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應用芬頓法降解四環素及其反應機制之研究

Degradation and Mechanism of Tetracycline by the Fenton Process

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應用芬頓法降解四環素(Tetracycline)及其反應機制之研究
Degradation and mechanism of Tetracycline by Fenton Process

本論文係李有儀君（B04602011）在國立臺灣大學生物環境系統工程學系完成之學士班學生論文，於民國 108 年 4 月 22 日承下列考試委員審查通過及口試及格，特此證明

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摘要

四環素(Tetracycline)是一種廣效性抗生素，因其抗菌範圍廣泛，除了常被用於治療人類疾病的藥物，也做為養殖漁業的藥浴或畜牧產業飼料添加劑，以預防疾病感染及提高生長速度，因此在自然水體及廢水中經常檢測出四環素的成分，使其成為廣受關注的新興污染物之一。根據前人研究顯示，四環素曾於廢水處理廠中被檢測出來，而較常見的處理方式有物理性活性碳吸附去除法及薄膜過濾法和化學氧化法處理。截至目前為止已有研究團隊針對光芬頓法及電芬頓法對含有四環素的廢水進行降解，雖然效果幾乎達到完全降解及礦化，但現地應用上較耗能且難以將此方法用於大規模的水體。另外，過去研究顯示，芬頓法在酸性環境下有良好的降解效率，但是要調整大規模水體的酸鹼值較為困難，增加現地應用的困難度，且廢水中通常含有多種可能影響芬頓法的有機共溶質。爰此，本研究旨在探討芬頓法試劑濃度以及共溶質分別在酸性及中性環境下對芬頓法降解四環素的影響，除了傳統芬頓法之外，本研究亦應用探討過一硫酸鹽(peroxymonosulfate, PMS)和過二硫酸鹽(peroxydisulfate, PDS)透過亞鐵離子活化並降解四環素，並比較不同氧化劑對降解效率的影響。

實驗結果顯示，芬頓試劑濃度越高，降解效率越高，在酸性環境下降解效率達到 79.6%，而在中性環境下達到 46.7%。比較不同氧化劑影響降解的實驗中，過氧化氫顯示最佳的去除效果，且 PMS 的氧化能力略高於 PDS。在酸性條件下當共溶質存在時，環糊精及腐植酸在 50 μM 的芬頓試劑濃度下可提高四環素的降解效率，但將過氧化氫的濃度提高至 100 μM 時，於共溶質存在下四環素降解減少，表示因為共溶質消耗的氫氧自由基多於生成。在中性環境下，共溶質會與四環素結合，在

反應過程中結合的分子會將四環素釋回溶液中，造成觀察到的降解效率有下降的趨勢。

關鍵字：高級氧化、有機共溶質、芬頓法、四環素、新興污染物

ABSTRACT

Tetracycline has been detected oftentimes in wastewater and natural water bodies. Previous studies showed high levels of removal efficiency of organic contaminants by Fenton process in the acidic environment, which, however, seemed impractical because of the infeasibility of pH adjustment. Meanwhile, wastewater usually contains a variety of NOMs that may affect the efficiency of the Fenton process. Thus, the present study aimed to investigate the effects of experimental parameters and coexisting organic materials on the degradation of tetracycline by Fenton process in the acidic and neutral environment. In addition to the classic Fenton process, the degradation process by peroxymonosulfate (PMS) and peroxydisulfate (PDS), that could also be activated by the ferrous ions, was also applied to the removal of tetracycline, and the degradation efficiencies from different oxidation processes were compared. The results showed that the higher Fenton reagent concentrations led to a higher degradation efficiency, which was up to 79.6% in acidic condition, and decreased to 46.7% in neutral condition. Among the investigated oxidants, hydrogen peroxide showed the best removal and PMS demonstrated a stronger oxidizing ability than PDS. At the presence of coexisting organic macromolecules, the overall degradation efficiency decreased with the magnitude of 5-10%. The α-cyclodextrin enhanced the degradation efficiency at the reagent concentrations of 50 μM both for ferrous ion and hydrogen peroxide in acidic condition. The tetracycline degradation was found decreasing at the presence of macromolecules, implying that the macromolecules consumed more •OH than produced.

Keywords: Advanced oxidation process, Coexisting organic macromolecules, Degradation, Fenton process, Tetracycline

CONTENTS

論文口試委員審定書 i

摘要 ii

ABSTRACT iv

CONTENTS v

LIST OF FIGURES viii

LIST OF TABLES x

Chapter 1 Introduction 1

- 1.1 Background 1
- 1.2 Research objectives 3

Chapter 2 Literature Review 4

- 2.1 Tetracycline antibiotics 4
  - 2.1.1 Emerging Contaminants 4
  - 2.1.2 Tetracycline antibiotics 4
  - 2.1.3 Environmental hazards caused by tetracycline antibiotics 5
  - 2.1.4 Tetracycline 5
- 2.2 The treatment of wastewater containing tetracycline 6
  - 2.2.1 Activated carbon adsorption 6
  - 2.2.2 Biological treatment 6
  - 2.2.3 Membrane filtration 7
- 2.3 Fenton process (Chemical oxidation method) 7
  - 2.3.1 Fenton process by H_2O_2/Fe(II) 7
  - 2.3.2 Photo-Fenton process 9
  - 2.3.3 Electro-Fenton process 9

2.4 Persulfate 9

2.4.1 Fe^{2+} activated PDS oxidation process 10

2.4.2 Fe^{2+} activated PMS oxidation process 11

2.5 Natural organic matter 13

2.5.1 Natural organic matter in water bodies 13

2.5.2 Citric acid 13

2.5.3 Humic substance 14

2.5.4 Cyclodextrin 15

Chapter 3 Materials and Methods 16

3.1 Research Framework 16

3.2 Chemicals and apparatus 17

3.2.1 Experimental chemicals and materials 17

3.2.2 Experimental equipment 18

3.3 Experimental procedures 19

3.3.1 Experimental process 19

3.3.2 Experimental solutions preparation 20

3.3.3 Analytical methods 20

3.3.4 Experimental procedures 22

Chapter 4 Results and Discussion 23

4.1 Degradation of tetracycline by Fenton process 23

4.1.1 Effect of reagents concentration in same $[Fe^{2+}]/[H_2O_2]$ ratio 23

4.1.2 Effect of ferrous ion 24

4.1.3 Effect of hydrogen peroxide 26

4.2 Effect of Fe^{2+} on tetracycline removal by persulfates 27

4.2.1 Degradation efficiency between different oxidants 27

4.3 Organic macromolecules on the tetracycline removal................................29

4.3.1 Effect of pH environment..29

4.3.2 Degradation efficiency at the presence of organic macromolecules ...30

4.4 Mechanism of Tetracycline degradation by Fenton....................................33

4.4.1 Tetracycline degradation by Classic Fenton process...........................33

4.4.2 Tetracycline degradation at the presence of organic macromolecules in acidic environment 36

Chapter 5 Conclusion 41

REFERENCE 42

APPENDIX A. Data analysis of the degradation of tetracycline by Fenton process 46

APPENDIX B. Data analysis of the degradation of tetracycline by Fe^{2+} activated persulfates 52

APPENDIX C. Data analysis of the degradation of tetracycline by Fenton process in neutral environment 55

APPENDIX D. Data analysis of the degradation of tetracycline by Fenton with coexisting macromolecules 56

LIST OF FIGURES

Figure 2.1 Reaction mechanism in Fenton process without the presence of target organic compounds. 8

Figure 2.2 The reaction mechanism in Fe^{2+} activated PDS without the presence of target organic compounds. 10

Figure 2.3 The reaction mechanism in Fe^{2+} activated PMS without the presence of target organic compounds. 12

Figure 2.4 Model of HA structure 15

Figure 3.1 Research framework....16

Figure 3.2 Experimental procedure of the present study....19

Figure 3.3 The calibration curve of Tetracycline. 21

Figure 4.1 The degradation efficiency of tetracycline by Fenton process in same reagents ratio. 23

Figure 4.2 The degradation efficiency of tetracycline by Fenton process in different Fe^{2+} concentration. 25

Figure 4.3 The degradation efficiency of tetracycline by Fenton process in different H_2O_2 concentration. 26

Figure 4.4 The degradation of Tetracycline by three oxidants in different reagents concentration ratio. 28

Figure 4.5 The degradation of Tetracycline by Fenton process in different pH. 29

Figure 4.6 The degradation of Tetracycline by Fenton process at pH 3 with coexisting organic macromolecules. 31

Figure 4.7 The degradation of Tetracycline by Fenton process at pH 7 with coexisting organic macromolecules. 32

Figure 4.8 The mass spectrum of tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started. 34

Figure 4.9 The mass spectrum of tetracycline degradation by Fenton Process at pH 7 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started. 35

Figure 4.10 The mass spectrum of citrate presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started. 37

Figure 4.11 The mass spectrum of humic acid presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started. 38

Figure 4.12 The mass spectrum of α-cyclodextrin presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started. 39

LIST OF TABLES

Table 3.1 Chemical and physical properties of Tetracycline 17

Table 3.2 Experimental chemicals 18

Table 3.3 Experimental equipment 18

Table 3.4 HPLC analytical condition of Tetracycline...21

Chapter 1 Introduction

1.1 Background

In the past few decades, contaminants of emerging concern (CEC) have become an important issue in assuring the environmental quality, and drug residues is one of the important categories that contribute the prevalence of emerging contaminants in the environment. When drug residues are released into the aquatic environment, they may pose a serious threat to the ecosystem and some of them are endocrine disruptors which impact the survival of biological communities in the water bodies. Moreover, these drug residues may enter human food chains through source water abstraction and drinking water distribution systems (Sauvé and Desrosiers, 2014). Meanwhile, tetracycline (TC), listed as the essential medicine by the World Health Organization, is known as a broad-spectrum antibiotic often used to treat a number of infections and become the first line treatment for many diseases. It is also used as the medicated bath of aquaculture and a feed additive of livestock farming to prevent disease and increase growth rate (Ocampo-Pérez et al., 2015). Due to its extensive application recently, tetracycline has been detected in natural water bodies and wastewater, becoming one of the emerging and concerning contaminants.

Many reported studies indicated the detection of tetracycline antibiotics in the effluents discharged from wastewater treatment plants in Taiwan and around the world. Although remained in a relatively low concentration range, the tetracycline residue in the ambient environment still attracts a lot of attention because it is difficult to biodegrade and may accumulate in the environment. TC was removed mostly through the activated carbon adsorption or membrane filtration, and might also be treated by chemical oxidation

(Lin et al., 2009; Zhang et al., 2015; Zhou et al., 2009). Previous studies showed that photo-Fenton process and electro-Fenton processes can almost completely degrade tetracycline in wastewater (Barhoumi et al., 2017; Yamal-Turbay et al., 2013). Since the photo- and electro-Fenton processes require additional energy input, they are considered relatively expensive and difficult to be installed for in-situ treatment. The classic Fenton process utilizes the reaction of hydrogen peroxide and ferrous ions to produce hydroxyl radicals to degrade the target contaminants (Fan et al., 2011). The Fenton process was found to be highly efficient at the condition of pH 3. For the real practice of wastewater treatment, maintaining the in-situ environment at acidic condition is highly infeasible, and many coexisting organic matters may compete with the target compounds for the oxidants, affecting the removal of tetracycline.

This study aimed to explore the tetracycline degradation by Fenton process both in acidic and neutral environment and to investigate the role of coexisting organic macromolecules in the degradation process. Tetracycline was selected as the target compound and the degradation efficiencies for the experimental results conducted at various concentrations of Fenton reagents were compared.

1.2 Research objectives

In this study, Fenton process and Fe^{2+} activated persulfate (PDS and PMS) were used to degrade tetracycline (TC). Humic acid, citrate, and α-cyclodextrin as the coexisting macromolecules were added in the degradation process. The experimental parameters were adjusted to investigate the tetracycline degradation rate under a variety of scenarios.

The objectives of the study are as follows:

1. The Fenton process is used to degrade the tetracycline both in the acidic and neutral environment and the removal efficiencies of different reagent dosages were observed to evaluate the optimal reagent ratio.
2. The Fenton Process at the optimal reagent dosage was applied to the Fe^{2+}-activated persulfate processes to investigate the degradation rate of tetracycline.
3. The tetracycline degradation by Fenton process at the presence of macromolecules in the acidic and neutral environment were investigated and the role of coexisting organic macromolecules was explored.

Chapter 2 Literature Review

2.1 Tetracycline antibiotics

2.1.1 Emerging Contaminants

Emerging contaminants can be classified into naturally-occurring or man-made substances, and they have been confirmed to be or suspected to be present in various environments. Meanwhile, pharmaceutical products and its derivatives have been identified as an important category of the concerning emerging contaminants. They may act as strong endocrine disruptors in the environment and have toxicity or long-term effects on the metabolism of living organisms. So far, very few studies established the complete monitoring systems for the emerging contaminants. Thus, emerging contaminants remain to have significant potential risks to human health and environmental ecology (Sauvé and Desrosiers, 2014).

2.1.2 Tetracycline antibiotics

Tetracycline antibiotics (TCs) are a group of broad-spectrum antibiotic compounds that have a common basic chemical structure of four aromatic/hydrocarbon rings. The first tetracycline antibiotic, chlortetracycline, was found from *Streptomyces aureofaciens (S. aureofaciens)* by Dr. Benjamin Duggar (Duggar, 1948). With the advancement of analytical chemistry, tetracycline antibiotics can be classified into natural and semi-synthetic categories. Tetracycline, chlortetracycline, oxytetracycline, and demeclocycline are often used TCs that exist in nature. Tetracycline exhibited strong activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, spirochetes, chlamydiae, rickettsiae, actinomyces, and Ameoba. Moreover, the low cost of tetracycline makes it not only being used as antibiotic for human diseases treatments but also served as aquaculture medicated bath and animal husbandry feed additives to prevent diseases and increase the growth rate (Ocampo-Pérez et al., 2015).

2.1.3 Environmental hazards caused by tetracycline antibiotics

Tetracycline antibiotics have chromaticity and antibacterial effects and it is difficult to be biodegraded in the ambient water bodies (Zhou et al., 2009). Moreover, environmental microorganisms present drug resistance due to a long-term exposure to antibiotics. Contaminant residues can be harmful to humans or organisms that are at the top of the food chains through biomagnification. Tetracycline antibiotics enter the environment via pharmaceutical use, animal husbandry behavior, and discarding unused or expired drugs, so their residues are often detected in the wastewater treatment plants. Lin et al. (2009) showed that TCs were detected in four secondary wastewater treatment plants in Taipei, Taiwan. The observed concentrations of TCs in surface water ranged from 0.11 to 4.2 μg/L, and those in the effluents of wastewater treatment plants (WWTPs) ranged from 46 to 1300 ng/L for tetracycline, 270 to 970 ng/L for chlortetracycline, and 240 ng/L for oxytetracycline (Ocampo-Pérez et al., 2015).

2.1.4 Tetracycline

Tetracycline was originally made from bacteria of the *Streptomyces* type. Because of its low production cost and wide range of antibacterial effects, tetracycline becomes the first line medicine for many diseases and also is listed in the WHO Model List of Essential Medicines. Tetracycline can penetrate the bacteria cell through the pore of plasma membrane by passive diffusion, and bind to the 16sRNA on the microbial ribosomal 30S subunit, inhibiting protein synthesis by blocking the attachment of charged aminoacyl-tRNA to the A site on the ribosome (Connell et al., 2003). Thus, the bacterial cells were unable to grow without proteins to achieve the antibacterial effects.

2.2 The treatment of wastewater containing tetracycline

2.2.1 Activated carbon adsorption

Activated carbon (AC) has a relatively large specific surface area and many suitable pores, and is widely used as an adsorbent for treating aqueous antibacterial agents (Hoseinzadeh Hesas et al., 2013). The target contaminants form a equilibrated surface layer on the AC particle. The contaminants are removed by adsorption through capturing the target compounds in the pores. However, the manufacturing process of activated carbon leads to high cost and uses non-renewable raw materials (Theydan and Ahmed, 2012). To regenerate the activated carbon, it is requited to wash the spent AC with high heat or chemicals to degrade the organic matters adsorbed on the AC surface. The study showed that the maximum adsorption of tetracycline by AC was 1121.5 mg/g (Zhang et al., 2015) and 1340.8 mg/g for oxytetracycline (Sun et al., 2012).

2.2.2 Biological treatment

The biological treatment process removes organic pollutants through microbial decomposition, however, tetracycline antibiotics exhibit an antibacterial effect which may inhibit the growth of the microorganisms. Thus, it is not suitable to treat TC-containing wastewater through biological treatment.

2.2.3 Membrane filtration

The pore size on the membrane surface and the structure of the membrane are the key factors controlling the efficiency in screening out particles or molecules in membrane filtration processes. They can be classified into reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) according to the particle size that can be screened out from small to large. However, the retained particles in the membrane may cause clogging after a long term operation period, which decreases the removal efficiency.

2.3 Fenton process (Chemical oxidation method)

2.3.1 Fenton process by H_2O_2/Fe(II)

Fenton process is an advanced oxidation process which catalyzes hydrogen peroxide by ferrous ion and produce hydroxyl radicals that have a strong oxidative ability to degrade the organic contaminants into carbon dioxide and water without contaminant selectivity (Fan et al., 2011). Fenton process is a chain reaction, which works best when the pH is close to 3. In the process, hydrogen peroxides react with ferrous ions to form hydroxyl radicals, ferric ions, and hydroxide ions. Hydrogen peroxide can still react with hydroxyl radicals to produce $\cdot HO_2$ radicals and reduce the ferric ions to ferrous ions, so the chain reactions remain until the hydrogen peroxide is consumed completely or by the addition of the quenching solution (Fan et al., 2011).

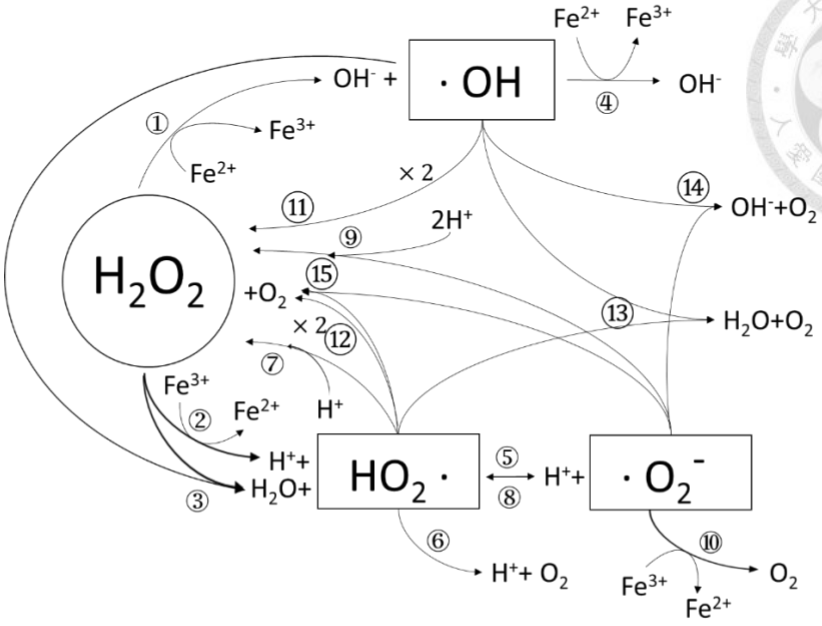


Figure 2.1 Reaction mechanism in Fenton process without the presence of target organic compounds.

The reaction equation of Fenton process:

Reaction	$K(M^{-1}S^{-1})$	Ref.
① $Fe^{2+} + H_2O_2 \rightarrow \cdot OH + OH^- + Fe^{3+}$	63	Metelitsa (1971)
② $H_2O_2 + Fe^{3+} \rightarrow \cdot HO_2 + H^+ + Fe^{2+}$	0.01	Walling and Goosen (1973)
③ $H_2O_2 + \cdot OH \rightarrow H_2O + \cdot HO_2$	2.7×10^{7}	Christensen et al. (1982)
④ $Fe^{2+} + \cdot OH \rightarrow OH^- + Fe^{3+}$	3.2×10^{8}	Stuglik and PawełZagórski (1981)
⑤ $\cdot HO_2 \rightarrow H^+ + \cdot O_2^-$	$1.58\times10^{-5}(S^{-1})$	Bielski et al. (1985)
⑥ $\cdot HO_2 + Fe^{2+} + H^+ \rightarrow H_2O_2 + Fe^{3+}$	1.2×10^{6}	Jayson et al. (1969)
⑦ $\cdot HO_2 + Fe^{3+} \rightarrow O_2 + H^+ + Fe^{2+}$	3.1×10^{5}	Rush and Bielski (1985)
⑧ $\cdot O_2^- + H^+ \rightarrow \cdot HO_2$	1.0×10^{10}	Bielski et al. (1985)
⑨ $\cdot O_2^- + Fe^{2+} + 2H^+ \rightarrow H_2O_2 + Fe^{3+}$	1.0×10^{7}	Rush and Bielski (1985)
⑩ $\cdot O_2^- + Fe^{3+} \rightarrow O_2 + Fe^{2+}$	5.0×10^{7}	Rothschild and Allen (1958)
⑪ $\cdot OH + \cdot OH \rightarrow H_2O_2$	4.2×10^{9}	Sehested et al. (1969)
⑫ $\cdot HO_2 + \cdot HO_2 \rightarrow H_2O_2 + O_2$	8.3×10^{5}	Bielski et al. (1985)
⑬ $\cdot OH + \cdot HO_2 \rightarrow H_2O + O_2$	1.0×10^{10}	Sehested et al. (1969)
⑭ $\cdot OH + \cdot O_2^- \rightarrow O_2 + OH^-$	1.0×10^{10}	Sehested et al. (1969)
⑮ $\cdot HO_2 + \cdot O_2^- (+ H^+) \rightarrow H_2O_2 + O_2$	9.7×10^{7}	Bielski et al. (1985)

2.3.2 Photo-Fenton process

The Fenton process may increase the production of ferric hydroxide sludge when treating real wastewater at neutral condition. In order to improve the disadvantage of Fenton process, photo-Fenton process was introduced to reduce the ferric ions to ferrous ions by photoelectric effect under UV light irradiation (Yamal-Turbay et al., 2013). However, the operating cost and energy spent for photo-Fenton process is high, making the treatment method difficult to apply to the *in situ* treatment.

2.3.3 Electro-Fenton process

The electro-Fenton process employs the same principle as the Fenton process that degrade the organic contaminants by strong oxidative hydroxyl radicals formed by the reaction between hydrogen peroxide and ferrous irons. The electro-Fenton process applies an electric field which can oxidize the organic pollutants at the anode and reduce the ferric ions to ferrous ions at the cathode to react with hydrogen peroxide continuously in the acidic environment (Nidheesh and Gandhimathi, 2012). As described in the previous paragraph, the application of the electro-Fenton process requires electrical energy and increase operation cost, thereby limiting the practical application of this method.

2.4 Persulfate

Although the Fenton process is effective in removing organic contaminants, there are several disadvantages such as iron sludge production, hydrogen peroxide consumption and pH dependency. Recent studies showed many successful cases in which peroxydisulfate (PDS, $S_2O_8^{2-}$) and peroxymonosulfate (PMS, SO_5^{2-}) were applied to on-site wastewater treatment (Kaur and Crimi, 2014; Zhao et al., 2014). Persulfates have the ability to decompose contaminants such as polychlorinated biphenyls (PCBs) (Fan et al., 2014; Fang et al., 2013), chlorophenol (Rastogi et al., 2009), polycyclic aromatic hydrocarbons (PAHs) (Gryzenia et al., 2009), and BTEX (Liang et al., 2008).

2.4.1 Fe^{2+} activated PDS oxidation process

As indicated in the study by (Wang et al., 2019), the reaction mechanism in Fe^{2+} activated PDS without the presence of target organic compounds was shown in Figure 2.2. In the reaction system, PDS is activated by Fe^{2+} to form sulfate radicals ($SO_4^{\bullet-}$) that may react with H_2O to form hydroxyl radicals or with PDS to form peroxydisulfate radicals.

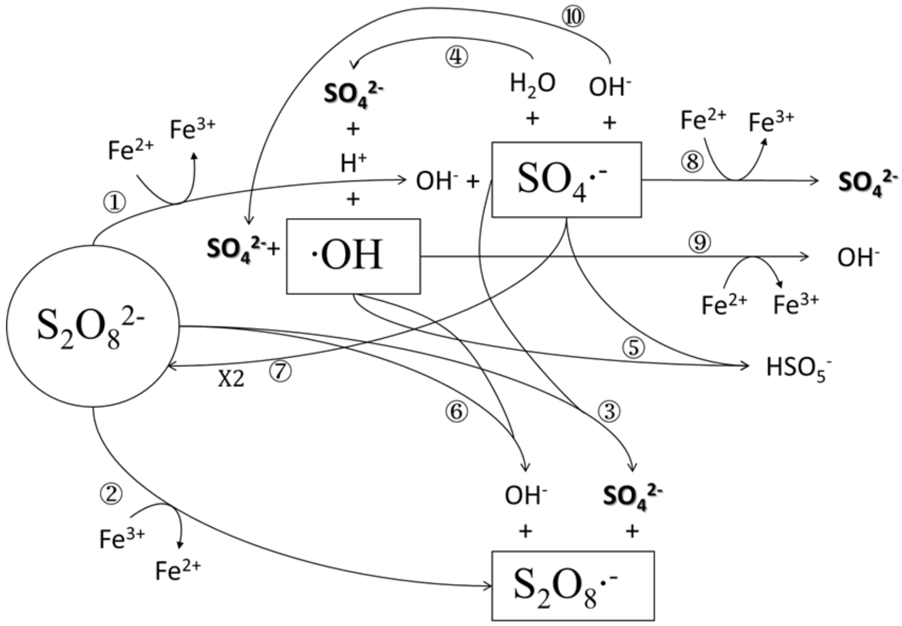


Figure 2.2 The reaction mechanism in Fe^{2+} activated PDS without the presence of target organic compounds.

The reaction equation of Fe2+ activated PDS oxidation process (Wang et al., 2019):

Reaction	$K(M^{-1}S^{-1})$
① $Fe^{2+} + S_2O_8^{2-} \rightarrow Fe^{3+} + SO_4^{\bullet -} + SO_4^{2-}$	17
② $Fe^{3+} + S_2O_8^{2-} \rightarrow Fe^{2+} + S_2O_8^{\bullet -}$	
③ $SO_4^{\bullet -} + S_2O_8^{2-} \rightarrow S_2O_8^{\bullet -} + SO_4^{2-}$	1.2×10^{6}
④ $SO_4^{\bullet -} + H_2O \rightarrow H^+ + SO_4^{2-} + HO\bullet$	200
⑤ $SO_4^{\bullet -} + HO\bullet \rightarrow HSO_5^-$	1.0×10^{10}
⑥ $HO\bullet + S_2O_8^{2-} \rightarrow S_2O_8^{\bullet -} + OH^-$	1.4×10^{7}
⑦ $\cdot SO_4^{\bullet -} + SO_4^{\bullet -} \rightarrow S_2O_8^{2-}$	8.1×10^{8}
⑧ $Fe^{2+} + SO_4^{\bullet -} \rightarrow Fe^{3+} + SO_4^{2-}$	4.6×10^{9}
⑨ $Fe^{2+} + HO\bullet \rightarrow Fe^{3+} + OH^-$	4.8×10^{8}
⑩ $SO_4^{\bullet -} + OH^- \rightarrow SO_4^{2-} + HO\bullet$ (Alkaline pH)	6.5×10^{7}
⑪ $Fe^{3+} + RH \rightarrow Fe^{2+} + R\bullet + H^+$	

2.4.2 Fe^{2+} activated PMS oxidation process

The reaction mechanism in Fe^{2+} activated PMS without the presence of target organic compounds was shown in Figure 2.3. PMS can activated by light or Fe^{2+} to form sulfate radicals ($SO_4^{\bullet -}$) and peroxymonosulfate radicals ($SO_5^{\bullet -}$), and these radicals may react with H_2O to form hydroxyl radicals. These radicals may react with each other or other species, resulting in the complexity in the PMS activation system.

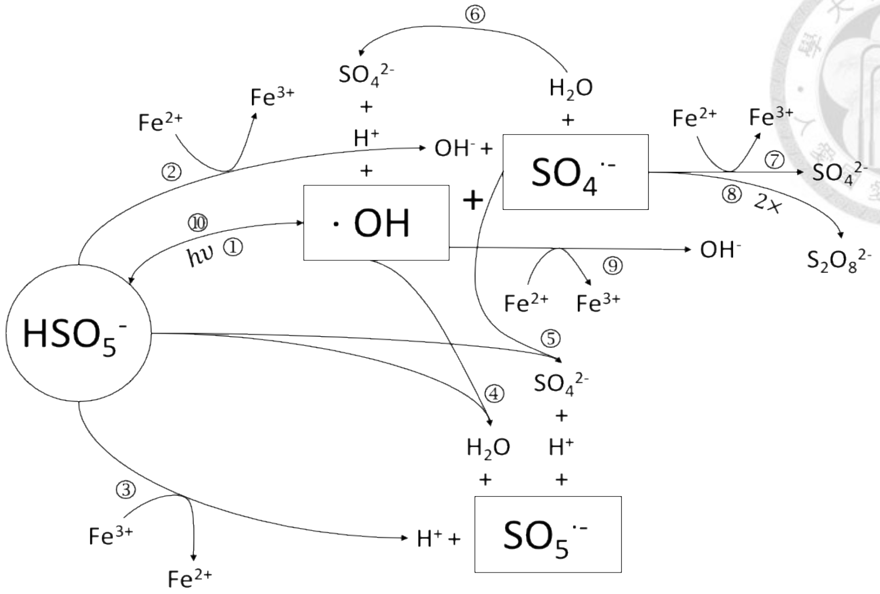


Figure 2.3 The reaction mechanism in Fe^{2+} activated PMS without the presence of target organic compounds.

The reaction equation of Fe2+ activated PMS oxidation process:

Reaction

① $HSO_5^- \rightarrow \cdot OH + SO_4^{\bullet-}$

② $HSO_5^- + Fe^{2+} \rightarrow OH^- + SO_4^{\bullet-} + Fe^{3+}$

③ $HSO_5^- + Fe^{3+} \rightarrow H^+ + SO_5^{\bullet-} + Fe^{2+}$

④ $HSO_5^- + \cdot OH \rightarrow H_2O + SO_5^{\bullet-}$

⑤ $HSO_5^- + SO_4^{\bullet-} \rightarrow SO_4^{2-} + H^+ + SO_5^{\bullet-}$

⑥ $SO_4^{\bullet-} + H_2O \rightarrow SO_4^{2-} + H^+ + \cdot OH$

⑦ $SO_4^{\bullet-} + Fe^{2+} \rightarrow SO_4^{2-} + Fe^{3+}$

⑧ $SO_4^{\bullet-} + SO_4^{\bullet-} \rightarrow S_2O_8^{2-}$

⑨ $\cdot OH + Fe^{2+} \rightarrow Fe^{3+} + OH^-$

⑩ $\cdot OH + SO_4^{\bullet-} \rightarrow HSO_5^-$

2.5 Natural organic matter

2.5.1 Natural organic matter in water bodies

The natural organic matter (NOM) includes all kinds of natural-occurring dissolved and particulate organic molecules. The sources of NOM in natural water bodies are the metabolic products, excretions and residues of animals and plants in the environment. The NOM in water can be classify by the size; (1) particulate organic matter (POM) is a kind of NOM with a particle size larger than 0.45μm, which can be removed by wastewater treatment and water purification processes; (2) dissolved organic matter (DOM) is a class of NOM with a particle size smaller than 0.45μm, which is difficult to remove by conventional wastewater treatment process (Leenheer and Croué, 2003).

2.5.2 Citric acid

Citric acid (2-Hydroxypropane-1,2,3-tricarboxylic acid) is a kind of organic acid found in plants and soil. It is a natural preservative produced by citrus fruits and commonly used as industrial chelating agents, household cleaners, and food additives. Meanwhile, the best operating pH of the Fenton process is 3 (Velásquez et al., 2014). However, when the pH increased, ferrous and ferric ions may precipitate and decrease the efficiency of Fenton process. To prevent the precipitation, chelating agent such as EDTA (Ethylenediaminetetraacetic acid), NTA (Nitrilotriacetic acid), and citrate was added to maintain the soluble form of Fe(II) and Fe(III) at neutral pH (Ling et al., 2017). Since EDTA is poor in biodegradability and environmentally persistent and NTA is a carcinogen, (Rastogi et al., 2009), the citrate is considered more suitable for use as a chelating agent in water and wastewater treatment.

2.5.3 Humic substance

Humic substances are weakly acidic (pKa= 3~5) and hydrophobic, which consist of mainly humic acid and fluvic acid. The molecular weight ranges from hundreds to tens of thousands Daltons, which makes humic substances a group of heterogeneous compounds with a lot of functional groups. In addition, humic substances are in negatively charged colloidal forms in natural water bodies and are difficult to decompose by microorganisms. Humic substances can be classified into the following three categories according to its solubility and molecular weight (Aiken, 1985):

- Humin: the molecular weight is up to 300,000 Da and is insoluble in any aqueous solution.
- Humic acid (HA): insoluble when $pH<2$ in aqueous solution, molecular weight between 1,000~100,000 Da.
- Fulvic acid (FA): the molecular weight is between 200~2,000 Da and containing more acidic functional groups (-COOH) and soluble in aqueous solution.

Humic acids are composed of molecules rich in both acidic functional groups such as carboxyl and phenolic groups, and hydrophobic moieties such as alkyl chains and aromatic cores (Ikeya et al., 2015). Hydrophilic active groups such as carboxyl and phenolic groups are easily dissociated in water to form negatively charged poly-anionic compounds, which cause humic acid to form stable complexes with metal ions (de Melo et al., 2016). However, the chemical composition of HAs is different depending on geographical region, climate and time, so it is difficult to accurately delineate its structure. A simulated structure of humic acid is shown in Figure 2.4 (Mirza et al., 2011).

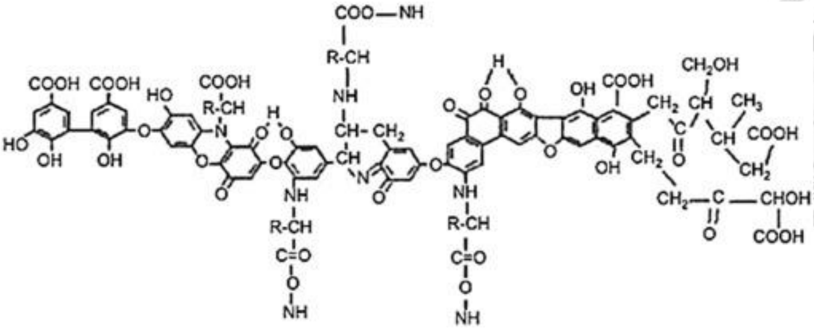


Figure 2.4 Model of HA structure

Based on its structural formula, humic acid can be divided into hydrophilic and hydrophobic parts. The hydrophilic part is composed of phenolic group and carboxyl group; the hydrophobic part is composed of aliphatic and aromatic. Hydrophilic phenolic groups and reactive functional groups such as carboxyl groups are easily to dissociate in water to form negatively charged poly-anions, which cause humic acid to form stable complexes with metal ions (De Melo et al, 2016).

2.5.4 Cyclodextrin

Produced from starch, cyclodextrins are a family of cyclic oligosaccharides consisting of a macrocyclic ring of glucose subunits. Cyclodextrins are used in food, pharmaceutical, and chemical industries, as well as agriculture and environmental engineering. Cyclodextrin is easily hydrolyzed under acidic conditions, and the interior of the toroid is regarded as less hydrophilic than the aqueous environment according to their structure, which enable them to host other hydrophobic molecules. The following three typical cyclodextrins contain six to eight glucose monomers in a ring, creating a cone shape:

- α-cyclodextrin: 6 glucose subunits
- β-cyclodextrin: 7 glucose subunits
- γ-cyclodextrin: 8 glucose subunits

Chapter 3 Materials and Methods

3.1 Research Framework

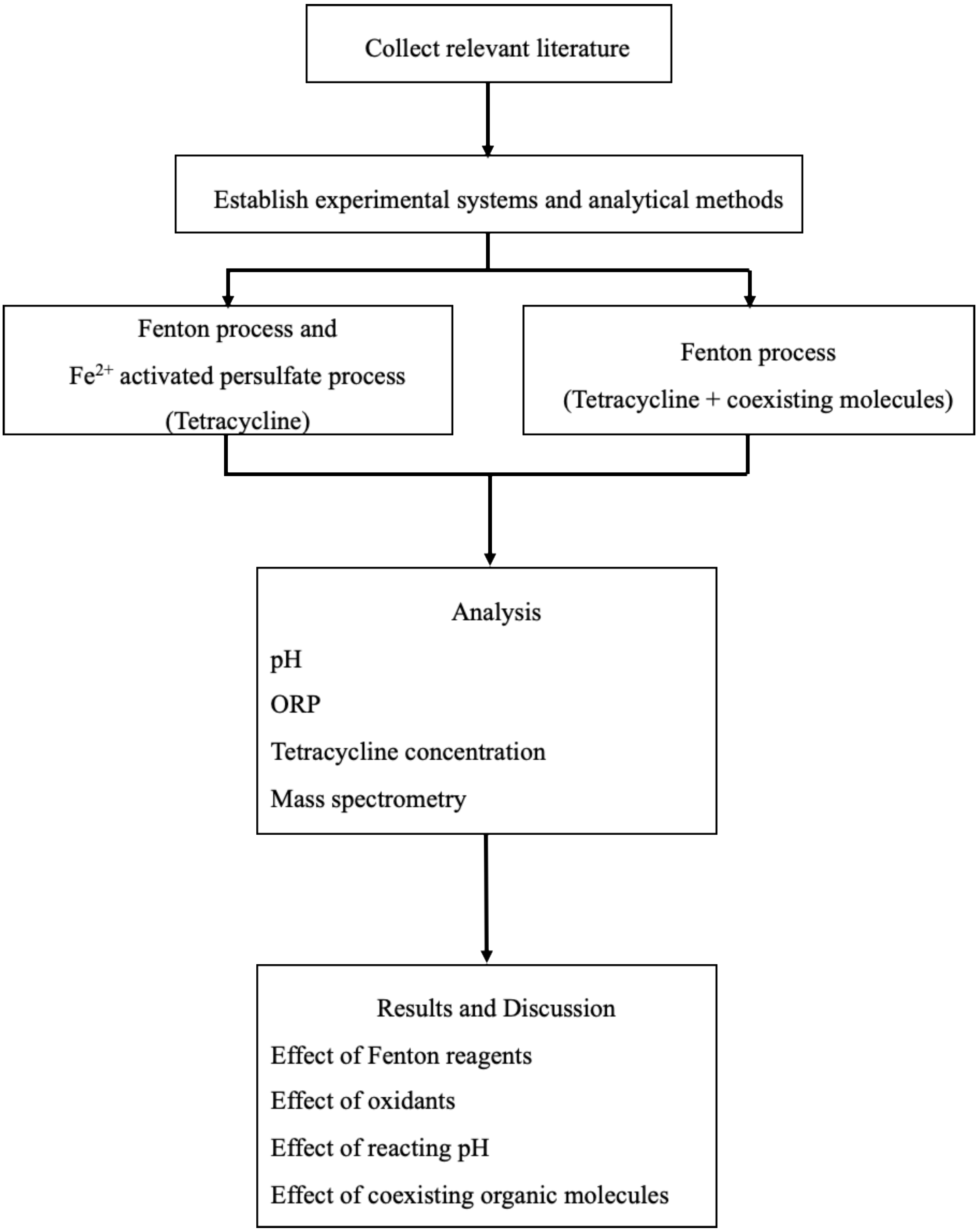


Figure 3.1 Research framework

3.2 Chemicals and apparatus

3.2.1 Experimental chemicals and materials

The chemical and physical properties, structure of tetracycline were listed in Table 3.1. All chemicals used were reagent grade, including hydrogen peroxide, ferrous sulfate, methanol, oxalic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium citrate, humic acid and cyclodextrin (details please refer to Table 3.2).

Table 3.1 Chemical and physical properties of Tetracycline

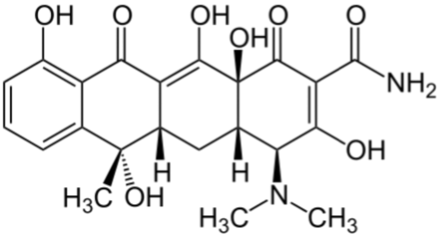
Chemical Name	**Tetracycline**
Molecular Formula	$C_{22}H_{24}N_2O_8$
Molecular Weight	444.44 g/mol
Water Solubility	231 mg/L
Melting Point	170~175℃
pKa	3.30
Structure	
Chemical Vendor	Sigma-Aldrich (Israel)

Table 3.2 Experimental chemicals

Chemical Name	Molecular Formula	Chemical Vendor	Country
Hydrogen peroxide	H_2O_2	Sigma-Aldrich	Germany
Ferrous sulfate	$FeSO_4$	Avantor	India
Sodium thiosulfate	$Na_2S_2O_3$	Alfa Aesar	Great Britain
Methanol	CH_3OH	Honeywell	Germany
Oxalic acid	$H_2C_2O_4$	Honeywell	Germany
Potassium dihydrogen phosphate	KH_2PO_4	Sigma-Aldrich	China
Dipotassium hydrogen phosphate	K_2HPO_4	Sigma-Aldrich	China
Sodium citrate	$C_6H_5O_7Na_3$	Sigma-Aldrich	India
Humic acid	-	Sigma-Aldrich	Switzerland
α-Cycoldextrin	$C_{36}H_{60}O_{30}$	Sigma-Aldrich	USA

3.2.2 Experimental equipment

Tetracycline was detected by HPLC. ORP and pH meters were employed during the experimental process to collect data. The precision of the electronic balance was up to 0.1 mg. The AOP experiments were conducted at the rotating speed of 600 rpm during the experiment.

Table 3.3 Experimental equipment

Name	Vendor	Country
High Performance Liquid Chromatography	Thermo Fisher	USA
pH meter	HOTEC	Taiwan
ORP meter	HOTEC	Taiwan

Electronic Balance	Shimadzu	Japan
Magnetic Stirrer	Clean	Taiwan

3.3 Experimental procedures

3.3.1 Experimental process

This study aimed to investigate the degradation of tetracycline by Fenton process in acidic and neutral environment to explore the reagent ratio of Fe^{2+} to H_2O_2 with best removal efficiency. The oxidant in Fenton process was replaced by persulfate and peroxymonosulfate in both pH conditions to investigate the degradation of tetracycline. Moreover, the addition of coexisting organic macromolecules, including citrate, humic acid and cyclodextrin, were conducted to explore the impact to the Fenton system.

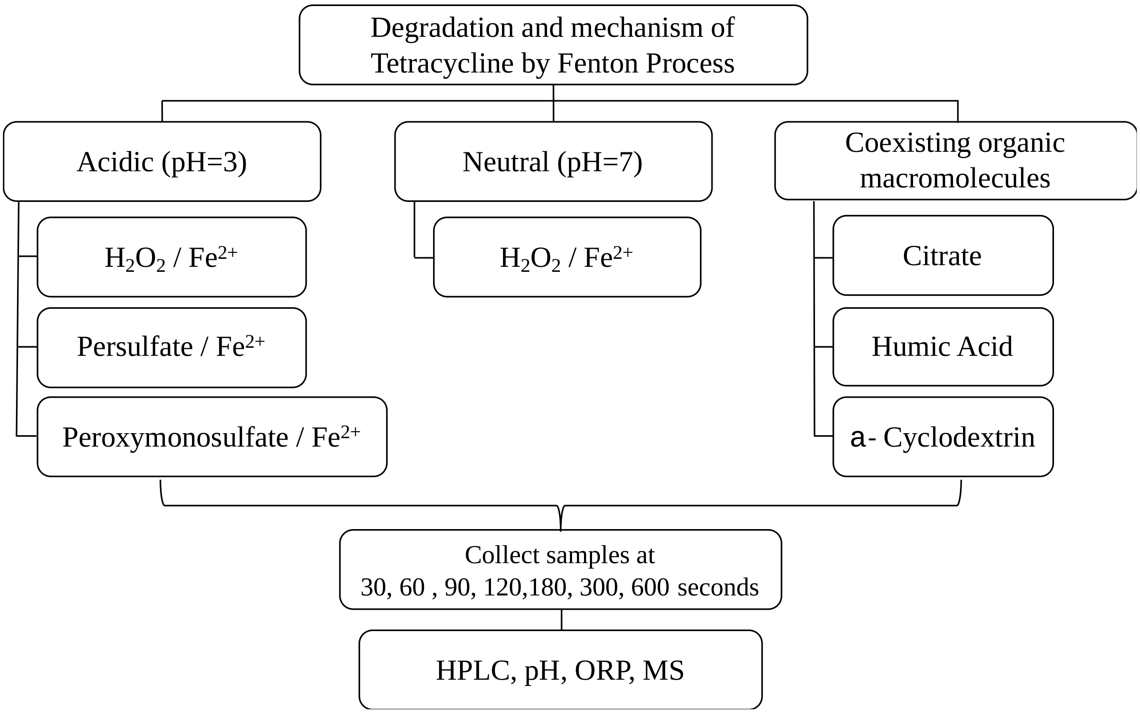


Figure 3.2 Experimental procedure of the present study

3.3.2 Experimental solutions preparation

Tetracycline solution was prepared every time immediately prior to experimentation. 25mg tetracycline powder were dissolved in 1L deionized water to make 100ppm tetracycline stock solution. The concentrations of tetracycline in the calibration curve preparation were dilute from the 100 ppm stock solution. For the experiments in neutral condition, 0.2 M 10 mL phosphate buffer was added to adjust the pH at 7.

In acidic Fenton system, a ferrous sulfate stock solution of 5 mM was prepared weekly, and the solution was acidified to pH 2 by sulfuric acid and was kept in 4℃ refrigerator. The H_2O_2 stock solution was dilute from 30% H_2O_2 to 5 mM and stored in brown bottle at 4℃ to prevent degradation. The humic acid stock solution was prepared in alkaline environment by NaOH addition, and stirred for an hour to fully dissolve the humic acid. The solution has to stand still for another hour, and filtered through the 0.45 μm glass fiber filter. The 1000 ppm humic stock solution has to store in dark bottle in 4℃ refrigerator.

3.3.3 Analytical methods

Tetracycline concentrations were quantified by Thermo Scientific Ultimate 3000 HPLC with Thermo Scientific Acclaim 120 C18 5μm 120Å (4.6 X 250mm) column and UV detector. The mobile phase condition was listed in Table 3.4. The calibration curve was showed in Figure 3.3.

Both the pH and the ORP value were measured by the electrode method. Before measurement, the pH meter were calibrated with the pH standard solution 4.01/ 7.00/10.01 and the ORP meter calibrated by ORP standard solution 220mV.

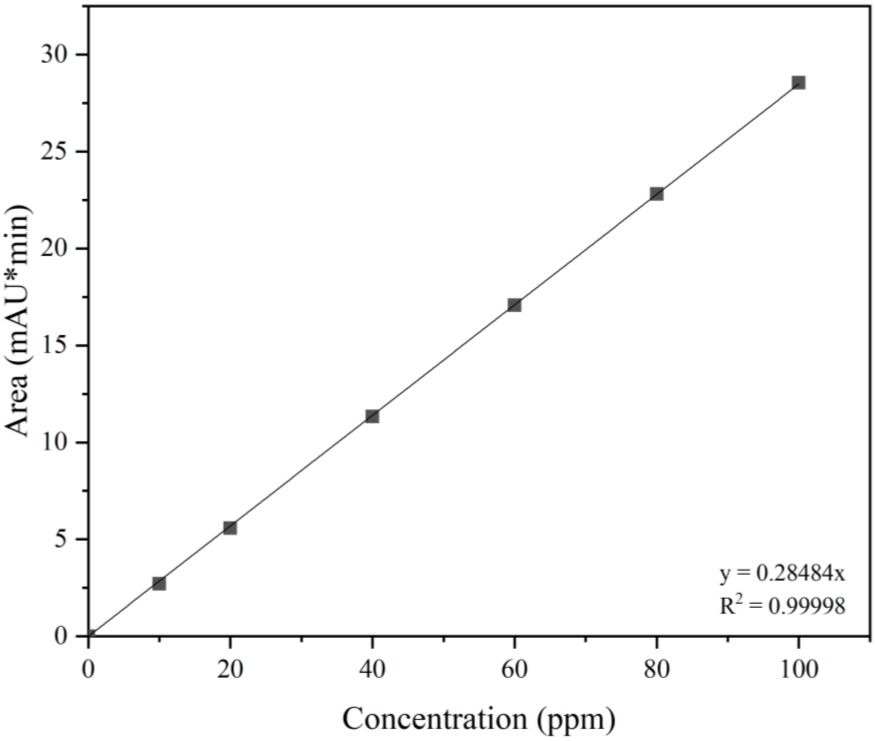


Figure 3.3 The calibration curve of Tetracycline.

Table 3.4 HPLC analytical condition of Tetracycline

Column Temperature	30°C	Flow rate	1.7 mL/min
Sample Volume	20 μL	Wavelength	360 nm
Mobile Phase Gradient	Time (min)	Methanol (%)	0.01M Oxalic acid (%)
	0	15	85
	4	30	70
	5	50	50
	6	75	25
	8	50	50
	9	30	70
	11	15	85

3.3.4 Experimental procedures

The initial concentration of tetracycline solution was 50 ppm. The acidic $FeSO_4$ was added in 500 mL tetracycline solution at the given experimental conditions. Sulfuric acid was used to adjust the pH and the magnetic stirrer at 600 rpm was used for mixing. The degradation experiment started by the addition of hydrogen peroxide, persulfate, or peroxymonosulfate into the solution, and the pH and ORP values were measured during the process. Samples were collected at the beginning and 30, 60, 90, 120, 180, 300, 600 seconds after the reaction taking place. All the samples were quenched by sodium thiosulfate immediately after collection, and were filtered through 0.22 μm filter and analyzed by HPLC.

The initial concentration of tetracycline solution was 50 ppm, in which phosphate buffer was added for pH control around 6.9 to 7. The Fenton process started by adding the oxidant and quenched by $Na_2S_2O_3$. Samples were collected at specific time and filtered before analyzed by HPLC.

The coexisting organic macromolecules were added in the initial tetracycline solution. In the acidic environment, the pH value was adjusted to 3; the pH was controlled around 6.9 to 7 by phosphate buffer in neutral experiments. The Fenton degradation started by adding hydrogen peroxide in the solution and the reaction was quenched by adding $Na_2S_2O_3$ when samples were collected. Samples were filtered and analyzed by HPLC.

Chapter 4 Results and Discussion

4.1 Degradation of tetracycline by Fenton process

4.1.1 Effect of reagents concentration in same [Fe^{2+}]/[H_2O_2] ratio

In the tetracycline degrade by Fenton process, the solution pH was around 3 and the initial concentration of tetracycline was 50 ppm. The Fenton reagents concentrations were 10, 30, 50, 70, 85, 100 μM at the [Fe^{2+}]/[H_2O_2] ratio of 1. The results are shown in Figure 4.1. During the first 30 seconds after the reaction took place, the degradation proceeded fast, and the reaction became slow after 30 seconds. The highest degradation percentage was 79.6% when Fe^{2+} and H_2O_2 were both 100 μM. The removal efficiencies were 10.0%, 35.0%, 44.3%, 63.8%, and 74.5% at the H_2O_2 concentrations of 10, 30, 50, 70, 85 μM, respectively. The more reagents added in the system, the higher degradation efficiency was observed.

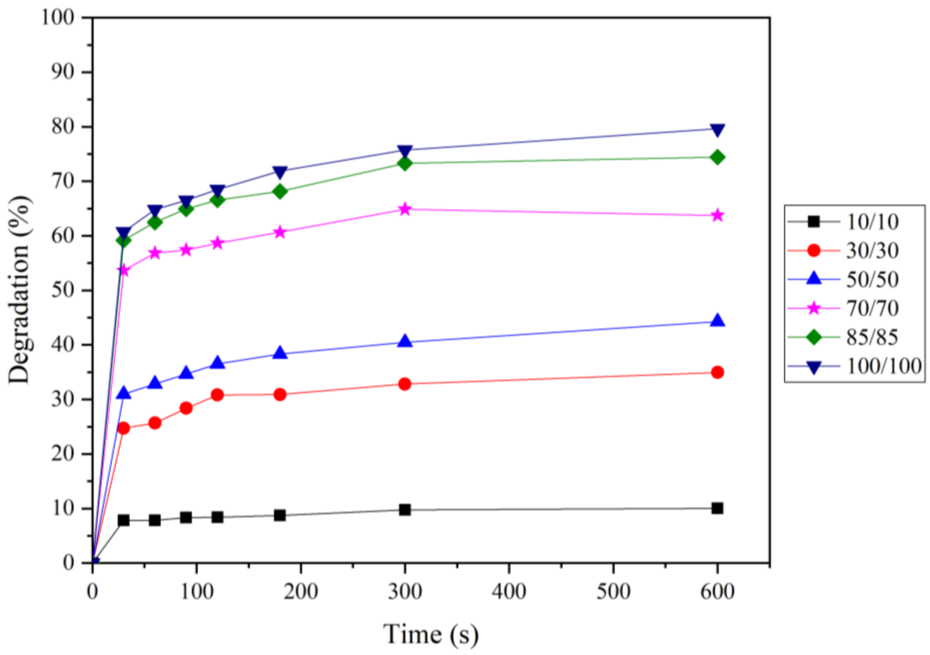


Figure 4.1 The degradation efficiency of tetracycline by Fenton process in same reagents ratio.

4.1.2 Effect of ferrous ion

Two hydrogen peroxide concentrations were chosen and the results were shown in Figure 4.2. During the first 30 seconds of the reaction, tetracycline has been degraded rapidly, and the decomposition became slow in the following 10 minutes. The removal efficiencies were 34.0%, 40.2%, 44.3%, 51.7%, 53.9%, and 58.3% at the Fe^{2+} concentrations of 10, 30, 50, 70, 85, 100 μM, respectively. As shown in Figure 4.2B, the removal efficiency was 79.6% after 10 minutes when the concentrations of both Fenton agents were 100 μM. The removal efficiencies were 49.2%, 56.7%, 71.0%, 69.0%, and 75.8% at the Fe^{2+} concentrations of 10, 30, 50, 70, 85 μM, respectively. The ferrous ions catalyzed the hydrogen peroxides to produce hydroxyl radicals, and the production rate increased when the ferrous ion concentration increased, making the degradation rate faster. However, excessive ferrous ions might interact with hydroxyl radicals and cause the consumption of hydroxyl radicals, making the enhancement in removal efficiency less obvious or exhibiting a decreasing fashion.

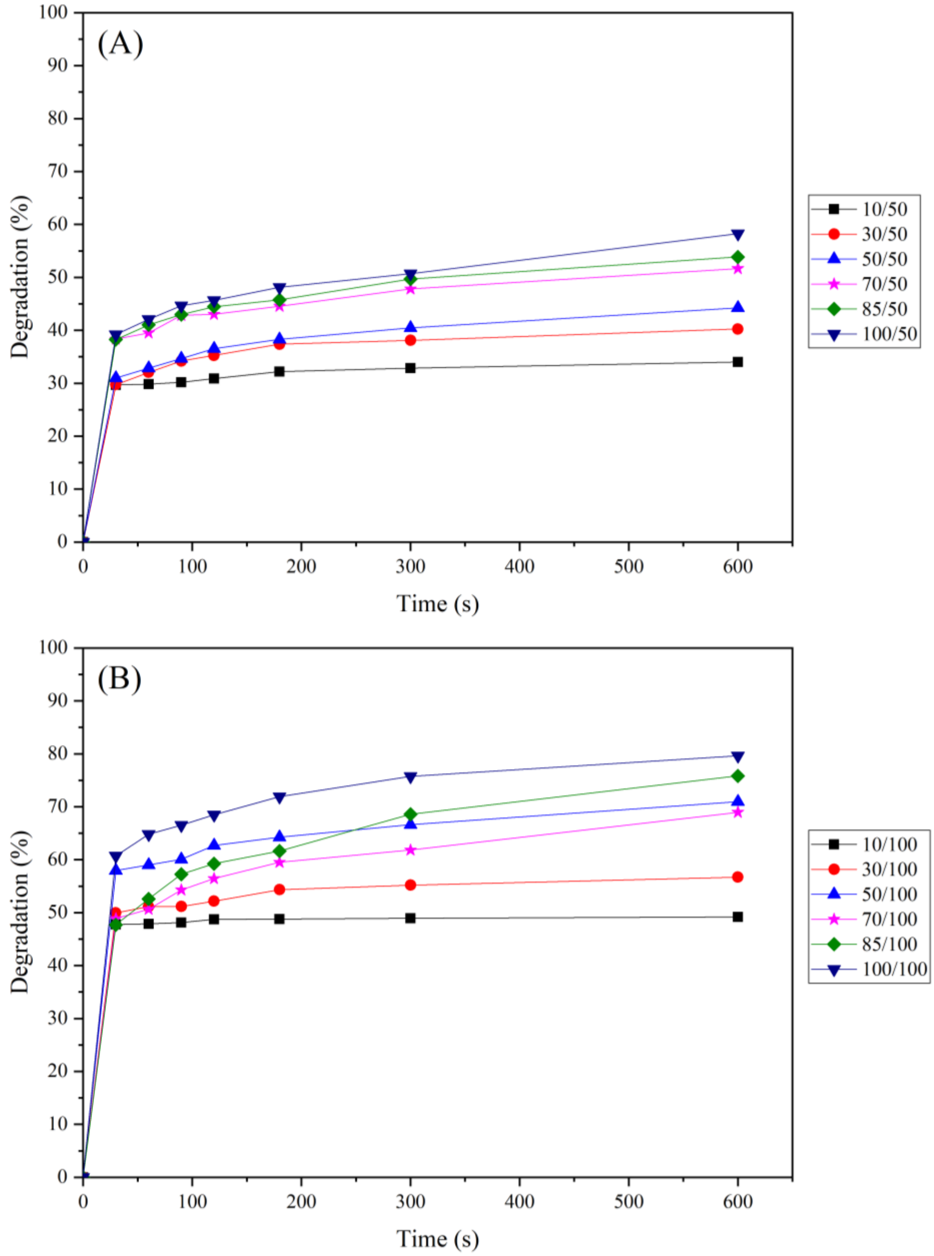


Figure 4.2 The degradation efficiency of tetracycline by Fenton process in different Fe^{2+} concentration. (A) $[H_2O_2]$ = 50 μM. (B) $[H_2O_2]$ = 100 μM.

4.1.3 Effect of hydrogen peroxide

At the ferrous ion concentration of 10 μM, the concentration of hydrogen peroxide was varied (i.e., $[Fe^{2+}]/[H_2O_2]$ ratios were 1:1, 1:5, and 1:10) in the degradation experiments and the results were shown in Figure 4.3. Because of the low concentration of ferrous ions, the reaction proceeded at the first 30 seconds and was slowed down afterwards. The maximum tetracycline removal was 49.17% when the hydrogen peroxide was 100 μM, 34.0% for 50 μM hydrogen peroxide, and 10.0% for 10 μM hydrogen peroxide, respectively. With the presence of Fe^{2+}, the more H_2O_2 concentration resulted in a more efficient tetracycline removal because of more hydroxyl radicals production.

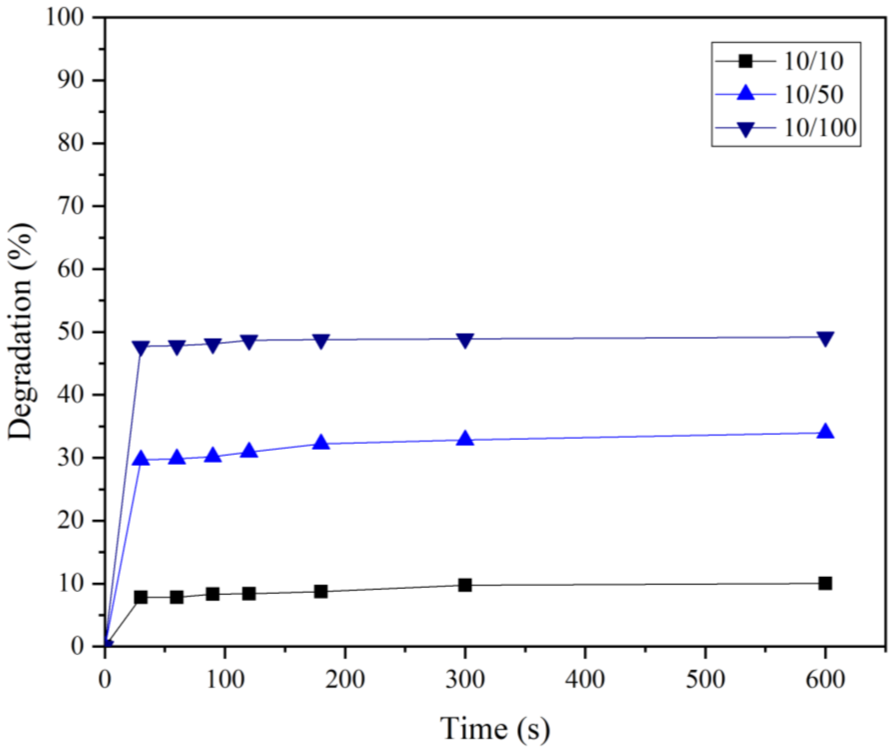


Figure 4.3 The degradation efficiency of tetracycline by Fenton process in different H_2O_2 concentration.

4.2 Effect of Fe^{2+} on tetracycline removal by persulfates

4.2.1 Degradation efficiency between different oxidants

In the PDS and PMS activated by Fe^{2+} experiments, the pH of the solution was controlled around 3. Three concentration combinations were applied and the results were shown in Figure 4.4. The results indicated that the degradation by hydrogen peroxide showed the best removal efficiency and the peroxymonosulfate showed a stronger oxidizing ability than peroxydisulfate. However, the removal efficiency increased in the first 30 seconds and remained stable until 10 minutes while the degradation reaction continued steadily in the case of hydrogen peroxide and peroxydisulfate. While the Fe^{2+} and PDS concentration were both 100 μM, the degradation pattern exhibited a different fashion from that in the Fenton process. (Figure 4.4C) The degradation could be classified into two part: (1) fast stage: from the reaction start to 90 seconds, ferrous ions activated persulfate and produced sulfate radicals to degrade tetracycline rapidly; (2) slow stage: the reaction constants of sulfate radicals with other intermediate compounds were higher than with the target compound. As the consumption of involving radicals increased, the degradation of tetracycline reduced.

Sulfate radicals produced in the Fe^{2+}/PDS and Fe^{2+}/PMS systems might react not only with target compounds but also with ferrous ions, oxidizing ferrous ions to ferric ions to decrease the sulfate radicals production, leading to a lower removal efficiency than that of the classic Fenton process. In addition, the reaction of reducing ferric ions to ferrous ions in the Fe^{2+}/PDS and Fe^{2+}/PMS systems impacts the degradation of tetracycline in the investigated system significantly.

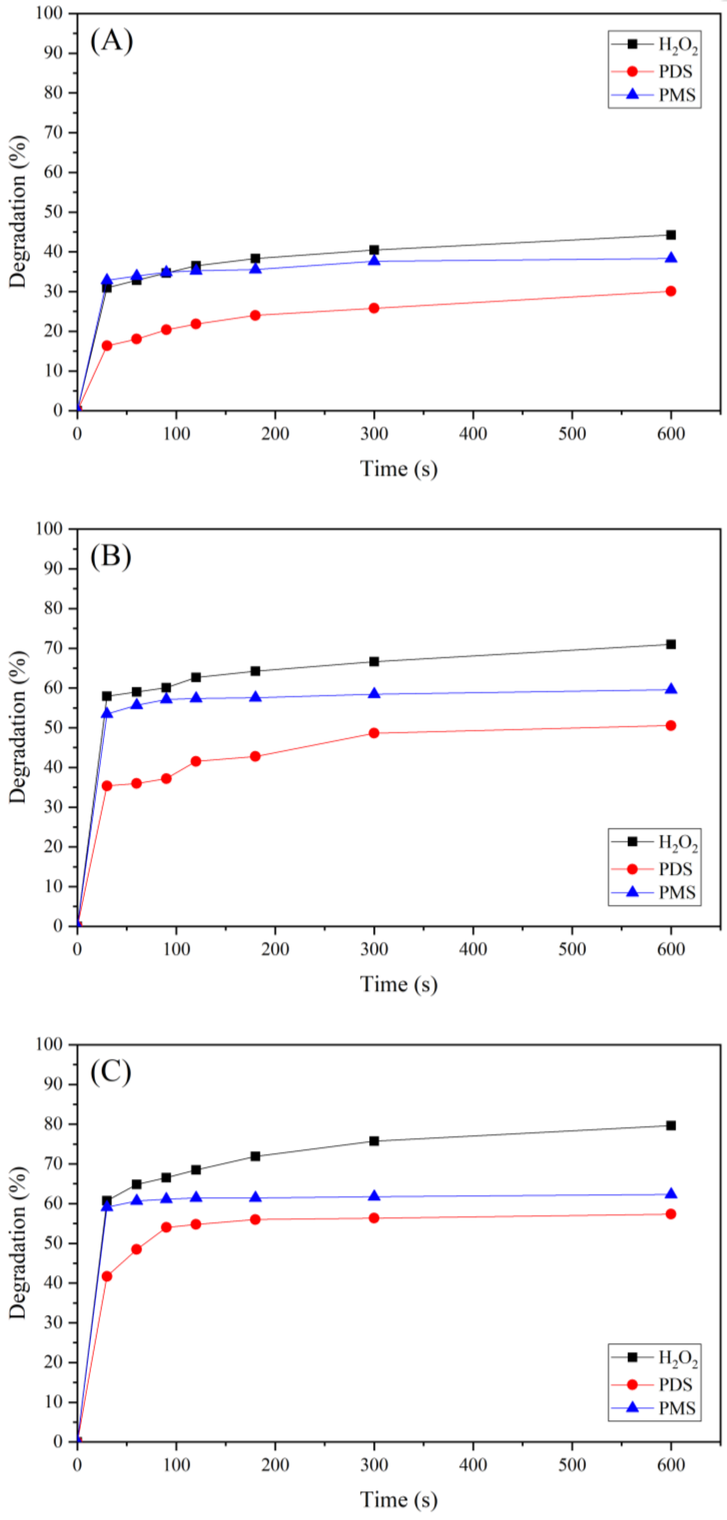


Figure 4.4 The degradation of Tetracycline by three oxidants in different reagents concentration ratio. (A) [Fe^{2+}] = 50 μM and [oxidants] = 50 μM. (B) [Fe^{2+}] = 50 μM and [oxidants] = 100 μM. (C) [Fe^{2+}] = 100 μM and [oxidants] = 100 μM.

4.3 Organic macromolecules on the tetracycline removal

4.3.1 Effect of pH environment

Fenton process was reported to have better treatment efficiency to operate in acidic environment, and the removal efficiency in neutral condition was still low. Two experiments were conducted to investigate the effect of pH and the results were shown in Figure 4.5. The degradation at neutral pH was decreased in general. At the first 30 seconds, the degradation was fast, and became slow afterwards. At [Fe^{2+}] = 50 μM, the higher H_2O_2 concentration resulted in better tetracycline removal. The maximum removal was found around 46.7% at neutral environment and 71.0% at acidic condition.

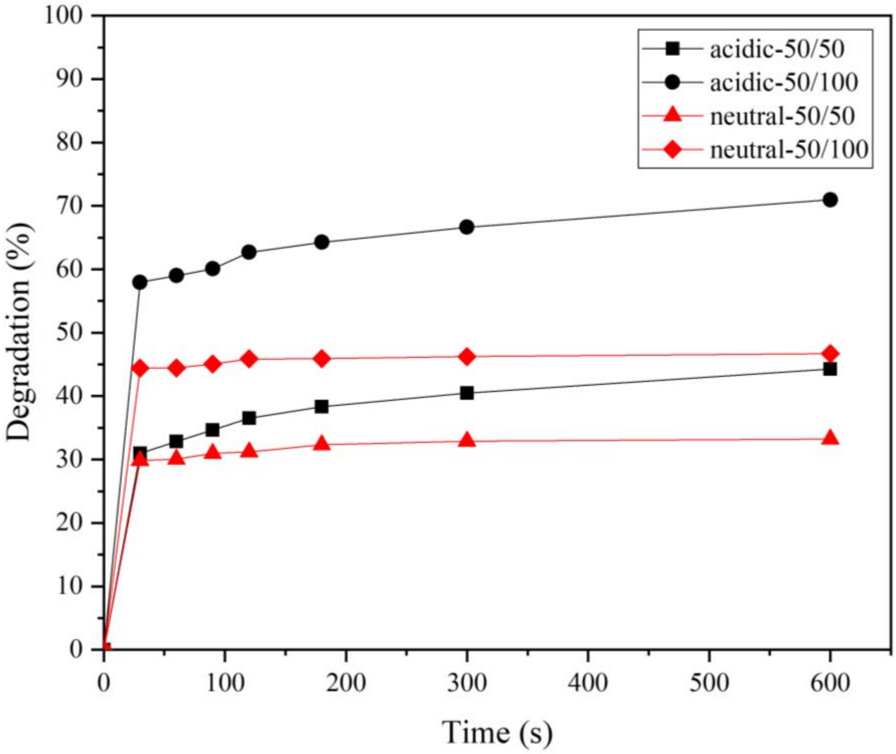


Figure 4.5 The degradation of Tetracycline by Fenton process in different pH.

4.3.2 Degradation efficiency at the presence of organic macromolecules

In this study, citrate, humic acid and α-cyclodextrin were added in the Fenton process to simulate impact of the coexisting organic macromolecules on the degradation. In the presence of citrate in acidic condition, the degradation efficiency was markedly lower than those in the absence of citrate (Figure 4.6). A coexisting organic macromolecule may compete with the target compound and facilitate the hydroxyl radical production, resulting in a possible change in organic removal efficiency. In Figure 4.6A, at the presence of α-cyclodextrin in $[Fe^{2+}]/[H_2O_2] = 50\ \mu M / 50\ \mu M$, the coexisting organic macromolecules have increased the degradation efficiency than that in the classic Fenton process, but the degradation efficiency decreased in $[Fe^{2+}]/[H_2O_2] = 50\ \mu M / 100\ \mu M$ (Figure 4.6B). The humic acid molecules may capture the tetracycline molecules in acidic condition, leading to the decrease in tetracycline concentration.

At the presence of coexisting organic macromolecules in neutral condition, the initial drop in tetracycline concentration implied that the macromolecules might chelate with tetracycline before degradation taking place (Figure 4.7). In Figure 4.7A, the competition between tetracycline and citrate or humic acid were obvious. While the coexisting organic molecules was degraded by hydroxyl radicals, the combined tetracycline molecules might be released back to the solution, causing the degradation efficiency to increase slightly.

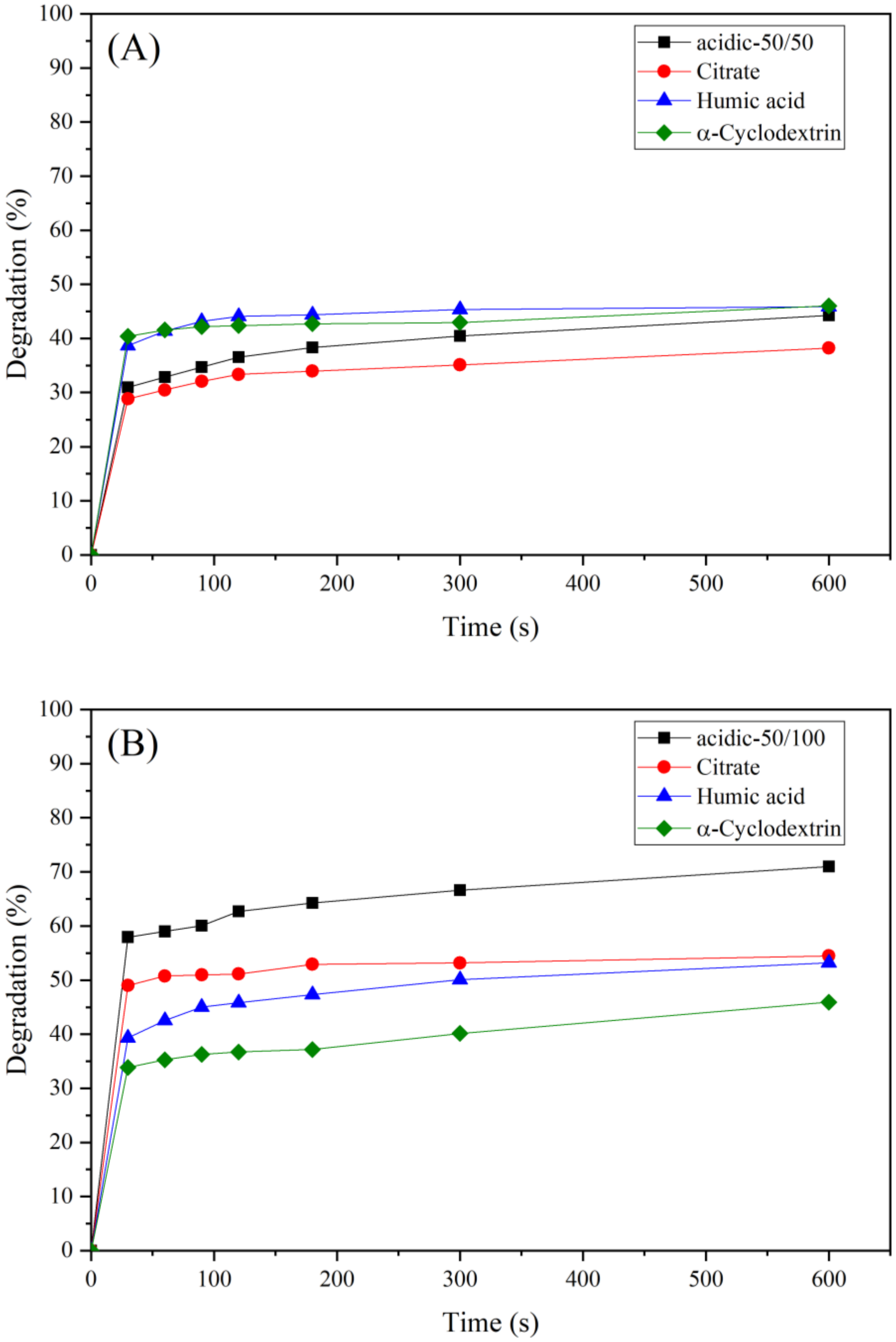


Figure 4.6 The degradation of Tetracycline by Fenton process at pH 3 with coexisting organic macromolecules. (A) $[Fe^{2+}]$ = 50 μM and $[H_2O_2]$ = 50 μM. (B) $[Fe^{2+}]$ = 50 μM and $[H_2O_2]$ = 100 μM.

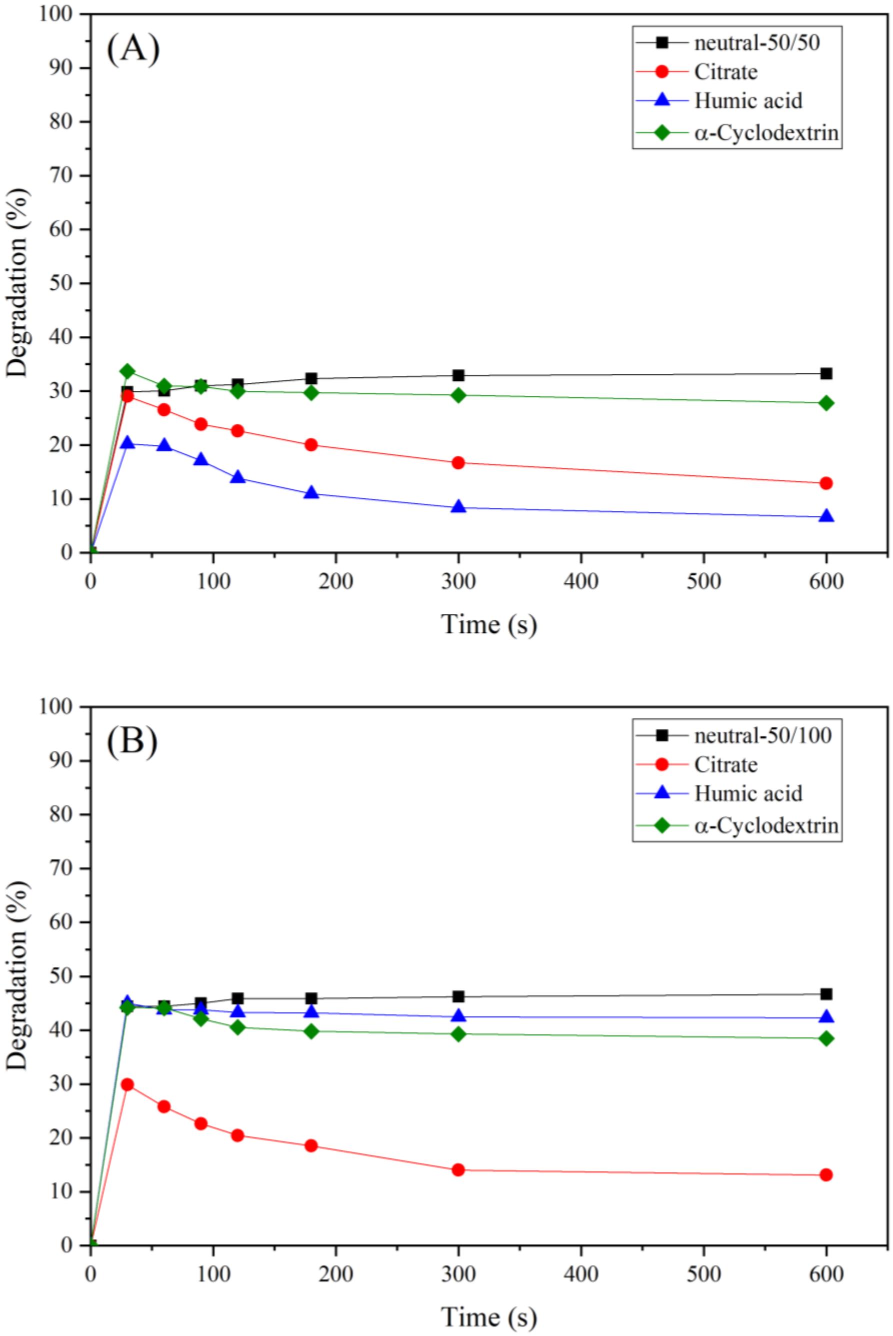


Figure 4.7 The degradation of Tetracycline by Fenton process at pH 7 with coexisting organic macromolecules. (A) $[Fe^{2+}]$ = 50 μM and $[H_2O_2]$ = 50 μM. (B) $[Fe^{2+}]$ = 50 μM and $[H_2O_2]$ = 100 μM.

4.4 Mechanism of Tetracycline degradation by Fenton

4.4.1 Tetracycline degradation by Classic Fenton process

In this study, tetracycline was degraded by classic Fenton process at pH 3 and 7 and the samples were collected at 0, 30, 300 seconds after the reaction took place, which were analyzed by ESI-MS using negative mode to investigate the m/z ratio between 150 to 1000. The m/z ratio of tetracycline was around 443.14. The results are shown in Figure 4.8 and Figure 4.9. The concentration of tetracycline decreased during the reaction, and it molecular structure decomposed to smaller intermediates. The peaks of m/z ratio between 200 to 300 were inferred as those intermediates, and the intensity increased as the reaction proceeded.

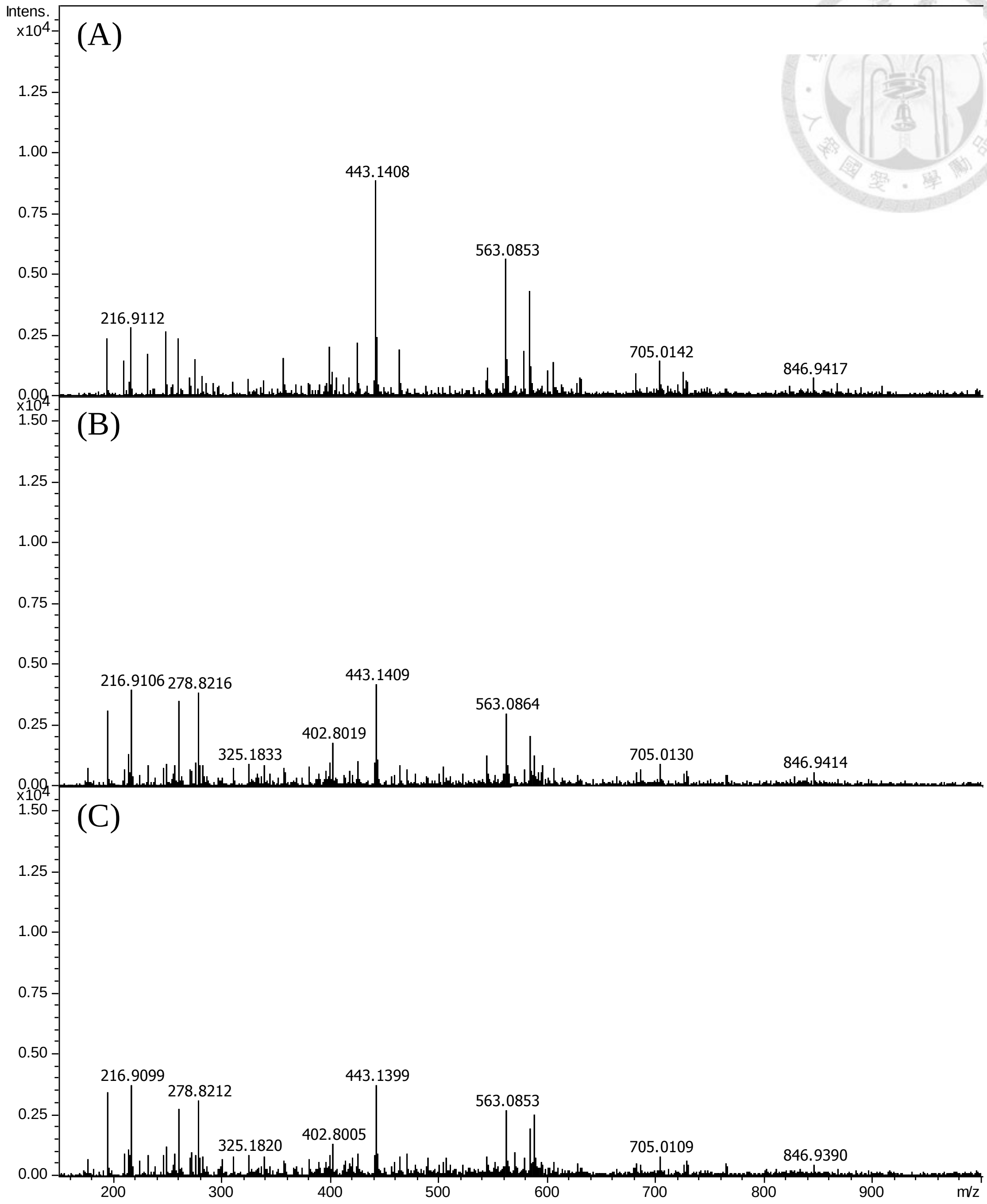


Figure 4.8 The mass spectrum of tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started.

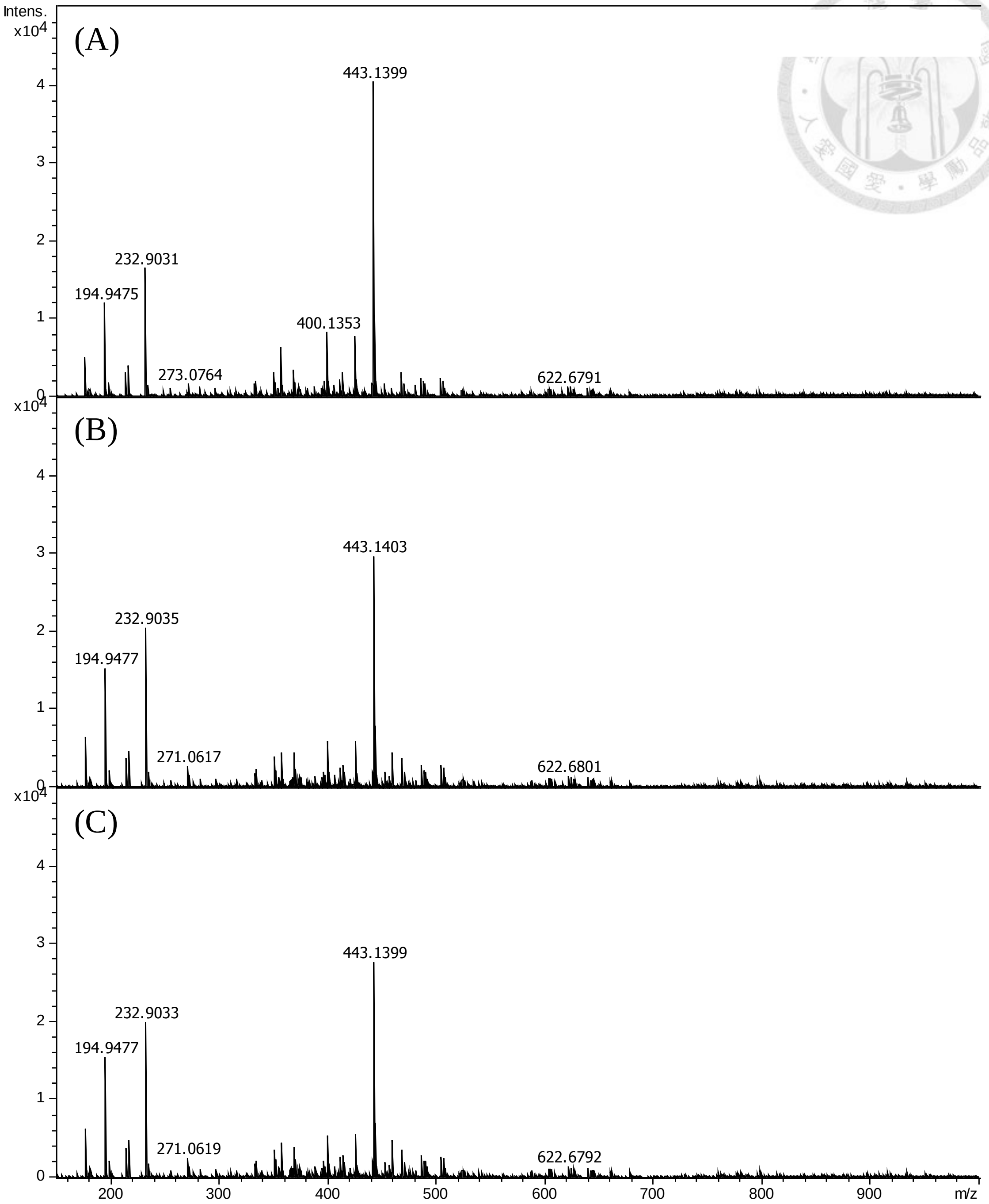


Figure 4.9 The mass spectrum of tetracycline degradation by Fenton Process at pH 7 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started.

4.4.2 Tetracycline degradation at the presence of organic macromolecules in acidic environment

In this study, citrate, humic acid and α-cyclodextrin were used as the coexisting organic macromolecules in the Fenton process to enhance the target compound degradation. Samples were collected at 0, 30, 300 seconds after the reaction took place. Analysis similar to those without co-existing macro-organicmolecules. The m/z ratio of tetracycline, citrate, α-cyclodextrin were around 443.14, 190.02, 971.28 under negative mode, respectively. In Figure 4.10, the intensity of tetracycline decreased significantly in the first 30 seconds and the decreasing trend changed to a slower fashion from 30 to 300 seconds. However, the intensity of citrate before reaction was lower than the others, indicating that citrate and tetracycline might form chelating complexes which increased the observed intensity. The humic acid is a macromolecule with various functional groups, so its respective signal might not be observed in Figure 4.11. Due to the huge molecular structure of humic acids, it might adsorb aqueous tetracycline, causing a decrease in tetracycline concentration before Fenton reaction taking place. However, in acidic environment the intensity of tetracycline mass spectroscopy exhibited a decreasing trend after the reaction took place. In Figure 4.12, the intensity of α-cyclodextrin has decreased, inferring that α-cyclodextrin might act as another target compound and consumed hydroxyl radicals.

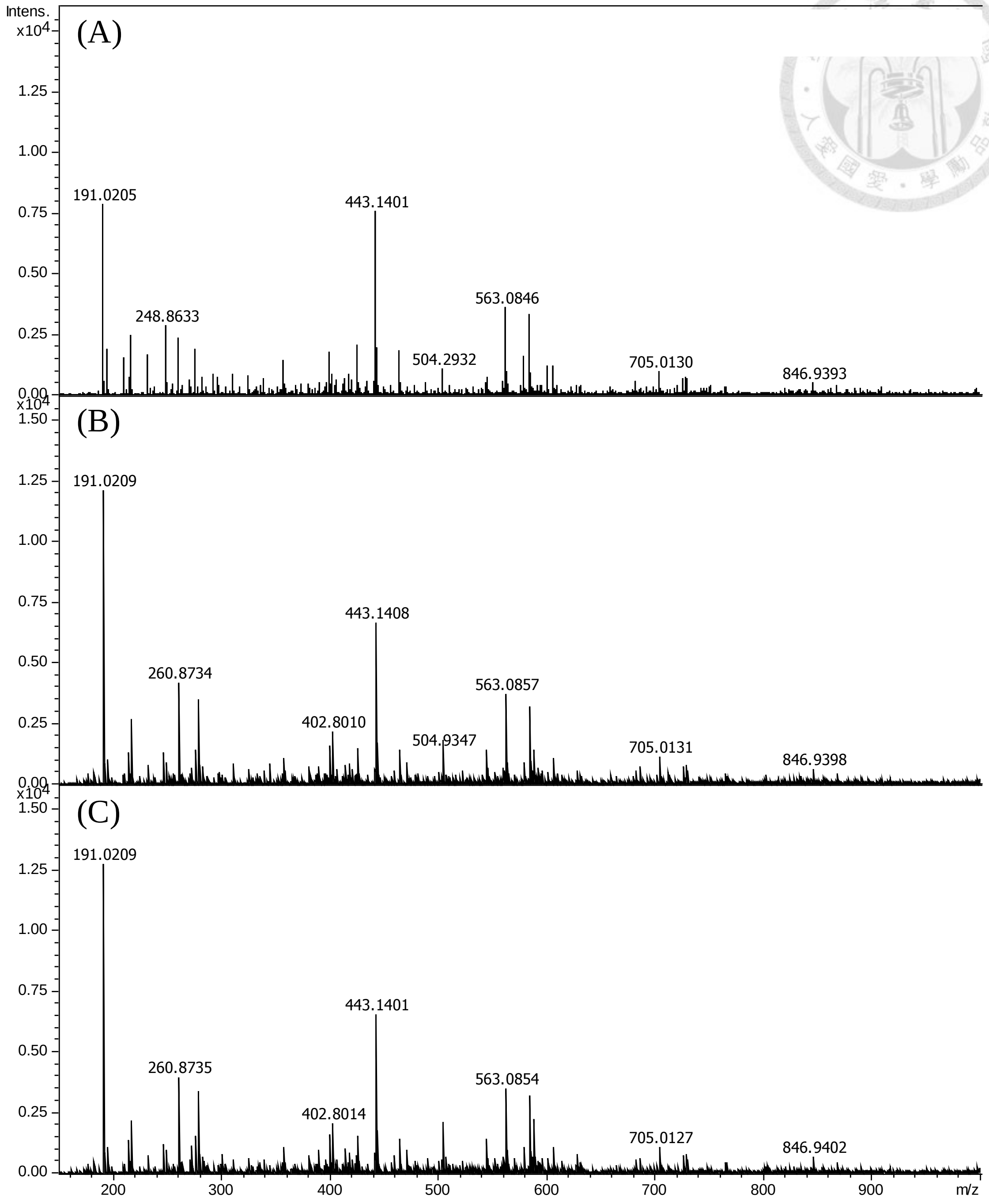


Figure 4.10 The mass spectrum of citrate presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started.

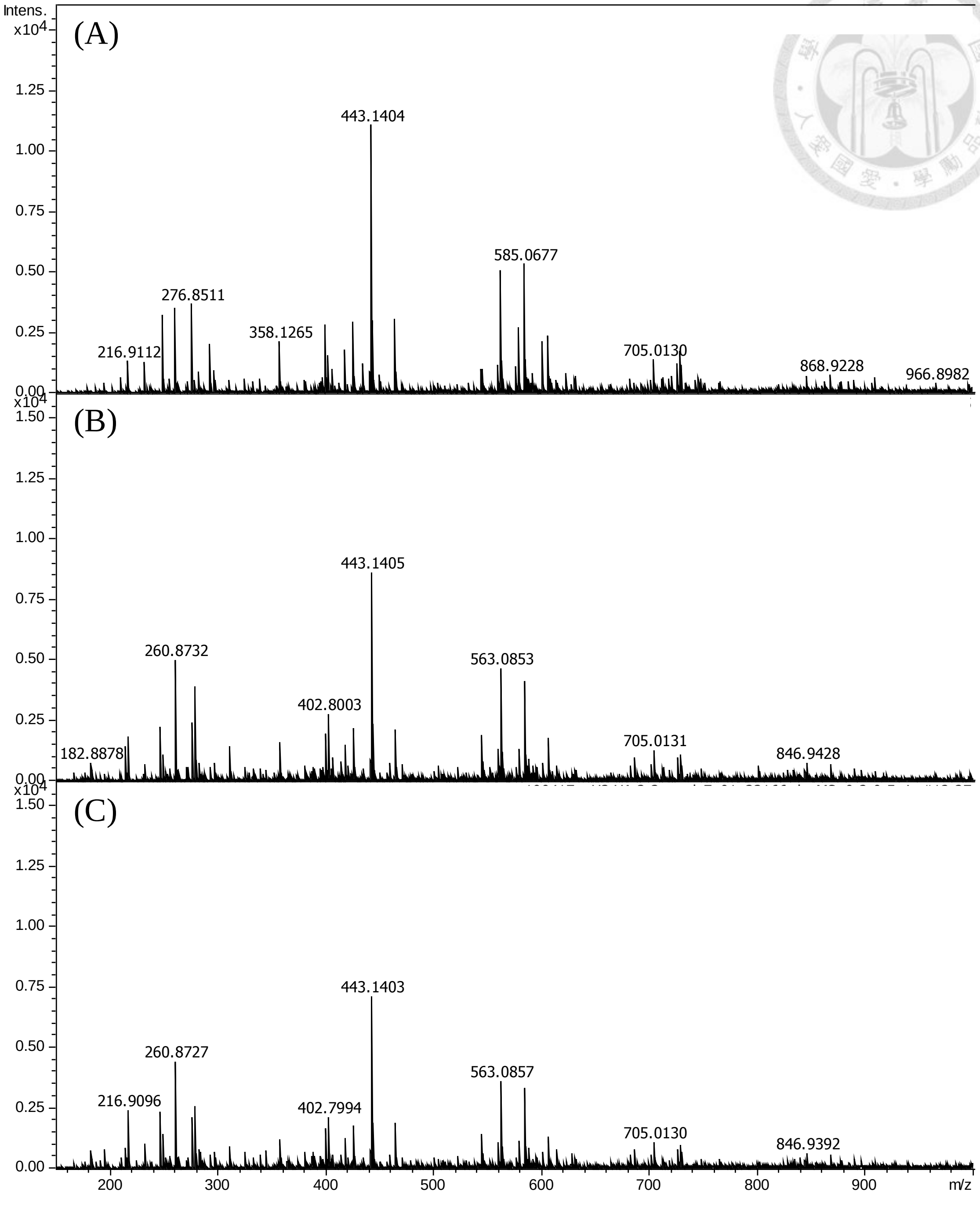


Figure 4.11 The mass spectrum of humic acid presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started.

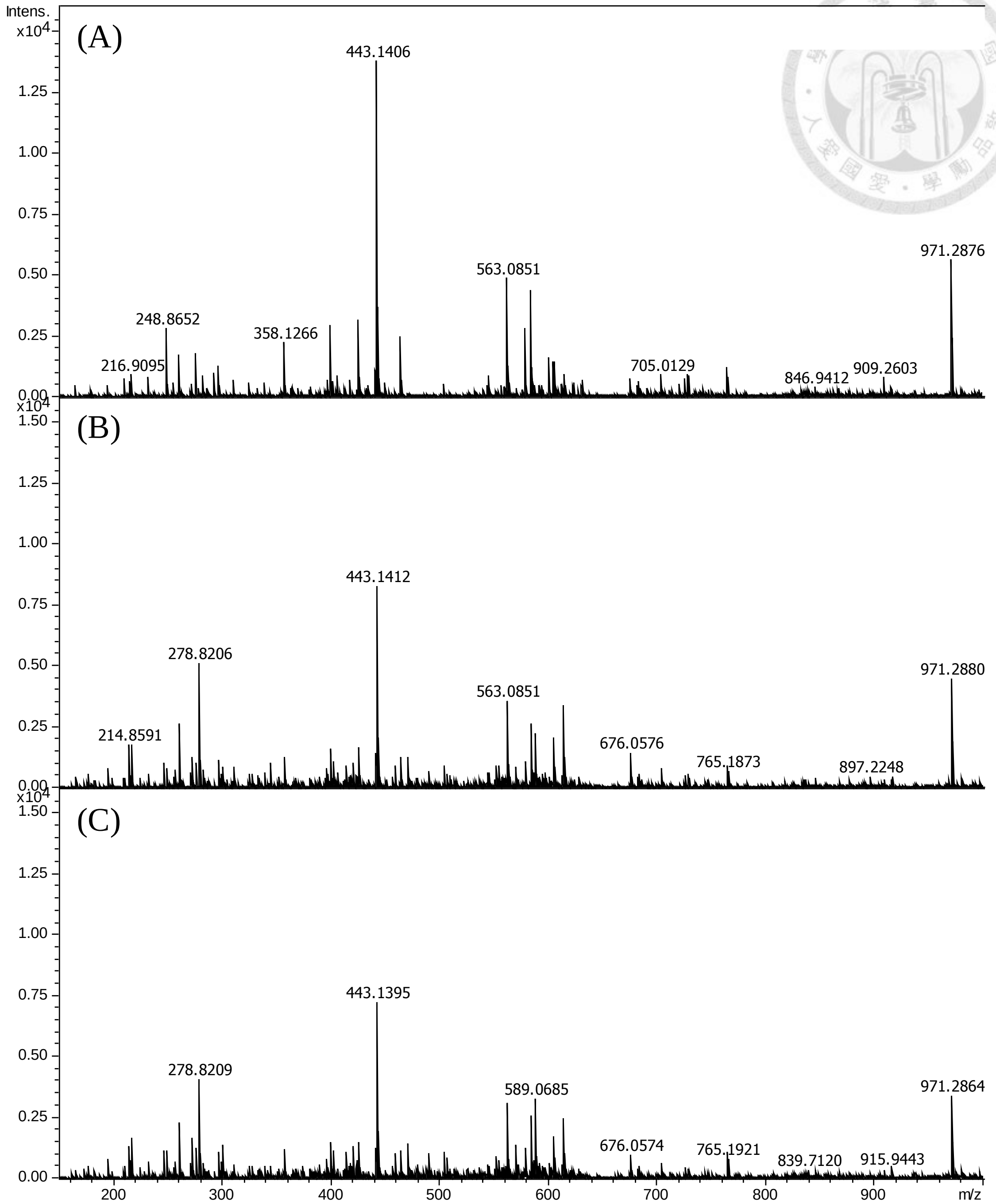


Figure 4.12 The mass spectrum of α-cyclodextrin presented tetracycline degradation by Fenton Process at pH 3 for samples collected before reaction (A), 30 seconds (B) and 300 seconds (C) after reaction started.

According to the literature, Wang and Wang (2018) compared the trimethoprim degradation by Fenton process to the Fe(II)-activated persulfate process. The result has showed that hydrogen peroxide caused a better degradation efficiency than that in the peroxydisulfate process, which was similar to the results obtained in this study. Moreover, many studies used UV-activated persulfate to degrade organic compounds. Delavaran Shiraz et al. (2018) proved that both UV and ferrous ions activated PDS at the same time and degraded catechol up to 99.1%. However, UV irradiation was not employed in this study, causing the degradation efficiency of Fe^{2+} activated PDS lower than that in classic Fenton process.

A previous study demonstrated that humic acid might act as another target compound, and facilitate the reduction of ferric ions to ferrous ions to increase hydroxyl radicals production, resulting in a higher degradation efficiency (Fan et al., 2013). However, in this study, the degradation efficiency decreased because macromolecules consumed the oxidants. Although the coexisting molecules competes with target compounds in the process, the suitable target compound may form the complex that assisted the electron transfer activity between ferric and ferrous ions to enhance the removal efficiency by generating more hydroxyl radicals.

Chapter 5 Conclusion

In the tetracycline degradation by Fenton process, the more reagents were added, the higher degradation efficiency occurred. The best reagent concentration ratio to operate Fenton process was $[Fe^{2+}]/[H_2O_2]$ = 100 μM /100 μM, causing 79.6% degradation of tetracycline in acidic condition. The concentration of hydrogen peroxide and ferrous ions affected the hydroxyl radical production.

Comparing to the degradation experiments of persulfate degradation, hydrogen peroxide showed the best removal efficiency, and peroxymonosulfate showed a stronger oxidizing ability than peroxydisulfate. Sulfate radicals may not only interact with target compound but also with ferrous ion. The degradation pattern of peroxydisulfate was different from that in Fenton process, in which fast stage and slow stage occurred in sequence.

In the neutral environment, the degradation efficiency by Fenton process decreased. The maximum removal was 46.7% when $[Fe^{2+}]/[H_2O_2]$ = 100 μM /100 μM. In the presence of coexisting organic macromolecules, citrate showed a lower removal efficiency than that without citrate; humic acid interacted with tetracycline in the beginning, leading to a decrease in tetracycline concentration; α-cyclodextrin showed a better removal efficiency in $[Fe^{2+}]/[H_2O_2]$ = 50 μM /50 μM in acidic condition. In neutral condition, macromolecules might chelate with tetracycline and the degradation efficiency increased slightly because the tetracycline molecules combined with coexisting organic molecules might be released back during the degradation process.

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APPENDIX A. Data analysis of the degradation of tetracycline by Fenton process

Tetracycline concentration (ppm)						
$[FeSO_4]$ = 10 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0	48.1382		48.7170			46.2000
30	44.3794		34.2679			24.1410
60	44.3606		34.1836			24.0896
90	44.1244		34.0146			23.9675
120	44.1013		33.6656			23.6971
180	43.9437		33.0272			23.6639
300	43.4365		32.7109			23.5932
600	43.3230		32.1562			23.4826

Tetracycline degradation efficiency (%)						
$[FeSO_4]$ = 10 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0	0.00		0.00			0.00
30	7.81		29.66			47.75
60	7.85		29.83			47.86
90	8.34		30.18			48.12
120	8.39		30.90			48.71
180	8.71		32.21			48.78
300	9.77		32.86			48.93
600	10.00		33.99			49.17

Tetracycline concentration (ppm)						
$[FeSO_4]$ = 30 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0		45.8872	45.6451			46.6009
30		34.5379	32.0763			23.3229
60		34.1148	31.0175			22.7682
90		32.8650	30.0336			22.7620
120		31.7407	29.5479			22.2876
180		31.7143	28.5839			21.2807
300		30.8239	28.2565			20.8756
600		29.8463	27.2757			20.1816

Tetracycline degradation efficiency (%)						
$[FeSO_4]$ = 30 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0		0.00	0.00			0.00
30		24.73	29.73			49.95
60		25.66	32.05			51.14
90		28.38	34.20			51.16
120		30.83	35.27			52.17
180		30.89	37.38			54.33
300		32.83	38.10			55.20
600		34.96	40.24			56.69

Tetracycline concentration (ppm)						
$[FeSO_4]$ = 50 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0			45.4421			45.3665
30			31.3720			19.0783
60			30.5097			18.6013
90			29.6874			18.1179
120			28.8528			16.9340
180			28.0351			16.2170
300			27.0485			15.1377
600			25.3342			13.1696

Tetracycline degradation efficiency (%)						
$[FeSO_4]$ = 50 μM						
Reaction time (s)	**H_2O_2 concentration (μM)**					
	10	**30**	**50**	**70**	**85**	**100**
0			0.00			0.00
30			30.96			57.95
60			32.86			59.00
90			34.67			60.06
120			36.51			62.67
180			38.31			64.25
300			40.48			66.63
600			44.25			70.97

Tetracycline concentration (ppm)						
$[FeSO_4]$ = 70 μM						
Reaction time (s)	H_2O_2 concentration (μM)					
	10	30	50	70	85	100
0			44.4190	44.7940		46.6152
30			27.3876	20.7632		23.8376
60			26.8686	19.3257		23.0105
90			25.4040	19.0744		21.3069
120			25.3066	18.5121		20.3151
180			24.6277	17.6177		18.8823
300			23.1797	15.7342		17.7943
600			21.4739	16.2359		14.4685

Tetracycline degradation efficiency (%)						
$[FeSO_4]$ = 70 μM						
Reaction time (s)	H_2O_2 concentration (μM)					
	10	30	50	70	85	100
0			0.00	0.00		0.00
30			38.34	53.65		48.86
60			39.51	56.86		50.64
90			42.81	57.42		54.29
120			43.03	58.67		56.42
180			44.56	60.67		59.49
300			47.82	64.87		61.83
600			51.66	63.75		68.96

Tetracycline concentration (ppm)						
$[FeSO_4]$ = 85 μM						
Reaction time (s)	H_2O_2 concentration (μM)					
	10	30	50	70	85	100
0			46.0775		44.6179	44.3596
30			28.4512		18.2099	23.2006
60			27.1741		16.7311	21.0238
90			26.2739		15.6412	18.9645
120			25.5950		14.9115	18.0735
180			25.0045		14.2169	17.0181
300			23.1772		11.9108	13.9421
600			21.2591		11.3999	10.7249

Tetracycline degradation efficiency (%)						
$[FeSO_4]$ = 85 μM						
Reaction time (s)	H_2O_2 concentration (μM)					
	10	30	50	70	85	100
0			0.00		0.00	0.00
30			38.25		59.19	47.70
60			41.03		62.50	52.61
90			42.98		64.94	57.25
120			44.45		66.58	59.26
180			45.73		68.14	61.64
300			49.70		73.30	68.57
600			53.86		74.45	75.82

Tetracycline concentration (ppm)						
[FeSO4] = 100 µM						
Reaction time (s)	**H_2O_2 concentration (µM)**					
	10	**30**	**50**	**70**	**85**	**100**
0			42.7009			43.5852
30			25.9660			17.1126
60			24.7249			15.3323
90			23.6380			14.5931
120			23.2067			13.7300
180			22.1496			12.2478
300			21.0496			10.5798
600			17.8164			8.8829

Tetracycline degradation efficiency (%)						
[FeSO4] = 100 µM						
Reaction time (s)	**H_2O_2 concentration (µM)**					
	10	**30**	**50**	**70**	**85**	**100**
0			0.00			0.00
30			39.19			60.74
60			42.10			64.82
90			44.64			66.52
120			45.65			68.50
180			48.13			71.90
300			50.70			75.73
600			58.28			79.62

APPENDIX B. Data analysis of the degradation of tetracycline by Fe^{2+} activated persulfates

Tetracycline concentration (ppm)			
[$FeSO_4$] = 50 μM, [oxidants] = 50 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	45.4421	45.5456	45.8665
30	31.3720	38.1063	30.8044
60	30.5097	37.3225	30.3180
90	29.6874	36.2653	29.8776
120	28.8528	35.6109	29.6975
180	28.0351	34.6120	29.5595
300	27.0485	33.8019	28.6199
600	25.3342	31.8519	28.3071

Tetracycline degradation efficiency (%)			
[$FeSO_4$] = 50 μM, [oxidants] = 50 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	0.00	0.00	0.00
30	30.96	16.33	32.84
60	32.86	18.05	33.90
90	34.67	20.38	34.86
120	36.51	21.81	35.25
180	38.31	24.01	35.55
300	40.48	25.78	37.60
600	44.25	30.07	38.28

Tetracycline concentration (ppm)			
[$FeSO_4$] = 50 μM, [oxidants] = 100 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	45.3665	44.6934	45.8792
30	19.0783	28.8883	21.3571
60	18.6013	28.6141	20.3459
90	18.1179	28.0635	19.6604
120	16.9340	26.1208	19.5639
180	16.2170	25.5751	19.4739
300	15.1377	22.9654	19.0635
600	13.1696	22.0977	18.5505

Tetracycline degradation efficiency (%)			
[$FeSO_4$] = 50 μM, [oxidants] = 100 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	0.00	0.00	0.00
30	57.95	35.36	53.45
60	59.00	35.98	55.65
90	60.06	37.21	57.15
120	62.67	41.56	57.36
180	64.25	42.78	57.55
300	66.63	48.62	58.45
600	70.97	50.56	59.57

Tetracycline concentration (ppm)			
[$FeSO_4$] = 100 μM, [oxidants] = 100 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	43.5852	43.9555	43.6411
30	17.1126	25.6191	17.8606
60	15.3323	22.6480	17.1479
90	14.5931	20.2063	16.9634
120	13.7300	19.8831	16.8298
180	12.2478	19.3377	16.8217
300	10.5798	19.1919	16.6921
600	8.8829	18.7506	16.4375

Tetracycline degradation efficiency (%)			
[$FeSO_4$] = 100 μM, [oxidants] = 100 μM			
Reaction time (s)	**oxidants**		
	H_2O_2	**PDS**	**PMS**
0	0.00	0.00	0.00
30	60.74	41.72	59.07
60	64.82	48.48	60.71
90	66.52	54.03	61.13
120	68.50	54.77	61.44
180	71.90	56.01	61.45
300	75.73	56.34	61.75
600	79.62	57.34	62.33

APPENDIX C. Data analysis of the degradation of tetracycline by Fenton process in neutral environment

Tetracycline concentration (ppm)		
[$FeSO_4$] = 50 μM		
Reaction time (s)	**H_2O_2 concentration (μM)**	
	50	**100**
0	33.7711	32.6075
30	23.6945	18.1256
60	23.6213	18.1225
90	23.3035	17.9222
120	23.2379	17.6528
180	22.8573	17.6452
300	22.6704	17.5312
600	22.5552	17.3815

Tetracycline degradation efficiency (%)		
[$FeSO_4$] = 50 μM		
Reaction time (s)	**H_2O_2 concentration (μM)**	
	50	**100**
0	0.00	0.00
30	29.84	44.41
60	30.05	44.42
90	31.00	45.04
120	31.19	45.86
180	32.32	45.89
300	32.87	46.24
600	33.21	46.69

APPENDIX D. Data analysis of the degradation of tetracycline by Fenton with coexisting macromolecules

Tetracycline concentration (ppm)			
[$FeSO_4$] = 50 μM, [H_2O_2] = 50 μM, pH = 3			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	42.8392	26.7809	45.5197
30	30.4878	16.4247	27.1379
60	29.7855	15.6997	26.5974
90	29.1013	15.2187	26.3139
120	28.5523	14.9793	26.2288
180	28.2972	14.8947	26.0744
300	27.8021	14.6321	25.9717
600	26.4715	14.5004	24.5688

Tetracycline degradation efficiency (%)			
[$FeSO_4$] = 50 μM, [H_2O_2] = 50 μM, pH = 3			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	0.00	0.00	0.00
30	28.83	38.67	40.38
60	30.47	41.38	41.57
90	32.07	43.17	42.19
120	33.35	44.07	42.38
180	33.95	44.38	42.72
300	35.10	45.36	42.94
600	38.21	45.86	46.03

Tetracycline concentration (ppm)			
[$FeSO_4$] = 50 μM, [H_2O_2] = 100 μM, pH = 3			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	37.7533	25.3552	46.5666
30	19.2449	15.3789	30.7894
60	18.5780	14.5616	30.1433
90	18.5057	13.9413	29.6613
120	18.4429	13.7338	29.4615
180	17.7635	13.3599	29.2577
300	17.6676	12.6563	27.8699
600	17.1852	11.8649	25.1777

Tetracycline degradation efficiency (%)			
[$FeSO_4$] = 50 μM, [H_2O_2] = 100 μM, pH = 3			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	0.00	0.00	0.00
30	49.02	39.35	33.88
60	50.79	42.57	35.27
90	50.98	45.02	36.30
120	51.15	45.83	36.73
180	52.95	47.31	37.17
300	53.20	50.08	40.15
600	54.48	53.21	45.93

Tetracycline concentration (ppm)			
$[FeSO_4]$ = 50 μM, $[H_2O_2]$ = 50 μM, pH = 7			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	36.7149	34.6254	33.2178
30	26.0559	27.6128	22.0342
60	26.9702	27.7711	22.9393
90	27.9579	28.6878	22.9657
120	28.4060	29.8313	23.2664
180	29.3589	30.8336	23.3464
300	30.5849	31.7295	23.5089
600	31.9730	32.3259	23.9782

Tetracycline degradation efficiency (%)			
$[FeSO_4]$ = 50 μM, $[H_2O_2]$ = 50 μM, pH = 7			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	0.00	0.00	0.00
30	29.03	20.25	33.67
60	26.54	19.80	30.94
90	23.85	17.15	30.86
120	22.63	13.85	29.96
180	20.04	10.95	29.72
300	16.70	8.36	29.23
600	12.92	6.64	27.82

Tetracycline concentration (ppm)			
$[\mathrm{FeSO_4}] = 50\ \mu\mathrm{M}$, $[\mathrm{H_2O_2}] = 100\ \mu\mathrm{M}$, pH = 7			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	36.1692	30.7822	32.6634
30	25.3652	16.9270	18.2243
60	26.8426	17.2960	18.2453
90	27.9790	17.2978	18.9099
120	28.7745	17.4549	19.4202
180	29.4703	17.4682	19.6607
300	31.0909	17.6979	19.8256
600	31.4282	17.7585	20.0957

Tetracycline degradation efficiency (%)			
$[\mathrm{FeSO_4}] = 50\ \mu\mathrm{M}$, $[\mathrm{H_2O_2}] = 100\ \mu\mathrm{M}$, pH = 7			
Reaction time (s)	**[Coexisting organic macromolecules] = 50 μM**		
	Citrate	**Humic acid**	**α-cyclodextrin**
0	0.00	0.00	0.00
30	29.87	45.01	44.21
60	25.79	43.81	44.14
90	22.64	43.81	42.11
120	20.44	43.30	40.54
180	18.52	43.25	39.81
300	14.04	42.51	39.30
600	13.11	42.31	38.48