REMOVAL OF OXYTETRACYCLINE FROM WATER USING OZONATION ALONE AND COMBINATION OF CATALYTIC OZONATION WITH ZINC OXIDE NANOPARTICLES

SUVITHRA NADESAN

UNIVERSITI SAINS MALAYSIA

2022

REMOVAL OF OXYTETRACYCLINE FROM WATER USING OZONATION ALONE AND COMBINATION OF CATALYTIC OZONATION WITH ZINC OXIDE NANOPARTICLES

by

SUVITHRA NADESAN

Thesis submitted in partial fulfilment of the requirement for the degree of

Bachelor of Chemical Engineering

June 2022

ACKNOWLEDGEMENT

The final year project is for the completion of degree of Bachelor of Chemical Engineering. Support and commitments from several individuals have contributed to the success in completion of my report 1. First and foremost, I would like to express my deepest gratitude to my supervisor, Professor Dr. Ahmad Zuhairi Abdullah for his invaluable guidance, assisting, advising and consistently providing me the knowledge throughout this final year project during this semester. I would like to express my sincere appreciation to the coordinator of EKC 499, Professor Dr.Mohd Roslee bin Othman for his arrangement on this subject. Apart from that, I would like to thank to all School of Chemical Engineering technicians, staff and postgraduate students who are willing to share their knowledge and assistance to carry out the experiment despite their busy schedule. I would like to take this opportunity to thank my family, friends and course mate for their endless support and encouragement throughout the time. The words of encouragement from them gave me the necessary motivation to do my final year project.

Suvithra Nadesan

July 2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE REVIEW

CHAPTER 3: MATERIALS AND METHODS

CHAPTER 4: RESULTS AND DISCUSSION

APPENDIX B: DETERMINATION OF MAXIMUM WAVELENGTH

APPENDIX C: CALIBRATION CURVE

LIST OF ABBREVIATIONS

•OH	Hydroxyl Radicals
AOP	Advanced Oxidation Processes
BOD	Biochemical Oxygen Demand
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
COP	Catalytic Ozonation Process
H_2O	Water
H_2O_2	Hydrogen Peroxide
HCL	Hydrochloride Acid
NaOH	Sodium Hydroxide
O_2	Oxygen
O_3	Ozone
OH	Hydroxide
OTC	Oxytetracycline
POP	Persistent Organic Pollutants
STP	Sewage Treatment Plants
TC	Tetracycline

UV	Ultraviolet Radiation
WWTP	Wastewater Treatment Plants
ZnO	Zinc oxide

LIST OF SYMBOLS

Symbol	Description	Units
C_t	Antibiotic concentration at instant time	mg/L
C_o	Initial antibiotic concentration	mg/L
k_{app}	Apparent rate constant	1/min
k	Constant of first order reaction rate	1/min
t	Reaction time	min
R^2	Correlation coefficient	-
λ	Wavelength	nm
V_1	Initial Volume	mL
V_2	Final Volume	mL
C_1	Initial concentration	mg/L
C_2	Final concentration	Mg/L

PENYINGKIRAN ANTIBIOTIK OXYTETRACYCLINE DARI AIR DENGAN PROSES PENGOZONAN SAHAJA DAN KOMBINASI PENGOZONAN PEMANGKIN MENGGUNAKAN NANOZARAH ZINK OKSIDA

ABSTRAK

Pencemaran Oxytetracycline (OTCs) adalah ancaman global yang semakin meningkat kepada biodiversiti akuatik dan daratan kerana penggunaannya yang tidak pernah berlaku sebelum ini dalam akuakultur, ternakan dan pencegahan penyakit manusia. Antibiotik seperti OTC menjadi ancaman serius kepada alam sekitar kerana penggunaannya yang tidak diambil kira dalam pertumbuhan haiwan subterapeutik dan rawatan manusia. Tujuan kajian ini adalah untuk membandingkan kecekapan pengozonan sahaja dan gabungan pengozonan dengan pemangkin dalam degradasi OTC sebagai bahan cemar bahaya alam sekitar dalam larutan akueus. Nanozarah zink oksida (ZnO) digunakan sebagai agen pemangkin dengan ozon (ZnO/O3). Pengaruh parameter operasi seperti kepekatan awal antibiotik (10,35,50) mg/l, suhu (15,25,35) °C, pH (3,7,11) dan dos mangkin (25,50, 100) mg disiasat dalam proses pengozonan ini. Bekalan ozon adalah malar sepanjang eksperimen iaitu 600 mg/j. Untuk kepekatan awal antibiotik, keputusan yang diperoleh menunjukkan bahawa ozon boleh merendahkan antibiotik OTC dengan lebih cepat pada kepekatan yang sangat rendah. Pada kepekatan 10 mg/l, kecekapan degradasi mencapai hampir 75.2% pada 20 minit pertama tindak balas. Pada pH 11, keputusan menunjukkan peningkatan beransur-ansur peratusan pada kadar penyingkiran OTC. Hampir lebih daripada 68. 1 % antibiotik didegradasi selepas 20 minit. Bagi berat pemangkin, jumlah yang lebih tinggi menunjukkan kecekapan degradasi yang paling tinggi. 100mg nanozarah ZnO merendahkan hampir 98.4% antibiotik dalam masa 20 minit pertama. Kadar penyingkiran tertinggi diperhatikan pada suhu 25°C dengan kecekapan degradasi 54.9 % pada 20 minit pertama. Keadaan optimum untuk keadaan tunggal ialah 10 mg/l pada pH=11 dengan suhu 25°C, selepas 120 minit, 95.8% penyingkiran OTC telah dicapai. Analisis kinetik menunjukkan bahawa pengozonan OTC dikenal pasti

sebagai tindak balas tertib pertama pseudo. Ditunjukkan bahawa kepekatan antibiotik 50 mg/l dengan pengozonan bermangkin mencapai kira-kira 99.5% manakala bagi 96.3% kadar penyingkiran bagi pengozonan tunggal pada keadaan pH=11, suhu 25°C pada 120 minit tindak balas. Keputusan menunjukkan bahawa pengozonan pemangkin adalah kaedah yang sangat berkesan untuk degradasi dan mineralisasi OTC dalam larutan akueus.

REMOVAL OF OXYTETRACYCLINE ANTIBIOTIC FROM WATER USING OZONATION ALONE AND CATALYTC OZONATION WITH ZINC OXIDE NANOPARTICLES

ABSTRACT

Oxytetracycline (OTCs) antibiotic pollution is a growing global threat to aquatic and terrestrial biodiversity due to its unprecedented use in aquaculture, livestock, and human disease prevention. Antibiotics like OTCs are becoming a serious threat to the environment due to their unaccounted use in sub therapeutic animal growth promotion and human treatment. Tetracyclines (TCs) are class of diverse antibiotics that have a wide range of applications in human, veterinary and aquaculture treatment. The purpose of this study was to compare the efficiency of sole ozonation and catalytic ozonation in the degradation of OTC as an environmental hazards contaminant in an aqueous solution. Zinc oxide (ZnO) nanoparticles were used as a catalytic agent with ozone (ZnO/O_3). The influence of operational parameters such as initial antibiotic concentration (10,35,50) mg/l, temperature (15,25,35) °C, pH (3,7,11) and catalyst dosage (25,50,100) mg were investigated in this ozonation process. The ozone supply was constant throughout the experiment which is 600 mg/h. For an initial concentration of antibiotic, the results obtained showed that ozone can degrade OTC antibiotic faster at very low concentration. This is because at higher concentration of antibiotics, there are more formation of intermediates that led to higher consumption of ozone and reaction time. At concentration of 10 mg/l, the degradation efficiency reached almost 75.2% at first 20 minutes of the reaction. At an initial pH of 11, the results showed a gradual increase of percentage on the removal rate of OTC. Almost more than 68. 1 % of antibiotic degraded after 20 minutes of reaction time. For the catalyst weight, higher amount showed the highest degradation efficiency. 100mg of ZnO nanoparticles degraded almost 98.37% of antibiotic within the first 20 minutes. The highest removal rate was observed at temperature of 25°C with degradation efficiency of 54.9 % at the first 20

minutes. The optimum condition for sole condition was 10 mg/l at pH=11 with temperature of 25°C, after 120 min, a 95.8 % of OTC removal was achieved. Concentration of 50 mg/L was used for other parameters to clearly observe the effect on the degradation of antibiotic. Kinetic analysis showed that the ozonation of OTC is identified as the pseudo first order reaction with linear relationship between OTC antibiotic and ozone. It is showed that antibiotic concentration of 50 mg/l with catalytic ozonation reached about 99.5% while for 96.3% of removal rate for sole ozonation at condition at pH=11, temperature of 25°C at 120 minutes reaction. The results demonstrated that catalytic ozonation with zinc oxide nanoparticles was a very effective method for OTC degradation.

CHAPTER 1: INTRODUCTION

Chapter 1 introduces the overview of this research and significance of advanced oxidation process (AOP) on the removal of oxytetracycline (OTC) from water. In general, this chapter summarizes the research background of antibiotic and the application of ozonation in order to remove the antibiotic from wastewater, the problem statement, and objectives of this final year project.

1.1 Research Background

Antibiotics are antibacterial chemicals with a complicated molecular structure. Antibiotics were first discovered in 1928 to treat bacterial infections, including many types of developing illnesses, and have since been effectively used in livestock husbandry as growth promoters and feed efficiency improvers (Baquero, 2008). As a result, they can be found as pollutants in wastewater, municipal sewage, and wastewater treatment plant influents and effluents (Kümmerer, 2009). Figure 1.1 shows the massive accumulation of antibiotics in the environment, and acquisition of antibiotic resistance in microorganisms coming in contact with an antibiotic (Kumar, 2020).

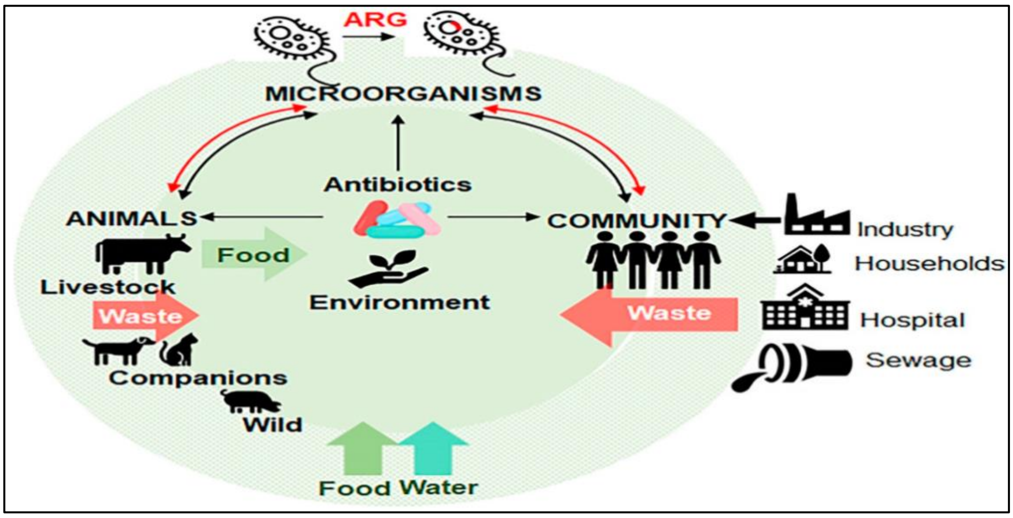


Figure 1.1: Schematic of major route of antibiotic dissemination in environment

Tetracyclines (TCs) are the most prevalent antibiotics, with a total of twenty compounds generated from a variety of Streptomyces species on the market as antibiotics (Chopra, 2001) .The basic constituent is tetracyclic ring structures with various hydroxyl, methyl, keto, and dimethylamino functional groups (Fiaz, 2021). OTC is a tetracycline antibiotic that is used to treat bacterial infections that are susceptible to it. Pharmaceutical antibiotics, like OTC antibiotics, are biologically active compounds that are water soluble but not biodegradable in nature (Fiaz, 2021). Table 1.1 shows the oxytetracycline physicochemical properties.

Table 1.1: Oxytetracycline physicochemical properties

Parameter	Value	Reference
Boiling point (°C)	not available	
Melting point (°C)	179-182 (decomposes)	Lewis 1997
pH	6.115 (saturated solution)	Lewis 1997
Density (kg/m^3)	not known	
Flash point (°C)	not available	
Stability	Stable in air, light sensitive	Lewis 1997
Solubility	Very slightly soluble in water; freely soluble in 3 N HCl and in alkaline solutions; sparingly soluble in alcohol	U.S. Pharmacopeia 1995
solubility - monoalkyl (C_8-C_{18}) trimethyl ammonium salt	1.0 mg/mL in water	Pfizer, Inc., MSDS[a] #116
solubility - dihydrate	0.6 mg/mL in water	Wassef 1983, p. 28, Weiss et al. 1956
solubility - hydrochloride salt	6.9 mg/mL in water	Wassef 1983, p. 28, Weiss et al. 1956

Around 70% of antibiotics consumed cannot be metabolized, including hospital effluents, wastewater treatment procedures, and sewage treatment plant discharges into the aquatic environment (STP). Domestic wastewater contains just 1 g L^{-1} of OTC, but hospital wastewater has 100 g L^{-1} of OTC (Hassan et al., 2021). OTCs are stable and resistant to oxidation in the environment, but they are unstable at high pHs, suggesting weak volatility and, as a consequence, limited degradability. Antibiotic residues accumulate in the environment's streams and soil, causing resistant species to arise. Antibiotics like OTC are becoming a serious environmental threat due to their unrestricted use in sub therapeutic animal growth promotion and human health (Landers et al., 2012). Long-term detrimental effects of OTC may result in ecological imbalance due to inadequate degradation of OTC in present therapeutic approaches. OTCs are linked to pollution because the development of antibiotic resistance has a negative influence on human health. As a result, several strategies are being studied in order to eliminate the OTC with a greater degrading efficiency (Boxall, 2004).

Advanced oxidation processes (AOPs) have made it possible to eliminate a variety of pollutants, including OTC, from wastewater. AOPs such as Fenton, photocatalytic oxidation, and ozonation were used to remove antibiotics. Catalytic ozonation is one of the most important AOPs for removing organic molecules quickly with a high removal rate (Psaltou et al., 2018). The combination of ozonation and catalysis has been used to improve the elimination of antibiotics and their byproducts. Catalysts employed with ozonation can be either homogeneous and heterogeneous. The advantage of heterogeneous catalyst is the easy separation of the catalyst from the solution and possibility to reuse the catalyst.

1.2 Problem Statement

Antibiotics are one of the most often prescribed medications in the world. Antibiotics are quite effective even at low doses (Fiaz et al., 2021) . Large volumes of antibiotics are released into the aquatic environment after metabolism because antibiotics take a long time to breakdown and some are non-degradable, resulting in antibiotic stability in wastewater for prolonged periods of time. Antibiotics left in wastewater for an extended length of time might cause bacteria to develop resistance as well as disturb the system when ingested by organisms (Li et al., 2008). If these excretions are not cleaned before being discharged into the aquatic environment, large levels of OTC will accumulate in the water ecosystem over time, causing to an increase of antibiotic resistance in bacteria and, as a consequence, a rise in drug resistant genes. Antibiotic resistance is a natural phenomenon in which bacteria develop resistance to antibiotics or antimicrobials as a result of mutations and bacteria's huge capacity to transfer genetic information (Harja a, 2017).Three different routes for releasing antibiotics into the environment can be distinguished: feed additives for stockbreeding and fish aquaculture, human and veterinary drugs, and environmental release during production shows in Figure 1.2 (Serwecinska, 2020).

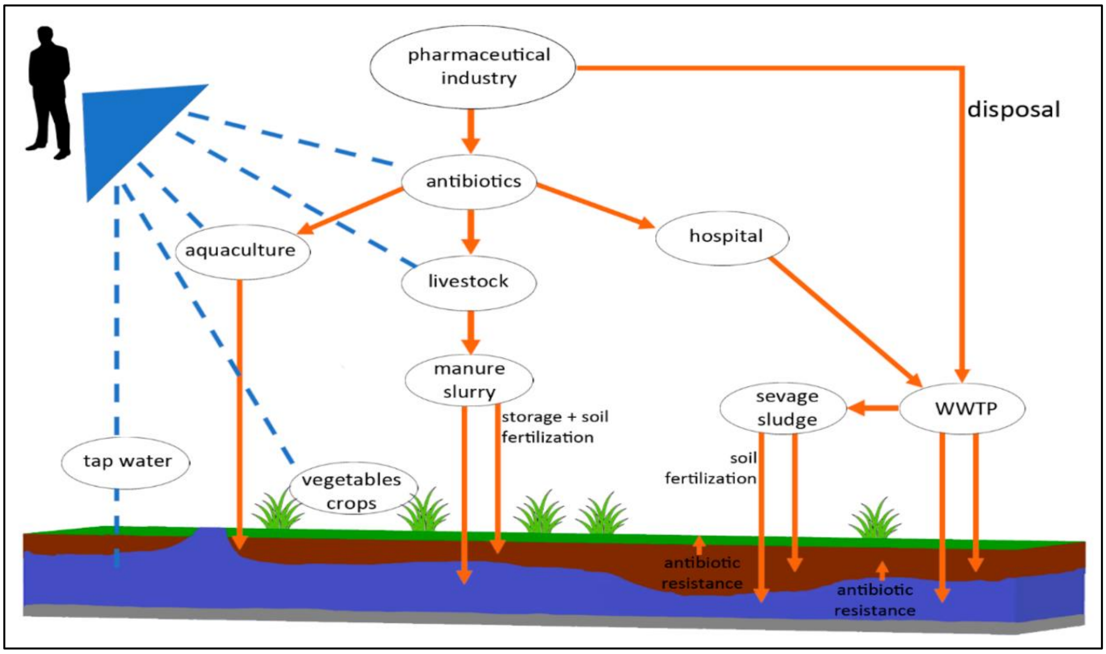


Figure 1.2: Route of antibiotic residues into the environment and human

Because the bacteria are subjected to antibiotics that do not kill them as a result of elective pressure, they develop resistance and constitute a serious health risk. In the longer term, antibiotics may make their way into the human body through food chain, presenting a significant risk to human health. Due to the inability of typical wastewater treatment processes to properly eliminate higher concentrations of OTCs, from wastewater is a serious challenge. Due to the ineffectiveness of traditional techniques of eliminating OTC antibiotics from wastewater streams were ineffective, improved treatment technologies were developed

(Cuerda-correa, 2020).One of the methods is advanced oxidation process, which is more cost effective than direct oxidation because to its lower energy consumption. Fenton, ozonation, and catalytic ozonation are some of the AOPs that have been utilized extensively to solve the issue. Several AOPs implementing the ozone to eliminate harmful components in wastewater. Ozone is a highly reactive oxidizing agent that may react directly with organic pollutants or disintegrate into free radicals to rapidly degrade organic compounds. When the ozone is employed, it does not remove the high level of mineralization and toxicity effectively (Deng , 2015).

As a consequence, ozonation is commonly combined with other technologies to expand its applicability and usage. Catalytic ozonation is an efficient method of breaking down OTC, resulting in high levels of elimination and mineralization.Therefore, in this study, the researcher study on the antibiotic degradation efficiency using sole ozonation and compare it with antibiotic degradation using combination of ozonation with other technologies as well. This study mainly to investigate the removal rate of OTC via sole ozonation and catalytic ozonation with using zinc oxide (ZnO) nanoparticles, through various operating variables, including temperature, initial OTC concentration, pH and catalyst dosage. From the best operational parameters of sole ozonation , a technique which are catalytic ozonation alone (ZnO/O_3) in order to compare the highest efficiency in the removal of oxytetracycline from aqueous solution.

1.3 Research Objectives

The objectives of this research are:

i. To investigate the effect of different pH on the degradation efficiency of oxytetracycline from water.
ii. To determine the initial oxytetracycline concentration that affects the degradation efficiency through ozonation alone.
iii. To find out the effect of temperature on oxytetracycline removal from water.
iv. To evaluate the effect of zinc oxide nanoparticles dosage and also as an adsorbent.
v. To compare the efficiency of OTC removal with sole ozonation (O_3) and catalytic ozonation using zinc oxide nanoparticles (O_3/ZnO).
vi. To find out the kinetic model of the ozonation process.

CHAPTER 2: LITERATURE REVIEW

The researcher addresses the sole ozonation and combination of catalytic ozonation with ultraviolet radiation for the degradation of antibiotics from water. In this chapter, the following subtopics are used for the literature review below. Wastewater, Antibiotics in Wastewater, Antibiotic Resistance, Advanced Oxidation Processes (AOPs), Ozonation of Antibiotics and Ozonation.

2.1 Wastewater

Wastewater is a polluted sort of water that is created by rainfall runoff and human activities. It is previously distilled water that originates from a number of sources, including industrial, residential, agricultural, and commercial activities, as well as sewage infiltration, sewer inflow, and surface runoff. Wastewater consists of 99% of water and 0.1% of contaminants which are nutrients, heavy metal, organic pollutant (Tuser, 2020).Generally, wastewater contained almost 10,000 organic compounds per year that are disposed from home and industrial areas across the world. All of these components must be treated appropriately and eliminated promptly, otherwise they might cause health problemto living organisms (Suwaiba, 2010)

2.2 Antibiotics

Antibiotics are antimicrobial substances having a complex molecular structure. Numerous ancient cultures employed natural treatments, including antibiotic compounds; nevertheless, pyocyanase, discovered by Rudolph Emmerich and Oscar Low in 1890, was the first antibiotic used in hospitals. Although pyrocyanase was proved to be resistant to cholera, typhoid, diphtheria, and anthrax, it was not effective in many individuals and was shortly discontinued. Antibiotics were discovered in 1928 to treat bacterial infections, which included a variety of developing ailments. They have subsequently been successfully employed in animal husbandry as growth boosters and feed efficiency enhancers (Nicolaou, 2018).

These discoveries paved the way for the development of the sulfonamide, penicillin, peptide, aminoglycoside, tetracycline, and amphenicol antibiotic families. Most of the antibiotics are still in use nowadays, and for each class, prototype antibiotic-derived derivatives have been produced. Antibiotics are one of the most often prescribed medications, which is unsurprising considering the diversity of antibiotic compounds available. The most frequently used antibiotic classes include cephalosporins, penicillins, macrolides, tetracyclines, aminoglycosides, and trimethoprim (Chopra & Roberts, 2001).Due to their widespread usage, these compounds are referred to be blockbuster drugs, and some have been extensively examined in recent years within the framework of potable water and wastewater treatment. Other antibiotics have been identified in low trace quantities in wastewater and sewage effluent.

2.3 Antibiotics in Wastewater

The most common antibiotic contaminations are wastewater effluent, bio solids, agricultural wastewater, and manure. Numerous antibiotics may be removed with variable degrees of efficacy depending on the content of the wastewater and the wastewater treatment plant's process train (WWTP). Antibiotics have the potential to contaminate the environment and even food crops. Researchers assessed 32 medicines in German wastewater treatment plants in one of the first studies on pharmaceuticals in wastewater and discovered that eighty %of the drugs examined were found in at least one wastewater treatment plant (Shraim et al., 2017). Although extensive research has been conducted on antibiotic detection in wastewater, it would be beneficial to gain a better understanding of the quantities detected in conventional municipal wastewater treatment plants in order to better understand the potential remediation of these growing pollutants in the environment using ozone or other processes. Due to the wastewater treatment process' incapability to remove all antibiotics, antibiotics may be found in effluent and released into the environment (Pruden et al., 2013).

Antibiotics may be detected in wastewater at amounts ranging from few milligrams per litre to hundreds of milligrams per litre. Although a trace of antibiotics and their metabolites may still be detected in surface waters after wastewater treatment, this concentration often declines (Hirsch et al., 1999). Due to their intricate structure, these antibiotics are difficult to dissolve and, as a consequence, are poorly biodegradable. Antibiotic pollution has become a more serious problem as a result of increased antibiotic usage, antibiotic resistance, and the adverse effects on aquatic environments and people. As a consequence, one of the most critical concerns in the area of water treatment is antibiotic removal from aqueous medium. Numerous contaminants are not cleared entirely by wastewater treatment systems (WWTPs). Numerous conventional procedures have been developed to remove contaminants from water. These conventional remedies are classified as physical, chemical, and biological. Table 2.1 summarizes the advantages and downsides of these conventional therapies (Cuerda-correa, 2020).

Table 2.1: Advantages and disadvantages of the different kinds of water treatment

	Physical or Physicochemical Treatment	Biological Treatment	Chemical Treatment
Kind of pollutant	Industrial (organic, inorganic, metals)	Industrial and domestic (low concentrations of organic and some inorganic)	Industrial (organic, inorganic, metals)
Methods	Filtration Adsorption Air flotation Extraction Flocculation Sedimentation	Anaerobic Aerobic Activated muds	Thermal oxidation (combustion) Chemical oxidation Ion exchange Chemical precipitation
Advantages	Low cost of capital Relatively safe Easy to operate	Easy maintenance Relatively safe elimination of the dissolved contaminants Easy to operate	High degree of treatment Elimination of the dissolved contaminants
Disadvantages	Volatile emissions High energetic cost Complex maintenance	Volatile emissions Require elimination of residual muds Susceptible to toxins or antibiotics	High costs of capital and operation. Difficult operation

This conventional method focuses on eliminating sediments and organic materials that may encourage microorganism development and water body eutrophication. However, due to their high costs and practical limitations, these treatments are not extensively employed at the moment. They are inefficient for removing chronic or growing pollutants in water, such as antibiotics, since many of them have complex structures and hence exhibit high chemical stability, complicating their ultimate elimination. Antibiotic compounds are researched in the aquatic environment for three reasons: to suppress microorganisms, to propagate antibiotic resistance, and alter molecular biology of bacteria. As a consequence, one of the industry's most significant challenges is extracting antibiotics from aqueous medium (Blaney, 2014).

2.4 Antibiotic Resistance

Antibiotic resistance develops as the drugs' minimum inhibitory concentration against bacteria increases. Although antibiotic resistance genes (ARGs) occur naturally in the environment, their increasing prevalence as a consequence of human activities has led scientists to classify ARGs as a new kind of environmental contaminant. ARGs spread from ambient bacteria to human diseases, impairing antibiotic efficacy and creating major health hazards. Antibiotic resistance occurs as a consequence of horizontal gene transfer of antibiotic resistance genes from other bacterial species, or alterations or mutations in the DNA of bacteria (Bello-López et al., 2019). These changes enable the bacteria to tolerate the antibiotic activity. This implies that when an antibiotic is administered, it kills all bacteria that have not mutated, while leaving antibiotic resistant germs alone.

Antibiotic-resistant bacteria may divide and multiply, resulting in an ever-increasing population of microorganisms resistant to antibiotics. Antibiotics or drugs meant to kill germs that are resistant to them no longer function, causing them to spread quickly and creating a public health danger. Recent study indicates that antibiotic-resistant bacteria have been discovered in a number of bodies of water. Antibiotic-resistant faecal coliforms and enterococci were detected in the influent and effluent of wastewater treatment plants (Gallert et al., 2005). Antibiotic resistance confers a survival advantage to microbes, making it difficult to eradicate the diseases they cause. Antibiotic-resistant bacteria infections are notoriously difficult to treat. As a consequence, physicians must use higher dosages of substitute antibiotics to treat ailments. Excessive doses may have adverse effects and contribute to the growth of antibiotic-resistant microorganisms.

2.5 Advanced Oxidation Processes

Advanced Oxidation Processes (AOPs), which are described as oxidation processes that produce significant amounts of hydroxyl radicals (OH) that implemented for water purification, were initially recommended in the 1980s. Later, the AOP concept was enhanced to sulphate radical oxidation processes (SO_4^{-1}). Unlike conventional oxidants like chlorine and ozone, which perform both functions of decontamination and disinfection, AOPs are used to eliminate contaminants in wastewater (Deng, 2015).The classification of AOP can also take into account the various potential forms of generating hydroxyl radicals. Photochemical and non-photochemical procedures can be differentiated in this manner (Kurt et al., 2017). Those containing Fenton, ammonia, ozone and hydrogen peroxide, electrochemical oxidation, cavitations, supercritical water oxidation, non-thermal plasma based electrical discharge; gamma-ray, x-ray, and electron-beam contain non-photochemical processes. Figure 2.1 shows the schematic diagram of different advanced oxidation processes (AOP) treatments.

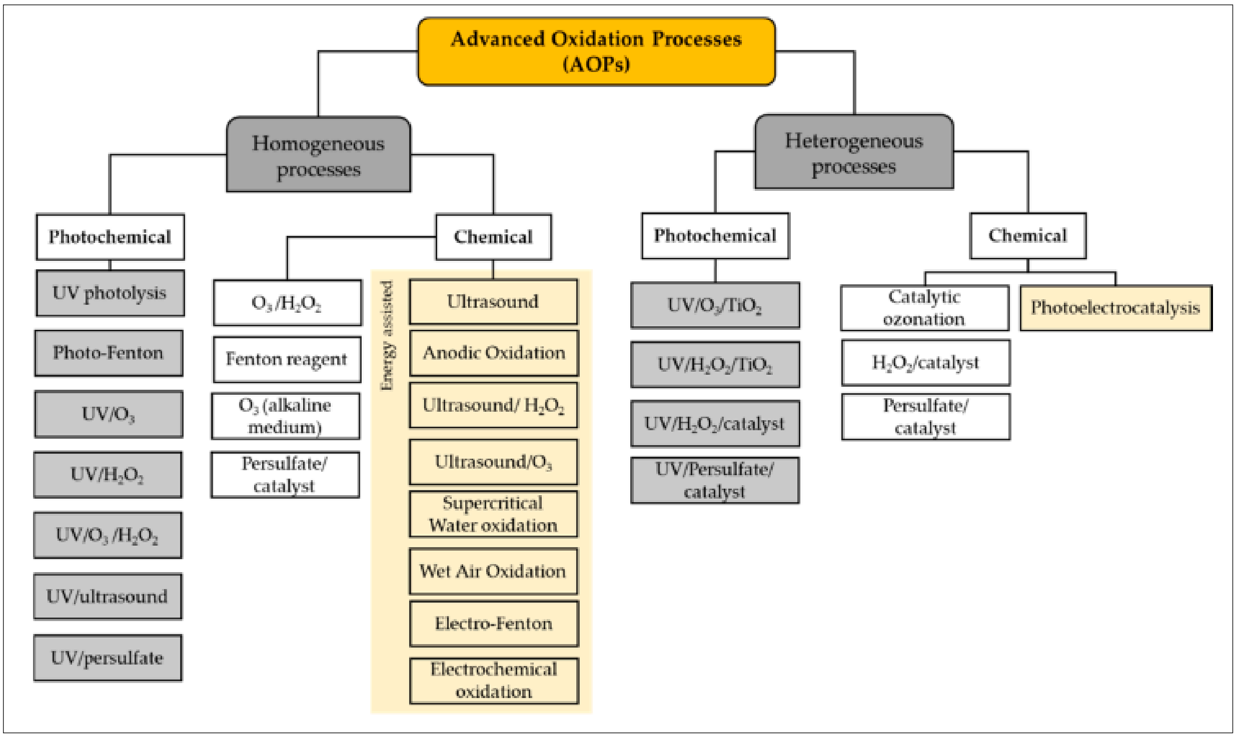


Figure 2.1 : Schematic diagram of different advanced oxidation processes (AOP) treatments.

These strategies have been researched for the purpose of eliminating micro pollutants from wastewater. The following table 2.2 summarizes the benefits and drawbacks of numerous non-biodegradable strategies for OTC breakdown (Safari et al., 2015; Huang et al., 2019; Fenton, 1894).

Table 2.2: A comparison of non-biodegradable tetracycline method

Non-biodegradable method	Advantages	Disadvantages
Ozonation	• High process efficiency • Lesser O3 consumption • Environmentally safe (no sludge production) • Improve efficiency by catalyst utilization	• Lesser solubility in water • Possible production of carcinogenic by product • Mass transfer limitation
Fenton process	• High performance • Simplicity • Non-toxic • Environmentally safe end product generation	• Strict pH range • High H2O2c consumption • Ferric sludge generation

Photolysis/photocatalysis	• Reaction conditions easily met • Complete decomposition of organic matter • Strong redox ability • Low cost • No adsorption saturation	• Inefficient visible light utilization • Rapid degradation of photo generated intermediates • Mass transfer limitations • Incomplete mineralization
Sonolysis/sonochemical oxidation	• Green or safe technique • No or negligible secondary pollution • Ultrasound waves clean catalysts surface thereby increasing catalyst efficiency	• Non selective mechanisms • High energy consumption • High maintenance cost

When AOPs are employed for wastewater treatment, these radicals as an oxidizing agent are expected to adequately destroy the wastewater contaminants, and change them to less and even harmless products in wastewater treatment. AOP is an oxidizing method for wastewater treatment that utilizes hydroxyl radicals as the principal oxidant to degrade and mineralize organic contaminants at pressures and temperatures found in nature. Although hydroxyl radicals (OH) have a greater oxidative reactivity than ozone and chlorine gas (E° = 2.8 V), their selectivity is limited. These highly reactive (OH) radicals are produced by H_2O_2 and ozone, as well as metal and semimetal catalysts.

2.6 Ozone Based Advanced Oxidation Process

The breakdown of tetracycline has been extensively investigated using ozone (O_3) a well-known oxidizing agent (Chopra, 2001). Since the late nineteenth century, ozone has been ozone has been widely employed in wastewater treatment. Ozone is a colourless gas with a strong odour. The current surge in popularity of ozone applications is mostly attributable to two factors: the lowered cost of ozone generating and the environmental benefits of ozone over chlorine (Rekhate, 2020). O_3 has oxidation potential of 2.07 V. (OH) is deduced from O_3 in certain situations in order to initiate indiscriminate oxidation (indirect mechanisms). Figure 2.2 illustrates the use of ozonation in conjunction with other approaches to boost hydroxyl radical production and hence treatment efficiency.

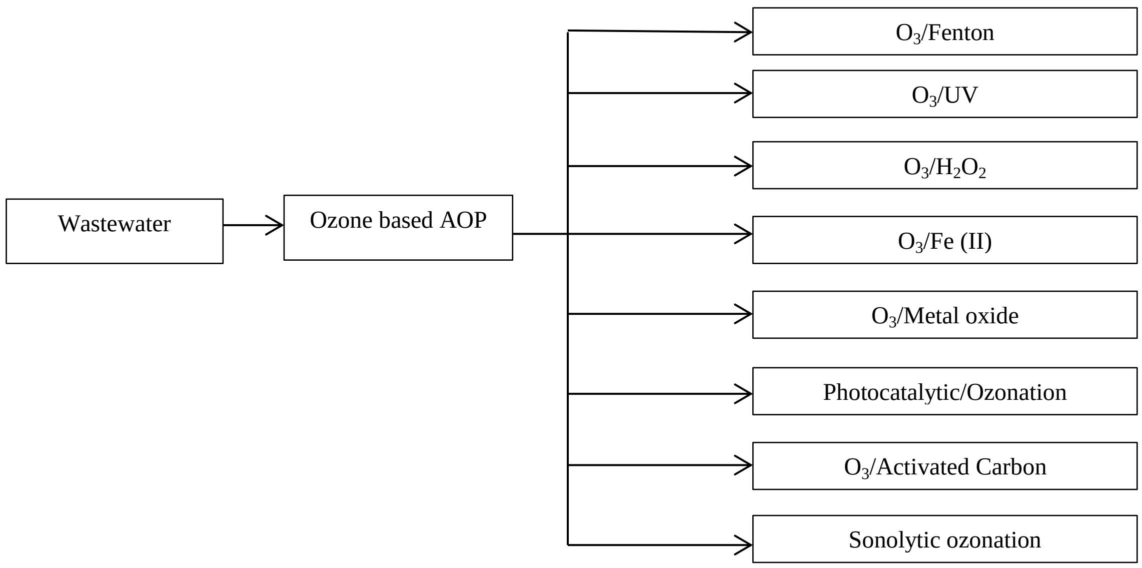


Figure 2.2: Ozone based AOPs

Because ozone exposure is often modest in ozone-based AOPs due to quick ozone breakdown, the bulk of antibiotic transformation occurs due to reactions with hydroxyl radicals. To explain the complicated (OH) generation, several precise processes have been proposed, and the overall reaction including (OH) formation is described as follows:

$$3O_3 + H_2O \rightarrow 2OH\cdot + 4O_2 \quad (2.1)$$

Typically, degradation rates in ozonation processes increase when pH rises, due to the release of free radicals from the breakdown of ozone in the base medium (reaction 2.3). The indirect oxidative reaction with ozone resembles the following:

$$OH^- + O_3 \rightarrow O_2 + HO_2^- \quad (2.2)$$

$$HO_2^- + O_3 \rightarrow O_3^- + HO_2 \quad (2.3)$$

$$HO_2 + O_2^- \rightarrow H^+ \quad (2.4)$$

$$O_2^- + O_3 \rightarrow O_3^- + O_2 \quad (2.5)$$

$$H^+ + O_3^- \rightarrow HO_3 \quad (2.6)$$

$$HO_3 \rightarrow OH + O_2 \quad (2.7)$$

At alkaline condition, the reaction is as shown as below:

$$OH + O_3 \rightarrow OH^2 + O_2 \quad (2.8)$$

2.7 Ozonation of Antibiotics

Due to the fact that antibiotics are a relatively class of pollutants, ozone has been effectively utilized to treat antibiotics in a variety of water sources, including wastewater, drinking water, and grey water treatment procedures. Because of the selective oxidation reactions offered by ozone over other commonly employed oxidants as well as the dual oxidant capabilities available from ozone’s principal decomposition product.

Antibiotics have already been studied in the treatment of wastewater, drinking water, and grey water, despite the fact that they represent as a new type of pathogen. Indeed, ozone has been successfully used to detoxify antibiotics as well as a variety of industrial pollutants in these water sources (Glaze, 1987; Hernández-Leal *et al.*, 2011). Due to the selective oxidation processes given by ozone over other commonly utilized oxidants, as well as the dual oxidant capabilities afforded by ozone's primary breakdown product, hydroxyl radicals, ozone is a suitable alternative for antibiotic transformation in water and waste water sources. (Ternes et al., 2003) published one of the first studies on antibiotic removal by ozonation after their investigation of ozonation of a variety of pharmaceutical and personal care products (PPCPs) in wastewater matrices. Among the chemicals examined were trimethoprim, sulfamethoxazole, clarithromycin, erythromycin, and roxithromycin. All five antibiotics were reduced to levels below quantification limits when ozone was added at a concentration of 5 mg/l, showing that macrolide, sulfonamide, and trimethoprim antibiotics effectively compete with organic matter for ozone reactions.

Adams *et al.*, (2002) demonstrated that after just 1.3 minutes of gaseous ozonation (2 %w/w) in Missouri River water, 95 %of seven antibiotics (carbadox, sulfachloropyradazine, sulfadimethoxine, sulfamethazine, sulfamethazine, sulfathiazole, and trimethoprim) were removed. A Swiss study discovered that macrolides and sulfonamides were effectively converted (>95 percent) into secondary effluents at pH 7 using ozone concentrations of 2 mg/l (Kraemer, 2019).

Okuda et al. (2008) observed that when ozone was added at a concentration of 3 mg/l, the overall mass concentration of pharmaceuticals, including numerous antibiotics, reduced from around 3800 ng/l to less than 50 ng/l. In less than 20 minutes, a supply of gaseous ozone containing 5.3 %ozone at a flow rate of 1.6 l/min was able to achieve a 90 %conversion efficiency for sulfonamide and macrolide antibiotics (Lin et al., 2009). All of these studies suggest that antibiotics react rapidly with ozone, even when DOM concentrations are substantially higher.

Numerous previous studies have shown that ozonation treatment may successfully remove antibiotics from drinking water and wastewater effluents (Adams et al., 2002; Hernández-Leal et al., 2011). (Adams et al., 2002) demonstrated that ozonation removed over 95% of various sulfonamides and trimethoprim from river water in around 1.3 minutes at a concentration of 7.1 mg. L^{-1}. Huber et al. (2005) reported that ozonation oxidized 90% to

95% of sulfonamides and macrolides in secondary wastewater effluents with a concentration greater than 2 mg. L^{-1}. Second-order rate constants for ozone and hydroxyl radicals have been reported for fluoroquinolones, sulfonamides, -lactams, macrolides, and trimethoprim (Dodd et al., 2009). The authors determined that at standard ozone disinfection dosages of 5-10 mg. 5-23 mg ozone L^{-1} DOC, antibiotics should be eliminated from river water and wastewater effluents 99 %of the time.

Organic molecules may be oxidatively destroyed either directly with molecular ozone (O3) or indirectly through hydroxyl radicals during ozone treatment (Staehelin, 1985). Numerous antibiotics, including sulfonamides, macrolides, fluoroquinolones, and tetracyclines, have been shown to be transformed primarily via direct ozone reaction during wastewater ozonation (Dodd et al., 2006), whereas cephalexin, penicillin, and N4-acetyl sulfamethoxazole, have been shown to be transformed primarily via hydroxyl radicals (Dodd et al., 2006). The relative dominance of the real oxidative pathway dictates the ratio of molecular ozone to hydroxyl radicals, the ensuing reaction kinetics, and the presence of organic matter (von Gunten, 2003; Elovitz et al., 2000).

Organic compounds may be degraded oxidatively during ozone treatment either directly or indirectly through hydroxyl radicals (Staehelin, 1985). Numerous antibiotics, including sulfonamides, macrolides, fluoroquinolones, and tetracyclines, have been shown to be transformed primarily by direct ozone reaction during wastewater ozonation whereas cephalexin, penicillin, and N4-acetyl sulfamethoxazole have been shown to be transformed primarily by hydroxyl radicals (Dodd et al., 2006). The ratio of molecular ozone to hydroxyl radicals, the resultant reaction kinetics, and the existence of organic matter would be determined by the actual oxidative pathway's relative superiority (von Gunten, 2003; Elovitz et al., 2000). Ozone and/or hydroxyl radicals inhibit antibiotics' bactericidal activity by attacking or modulating their pharmaceutically active functional groups, including Netheroxime and dimethylamino macrolide groups (Lange et al., 2006; Dodd et al., 2009), sulfonamide aniline moieties , penicillin thioether groups, and cephalosporin unsaturated bonds (Huber Dodd et al., 2009).

Due to their great resistance to oxidative attack, compounds with electron-rich aromatic systems, such as hydroxyl, amino, acylamino, alcoxy, and alkyl aromatic compounds, as well as those with deprotonated amine and non-aromatic alkene groups, were strongly removed by ozonation (Dickenson et al., 2009). However, when ozone is used to oxidize antibiotics, the risk of changing into molecules that remain biologically active and resistant to further ozonation is a significant issue. According to Dodd et al. (2009), the ozonation products of -lactam antibiotics retain biological activity after first oxidation events; however, hydroxyl radicals or ozone may be further destroyed if the residual ozone concentration is sufficient. However, in the case of roxithromycin, the major ozonation products have been retained by the bactericidal dimethylamino groups and are particularly resistant to further breakdown at extremely high ozone exposures (Radjenovic et al., 2009).

2.8 Catalytic Ozonation

Ozonation is a green technique that eliminates or degrades a large number of dangerous organic components found in wastewater, hence increasing the amount of biodegradable molecules (Mecha et al., 2016). Ozone interacts directly with other molecules through O3 molecules and/or indirectly via hydroxyl radicals (OH). As a consequence, enhanced ozone-based oxidation techniques were employed to increase the ozone reactivity. Ozone has lately been explored in combination with heterogeneous catalysts in liquid phase processes involving aniline, phenol, formic acid, and cyanide ions. Mineralization rates of organic and inorganic compounds considerably increase in the performance of the oxidation process. Due to the wide surface area and porous structure of activated carbon (AC), it has a high adsorption capacity for certain compounds, enabling it to be employed successfully in the degradation of aqueous or gaseous contaminants. Additionally, the usage of activated carbon may expedite the breakdown of O_3 and result in an increase in the concentration of active radicals. Catalysts may be homogeneous or heterogeneous when employed in conjunction with ozonation.

The advantage of a heterogeneous catalyst is that it may be easily separated from the liquid and reused. Metal oxide nanoparticles, such as zinc oxide (ZnO) nanoparticles, have a high surface area and a cheap manufacturing cost, which makes them perfect for water and wastewater treatment. Additionally, ZnO exhibits antibacterial, adsorptive, and toxicant degradation-friendly properties. Titanium dioxide (TiO_2) is mainly used as a photo catalyst. To enhance the photocatalytic system's performance, the reaction environment should be supplemented by a strong oxidant species such as hydrogen peroxide in the presence of Fe (II) ions or ozone. Additionally, TiO_2 has been studied as a pollutant absorber in wastewater. Activated carbon (AC) has a large surface area and a porous structure, which enables it to have a high adsorption capacity for certain compounds, enabling for effective removal of aqueous and/or gaseous contaminants. Additionally, the use of activated carbon may increase the rate of O_3 breakdown, resulting in a larger concentration of active radicals. Catalysts may be homogeneous or heterogeneous when employed in conjunction with ozonation. The benefit of heterogeneous catalysts is their ease of separation from the liquid and their reusability (Fiaz et al., 2021).

2.10 Benefits of ozone (O_3) based treatment

In general, chlorine is employed as a significant disinfectant in most water treatment plants across the globe. However, current research suggested that while chlorine combines with any organic molecule present in water, produce by products comprise THMs for instance chloroform. THMs enter the body and increase the formation of free radicals which are particularly carcinogenic. According to the U.S Council of Environmental Quality, the risk of cancer among people consuming chlorinate water is up to 93% percent higher compared to people who drink water with no chlorine. Therefore, the United States has included ozone (O_3) as a disinfectant in over 280 main water treatment plants. O_3 is more than twice as powerful as chlorine and performs 3000 times faster (Shah et al., 2016). O_3 is used as oxidant or in combination with other oxidants or energy (AOPs) in treatment of ground, surface, or wastewater. The ozone based treatment technologies have general goal is to optimise the utilisation of O_3 for improved disinfection or elimination of existing pollutants from water (Rosal, 2008). Ozone based water treatment has various advantages which summarized are as follow:

i. It has high oxidizing capacity and short reaction time by allowing the microbes (germs) comprising viruses to destroy in seconds.
ii. Ozone based treatment supply oxygen to the water.
iii. Ozone able to remove the colour, odour and taste with no chemicals.

O_3 is an extremely reactive gas which may oxidise the bacteria molds, organic substance furthermore other pollutants present in water. O_3 changes reverse into the oxygen rapidly, furthermore leaves no trace one time it has been utilized. Operating cost are low because of the oxygen supply in the off-gas for tanks of activated sludge.

CHAPTER 3: MATERIALS AND METHODS

3.1 Overview of research methodology

This research is mainly focused on the experiment of removal of oxytetracycline from water using sole ozonation and combination of catalytic ozonationusing ZnO nanoparticles. Figure 3.1 shows the flow diagram of research project for ozonation of oxytetracycline from water to achieve highest removal efficiency. The experiment is carried out in the SCE Integrated Research Laboratory in USM. After the determination of maximum wavelength and calibration curve of oxytetracycline concentration, the best of each operating parameters will be obtained. The data will be collected to do comparison of sole ozonation and catalytic ozonation with zinc oxide nanoparticles.

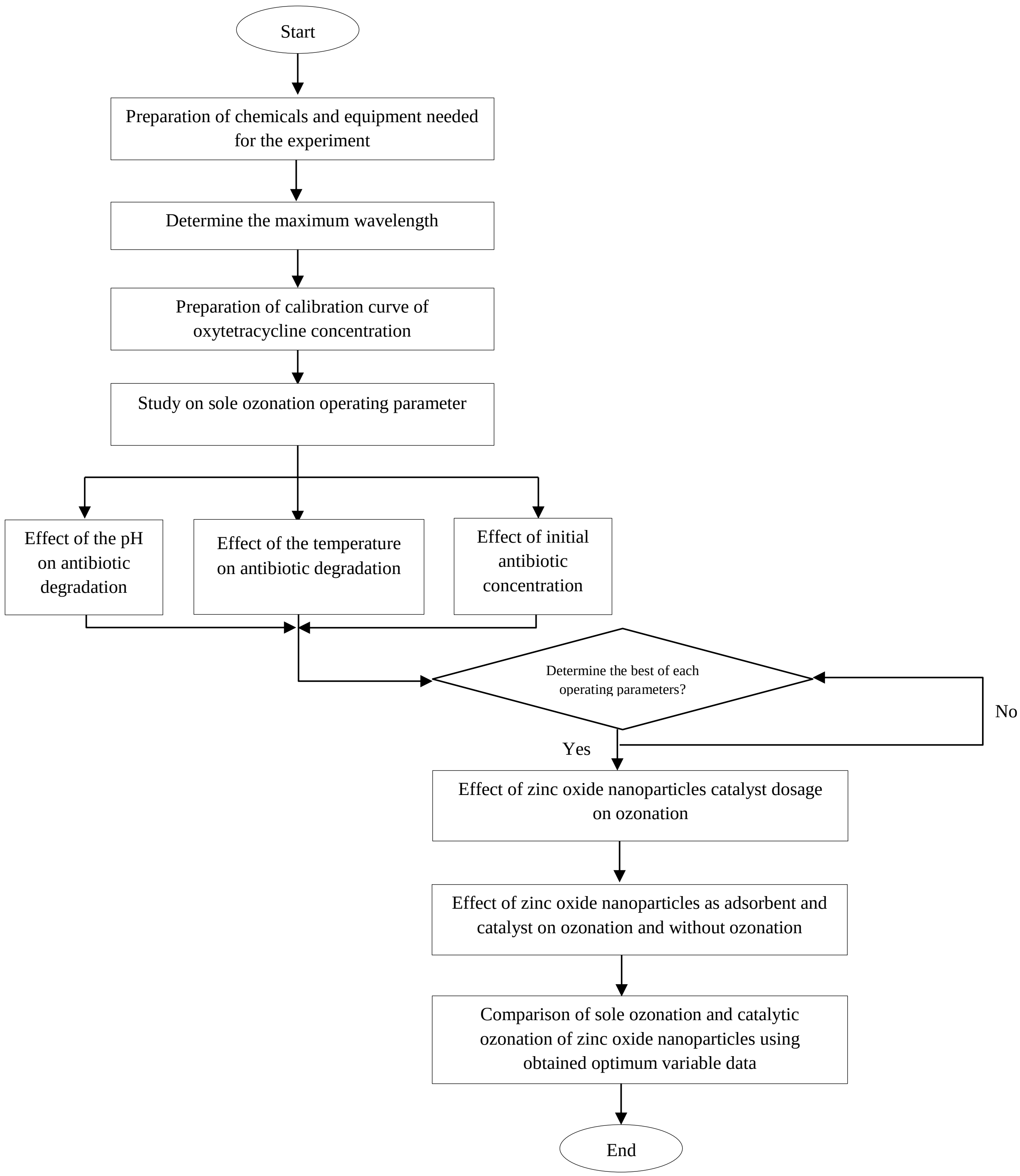
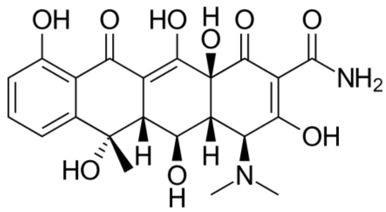


Figure 3.1: Flow diagram of research project

3.2 Chemical structure and Relevant Data for Oxytetracycline

The list of chemical structure and relevant information for oxytetracycline are shown in Table 3.1.

Table 3.1: Chemical structure and relevant data for oxytetracycline

Name	Oxytetracycline
Formula	$C_{22}H_{24}N_2O_9$
Structure	
Molecular weight,MW (g mol^{-1})	460.434
Maximum wavelength, λ_{max}(nm)	296

3.3 Materials and Chemicals Required

The list of materials and chemical used in this study are shown in Table 3.2.

Table 3.2: Materials and chemicals used for the experiment

Materials	**Molecular Formula**	**Purpose**
Oxytetracycline Powder	$C_{22}H_{24}N_2O_9$	Used as the main antibiotic for the experiment
Distilled Water	H_2O	To dilute the antibiotic solution to get desired concentration
Hydrochloric acid	HCl	To decrease the pH of the solution
Sodium Hydroxide	NaOH	To increase the pH of the solution
Zinc oxide nanoparticles	ZnO	Used as catalyst and adsorbent for the antibiotic solution

3.4 Equipment and Facilities Required

The list of equipment and facilities required in this study is listed out in Table 3.3.

Table 3.3: Equipment and facilities required

Equipment	Usage
SPARRO Ozone generator	To supply ozone to the antibiotic solution
UV/Vis spectrophotometer (UV-1800 SHIMADZU)	To study the concentration antibiotics
Electronic balance	Weighing the antibiotics powder
pH Meter (pH 2700 EUTECH)	To check the pH of antibiotics solution before the experiment was carried out.
Magnetic stirrer	To mix the solution thoroughly
Thermometer	To measure the temperature

3.5 Preparation of antibiotic stock solution

The oxytetracycline antibiotic stock solution will be prepared to determine the maximum wavelength and the calibration curve for the concentration. Oxytetracycline stock solution of 1000 mg/L is prepared by dissolving 1000 mg of antibiotic powder in a 1L of volumetric flask. The desired concentration of solutions is then prepared by diluting the stock solution with distilled water. After the stock solution is prepared, the known desired concentration solution is prepared by using the following formula:

$$c_1 \, x \, V_1 = c_2 \, x \, V_2 \qquad (3.1)$$

Where

$c_1 = initial\ concentration$

$V_1 = initial\ volume\ (stock\ volume)$

$c_2 = final\ concentration$