in a low  $\text{H}_2\text{O}_2$  dosage range (12.5, 50, and 150 g  $\text{H}_2\text{O}_2$ / kg TS) by applying high  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio. Due to the possible interference of high iron concentration at 1.22  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio on COD test, the effect of oxidant dose on the organic matter solubilization was evaluated by dissolved organic carbon content (DOC) instead of COD of the sludge samples. The results

for antibiotic degradation and normalized DOC values are presented in Figure 4.5 as a function of  $\text{H}_2\text{O}_2$  dosage.

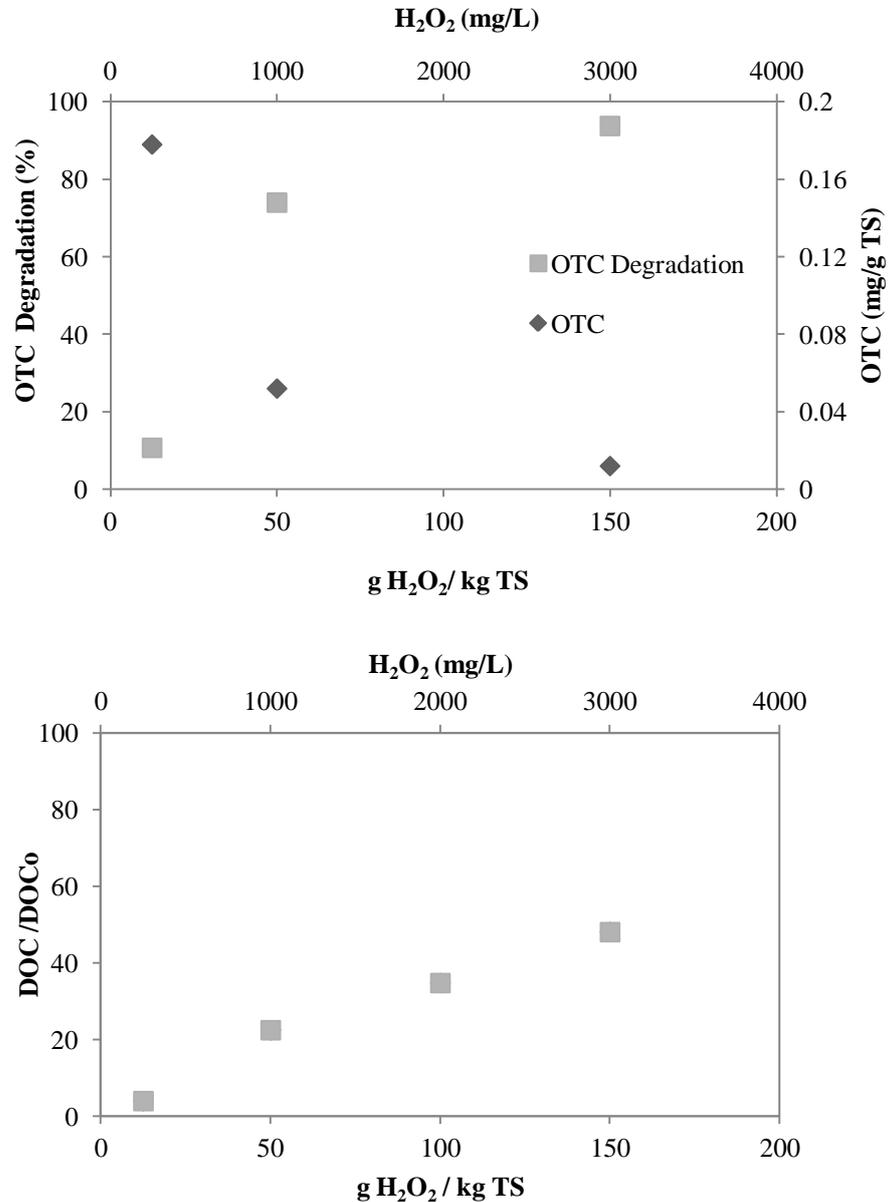


Figure 4.5. Effect of  $\text{H}_2\text{O}_2$  dosage on OTC degradation and organics solubilization.

Experimental conditions:  $\text{TS}=20$  g/L;  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]=1.22$ ;  $\text{pH}_i=3$  and  $T=25$  °C.

A considerable portion of the organics is strongly associated with the solid phase of the sludge (Table 4.1) and less than 1% was soluble as mentioned previously. Similarly, 97% of spiked OTC adsorbed on the solid phase. The application of lowest dose of  $\text{H}_2\text{O}_2$  (12.5 g/kg TS) provided only 10.7% OTC degradation as illustrated in Figure 4.5. By

increasing the oxidant dose to 150 g/kg TS, the degradation of antibiotic increased to 94%. Besides, a noticeable DOC increase (48 times) in the supernatant of the sludge was achieved under these experimental conditions. This result clearly indicated that the consumption of oxidants with sludge components resulted in insufficient oxidant concentration (Flotron et al., 2003) to degrade the target contaminant. Due to the high reactivity of both hydrogen peroxide and produced oxidative radicals, only the dissolved pollutants are probably oxidized and caused the consumption of them. However, high peroxide dosage might provide desorption of contaminants as suggested by Watts et al., (1994) as well as the increasing  $\text{OH}\cdot$  radical concentration.

Depending upon the applied  $[\text{Fe}^{2+}]/[\text{H}_2\text{O}_2]$  ratio the pH value of the sludge was reduced. In order to evaluate the contribution of acidification on overall solubilization during Fenton oxidation a series of the control experiments were conducted by the acidification of the sludge without addition of Fenton's reagent since it is known that acid pre-treatment can provide solubilization of sludge by means of cell lysis (Devlin et al., 2011). In Figure 4.6, COD solubilization achieved within two hours treatment period is shown as a function of pH in the range from 6.5 to 1 by the addition of  $\text{H}_2\text{SO}_4$ .

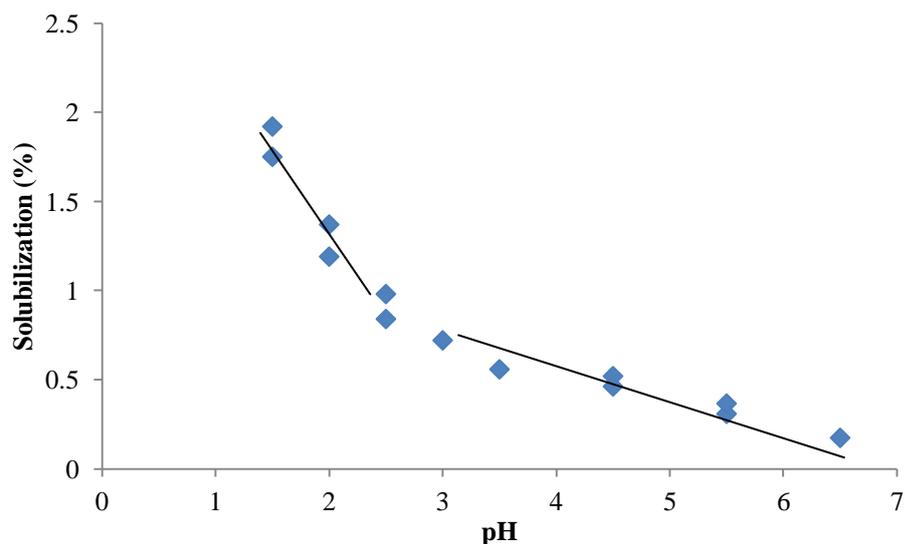


Figure 4.6. Effect of acidification of WAS on COD solubilization.

Experimental conditions: TS= 20 g/L; T= 25 °C; Reaction Time= 120 minutes.

By the acidification of sludge to a pH value of 3 and 1, the achieved COD solubilizations were 0.5 and 2.5%, respectively and these values are higher than that of the Devlin's investigation (Devlin et al., 2011) due to the higher TS content of the sludge. In order to clarify the contribution of acidification on COD solubilization during the Fenton process the solubilization values achieved by both acidification and Fenton process are listed in Table 4.5. This table also represents the pH value attained by the addition of Fenton's reagent. Acid solubilization achieved at the same pH with the Fenton oxidation was estimated from the data presented in Figure 4.6.

Table 4.5. COD solubilization achieved by Fenton oxidation and acidification

| [Fe <sup>+2</sup> ]/[H <sub>2</sub> O <sub>2</sub> ] | pH  | COD solubilization (%) |               |
|--|-----|------------------------|---------------|
|  |     | Fenton Oxidation       | Acidification |
| <b>0</b>   | 3   | 0.95                   | 0.58          |
| <b>0.02</b>  | 2.8 | 1.73                   | 0.71          |
| <b>0.04</b>  | 2.6 | 2.19                   | 0.86          |
| <b>0.20</b>  | 2.6 | 2.56                   | 0.92          |
| <b>0.40</b>  | 2.4 | 4.97                   | 1.06          |
| <b>0.80</b>  | 2.1 | 6.15                   | 1.28          |
| <b>1.22</b>  | 1.7 | 11                     | 1.59          |

H<sub>2</sub>O<sub>2</sub> dosage: 150 g/kg TS; Reaction time: 120 minutes

As can be seen from Table 4.5 the decrease in the pH value is not remarkable for the [Fe<sup>+2</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratios smaller than 0.4 and acidification did not cause substantial solubilization for each case. Therefore, obtained solubilization was because of increased hydroxyl radical concentration rather than the acidification.

#### 4.2.4. Interaction between H<sub>2</sub>O<sub>2</sub> dosage and [Fe<sup>+2</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratio

In order to clarify interaction between H<sub>2</sub>O<sub>2</sub> dosage and the [Fe<sup>+2</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratio contour plot of OTC degradation was drawn by using Minitab 16 statistical program (Figure 4.7).

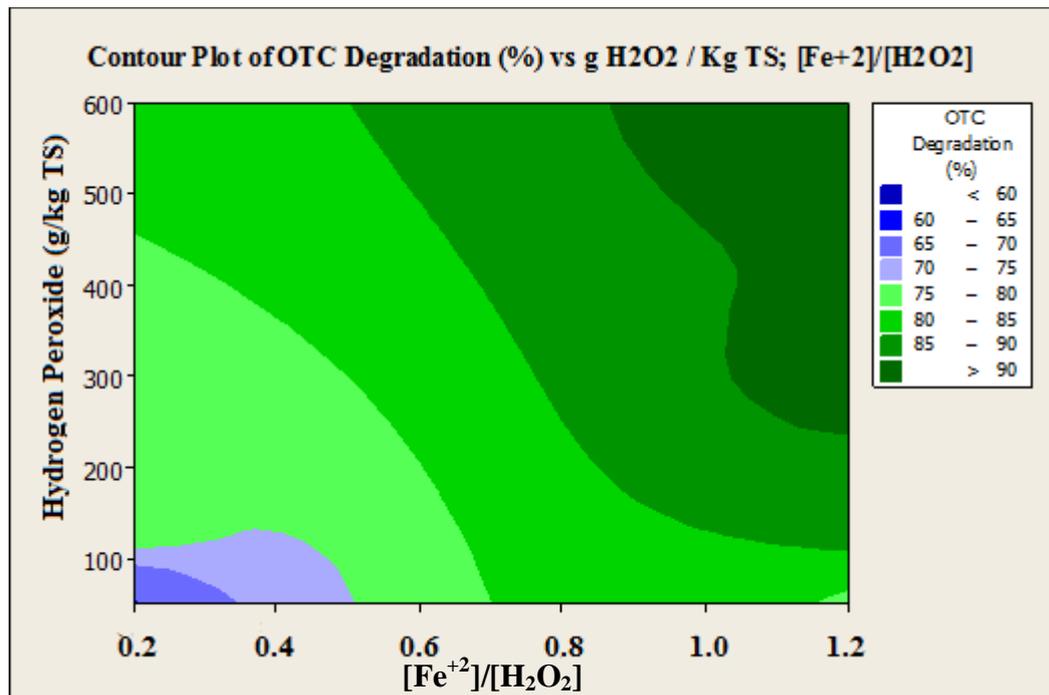


Figure 4.7. Contour Plot of OTC degradation vs. H<sub>2</sub>O<sub>2</sub> dosage and [Fe<sup>2+</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratio. Experimental conditions: TS = 20 g/L; pH<sub>i</sub>= 3; T= 25 °C; reaction period=120 minutes.

As Figure 4.7 shows that at low H<sub>2</sub>O<sub>2</sub> dosages (< 150 g/kg TS) the effect of [Fe<sup>2+</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratio was significant on OTC degradation. However, at higher H<sub>2</sub>O<sub>2</sub> dosages this effect was observable at only for low [Fe<sup>2+</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratios (<0.4) and further increasing the ratio did not alter OTC degradation significantly.

#### 4.2.5. Effect of Total Solid Content on Fenton Oxidation Process Efficiency

The effect of sludge matrix on Fenton oxidation performance was investigated by changing the total solid content of the sludge. The results obtained by the application two different H<sub>2</sub>O<sub>2</sub> dosages (250 and 3000 mg/L). OTC degradation and normalized DOC values are demonstrated as a function of total solid content of the sludge in Figure 4.8.

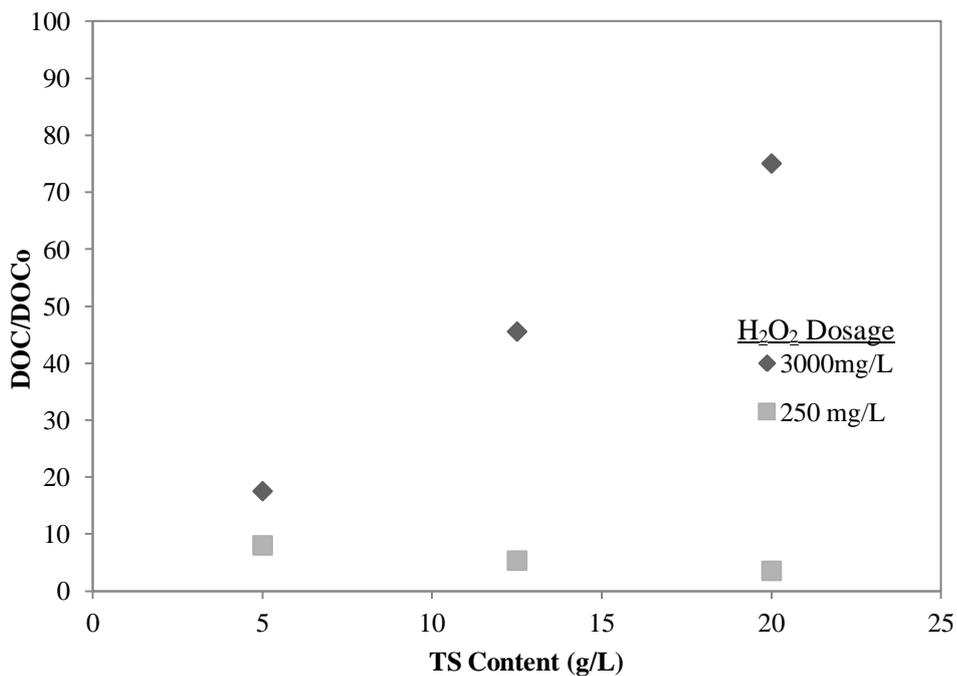
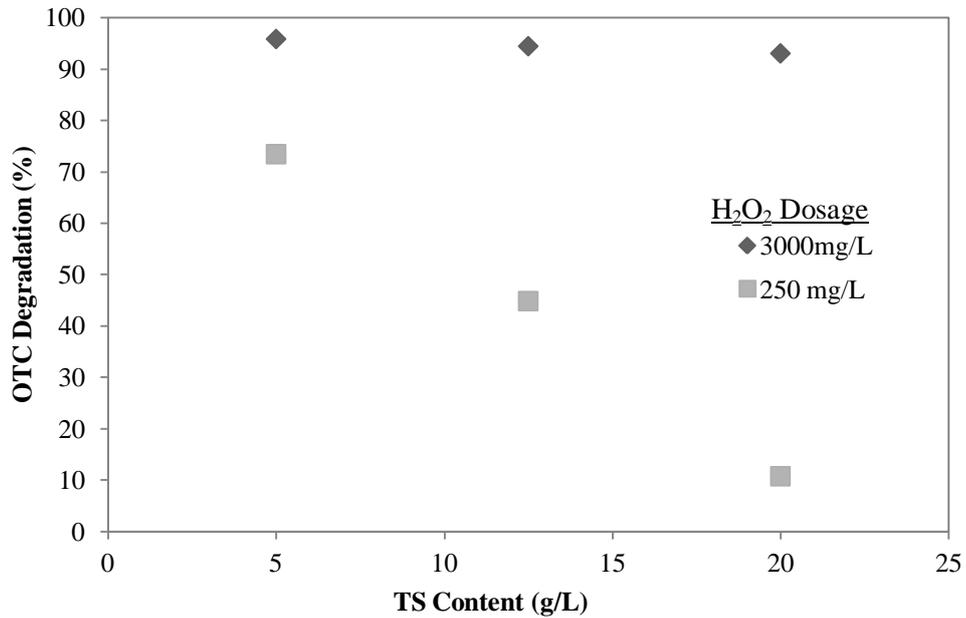


Figure 4.8. Effect of TS content on antibiotic degradation and organics solubilization.

Experimental conditions:  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]=1.22$ ;  $\text{pH}_i=3$ ;  $T=25^\circ\text{C}$ .

At lower concentration of H<sub>2</sub>O<sub>2</sub> (250 g/L) increasing TS content from 5 g/L to 20 g/L resulted in a remarkable reduction of OTC removal from 74% to 10% (Figure 4.8) although H<sub>2</sub>O<sub>2</sub> utilization rate increased as shown in Figure 4.9. Similarly, organic matter solubilization was adversely affected by the change of TS content of the sludge because

$\text{H}_2\text{O}_2$  was completely consumed at the end of the 120 minutes treatment period (Figure 4.9). While DOC in the supernatant of the sludge with 5 g/L TS exhibited 8-fold increase it reduced to 3.5-fold at high TS content (Figure 4.8). The competition between the solubilized and particulate compound for oxidant could result in lower treatment performance. Hence, a rapid consumption of the oxidant was observed without achievement of high removal of antibiotic. Besides the lack of sufficient oxidant, interfered mass and oxygen transfer due to the more viscous sludge properties as suggested by Pham et al. (2009) could be the reasons of reduced treatment efficiency of Fenton process in the presence of high total solid content.

On the other hand, when high amount of  $\text{H}_2\text{O}_2$  (3000 mg/L) was applied to the sludge, regardless of TS content considerably high OTC removal (94-96%) was achieved and residual  $\text{H}_2\text{O}_2$  in the medium was detected until the end of the reaction as represented in Figure 4.9. High concentration of  $\text{H}_2\text{O}_2$  could provide desorption of antibiotic as well as enhancement of  $\text{OH}\cdot$  radical production as mentioned before. Hence, the competition between particulate and dissolved matter for oxidants could be reduced. In contrast to the results obtained by low  $\text{H}_2\text{O}_2$  dosage, DOC release increased from 17 to 75 times by increasing TS content (Figure 4.8). However, residual  $\text{H}_2\text{O}_2$  (5% of the initial concentration) at the end of 120 minutes was not sufficient to degrade sorbed OTC on sludge particles and the complete degradation of OTC was not obtained.

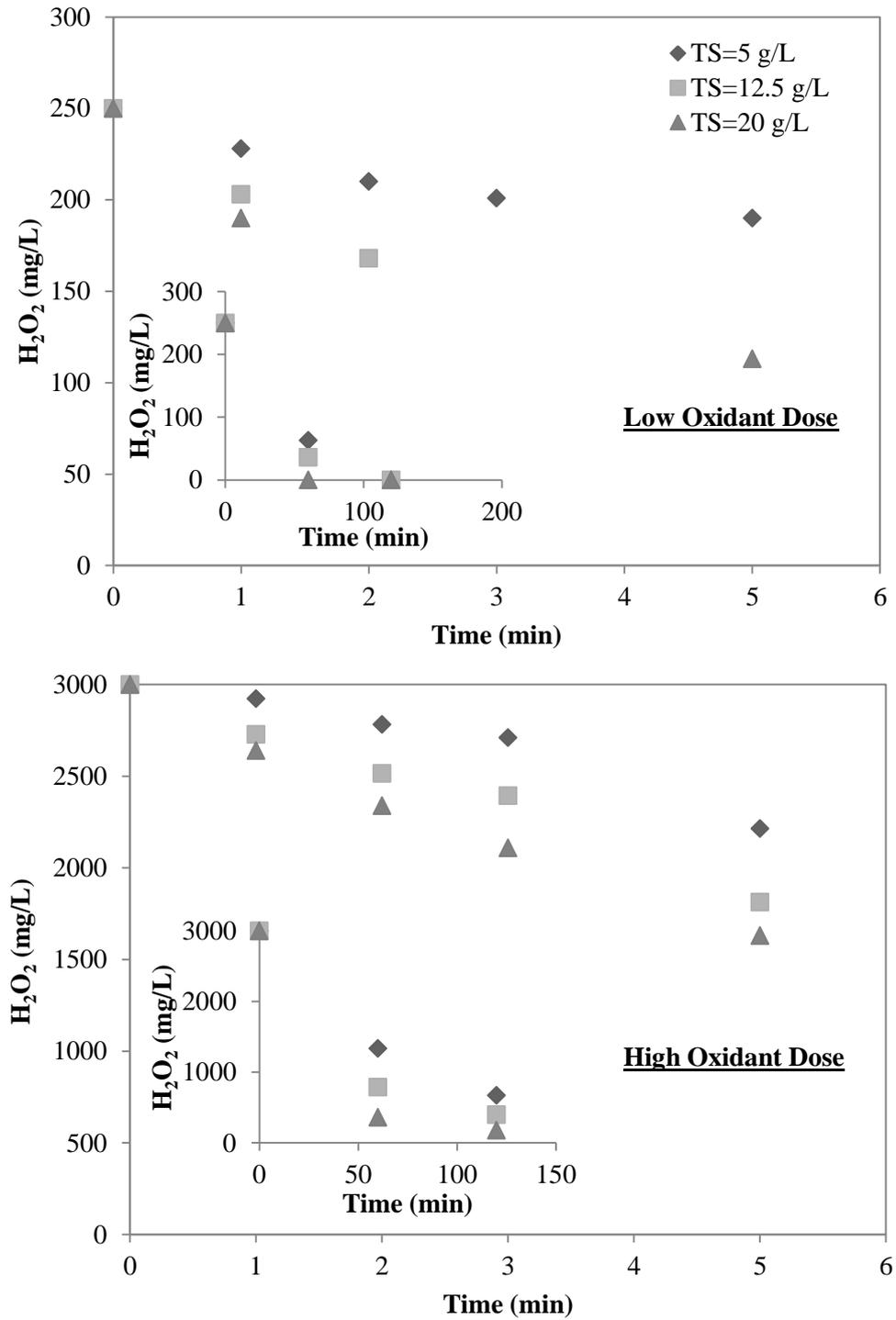


Figure 4.9. Effect of TS content on  $\text{H}_2\text{O}_2$  utilization kinetics at different  $\text{H}_2\text{O}_2$  dosages.

Experimental conditions:  $[\text{Fe}^{2+}]/[\text{H}_2\text{O}_2] = 1.2$ ; initial  $\text{pH}_i = 3$ ;  $T = 25^\circ\text{C}$ .

#### 4.2.6. Effect of Temperature on Fenton Oxidation Process Efficiency

Temperature is known to be another effective factor on  $\text{H}_2\text{O}_2$  utilization rate and Fenton Oxidation Process (Neyens and Baeyens, 2003). Therefore, the effect of temperature between 20-40 °C was examined by keeping other parameters constant (Figure 4.10). Due to the instability of OTC at higher temperatures, these were not studied.

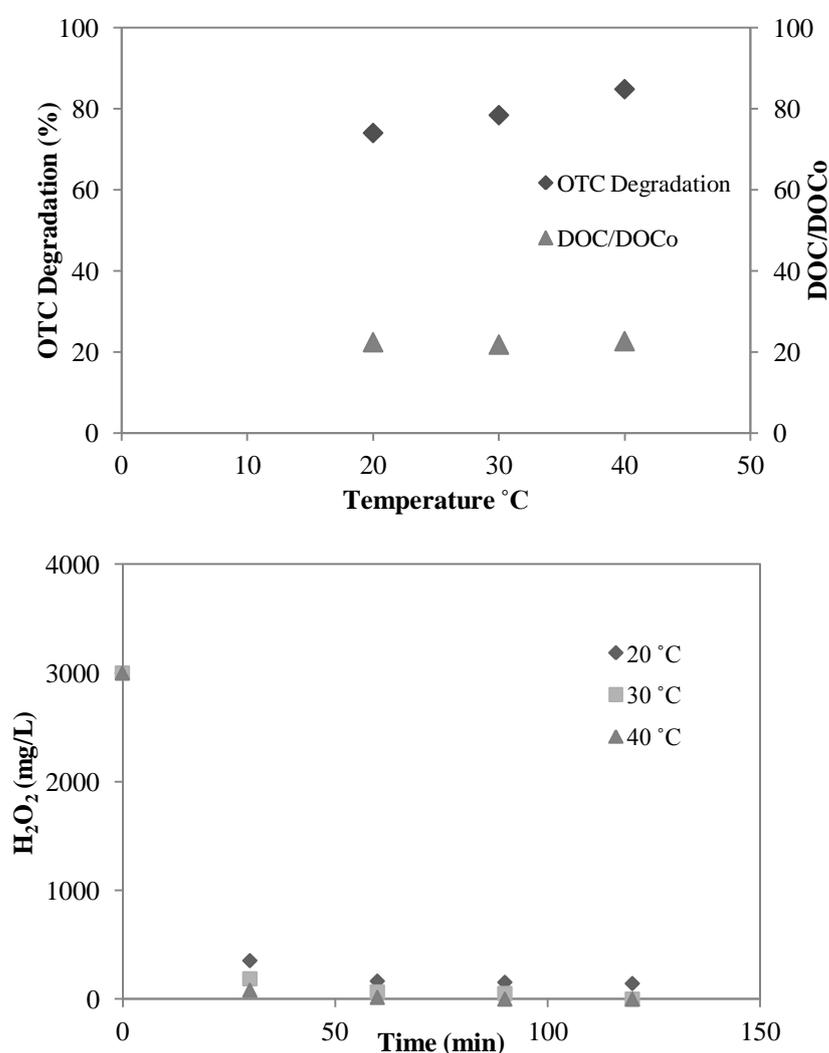


Figure 4.10. Effect of temperature on Fenton oxidation efficiency. Experimental conditions:  $\text{H}_2\text{O}_2=150$  g/kg (3000 mg/L);  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]=1.22$ ;  $\text{pH}_i=3$ .

The temperature in the range tested in this study seems to have minor effect on OTC degradation which exhibited 10% improvement by increasing the reaction temperature

from 20 to 40 °C. In accordance to enhancement of antibiotic degradation  $H_2O_2$  utilization exhibited a slight increase by the temperature increase (Figure 4.10). On the other hand, solubilization achieved at each investigated temperature was approximately same.

#### 4.2.7. Effect of Fenton Oxidation on Metal and Nutrient Release

As a result of the solubilization of the sludge components, the release of metals and nutrients into soluble phase are expected during Fenton oxidation process. Their solubilizations are of prime importance for the separation of this regulated pollutant, heavy metals and also the recovery valuable nutrients. Actually, the combined use of an inorganic acid (sulfuric acid) and oxidant is a patented metal leaching technique (Blais et al., 2005).

Figure 4.11 displays metal solubilization from the sludge at the end of two hours Fenton oxidation under the conditions in which higher OTC degradation and solubilization were achieved.

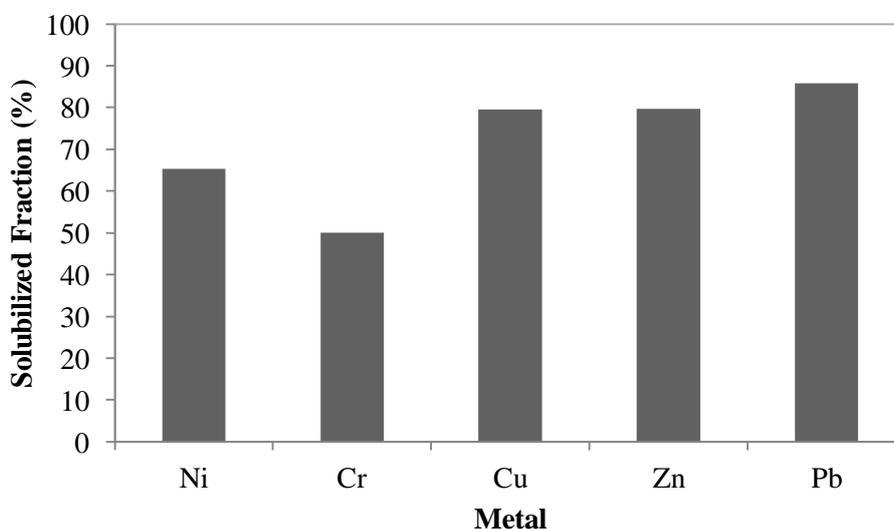


Figure 4.11. Effect of Fenton oxidation on heavy metal solubilization. Experimental conditions:  $H_2O_2= 150$  g/ kg TS;  $[Fe^{+2}]/[H_2O_2]= 0.8$ ; TS= 20 g/L;  $pH_i=3$ ;  $T=25$  °C.

Fenton oxidation of sludge resulted distinguishable changes for some of the metals' concentrations found in the sludge samples (Figure 4.11). While Cu, Zn, and Pb were highly solubilized Cr exhibited relatively lower solubilization by Fenton oxidation.

Extraction of metals from the sludge depends on organic fraction, exchangeable metal fraction and carbonate fraction which are susceptible to changes in pH (Tessier et al., 1979). It is known that the use of inorganic acids did not provide high yield for metal solubilization (Chang and Liu, 1998; Lo and Chen, 1990; Mitani et al., 1991) especially for copper and lead (Blais et al., 1992). However, 80% release was achieved for copper in this study despite of its high ability for binding to organics. It is obvious that the organic matter degradation by Fenton oxidation caused the release of the metals (Dewil et al., 2007).

Besides metals, nutrient solubilization was investigated at the condition that highest OTC and solubilization were obtained and the results are listed in Table 4.6. Initial concentrations of soluble phosphorus and ammonia of raw sludge were 12.5 and 2.9 mg/L, respectively.

Table 4.6. Solubilization of ammonia and phosphate by Fenton oxidation

|                          | <b>Soluble Ammonia</b><br>(N mg/L) | <b>Soluble Phosphorous</b><br>(P mg/L) |
|--------------------------|------------------------------------|--|
| Raw Sludge               | 2.9                                | 12.5                                   |
| Fenton Treated<br>Sludge | 27.8                               | 44.25                                  |

As seen from the table, ammonia and soluble phosphorous concentrations were increased by approximately by 10 and 3 times, respectively. This can be attributed to disintegration of sludge particles obtained by Fenton oxidation since destruction of activated sludge particles by pre-treatment is known to increase the release of ammonia and phosphorous (Carrere et al., 2010).

#### **4.2.8. Effect of Fenton Process on Physical Properties of Sludge**

Fenton Oxidation is known to be effective on various physical characteristics of sewage sludge such as filterability, dewaterability, particle size distribution, CST, and SVI. (Neyens et al., 2003; Kim et al., 2010; Erden and Filibeli, 2010; Pham et al., 2010). Regarding this, some analysis was conducted in order to investigate the impact of Fenton oxidation on sludge physical characteristics. Sludge settleability was examined by SSV

(Settled Sludge Volume) measurements. Related data for raw and treated sludge samples with different TS contents are demonstrated on Figure 4.12.

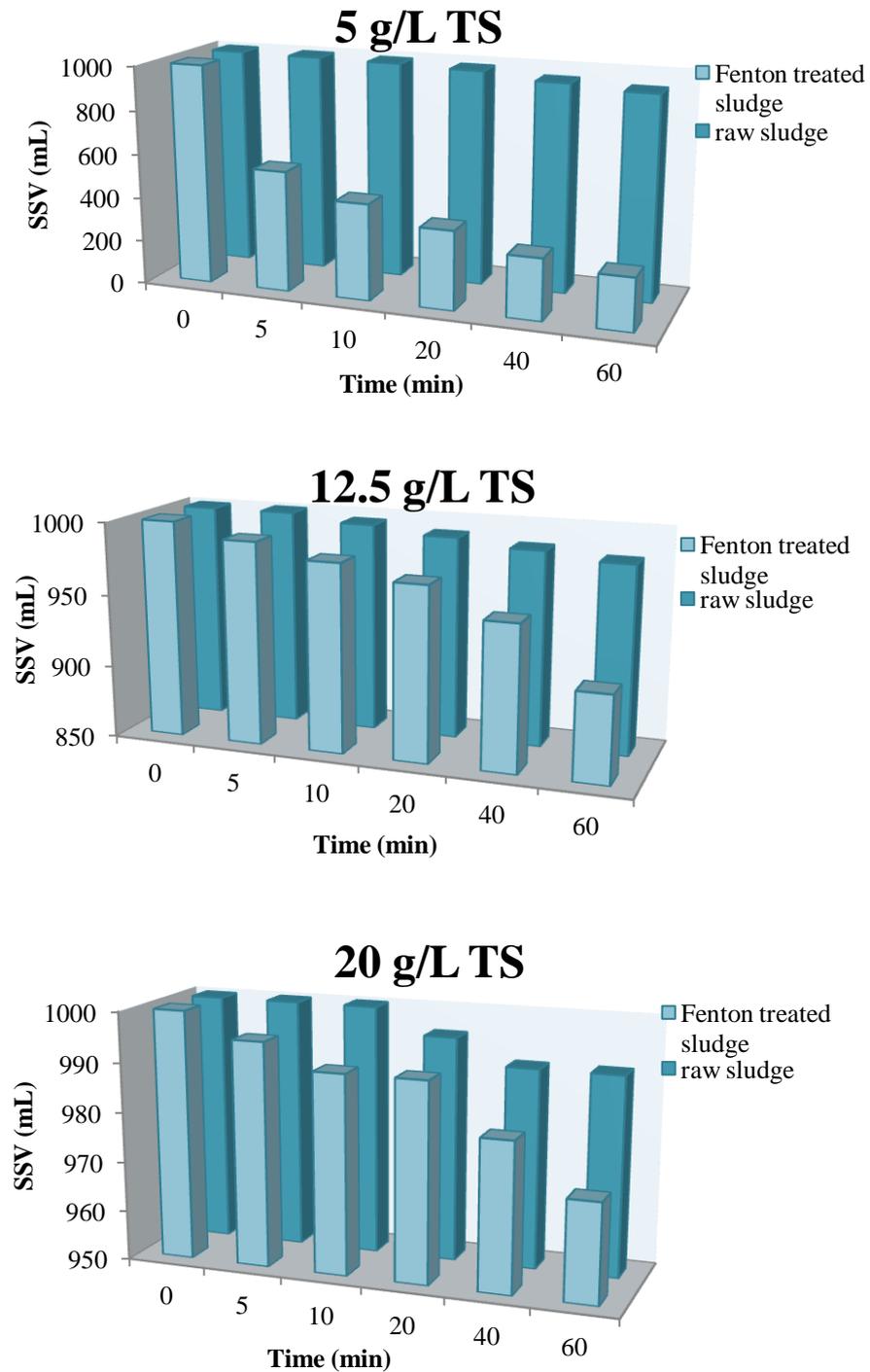


Figure 4.12. SSV values of raw and treated sludge at different TS contents. Experimental conditions:  $\text{H}_2\text{O}_2$  dosage= 150 g/kg TS;  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]=0.8$ ;  $\text{pH}_i=3$ ;  $T=25\text{ }^\circ\text{C}$

As it is demonstrated by Figure 4.12, for the sludge samples with 5 g/L TS content settled sludge volume in 60 minutes was decreased from 930 mL to 240 mL showing 74% enhancement of settleability. High concentrations of ferrous cations are known to improve settleability of sludge (Jin et al., 2003). Iron content of Fenton reagent is obviously a reason for increased settleability of the sludge. However, at 12.5 g/L TS content only 8% enhancement in sludge settleability was observed with the same ferrous iron dose. At higher TS content (20 g/L) there was no significant enhancement (3%) and settled volume of raw and treated sludge samples were very close each other (990 and 970 mL/60min).

Besides the settleability, dewatering capacity of sewage sludge was predicted by CST. According to the measurement applied to raw and treated sludge samples, it was observed that Fenton oxidation resulted only 1% (data not shown) decrease of CST value (from 10.2 s to 10.1 s) at 150 g/kg TS H<sub>2</sub>O<sub>2</sub> dosage and 0.2 [Fe<sup>+2</sup>]/ [H<sub>2</sub>O<sub>2</sub>] ratio at pH 3. A higher [Fe<sup>+2</sup>]/ [H<sub>2</sub>O<sub>2</sub>] ratio of 0.8 resulted in 3.5% CST reduction which corresponded to good dewatering characteristics (Neyens et al., 2003).

Comparison of raw and treated sludge particle size distribution revealed that Fenton oxidation caused a variation on particle size distribution of the treated sludge at 150 g/kg TS H<sub>2</sub>O<sub>2</sub> dosage with [Fe<sup>+2</sup>]/ [H<sub>2</sub>O<sub>2</sub>] ratio of 0.2 (Table 4.7). Particle size distribution diagram are presented in Appendix B.

Table 4.7. Particle size distribution of raw and treated sludge

|                              | Particle Size Distribution (um) |            |             |
|------------------------------|---------------------------------|------------|-------------|
|                              | (d0.1)                          | (d0.5)     | (d0.9)      |
| <b>Raw Sludge</b>            | 18.8 ± 3.1                      | 54.7 ± 3.3 | 132.1 ± 5.3 |
| <b>Fenton Treated Sludge</b> | 15.2 ± 1.8                      | 48.5 ± 3.1 | 109.4 ± 6.2 |

Fenton oxidation resulted in a reduction of mean sludge particle size as well as at (d0.9) and (d0.1). By the disintegration of sludge particles and the solubilization of organics the viscosity of sludge was decreased by 26% (data not shown). These results proved that Fenton oxidation was efficient for sludge reduction as well as antibiotic degradation.

## 5. CONCLUSIONS

The present study was undertaken to ascertain the effect of Fenton oxidation on the antibiotic degradation found in synthetically contaminated WAS. Sludge solubilization, metal and nutrient release and changes in physical properties were studied in order to explain Fenton oxidation efficiency.

1. The chemical characterization indicated that the concentration of a tetracycline group antibiotic, OTC in 15 different waste activated sludge samples was under the detection limit of applied analysis method in which limit of detection and recovery values were 0.01 mg/g and 86%, respectively. In addition, heavy metal concentrations in the sludge did not exceed limit values that are specified in Turkish legislation for the land application of the sludge.

2. The treatment of synthetically contaminated waste activated sludge by Fenton oxidation process revealed that OTC degradation depended on hydrogen peroxide dose,  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio, and total solids content. Temperature in the range between 20 and 40 °C did not influence the Fenton oxidation process efficiency in terms of DOC solubilization, however a minor effect on antibiotic degradation was observed.

3. Increasing  $\text{H}_2\text{O}_2$  oxidant dosage from 12.5 g/kg TS to 150 g/kg TS resulted in an increase in OTC degradation from 10 to 94% at  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio of 1.22. At low dosages of  $\text{H}_2\text{O}_2$ , sludge components consumed the oxidant hence, caused the insufficient amount of remaining oxidant for the degradation of strongly sorbed OTC. Besides the peroxide dose,  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio is an important parameter that affected efficiency. Indigenous iron content of the sludge (5.6 g/L) did not catalyze hydrogen peroxide decomposition to produce reactive radicals. High  $\text{H}_2\text{O}_2$  dose and  $[\text{Fe}^{+2}]/[\text{H}_2\text{O}_2]$  ratio were required for high process efficiency.

4. Rapid consumption of  $\text{H}_2\text{O}_2$  by sludge components caused the slowing down of OTC degradation during treatment period; however, COD released continued

due to the acidic conditions in Fenton oxidation process. The intermitted addition of hydrogen peroxide at 150 g/kg TS dosage did not enhance the degradation of OTC and solubilization.

5. An increase in total solid content of the sludge from 5 g/L to 20 g/L resulted in reduction of OTC degradation from 74% to 10% during the Fenton oxidation with 12.5 g/kg TS H<sub>2</sub>O<sub>2</sub> dosage and [Fe<sup>+2</sup>]/[H<sub>2</sub>O<sub>2</sub>] ratio of 0.8 due to the lack of sufficient oxidant. The settleability treated sludge was also decreased by increasing total solid content, whereas Fenton oxidation was enhanced settleability of the sludge at low solids content. At high H<sub>2</sub>O<sub>2</sub> dosage (150 g/kg TS) higher OTC degradation was achieved regardless of TS content due to the desorption ability of hydrogen peroxide for organic contaminants including OTC.

6. Beside the antibiotic degradation and COD solubilization, metal and nutrient release from the sludge were obtained by the application of Fenton oxidation process. While Fenton oxidation process with 150 g H<sub>2</sub>O<sub>2</sub>/ kg TS; and [Fe<sup>+2</sup>]/[H<sub>2</sub>O<sub>2</sub>]= 0.8 yielded about 85% solubilization of Cu, Zn, and Pb it provided 50% Cr solubilization. Ammonia and phosphate release was also achieved as concentrations in soluble phase were increased by Fenton oxidation.

7. Fenton oxidation provided an enhancement of dewaterability of the sludge which was evaluated by 26% reduction in viscosity. A reduction of particle size distribution was also obtained by Fenton oxidation.

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**APPENDIX A**  
**HPLC Chromotograms of OTC**

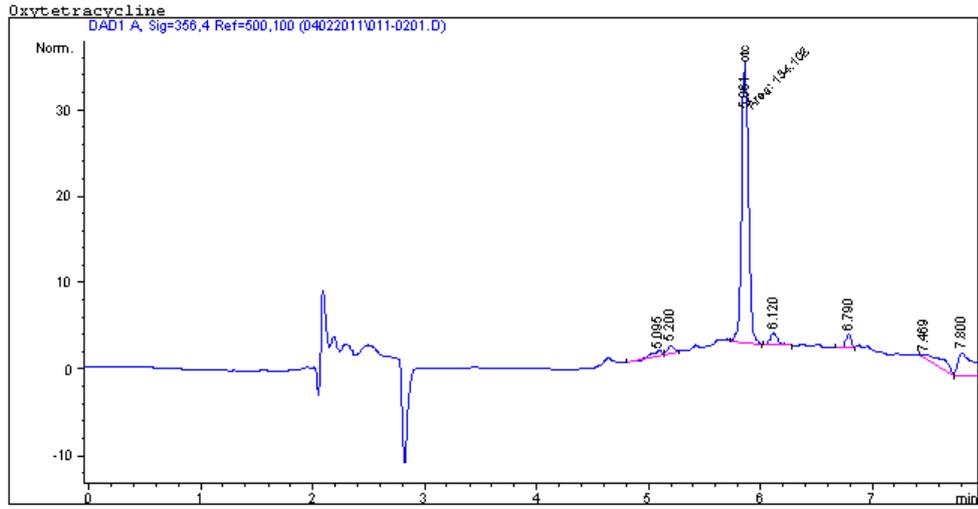


Figure A.1. HPLC Chromotogram of Untreated Sludge Sample.

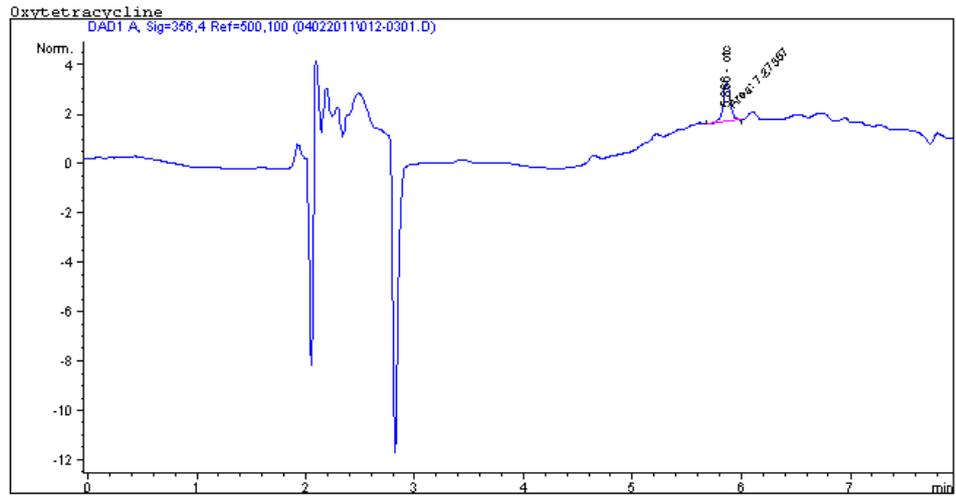


Figure A.2. HPLC Chromotgram of treated sludge sample.

**APPENDIX B****Particle Size Distribution Diagrams of Raw and Treated Sludge**

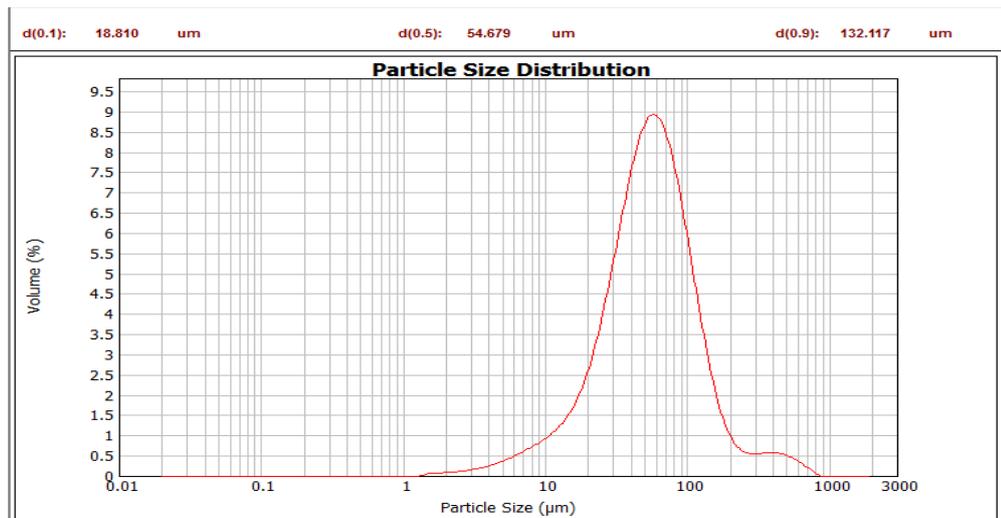


Figure B.1. Particle size distribution diagram of untreated sample.

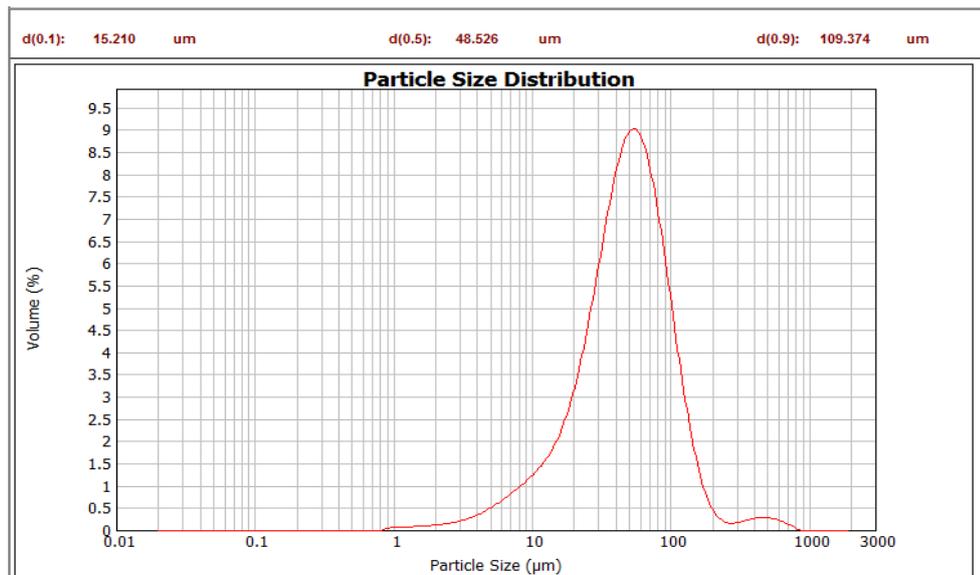


Figure B.2. Particle size distribution diagram of treated sample.