DEGRADATION OF OXYTETRACYCLINE FROM AQUACULTURE WASTEWATER USING SOLE OZONATION AND CATALYTIC OZONATION IN THE PRESENCE OF ACTIVATED CARBON

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by

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Bohesharvind A/L Naraiyanan Murthi

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LIST OF SYMBOLS

C_1	Initial concentration
C_2	Final concentration
V_1	Initial Volume
V_2	Final volume
V_W	Volume of water added
C_o	Initial antibiotic concentration
C_t	Antibiotic concentration at specified time
k	Kinetic rate constant
t	Reaction time
E_{E0}	Energy consumption
W	Power of ozone generator
V	Volume of treated water

LIST OF ABBREVIATIONS

COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
TOC	Total Organic Content
NOC	Natural Organic Content
DOC	Dissolved Organic Content
H_2O_2	Hydrogen peroxide
AOPs	Advanced Oxidation Process
O_3	Ozone
AC	Activated Carbon
UV-vis	Ultraviolet visible light
λ_{max}	Maximum wavelength
OH•	Hydroxyl radical
NaOH	Sodium hydroxide
OTC	Oxytetracycline
UV	Ultraviolet

pH	Potential of Hydrogen
$UV/H_2O_2/Fe^{2+}$	Photo-Fenton
H_2O_2/Fe^{2+}	Fenton
HO_2^-	Hydroperoxyl
TiO_2	Titanium dioxide
H^+	Hydrogen ion
Fe^{3+}	Iron (III)
e^-	electron
H_2O	Water
E°	Standard reduction potential

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ABSTRAK

Proses pengoksidaan lanjutan, seperti ozonasi, mendapat perhatian kerana keupayaan untuk mendegradasi antibiotik. Walau bagaimanapun, penghasilan ozon diketahui berintensifkan tenaga dan sangat dipengaruhi oleh keadaan sekitarnya. Oleh itu, kecekapan ozonasi tunggal dan ozonasi pemangkin untuk merawat air sisa akuakultur sintetik yang mengandungi oxytetracycline telah dikaji. Sepanjang eksperimen, kadar aliran ozon telah ditetapkan pada 500 mg/jam dan ozonasi tunggal dijalankan selama 120 minit manakala ozonasi pemangkin dijalankan selama 60 minit, Faktor -faktor yang mempengaruhi proses termasuk kesan suhu (13 °C, 23 °C, 33 °C), pH awal (3, 5, 7, 9, 11), kepekatan awal larutan oxytetracycline (10 mg/L, 30 mg/L , 50 mg/L) dan dos karbon aktif awal (75mg/l, 125 mg/l, 175mg/l, 225mg/l, 275mg/L) telah dikaji. PH 9 (sedikit alkali) mencapai kecekapan degradasi optimum sebanyak 91.2% dalam 60 minit ozonasi. Dalam keadaan pH ini, lebih banyak radikal hidroksil dan ozon bertindak balas dengan oxytetracycline tanpa dirosakkan oleh pemulung radikal hidroksil. Suhu optimum didapati 23 °C dengan kecekapan degradasi sebanyak 88.5% kerana memberikan keseimbangan yang baik untuk kesan yang bertentangan antara kadar ozonasi dan kelarutan ozon dalam larutan akueus. Degradasi oxytetracycline menurun dengan peningkatan kepekatan awal larutan oxytetracycline kerana bekalan ozon tidak mencukupi. Kecekapan degradasi meningkat dengan peningkatan dos karbon aktif kerana terdapat lebih banyak tapak spesifik. Dos karbon aktif yang optimum didapati 225 mg/ L kerana memberikan degradasi oxytetracycline tertinggi (98.6%). Walau bagaimanapun, peningkatan selanjutnya

dalam dos karbon aktif sehingga 275 mg/L menghasilkan kecekapan degradasi yang serupa (99.2%) kerana ketepuan tapak spesifik karbon aktif. Analisis kinetik antibiotik menunjukkan bahawa ozonasi tunggal oxytetracycline mengikuti reaksi urutan pertama selama 60 minit pertama manakala reaksi urutan kedua untuk jumlah masa reaksi selama 120 minit. Ozonasi pemangkin menunjukkan degradasi yang lebih baik daripada ozonasi tunggal hanya dalam 30 minit. Sebagai contoh, kecekapan degradasi oxytetracycline untuk ozonasi pemangkin dan ozonasi tunggal adalah 98.6% dan 73.0%, masing-masing. Secara ringkasnya, ozonasi pemangkin terbukti jauh lebih berkesan daripada ozonasi tunggal.

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ABSTRACT

Advanced oxidation processes, such as ozonation, are gaining attention due to their capability to degrade antibiotics. However, generation of ozone is known to be an energy intensive process and greatly affected by the surrounding conditions. Hence, the efficiency of sole ozonation and catalytic ozonation to treat synthetic aquaculture wastewater containing oxytetracycline was studied. Throughout the experiment, the ozone flowrate was held constant at 500 mg/h and the sole ozonation was carried out for 120 minutes while catalytic ozonation was carried out for 60 minutes. Factors affecting the process including the effect of temperature (13℃, 23℃, 33℃), initial pH (3, 5, 7, 9, 11), initial concentration of oxytetracycline solution (10 mg/L, 30 mg/L, 50 mg/L) and initial activated carbon dosage (75mg/L, 125 mg/L, 175mg/L, 225mg/L, 275mg/L) was studied. The pH 9 (slightly alkaline) achieved the optimum degradation efficiency of 91.2% within 60 minutes of ozonation. In this pH condition, more hydroxyl radicals and ozone reacted with oxytetracycline without being damaged by hydroxyl radical scavengers. The optimum temperature was found to be 23 ℃ with a degradation efficiency of 88.5% as it provided a good balance for the opposing effect between the rate of ozonation and solubility of ozone in aqueous solution. The oxytetracycline degradation decreased with the increase in initial oxytetracycline concentration due insufficient ozone supply. The degradation efficiency increased with the increase in activated carbon dosage as more specific sites were available. The optimum activated carbon dosage was found to be 225 mg/L as it gave the highest degradation of oxytetracycline (98.6%). However, further increase

in activated carbon dosage up to 275 mg/L resulted in similar degradation efficiency (99.2%) due to the saturation of specific site of activated carbon. The antibiotic kinetic analysis shows that the sole ozonation of the oxytetracycline follows the first order reaction for the first 60 minutes while second order reaction for total reaction time of 120 minutes. The catalytic ozonation showed a better degradation than sole ozonation in just 30 minutes. For instance, the oxytetracycline degradation efficiency for catalytic ozonation and sole ozonation were 98.6% and 73.0%, respectively. In a nutshell, catalytic ozonation was proven to be much more effective than sole ozonation.

CHAPTER 1

INTRODUCTION

1.1 Overview of introduction

This chapter includes a background study of aquaculture and its importance to humans and the economic development of a country. Besides, the numerous sources of antibiotics were discussed, as are their negative implications. Furthermore, a brief introduction of the advanced oxidation process as a potential technology for antibiotic removal was presented. In addition to that, this chapter also summarizes the problems associated with excessive usage of antibiotics and the various approaches taken to overcome the above issues. Moreover, the pros and cons of each method were summarized. Lastly, the best method for the present study was stated together with its major objectives.

1.2 Aquaculture industry

The human population is growing rapidly, from 3 billion in the early 1960s, and it is expected to reach 9.7 billion by 2050 (Silva et al., 2021). As a result, the demand for animal-derived foods, especially fish, has increased tremendously over the past decades. In fact, the annual fish consumption rate is twice the population growth (Silva et al., 2021). This could be due to the numerous health benefits associated with the consumption of fish. As per scientific research, fish is known to be highly rich in protein and omega-3 fatty acids, which are essential nutrients for muscle development and are highly effective in reducing bad cholesterol. Subsequently, the worldwide decline of fish stocks owing to increased consumption has resulted in an evolution in the aquaculture industry, which has a high potential for contributing to the country's fish demand. As example, worldwide fish aquaculture production has increased dramatically, reaching a high of 171 million tons in 2016 (Yu et al., 2020). Based on the United Nations Food and Agriculture Organization, the aquaculture industry accounts for 50% of the world's fish production for the food sector and is known to be more than any other animal-based production combined together, and its production capacity is expected to rise up to 62% by 2030 (Rahman et al., 2021). Aside from high-quality fish production, this industry has significant beneficial impacts, such as reduced reliance on natural resources and economic development. Aquaculture, for example, was estimated to employ 18.7 million people in 2015 (Silva et al., 2021).

In Malaysia, aquaculture has evolved into a development engine that helps in the advancement of our country's economy and has grown at an average rate of 10% annually for the last 5 years, according to the Food and Agriculture Organization (FAO). For instance, gross domestic product (GDP) growth is expected to increase by 3.5% from 2016 to 2020 (Nizam et al., 2020). The aquaculture industry in Malaysia can be divided into several sectors. Namely, brackish water, freshwater, seaweed, ornamental and aquatic plants. The work forces for each sector are shown in **Figure 1.1** (Kurniawan et al., 2021).

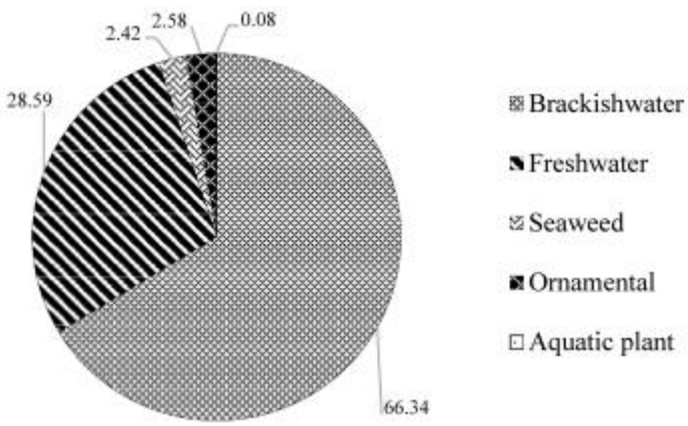

Figure 1.1: Total workforce percentage of brackish water, freshwater, seaweed, ornamental, and aquatic plant in Malaysia (Kurniawan et al., 2021)

In 2019, brackish water aquaculture in our country produced 224, 171 tons of fish worth more than RM 2.3 billion (Kurniawan et al., 2021). The most significant cultured species in brackish water are anadara granosa, shrimp, panaeus monodon, and other marine fishes that account for 54%, 22.4%, 17.3%, and 6.3%, respectively. Meanwhile, freshwater aquaculture contributed 105,101 tons of fish in the same year, worth more than $711 million. oreochrmois niloticus, catfish, carps, and other species are the most important freshwater cultured species, accounting for 44.7%, 36.7%, 10.08%, and 8.52%, respectively. Other than that, seaweed, ornamental fish, and aquatic plant aquaculture contribute 174, 083 tons worth RM 52 million, 325, 328, 503 pieces worth RM 350, 326, and 117, 006 bundles worth RM 19,924 per year, respectively (Kurniawan et al., 2021).

On the other hand, this sector has faced a huge drawback. In the cultured fish, for example, bacterial infections developed. As a result, fisheries experienced massive stock losses. To meet global fish demand, this industry has been subjected to the requirement of intensification, with the goal of reaching 102 million tons of production by 2025 in order to maintain current consumption levels (Silva et al., 2021). In this regard, antibiotics are commonly used to prevent and treat infections in aquaculture.

1.3 Antibiotics

Antibiotics are drugs that are being used worldwide as medicine to treat bacterial infections and are able to work efficiently even at low concentrations. However, it is very difficult for the fish to completely metabolize antibiotics. Subsequently, a huge quantity of unmetabolized antibiotics are thrown into the surrounding aquatic environment. As shown below in **Figure 1.2**, the major sources of antibiotics are aquaculture, hospitals, households, and industries (Mahdi et al., 2021).

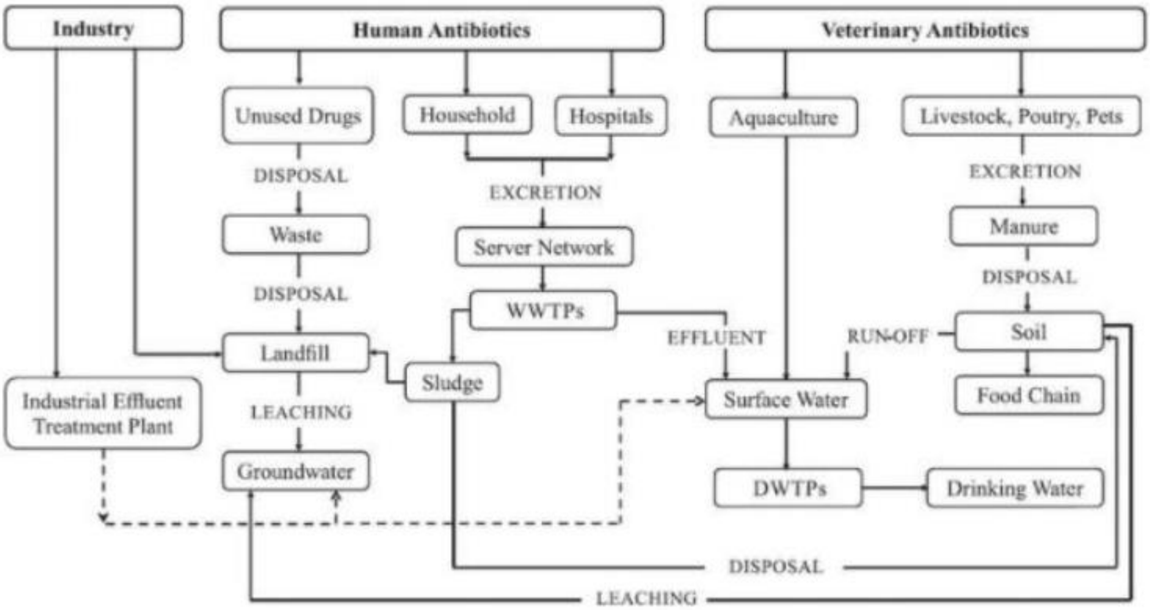

Figure 1.2: Pathways of antibiotics to the environment (Mahdi et al., 2021).

Following that, antibiotics remain for a long period due to their high resistance towards biodegradation and cause severe negative implications for the environment. Most notably, a high concentration of antibiotics in an environment contributes to the development of antibiotic resistance pathogens. The term ‘antibiotic resistance’ refers to the capacity of infection-causing microorganisms to withstand the effects of infection-fighting antibiotics. Indeed, antibiotic resistance gives microorganisms a survival benefit, making it almost impossible to eradicate the infections they cause (Leal et al., 2018). As a result, higher doses of substitute antibiotics are needed to treat the particular illness, which may result in severe side effects and promote the ability to produce more antibiotic-resistant bacteria strains (Silva et al., 2021). The development of antibiotic resistance bacteria could lead to inhibition of natural antibiotics in the body. Antibiotic-resistant bacteria, for example, produce lactamases that hydrolyze the penicillin-lactam ring, rendering the antibiotic inactive. Furthermore, an excess of antibiotic in fish tissue may act as a protein synthesis inhibitor by reacting with the ribosome-messenger complex and preventing the binding of the tRNA and mRNA complex. Besides, it also acts as a toxin for red blood cells (Leal et al., 2018).

Moreover, several research has shown excessive and pro-longed usage of antibiotics could remain in the fish tissue, resulting in nephrotoxicity and liver damage (Mog et al., 2020). Thus, it will be a threat for those who consuming it on regular basis as fish product are contaminated by antibiotics. In detail, there is scientific discovery about the transmission of antibiotic resistance genes from the fish to humans which will subsequently results in the development of highly resistance bacteria or pathogen in human body. This process is known as horizontal gene transfer (Mog et al., 2020). For instance, plasmids harboring resistance factors from fish pathogen and bacteria can be easily transmitted not only to bacteria of the same genus but also to E. coli which is an essential bacteria that helps to digest the food in the small intestine of humans. As a result, the E. coli could be transform into a pathogenic bacteria and become extremely harmful to human (Leal et al., 2018).

1.4 Traditional wastewater treatment and advanced oxidation process

Traditionally, wastewater treatment includes physical, biological, and chemical processes as shown in **Table 1.1**. These processes mainly emphasize the removal of inorganic materials, heavy metals, and organic compounds (Correa et al., 2019). These processes are being widely applied in wastewater treatment plants all over the world.

Table 1.1: Classification of traditional treatment processes with its respective advantageous and disadvantageous (Correa et al., 2019)

Types of treatments	**Physical treatments**	**Chemical treatments**	**Biological treatments**
Methods	Filtration Adsorption Air flotation Extraction Flocculation sedimentation	Thermal oxidation Chemical oxidation Ion exchange Chemical precipitation	Anerobic Aerobic Activated muds
Advantages	Low capital cost Relatively safe Easy operability	High degree of treatment Elimination of dissolved contaminants	Relatively safe Easy maintenance Easy operability Elimination of dissolved contaminants
Disadvantages	Volatile emissions High energy requirement High maintenance cost	High capital cost High operation cost Operational difficulties	Volatile emissions Vulnerable to antibiotics Extra purification step for mud removal

Traditional methods, on the other hand, are no longer of interest due to their high cost and operational difficulties. Furthermore, they are ineffective for antibiotic treatment. Antibiotics are emerging pollutants with a complex structure and high chemical stability. Also, it is non-biodegradable and degrades at a slower rate. As a result, a more modern approach is required. For instance, advanced oxidation processes (AOP) is a type of highly advanced wastewater treatment technology that involves the in-situ generation of strong oxidants, specifically hydroxyl radicals, which react quickly with most organic substances such as antibiotics by oxidizing them into less harmful substances (Correa et al., 2019). Indeed, hydroxyl radicals are the most advantageous of the strong oxidants because they have several important properties, such as non-toxicity, non-corrosiveness, and a low life span, which allows them to degrade into water and oxygen quickly in the absence of pollutants. Furthermore, it generates no additional waste and is produced by assemblies that are very simple to manipulate (Tao et al., 2020). In this regard, there are various oxidation agents which are represented in **Table 1.2** (Correa et al., 2019).

Table 1.2 : Oxidizing agent in aqueous media and their respective standard reduction potential value (Correa et al., 2019).

Oxidizing agent	Standard reduction potential, E° (V)
Fluorine	3.05
Hydroxyl radical	2.8
Sulfate radical anion	2.6
Ferrate	2.2
Ozone	2.08
Peroxydisulfate	2.01
Hydrogen peroxide	1.76
Permanganate (a)	1.67
Hydroperoxyl radical (a)	1.65
Permanganate (b)	1.51
Hydroperoxyl radical (b)	1.44
Dichromate	1.36
Chlorine	1.36
Manganese dioxide	1.23
Oxygen	1.23
Bromine	1.07

Most importantly, hydroxyl radicals were the second known reactive species with the highest oxidant power of 2.8 V after fluorine, as shown in **Table 1.2**, and they act in a non-selective manner on organic contaminants in wastewater, ideally resulting in the complete mineralization of organic contaminants into carbon dioxide and water (Correa et al., 2019). Although fluorine possesses a much higher oxidant power, its use in wastewater treatment is not applicable due to its toxicity. Indeed, the use of fluorine in aquaculture wastewater for the removal of antibiotics could result in a dangerous situation as this oxidizing agent inhibits cellular respiration as it remains in the treated water in the form of fluoride ions (Tao et al., 2020).

Following that, advance oxidation process (AOP) can be categorized based on the source of hydroxyl radicals. Namely, photolysis, ozone-based processes, hydrogen peroxide-based processes, heterogenous photocatalysis, sono-chemical oxidation, and electrochemical oxidation. This classifications of advance oxidation process are summarized and is as shown in **Table 1.3** (Correa et al., 2019).

Table 1.3 : Classification of Advanced Oxidation Process (AOP) (Correa et al., 2019).

Generic name	**Sources of hydroxyl radicals**
Photolysis	UV
Ozone-based processes	O_3 O_3/UV O_3/ H_2O_2 O_3/UV/ H_2O_2
Hydrogen peroxide-based processes	H_2O_2/UV H_2O_2 /Fe^{2+}

	H_2O_2 /Fe^{3+} H_2O_2 /Fe^{2+}/UV
Heterogenous photocatalysis	TIO_2/ UV TIO_2/ UV/ H_2O2
Sono-chemical Oxidation	Ultrasound (20 kHz to 2 MHz)

The advanced oxidation process was known to be very effective in treating antibiotics as compared to most of the conventional processes. However, there are variety of methods in the advanced oxidation process. A brief overview of all the available methods will be discussed in this section. First and foremost, photolysis involves the irradiation of ultraviolet radiation (UV), which will then be absorbed in the form of photons by organic molecules such as antibiotics. Subsequently, the weak bonds of organic molecules will undergo homolysis to form ozone or hydrogen peroxide, which will then produce free radicals such as hydroxyl radicals (Correa et al., 2019). Also, free radicals can also be produced by electron transfer from an organic molecule in an excited state to molecular oxygen. These free radicals are responsible for the degradation of organic molecules (Mahdi et al., 2021).

Besides, the ozonation process is very complex and takes place mainly in two pathways. Direct ozonation, for instance, occurs in an acidic or neutral environment with dissolved ozone. Meanwhile, indirect ozonation prevails under alkaline conditions, which results in the formation of hydroxyl radicals (Rahmah et al., 2017). Hydrogen peroxide, for example, will result in the production of hydroxyl radicals in all environmental conditions. Heterogenous catalysts such as titanium dioxide, on the other hand, are able to perform both oxidation and adsorption simultaneously. Indeed, the oxidation process begins with UV absorption, which excites the electrons, followed by oxygen molecule adsorption onto the adsorbent surface, where the excited electron is transferred, resulting in the formation of superoxide anion, which is then converted into hydroxyl radicals and degrades the organic compound (Correa et al., 2019). At the same time, organic molecules can also be degraded directly by the action of excited electrons.

Lastly, sono-chemical oxidation is the use of ultrasound at a frequency of 20 kHz to 2 MHz (Abdurahman and Abdullah, 2020). The ultrasonic waves will propagate through the wastewater and result in cavitation, which causes the implosion of microbubbles and, consequently, results in the release of a large amount of energy to convert water into hydrogen peroxide and finally into hydroxyl radicals (Wang et al., 2019).

1.5 Problem statement

The emergence of antibiotic resistance genes in aquaculture wastewater and their proclivity to be transmitted to humans is causing widespread alarm around the world (Li et al., 2019). Therefore, numerous approaches, including conventional and modern methods, are being investigated to overcome the aforementioned issue.

Treatment processes for aquaculture wastewater that primarily rely on traditional methods such as flocculation, precipitation, and biological systems are less effective due to the complex structure, high chemical stability, and high resistance to biodegradation of antibiotics (Correa et al., 2020). On the other hand, adsorption processes using carbon-based adsorbents such as activated carbon and graphene oxide have recently gained attention for removing antibiotics because of their simplicity, convenience, and high removal efficiency for certain antibiotics (Yu et al., 2020). For instance, activated carbon alone was capable of removing 99.9% of oxytetracycline at a low initial antibiotic concentration of 10mg/L. Meanwhile, as the initial oxytetracycline concentration increased up to 400mg/L, the removal percentage reduced to 49.21% (Nayeri and Mehrabi, 2019). This shows that activated carbon acts as a good adsorbent for oxytetracycline at low concentrations but becomes ineffective at higher oxytetracycline concentrations. The further increase in adsorbent dosage leads to higher cost requirements and a more difficult recovery procedure. Thus, industrial waste containing a high concentration of antibiotics with complex structures makes them difficult to adsorb. In short, adsorption alone may not be sufficient due to the extreme resistance to biodegradation of antibiotics. Hence, it requires degradation of these antibiotics into simpler compounds for better and more effective adsorption (Correa et al., 2020). The prolonged contact time between antibiotics and adsorbents resulted in a desorption process, which in turn increased the antibiotic concentration in the wastewater. Thus, adaptation to modern technology is extremely important.

In this context, advanced oxidation processes are gaining attention worldwide as they involve the degradation of antibiotics that are highly non-biodegradable, rendering them less complex and making them susceptible to biodegradation. In more detail, ozonation is a good choice for the degradation of non-biodegradable substances such as oxytetracycline due to the presence of hydroxyl radicals that oxidized the conventionally resistant non-biodegradable organic pollutants while also improved the wastewater biodegradability (Grieser et al., 2015). Based on a study by Wang et al., 2020, the by-products from the partial ozonation process of oxytetracycline are found to be more toxic than its parent compound. This demonstrates that ozonation alone was ineffective in because the by-products are far more dangerous. Subsequently, more ozone was needed for complete mineralization of these substances. However, a vast amount of energy was required to achieve the complete mineralization of antibiotics due to the low mass transfer rate of ozone. Thus, ozonation and adsorption have both pros and cons.

Recently, the coupling of adsorbent with ozonation became a topic of interest since the adsorbent, such as activated carbon, promoted the mass transfer of ozone into hydroxyl radicals, which resulted in greater removal of antibiotics (Utrilla et al., 2018). Furthermore, the activated carbon provided a specific site for the interaction between ozone and pollutants. Thus, catalytic ozonation efficiency increases with the increase in adsorbent dosage. However, at excess adsorbent dosage, the concentration per unit specific area of both ozone and pollutants will be greatly reduced, leading to poor catalytic ozonation performance (Nie et., 2021). Thus, the optimum catalyst dosage is very important to be determined. On the other hand, ozone helps to degrade the antibiotics into simpler compounds, thus increasing the adsorption of the degraded antibiotics onto an adsorbent, resulting in a lower requirement of adsorbent.

In this present study, sole ozonation and catalytic ozonation were investigated by manipulating the initial concentration of oxytetracycline, initial activated carbon dosage, initial pH of the oxytetracycline solution, and the temperature of the oxytetracycline solution as these factors significantly influenced the degradation of antibiotics.

1.6 Research objectives

1. To analyze the effects of the different initial concentrations of antibiotic solutions on the antibiotic degradation efficiency using sole ozonation.
2. To investigate the effect of the different initial pH of the antibiotic solutions on the antibiotic degradation efficiency using sole ozonation.
3. To understand the effects of the different temperatures of antibiotic solutions on the antibiotic degradation efficiency using sole ozonation
4. To study the effect of the different activated carbon dosages on the antibiotic degradation efficiency via catalytic ozonation.

5. To evaluate the efficiency of sole ozonation and catalytic ozonation on the degradation of the antibiotic.

1.7 Sustainability of the wastewater treatment

The United Nations Member States in 2015 developed 17 Sustainable Development Goals (SDGs) with the aim of providing peace and prosperity for people and the planet by promoting health and education, decreasing inequality, and stimulating economic development, all while combating climate change and striving to protect our oceans and forests. This study is relevant to SDG 6 (clean water and sanitation). Indeed, ozonation and adsorption processes were able to degrade and remove antibiotics and their by-products as well as other toxic substances to a great extent. Consequently, we will be able to access clean water, and water pollution will be greatly reduced in the future. In relation to that, clean water is able to ensure the avoidance of exposure to countless diseases and helps to promote better health, which is aligned with SDG 3 (good health and well-being). The addition of activated carbon to the ozonation process helped to reduce the energy consumption of the overall process by enhancing the ozone mass transfer, which is known to be the rate-limiting step that is highly related to SDG 12 (responsible consumption and production). Water-related ecosystems are also preserved and conserved, which aligns with SDG 14 (life below water) due to the removal of highly non-biodegradable antibiotics. In addition, the removal of excessive antibiotics also results in reducing the development of antibiotic resistance genes in fish, which in turn helps to promote fish production worldwide as fish are rich in omega-3-fatty acids that possess numerous health benefits, such as reducing cholesterol levels in our bodies. Thus, the economy of the country will be boosted without adversely affecting the environment or its people, and this is aligned with SDG 8 (decent work and economic growth).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of literature review

This chapter includes the misuse of antibiotics in aquaculture wastewater, followed by its negative implications. Besides, various approaches, including traditional methods such as adsorption and modern technology such as advanced oxidation processes, particularly ozonation, as well as combinations of these methods, were discussed. Moreover, a brief comparison of all the methods was performed prior to choosing the most promising technology to treat antibiotics, particularly oxytetracycline, from wastewater.

2.2 Antibiotics in aquaculture wastewater

Antimicrobial agents like antibiotics have significantly revolutionized contemporary medicine, saving countless lives and enhancing life expectancy. According to global estimates, the annual use of antibiotics for the treatment of bacterial infections in aquaculture is between 100,000 and 200,000 tons (Yu et al., 2020). In detail, 80% of antibiotics used in aquaculture, however, were not ingested, and 75% were unable to be digested by the fish, resulting in their secretion into the surrounding aquatic environment, which included agriculture farms, rivers, and lands (Li et al., 2019). Consequently, the aquatic environment, rivers, farms, and lands will be contaminated as the antibiotics are non-biodegradable and remain in the surface water for a long time (Rahmah et al., 2020). As a result, the drinking water may be contaminated with some amounts of antibiotics. As reported by Mog and Manu (2020), since a low concentration of antibiotics is very effective, the risk of developing antibacterial resistance bacteria in the human body is also a major problem, as reported by Mog and Manu (2020). As a result, several techniques, both conventional and modern, are being researched to overcome the aforementioned problem.

2.3 Traditional wastewater treatment methods

Traditional wastewater treatments, such as flocculation, precipitation, and biological systems, were less successful in treating antibiotic solutions as the antibiotics were substances with complex structures, high chemical stability, and high resistance towards biodegradation (Correa et al., 2019). However, conventional approaches such as the adsorption process have gained much attention recently due to their capability of removing antibiotics, particularly oxytetracycline. In this regard, carbon-based adsorbents such as graphene oxide and activated carbon are effective in removing antibiotics (Nayeri et al., 2019; Hadki et al., 2021). Indeed, activated carbon was able to remove 99.9% of oxytetracycline at a pH, initial oxytetracycline concentration, and contact time of 9, 10 mg/L, and 60 minutes, respectively, when the adsorbent dosage was 1.5 g/L (Nayeri et al., 2019). In another study, graphene oxide was demonstrated to remove 87% of oxytetracycline at a pH, an initial oxytetracycline concentration, and contact time of 5, 10 mg/L, and 60 min, respectively, when the adsorbent dosage was 0.3 g/L. The addition of boron to graphene oxide leads to the 100% removal of oxytetracycline under the same optimum conditions (Hadki et al., 2021).

Other than that, pH had a great influence on removal efficiency. Typically, a pH range from 5 to 9 shows the highest removal efficiency (Nayeri et al., 2019). This suggests that slightly acidic, neutral, or slightly alkaline conditions are preferable. Generally, oxytetracycline is an amphoteric molecule (Li et al., 2019), as shown in **Figure 2.1**.

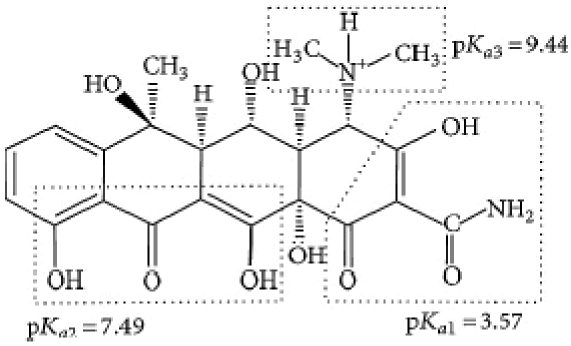

Figure 2.1 : Molecular structure of oxytetracycline at different pH (Li et al., 2019).

At extremely acidic conditions with a pH of less than 5, oxytetracycline was in cationic form, which means its surface had been positively charged. On the other hand, the negative charge on the surface of the adsorbent was reduced due to the protonation effect of hydrogen ions present in the solution, and this weakened the electrostatic attraction with cationic oxytetracycline. At extremely basic conditions with a pH greater than 9, oxytetracycline was in an anionic form, which means its surface was negatively charged. Thus, there will be an electrostatic repulsion occurring between anionic oxytetracycline and the negatively charged adsorbent surface, which causes a decreased oxytetracycline adsorption efficiency (Silva et al., 2021). At pH 7, oxytetracycline will be present in a zwitterionic form, indicating that the positive and negative charges are nearly equal, resulting in a net charge of zero. Not only that, at pH 5 or pH 9, the oxytetracycline could contain a slight positive charge or a slight negative charge, respectively, or could be similar to that at pH 7. Non-electrostatic interaction between the benzene rings of the carbon-based adsorbent and double bonds of the oxytetracycline occurs predominantly under this condition, resulting in greater adsorption (Correa et al., 2019; Hadki et al., 2021).

In addition to their high removal efficiency under optimized conditions, these adsorbents, including both activated carbon and graphene oxide, left behind less by-products at the end of the adsorption process. Most importantly, activated carbon is cheap, non-toxic, and environmentally friendly (Nayeri et al., 2019). On the other hand, the reaction time is quite long for the removal of very small amounts of antibiotics, which acts as a barrier for the use of this method at an industrial level, which could be due to the complex structure of antibiotics and their high chemical stability. Besides, it is also proven that an increase in oxytetracycline concentration resulted in a declination of removal efficiency beyond the optimum antibiotic concentration. For instance, when the concentration of oxytetracycline was increased from 5 mg/L to 10 mg/L, the removal efficiency increased from 79.74% to 86.70%, followed by a decreased from 86.70% to 75.20% when the initial concentration of antibiotic was increased from 10 mg/L to 50 mg/L (Hadki et al., 2021). In short, the increase in initial antibiotic concentration resulted in increase in the antibiotic removal efficiency until the optimum antibiotic concentration of 10 mg/L. Thus, the frequency of effective collision between the nano graphene oxide and oxytetracycline increase as the antibiotic concentration was increased, resulted in greater adsorption. Beyond that, the further increase in antibiotic concentration led to insufficient nano graphene oxide adsorption as its concentration was fixed at 0.3 mg/L, which resulted in poor adsorption performance. In more detail, the nano graphene oxide adsorbent became saturated and no longer adsorbed the oxytetracycline. Thus, the removal efficacy of oxytetracycline was greatly reduced. Most significantly, prolonged contact time between the adsorbent and antibiotic resulted in a desorption process, leading to poor overall adsorption performance (Li et al., 2019). Thus, the optimum contact time needs to be determined, and it will be achieved when the concentration of oxytetracycline reaches equilibrium between the surrounding liquid phase and the adsorbent solid phase. By considering the above issues regarding adsorption processes, there is current research that emphasizes modern technology, such as advanced oxidation processes.

2.4 Advanced oxidation processes

Modern approaches, like advanced oxidation processes, have gained much attention due to their capability of oxidizing antibiotics into less harmful substances, which, ideally, resulted in a complete mineralization to carbon dioxide and water as shown in Equation 2.1 below (Correa et al., 2019).

$$R - H \ + \ \mathrm{O\,H\cdot} \ \rightarrow \ \mathrm{H_2O} + \ \mathrm{CO_2} \tag{2.1}$$

In depth, the advanced oxidation process were divided into various types as shown in **Figure 2.2** below, and all the methods were discussed and compared to pick the best out of it for the current study.

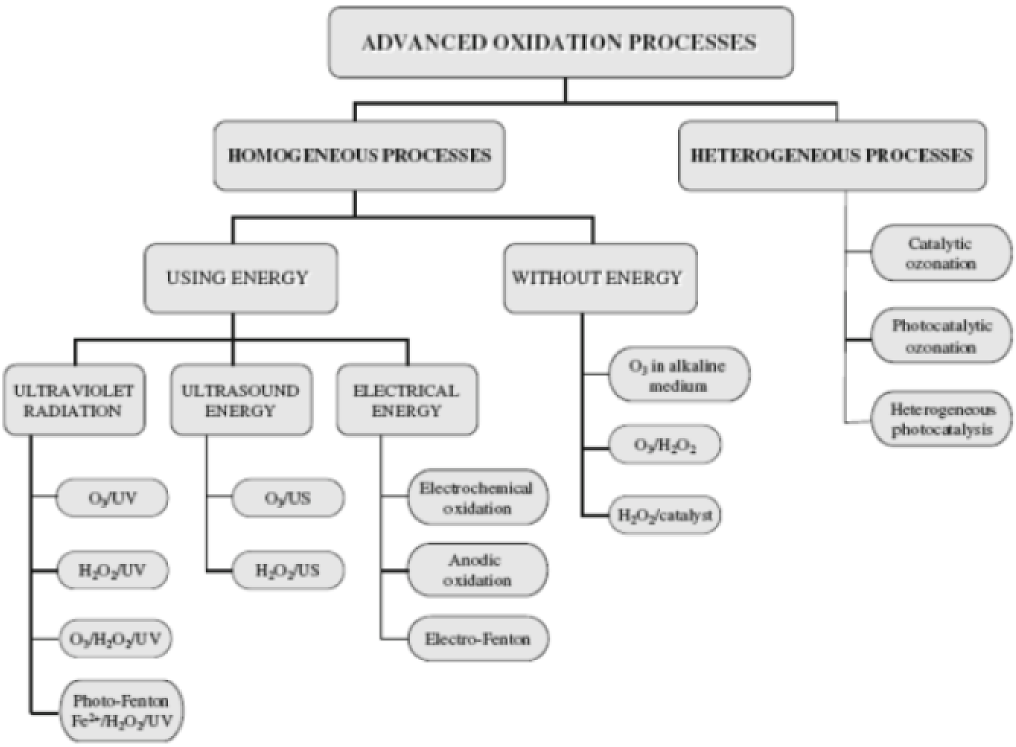

Figure 2.2: Classifications of advanced oxidation processes (Correa et al., 2019).

The efficacy of advanced oxidation processes was determined by the generation of hydroxyl radicals that possessed a standard reduction potential of 2.80 V and were non-selective as well as extremely reactive, with a reaction rate constant of approximately 109 $Lmol^{-1}S^{-1}$ (Ribeiro et al., 2015). In this regard, ozonation was the best choice among other methods of advanced oxidation processes. In fact, ozonation was able to degrade both organic and inorganic pollutants directly by attacking the -C = C- double bonds or -N = N- double bonds, which cannot be observed in other methods under acidic or neutral conditions. Besides, it also possesses a standard reduction potential value of 2.08 V, which is greater than the standard reduction potential of 1.76 V of hydrogen peroxide (Correa et al., 2019). This shows that ozonation possessed a better oxidizing capability than hydrogen peroxide. In the basic environment, both ozone and hydrogen decompose into hydroxyl radicals to degrade antibiotics. Both species have a short life span, which means they decompose into water and oxygen in the absence of pollutants. Consequently, no further chemical treatment was necessary for the recovery or separation of ozone and hydrogen peroxide from the treated wastewater, as reported by Moshin and Mohammed (2021).

On the other hand, heterogeneous photocatalysis systems, which commonly use titanium dioxide, require an additional recovery step for this semiconductor upon completion of the reaction. Besides, this photocatalysis requires the assistance of ultraviolet radiation to produce hydroxyl radicals. For large scale operations, this technology was not feasible due to the fact that a consistent and even distribution of ultraviolet radiation was very difficult to achieve. Besides, the production of hydroxyl radicals is known to be a very slow process. Besides, the large bandgap width of this semiconductor is not suitable for ultraviolet radiation absorption, which requires an additional element to reduce its band gap width so that it is suitable for ultraviolet radiation absorption (Correa et at., 2019). Moreover, the sono-chemical oxidation process, which involves the use of ultrasound for the sonolysis of water to form hydroxyl radicals, operates at a pressure and temperature of 100 MPa and 5000 °C (Abdurahman and Abdullah, 2020). This method is much more energy consuming than any of the other previously addressed methods.

In summary, the ozonation process results in greater advantages as compared to advanced oxidation processes in terms of the degradation of antibiotics and energy consumption. Thus, this study will focus more on ozonation.

2.5 Ozonation process

There is some research on the use of ozonation alone in treating antibiotics. In fact, Uslu and Balcioglu (2008) conducted a study on the effect of an excess amount of ozone on the degradation of oxytetracycline. This study shows that ozone alone can achieve 97% of oxytetracycline within 5 minutes at a pH and ozone concentration of 8.5 and 32.7 mg/L, respectively. Besides, the increase in ozone concentration and pH results in greater removal efficiency. Indeed, the ozone decomposition rate increases with pH and ozone concentration, resulting in a greater formation of hydroxyl radicals. At the same time, phenolic groups of oxytetracycline deprotonate at a pH of 8.5, becoming a strong nucleophile, enabling a fast reaction with electrophilic ozone (Wang et al., 2019). Recently, Dawood and Abdulrazzaq (2021) conducted an experiment on microbubble ozonation of oxytetracycline. Under optimal pH, ozone flow rate, and contact time of 7.2, 13.3 mg/min, and 40 minutes, respectively, 98.96% of tetracycline was removed. Most notably, pH has a significant impact on degradation efficiency.

As addressed previously, oxytetracycline is an amphoteric molecule with various functional groups. Thus, this molecule exhibits different properties at different pHs. Similarly, ozonation is also affected by the changes in pH. In depth, ozonation can proceed in two major pathways. First and foremost, direct ozonation prevails mainly in acidic medium with dissolved ozone. This process is known to be selective and slow with a reaction rate constant in the range of (1^6 – 10^6) $M^{-1}s^{-1}$ (S. N. Malik et al., 2020) and the degradation of tetracycline is minimal since insignificant hydroxyl radicals are present to degrade the tetracycline (Correa, et al., 2019). In more detail, the ozone molecule can attack the oxytetracycline in two major ways due to the unique structure of ozone as shown in **Figure 2.3**. Namely, electrophilic reactions and nucleophilic reactions. Based on **Figure 2.3**, it is well known that ozone possesses a negative and positive charge, which indicates it could act as a nucleophile and an electrophile.

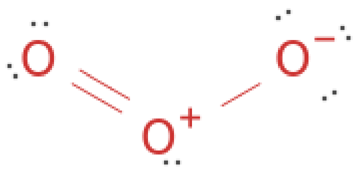

Figure 2.3: The structure of ozone

To further emphasize on this, electrophilic reaction occurs in a molecular solution with a high electron density or in a solution with aromatic compounds. For instance, the phenol group of oxytetracycline could possibility reacts with ozone as depicted in **Figure 2.4** below.

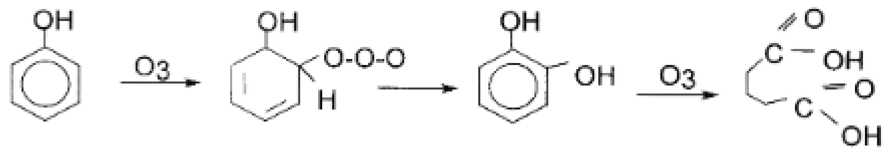

Figure 2.4: Electrophilic reaction between oxytetracycline's phenolic group and ozone

Based on **Figure 2.4**, the very first attack of the ozone molecule is targeted at the least deactivated meta position of oxytetracycline, causing phenol to react quickly with ozone, as illustrated in **Figure 2.4**, resulting in the creation of ortho and para-hydroxylated by-products. This molecule was subsequently exposed to further ozonation, which resulted in the formation of aliphatic products having carboxyl and carbonyl functionalities. The carbonyl and carboxyl groups of tetracycline molecules, on the other hand, are the primary targets of nucleophilic reactions. This reaction was considered to be much slower than electrophilic reactions. Secondly, under basic conditions, the ozone molecule will undergo indirect oxidation via the generation of hydroxyl radicals which occurs in a series of steps as shown in **Table 2.1.**

Table 2.1: Mechanism for indirect ozonation for the production of hydroxyl radicals

Chain initiation	$O_3 + OH^- \rightarrow HO_2^- + O_2$	(2.2)
Chain propagation	$O_3 + HO_2^- \rightarrow HO_2 + O_3^-$	(2.3)
	$HO_2 \rightarrow H^+ + O_2^-$	(2.4)
	$O_2^- + O_3 \rightarrow O_3^- + O_2$	(2.5)
	$O_3^- + H^+ \rightarrow HO_3$	(2.6)
Chain termination	$HO_3 \rightarrow HO\cdot + O_2$	(2.7)

In relation to that, high pH promotes ozone breakdown into free radicals, which behave as reactive species, oxytetracycline degradation rates are predicted to rise with pH. However, oxytetracycline degradation was reduced greatly when the pH was raised above 10. This might be attributed to additional degradation of by-products and oxytetracycline at the same time as they become important hydroxyl radical scavengers, lowering hydroxyl's selectivity towards oxytetracycline as reported by Dawood and Abdulrazzaq (2021). Furthermore, in extremely alkaline conditions, a fast-side reaction occurs, resulting in the formation of hydroperoxyl radicals as shown in Equation 2.2, which damages the hydroxyl radicals and leads to a reduction in their oxidation capacity (Correa et al., 2020).

$$HO\cdot + O_3 \rightarrow HO_2\cdot + O_2 \quad (2.8)$$

Under neutral, slightly basic, or slightly acidic conditions, the oxytetracycline's undergone both direct ozonation and indirect ozonation in the presence of dissolved ozone and hydroxyl radicals, respectively (Lu et al., 2020). As a result, the greatest removal of oxytetracycline will be detected (Li et al., 2019). Ozonation, on the other hand, could lead to the formation of more toxic substances when the parent molecule, such as oxytetracycline, degrades. This scenario arises as a result of a lack of contact time between ozone and contaminants, particularly when tetracycline concentrations are high (Li et al., 2008). As a result, oxytetracycline undergoes partial degradation, yielding toxic byproducts such as 4-epi-OTC (EOTC), 2-acetyl-2-decarboxy-amido-OTC (ADOTC), -apo-OTC, and -apo-OTC. Among some of the by-products, 4-epi-OTC is the most toxic and detrimental to aquatic organisms (Li et al., 2019). As a result, ozonation alone is not appropriate to be used.

2.5 Catalytic ozonation

Recently, Moshin and Ahmed (2021) conducted a semi-batch experiment on the degradation of antibiotics, particularly tetracycline, using ozonation alone and catalytic ozonation in the presence of a zinc oxide catalyst. In this experiment, the pH was varied from 3 to 11, the initial concentration of oxytetracycline was changed from 10 to 100 mg/L, the temperature was adjusted from 15 to 35, the zinc oxide catalyst loading was altered from 25 to 200 mg/L, and the flowrate of ozone was regulated from 0.138 to 1.388 mg/s. After 35 minutes at room temperature, the removal efficiency of tetracycline reached 94 % at optimal pH and an ozone flowrate of 7 and 1.38 mg/s, respectively, with a zinc oxide catalyst loading of 100 mg/L. However, sole ozonation was only able to reach a degradation efficiency of 81%. Thus, the use of zinc oxide catalyst aids in the elimination of antibiotics by catalyzing the conversion of ozone to hydroxyl radicals as well as providing an active site for improved interaction between ozone and tetracycline. This ensures that ozone and oxytetracycline have enough interaction time in a confined space, resulting in increased degradation of oxytetracycline (Li et al., 2019). On the other hand, excess catalyst dose results in a decrease in ozone and oxytetracycline concentration per unit area, resulting in poor catalytic ozonation performance. Moshin and Ahmed (2021) have found that the removal efficiency of oxytetracycline increases with the increase in catalyst dosage from 25 mg/L. Beyond that, the oxytetracycline removal percentage decreases as the catalyst dosage increases from 100 mg/L to 200 mg/L. Besides, temperature is also an important factor affecting the removal efficiency. The efficiency of ozonation decreases as temperature rises, which might be owing to a decrease in ozone stability and solubility at high temperatures (Li et al., 2019).

Most importantly, ozonation coupled with activated carbon results in a 100% removal efficiency of oxytetracycline at a pH of 7 and an activated carbon dosage of 1.5 g/L.