

CHAPTER II

LITERATURE REVIEW

2.1 Pharmaceutical waste

Pharmaceutical waste is any refuse generated during drug manufacturing, research, or patient care in hospitals and clinics (Jang 2019). Since the Covid-19 pandemic began, that refuse has soared. Facilities and households used far more medicine in their fight against the virus, leaving behind larger amounts of unused or expired products. Numerous studies track this spike, noting that the overall volume of medical and pharmaceutical waste rose sharply during lockdowns and vaccination campaigns (Nzediegwu and Chang 2020; H. L. Zhao et al. 2021). Careful management is therefore vital; throwing these materials away carelessly can pollute water, soil, and air, while also creating new pathways for infections. The waste often contains dangerous items-such as live pathogens, leftover pain relievers, antivirals, antibiotics, antiparasitics, cytotoxic agents, toxic solvents, heavy metals, and even human tissue-making safe disposal both complex and urgent (Wei et al. 2021).

Pharmaceutical wastewater originates mainly from a few important sources, the most significant of which are drug production plants. These manufacturing facilities stand out as the largest single producers of such contaminated effluent. Wastewater from these facilities contains high concentrations of active pharmaceutical ingredients (APIs), solvents, reaction intermediates, and by-products. The pharmaceutical production process, whether batch or continuous, results in effluents with varying concentrations of APIs. In some cases, concentrations can range from micrograms per liter ($\mu\text{g/L}$) to milligrams per liter (mg/L), significantly higher than those found in municipal wastewater. Even at very low concentrations (ng/L), the presence of these chemical compounds can harm ecosystems and pose health risks to living organisms (Khan et al. 2021). A large portion of pharmaceutical waste is hazardous and difficult to treat in conventional wastewater treatment plants (WWTPs) used by the pharmaceutical industry.

Traditional treatment methods are often ineffective at removing contaminants due to the high levels of biochemical pollution, including biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solution (TSS), pH, secondary metabolites, and other chemical substances. (A. K. Thakur et al. 2023).

The pharmacological properties of pharmaceuticals pose potential threats to the environment, both in the short and long term, including toxicity and the development of antibiotic resistance in microorganisms (Guyomard-Rabenirina et al. 2017; Uluseker et al. 2021). However, at present, these contaminants are not regulated by environmental laws (Kosma et al. 2020). The pharmaceutical waste quality standards established by the Ministry of Environment and Forestry, outlined in Ministerial Regulation No. 5 of 2014, only cover macro parameters. However, micro parameters also consist of hazardous pollutants that, if accumulated in water bodies, can have toxic effects on the environment and human health, as well as contribute to the spread of harmful diseases. The pharmaceutical waste quality standards according to Ministerial Regulation No. 5 of 2014 are shown in Table 2.1.

Table 2.1 Pharmaceutical industry waste quality standards

Parameter	Threshold limit concentration (mg/L)
BOD	75
COD	150
TSS	75
pH	6-9

2.2 Antibiotics

Antibiotics are chemical substances produced by fungi and bacteria that have the ability to kill or inhibit the growth of microbes. Antibiotics are derived from various species of microorganisms (bacteria, fungi, and actinomycetes) that can suppress the growth or kill other microorganisms (Zhuan and Wang 2020). Based on their selective toxicity, antibiotics are divided into two categories: bacteriostatic antibiotics, which inhibit bacterial growth, and bactericidal antibiotics, which kill bacteria. Additionally, based on their activity spectrum, antibiotics are classified

into two groups: broad-spectrum antibiotics and narrow-spectrum antibiotics. Broad-spectrum antibiotics are designed to hinder the growth of, or directly kill, a wide variety of both Gram-positive and Gram-negative bacteria; tetracycline is a classic example. In contrast, narrow-spectrum drugs target a much smaller set of pathogens, with penicillin G often cited as a representative agent (Coyne et al. 2019).

Researchers sort these drugs into broad classes such as macrolides, beta-lactams, quinolones, tetracyclines, sulfonamides, arspenamines, polypeptides, aminoglycosides, amphenicols, lipopeptides, oxazolidinones, glycopeptides, streptogramins, ansamycins, and lincosamides (Wang et al. 2018). Within macrolides, agents like erythromycin, azithromycin, clarithromycin, and roxithromycin are noted for their biliary excretion and their mechanism of blocking bacterial protein production. Beta-lactams mainly disrupt the synthesis of the bacterial cell wall, and amoxicillin stands out as one of the most frequently prescribed compounds within this family (Anastopoulos et al. 2020).

The quinolone group originates from nalidixic acid and is considered a modification of the quinolone molecule (Ikram et al. 2020). The tetracycline family includes chlortetracycline, tetracycline, lymecycline, oxytetracycline, clomocycline, methacycline, demethylchlortetracycline, rolitetracycline, doxycycline, minocycline, and tertiary-butyl-glycylamidinominocycline. All of these members work by blocking protein fabrication inside the target bacteria (Tao et al. 2018). Sulfonamides include sulfadiazine, sulfamethazine, sulfachlopyridazine, sulfamethizole, sulfamethoxazole, sulfadimethoxine, sulfamerazine, sulfamoxole, sulfapyridine, sulfathiazole, and sulfisoxazole (Fekadu et al. 2019).

2.2.1. Amoxicillin (AMX)

Amoxicillin (AMX) is also known by other names such as *D(-)-α-amino-p-hydroxybenzylpenicillin*, amoxicillin, or amoxixiline. The chemical formula of amoxicillin is. (2S,5R,6R)-6[[[(2r)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimetyl-7oxo-4-thia-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid. AMX

has the chemical formula $C_{16}H_{19}N_3O_5S$ and is one of the most commonly used antibiotics. AMX belongs to the penicillin group of beta-lactam antibiotics (Yang et al. 2017). The structure of AMX is amphoteric due to its three main functional groups, such as $-NH_2$, $-COOH$ dan $-OH$. AMX belongs to the beta-lactam group and is frequently used in the treatment of bacterial infections. The chemical structure of AMX is shown in Figure 2.1.

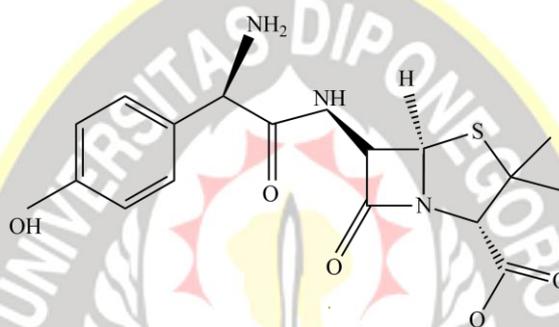


Figure 2.1 Chemical structure of AMX

AMX is commonly prescribed in children for the treatment of pneumonia and other diseases including bacterial infections of the ears, sinuses, throat, urinary tract, skin, abdomen and blood. AMX is formulated in the form of conventional capsules, tablets, powders for oral suspension, and dispersible tablets. AMX has a high consumption rate and produces continuous emissions, so it is often detected in some environmental samples. AMX has the properties of being difficult to decompose even in low concentrations, low toxicity levels and cannot be removed by simple methods such as activated carbon adsorption, electrolysis and biological methods (H. Liu et al. 2022).

AMX as a potent antibiotic works by increasing and deactivating binding proteins located in the inner membrane of the bacterial cell wall. The inactivation process interferes with the cross-linking of peptidoglycan chains that are necessary for bacterial cell wall strength. This will interfere with the synthesis of the bacterial cell wall and result in the weakening of the bacterial cell wall and cause cell lysis (Anastopoulos et al. 2020).

The large use of AMX in medicine results in an increase in AMX waste. More than 86% of the compounds in AMX are not metabolized and excreted through the

urine of the human body. AMX has also now been detected in many water samples, domestic and industrial wastewater and surface water at g/L concentration levels (Kimosop et al. 2016). AMX was found in the amount of 10-60 mg/L on the surface of the water body (Pourmoslemi, Mohammadi, Kobarfard, and Assi 2016).

2.2.2. Fluroquinolone group

Ciprofloxacin (CIP) is one of the types of antibiotics of the fluoroquinolone class that is most often found in sewers, water bodies, (Danner et al. 2019) and even in WWTP effluent (Guerra et al. 2014) A total of 5.6 mg/L and $3 - (7 \times 10^4)$ mg/L CIP were found in WWTP effluents (Batt et al. 2007) and even on the surface of the water body itself reached $(2.45 - 6.3) \times 10^4$ mg/L (Igwegbe et al. 2021).

Levofloxacin (LEV) is a type of antibiotic from the third-generation fluorokuinolone group that is commonly used in the treatment of bacterial infections. LEV has a chemical formula of $C_{18}H_{20}FN_3O_4$. The chemical name of levofloxacin is Acid(-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinil)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate. The chemical structure of CIP and LEV can be seen in figure 2.2.

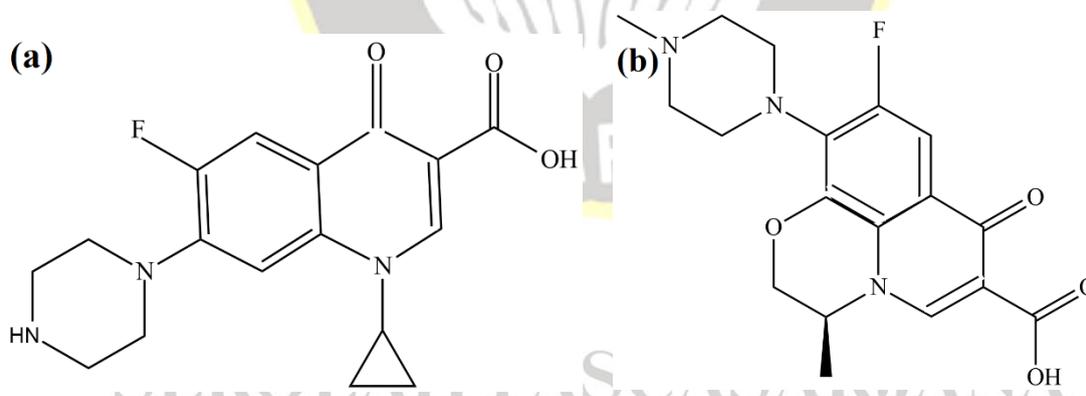


Figure 2.2 Chemical Structure of the fluoroquinolone group (a) Ciprofloxacin, and (b) Levofloxacin

LEV exhibits specific activity in fighting gram-negative, gram-positive bacteria and intracellular pathogens (Lu et al. 2019). Due to its effectiveness, LEV is used in the treatment of bacterial infections and even HIV disease. However, the metabolism of LEV in the human body is small. As much as 15-20% of LEV is

metabolized by the body and the rest will be excreted into the environment through urine as a parent compound (Gong et al. 2020). LEV is dangerous in water because they pose a serious threat to health caused by bacterial resistance and gene exchange. In addition, LEVs also have a negative impact on ecosystems (Zhang et al. 2023).

2.2.3. Tetracycline group

Tetracycline (TC) includes a major class of antibiotics that are often used in hospital treatment due to their extensive antibacterial activity against a wide range of gram-positive and gram-negative bacteria (Grossman 2016). After treatment, more than 70% of TC is released in its active form into the environment. Recent studies have revealed that tetracycline (TC) now appears in surface water and even groundwater at levels approaching 20 mg/L, and half of the 139 rivers examined across the United States show detectable TC (Chang et al. 2014). The mean concentration recorded in U.S. surface waters is 1.34 mg/L (Safari et al. 2015).

Doxycycline (DOX), a member of the tetracycline family, offers broad-spectrum antimicrobial action and is deployed in both human medicine and veterinary practice. Its molecular formula is $C_{22}H_{24}N_2O_8$. As a second-generation compound, DOX generally carries lower toxicity than the first-generation tetracyclines. The structures of TC and DOX are illustrated in Figure 2.3.

Because of its wide spectrum, DOX is routinely prescribed to prevent and treat infections caused by both gram-positive and gram-negative bacteria (Bakhsheshi-Rad et al. 2018). Consequently, concentrations can reach 6.7 $\mu\text{g/L}$ in treated wastewater and 1.5 mg/kg in raw sewage sludge (Borghi et al. 2015). DOX is one of the main sources of antibiotic contamination in waters, so it is a threat to the environment, especially the balance of aquatic ecosystems (Liu et al. 2021).

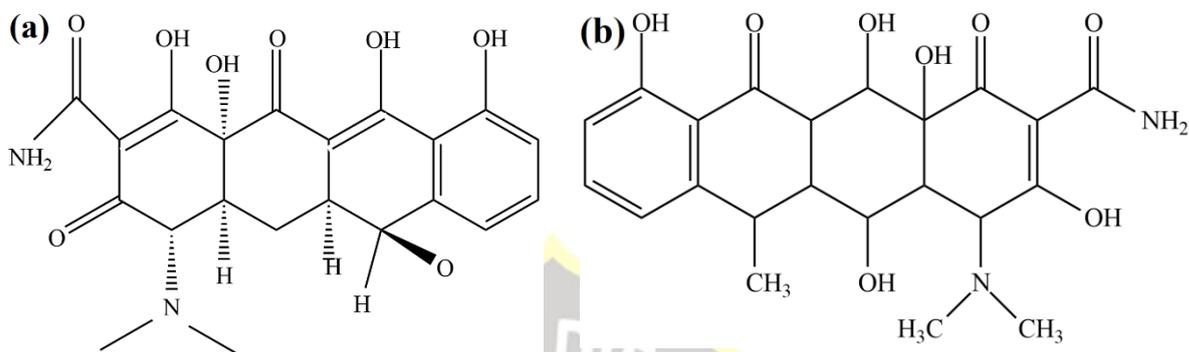


Figure 2.3 Chemical structure of tetracycline antibiotics (a) Tetracycline, and (b) Doxycycline

2.3 Antibiotic Pollution

Antibiotics have resistant and persistent properties when they are in the aquatic environment even in very small concentrations. The properties of antibiotics as one of the harmful and toxic compounds will cause derivative effects if consumed continuously. Clean water that is free of antibiotics and resistant bacteria is a world problem that must be overcome immediately and in Southeast Asia itself does not have regulations related to these problems (Kurniawan and Mariadi 2019).

The sources of antibiotic pollution in the aquatic environment in Southeast Asia can be classified into several main groups including waste from hospitals and the pharmaceutical industry, waste from aquaculture and livestock, municipal waste that is not properly treated, waste from WWTP and leachate landfills. During the production and application process, antibiotics can enter the environment directly or indirectly into the environment through various pathways. Antibiotics can be discharged into the environment during production through pharmaceutical industry waste or residual fermentation (Bai et al. 2022).

Antibiotics from the pharmaceutical industry, hospitals, wastewater treatment plants, livestock farming are the most potential contributors that cause antibiotics to enter the environment. Antibiotics enter the environment through transfer in water, be it surface water, drinking water and groundwater, sediments, soils as well as through the food chain (Bai et al. 2022). In general, the concentration of antibiotics in the environment varies greatly from ng/L to mg/L. Different levels

of antibiotic concentrations depend on the source of antibiotic waste (Anh et al. 2021).

The presence of antibiotics in the environment results in pollution and a decrease in the quality of waters. Antibiotic contamination results in an increase in the number of bacteria that experience antibiotic resistance (ARGs) in the environment. Even in small amounts, antibiotics in the waters will inhibit and kill non-pathogenic microorganisms that help the ecosystem function. Antibiotic pollution occurs in one of them due to the inability of WWTP to degrade antibiotics. A study conducted on one of the hospitals in Indonesia, namely Prof. Dr. R. D Kandou Manado Hospital, showed the presence of antibiotics contained in the liquid waste disposal system at the hospital. In addition, there are several bacteria including *Streptococcus sp*, *Escherichia sp* and *Staphylococcus sp* that are resistant to Clindamycin antibiotics (Kusuma et al. 2022).

Antibiotic contamination causes antibiotic resistance. An initial study conducted by Kristanto and Koven (2019) found that there was an event of *E Coli* bacterial antibiotic resistance in a hospital WWTP in Jakarta, Indonesia. Antibiotic resistance of *E Coli* bacteria includes 3 types of antibiotics, namely Meropenem, Ciprofloxacin and Cefixime. WWTP water contains 4.6×10^4 CFU *E Coli* and the percentage of antibiotic resistance was found in meropenem was 3.8%, in Ciprofloxacin by 53.8% and in Cefixime by 56.3%. The antibiotic resistance event found was caused by antibiotic contamination in the hospital WWTP. As a result, the antibiotic becomes resistant and reduces its effectiveness in killing bacteria.

In Indonesia, antibiotics found in urban waste in the Jakarta area ranged from 17 to 1489 ng/L with an average of 607 ng/L. The main compounds found were Sulfamethoxazole (SMX), Lincomycin (LIN), Erythromycin (ERY) and Tetramethylpiperidine (TMP) (Shimizu et al. 2013). Antibiotics were also found in hospital WWTP effluent at one of the hospitals in Palembang, West Sumatra, Indonesia. The results of the study showed that 5 antibiotic substances were detected out of 126 types of antibiotics that were screened. The 5 antibiotics include Ciprofloxacin, Lincomycin, Metronidazole, Netilmicin and

Ofloxacin/Levofloxacin with the highest intensity (9.00 E+04) owned by Ciprofloxacin (Kurniawan et al. 2019).

Antibiotic contamination is also found in the world of food, especially fishery cultivation. The problem of antibiotic contamination is closely related to the unsupervised and uncontrolled use of antibiotics so that antibiotics accumulate in animal tissues/organs. Pawestri et al. (2019) in their study showed that 24 samples of tilapia meat marketed in Yogyakarta were detected to contain tetracycline antibiotic residues, with 19 samples being above the threshold limit determined by BSN. Antibiotic contamination in the food world has become widespread. Many research studies have been conducted to detect the level of antibiotic residues in the food world. The results of the study show that antibiotic residues have been found in many cultivated fish and livestock (Pawestri et al. 2019)

2.4 The Impact of Antibiotic Pollution

Since the invention of antibiotics 70 years ago, millions of people have been spared disease. The potential of antibiotics to treat or prevent disease has led to an increase in their use. Not infrequently, many abuse the use of antibiotics. Indonesia as a developing country with the use of antibiotics of 30%-80% is consumed freely not based on the indications experienced by patients (Kurniawan et al. 2019). Studies on antibiotic use have been conducted in various health care agencies and in community-based surveys. In Indonesia, antibiotics require a prescription to be shared. However, a study (Thobari et al. 2019) showed a high rate of antibiotic administration in pharmacies without a prescription. In addition to being consumed by humans, antibiotics are also given to livestock. Antibiotics are not only used in medical medicine, but in other sectors (Kristanto and Koven 2019).

Antibiotic misuse is the cause of the emerging problem of antibiotic resistance due to microbes (AMR) that has evolved. AMR is a condition in which microorganisms are able to survive the therapeutic dose of antimicrobial compounds, so that microorganisms are still able to thrive. AMR can also reduce the efficacy of drugs, increase the risk of disease spread, aggravate, and cause death in treatment in humans, animals, fish, and plants (Coordinating Minister for PMK

RI Regulation Number 7 of 2021). The rise of antibiotic resistance, especially in Indonesia, has the potential to disrupt the achievement of national development targets in the fields of disease control and food security and national security.

2.4.1. Antibiotic resistance in hospitals

The extent of antimicrobial resistance (AMR) at the national level in Indonesia remains uncertain, as AMR surveillance is still limited and does not yet provide a comprehensive picture. Monitoring of antibiotic resistance in hospitals began in 2002 through various methods, including the detection of Extended-Spectrum Beta-Lactamase (ESBL) and methicillin-resistant *Staphylococcus aureus* (MRSA). Several surveys and studies conducted in hospitals, such as at Dr. Soetomo General Hospital in Surabaya, have contributed to the data. Research on MRSA epidemiology in 2014 found that the prevalence of MRSA among patients in both surgical and non-surgical wards was 8.0% and 8.2%, respectively (Regulation of the Coordinating Minister for PMK RI Number 7 of 2021).

Research conducted by Rukmini et al. 2019 reported the occurrence of Antimicrobial Resistance in Indonesia (AMRIN) at Dr. Soetomo Hospital and Dr. Kariadi Hospital proving that there are already dangerous multi-resistant bacteria such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamases-producing bacteria(ESBL). Meanwhile, at H. Adam Malik Hospital, bacterial resistance was found to be resistant to antibiotics such as ampicillin, gentamicin and cefotaxime. The most bacteria that experience antibiotic resistance are gram-negative bacteria, and the most species are experienced by *Staphylococcus sp*, *Pseudomonas sp* and *Enterobacter sp* (Rukmini et al. 2019).

The 2019 and 2020 Indonesian AMR (*Indonesia Antimicrobial Surveillance System / INASS*) surveillance was followed by 20 hospitals, the proportion of *E. coli* and *K. pneumoniae* bacteria that cause bloodstream infections and resistance to third-generation cephalosporin antibiotics and fluoroquinolone groups increased. In 2020, *E. coli* was resistant to antibiotics of the third-generation cephalosporin group and fluoroquinolone at 66.7% and 65.6%. Meanwhile, *K. pneumoniae* is resistant by 74.4% and 53.2%. This data has been reported to the Global

Antimicrobial Resistance Surveillance System (GLASS) platform (Coordinating Minister for PMK RI Regulation Number 7 of 2021).

2.4.2. Antibiotic resistance in farm animals

Antibiotic contamination is also found in the world of animal husbandry, especially in livestock. The problem of antibiotic contamination is closely related to the unsupervised and uncontrolled use of antibiotics so that antibiotics accumulate in animal tissues/organs. Pawestri et al. (2019). Monitoring of antibiotic resistance in livestock is carried out by the Ministry of Agriculture using Antimicrobial Susceptibility Testing (AST) on livestock and their products (Coordinating Minister for PMK RI Regulation Number 7 of 2021).

The results of the survey showed that testing of livestock products (chicken meat) against *E. coli* bacterial isolates showed that *E. coli* was resistant to tetracycline by 80.6% in 2011. Meanwhile, in 2012, the highest resistance to enrofloxacin was 94.38%. A survey was also conducted on *Salmonella* bacterial isolates which showed that of the 12 isolates tested, all of them were resistant to erythromycin, 8.33% were resistant to enrofloxacin, 16.67% were resistant to nalidic acid and 25% were resistant to tetracycline (Coordinating Minister for PMK RI Regulation Number 7 of 2021).

2.4.3. The Impact of Antibiotics on the Environment

Antibiotics that enter the aquatic environment have the potential to affect the ecosystem of microorganisms. Antibiotics are considered to be ecological factors that drive microbial evolution by altering the structure of microbial communities, inhibiting or improving their ecological functions as well as influencing drug resistance mechanisms (Polianciuc et al. 2020).

Exposure to antibiotics in the environment leads to reduced aquatic microbial diversity (Kraemer et al. 2019). The loss of microbial diversity in the waters that are the basis of the aquatic ecosystem results in a decrease in the performance of the aquatic ecosystem. Aquatic ecosystems that have important functions in water cycling, nutrient cycling, decomposition and primary productivity in various

environments will be damaged (Eckert et al. 2019; Grenni et al. 2018). In fact, aquatic microbes have an important role in carbon cycling and primary productivity. Not only that, exposure to antibiotics will alter the structure of microbial communities leading to loss of biomass and reduction of microbial activity including nitrification, denitrification and respiration (Cycoń et al. 2019; Thiele-Bruhn and Beck 2005) Antibiotics in waters can also affect the activity of bacterial enzymes including dehydrogenase, phosphatase and urease which are considered important indicators of soil and water activity. Thus, exposure to antibiotics will have an impact on increasing the abundance of parasites and pathogens in soil and water environments (Kraemer et al. 2019)

In addition, the presence of antibiotics in the waters will cause eutrophication and pose a health risk to humans. Antibiotics that are in the environment at low concentrations can accumulate in the human population through long-term exposure through drinking water or food (Drury et al. 2013) Therefore, exposure to antibiotics in the environment is very dangerous for both the aquatic environment, animals, ecosystems and even humans as environmental users.

The impact of antibiotics on aquatic ecosystems depends on the concentration, bioavailability, time of exposure and addition of substrates such as metals. Changes in ecological function caused by exposure to antibiotic residues include nitrogen transformation processes, such as oxytetracycline antibiotics that are able to inhibit the nitrification process in surface water (Klaver and Matthews 1994) In addition, antibiotics are also able to inhibit the process of methanogenesis. Previous research case studies related to exposure to sulfamethoxazole and ofloxacin antibiotics have shown that contamination of these two types of antibiotics slightly inhibits the anaerobic digestion of methanogens (Halling-Sørensen 2001)

In another study, based on phospholipid fatty acid analysis, it was found that broad-spectrum antibiotics of the ciprofloxacin type favored the presence of sulfate-reducing bacteria and gram-negative bacteria while reducing the number of gram-positive bacteria. Ciprofloxacin is able to modify the structure of bacterial

communities at concentrations as low as 20 µg/mL in sediments. Even antibiotics with low bioavailability are still able to modify microbial communities (Fountoulakis et al. 2004)

2.5 Antibiotic Degradation Technology

Antibiotic removal technology including physical, chemical and biological processes has been widely applied. Several conventional biological processes for degrading antibiotics in waters have been carried out, but they have relatively low degradation efficiency and are still ineffective (Wang et al. 2019) even contributing to a selective increase in the prevalence of antibiotic resistance (Czekalski et al. 2012) Among these methods is the adsorption method. Adsorption is one of the physics methods used in antibiotic degradation by utilizing materials that function to absorb antibiotics. Adsorption of antibiotics in water has been performed by (Zhang et al. 2016) using activated carbon powder and activated carbon granules. This method has disadvantages, namely the production of adsorbent materials that are complicated and expensive and produce products in the form of secondary waste (Kutuzova et al. 2021)

Another method of antibiotic removal is coagulation. The coagulation process utilizes the physical chemical process of mixing coagulant materials into wastewater. The coagulant materials used are anionic surfactants, Sodium dodecyl sulfate (SDS), cationic polyelectrolytes, polyallylamine hydrochloride (PAH) which can remove antibiotics in water (Saitoh et al. 2017) The membrane separation method can also be used for antibiotic degradation. Membrane-separation processes classify according to pore size and operating principle; microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), dialysis, electro dialysis, reverse osmosis (RO), and forward osmosis (FO) have each been applied to recover antibiotics. Successful application, however, hinges on matching feed stream characteristics with membrane chemistry, pore morphology, surface area, and solute load, as Zhao et al. (2018) underscore (Zhao et al. 2018).

Concurrently, researchers are advancing antibiotic-degradation strategies through processes grouped under the umbrella of advanced oxidation processes (AOPs) (Hunge et al. 2022; Li et al. 2019; Márquez Brazón et al. 2016; Shang et al. 2018; Yang et al. 2020). Among all available technologies, AOPs have proven notably effective at removing pharmaceuticals and other recalcitrant, toxic organics from both waters and waste streams (Wang et al. 2019)

The AOP approach fundamentally relies on generating strong oxidants that rapidly break down contaminants. It has found wide acceptance in wastewater treatment because such oxidation can mineralize a broad range of organic pollutants, including residual drugs. Widely used AOP schemes include Fenton reactions, photocatalytic systems, ozonation, electrochemical routes, and photolytic processes (Kutuzova et al. 2021). In many cases, semiconductor photocatalysts serve as the active material within these configurations. Photocatalytic reactions may take place in semiconductor solids, substances whose electrical conductivity sits between that of metals and insulators (Dong et al. 2017)

2.6 Photocatalyst

Photocatalysts are solid catalytic materials that initiate chemical reactions only when illuminated by photons, including sunlight (Pang et al. 2021) The materials that can be used in the photocatalyst process are semiconductor materials such as TiO_2 , ZnO , Fe_2O_3 , CdS , ZnS , and Bi_2O_3 . The photocatalytic process starts with photoexcitation: light striking the semiconductor promotes electrons from the valence band to the conduction band, leaving behind holes. The energy of the excited electrons generates a pair of mobile negative charges and mobile holes, collectively referred to as photo-excited carriers in semiconductors (Kurt et al. 2017).

Because of their high quantum efficiency and repeatable performance, photocatalysts are gaining prominence as agents for transforming organic waste. They can mineralize stubborn pollutants, such as antibiotics, into harmless ions and

gases. Accordingly, researchers are actively engineering new photocatalytic materials to clean polluted water more effectively.

(Van Thuan et al. 2022) Some of the requirements that must be met by an effective photocatalyst system are that they are able to absorb high sunlight, have an appropriate energy band gap value (1.5–2.8 eV), long-term charge carrier separation, high phototransporter mobility, suitable physical and chemical properties, sufficient band gaps and alignment to meet the kinetic requirements of the target reaction (Li et al. 2022; Oladipo 2021).

The mechanism of the photocatalyst is illustrated according to figure 2.4. When exposed to light of the appropriate wavelength, an electron (e^-) in the valence band absorbs the photon's energy so that excitation occurs to the conduction band. This results in the formation of holes (h^+) on the valence band and charge-carrying pairs (e^- and h^+) on the surface of the photocatalyst. Highly reactive and h^+ electrons on the surface of photocatalysts tend to undergo reduction and oxidation reactions to produce hydroxyl ($\cdot OH$) and superoxide ($\cdot O_2^-$) radicals in succession (Oladipo and Mustafa 2023)

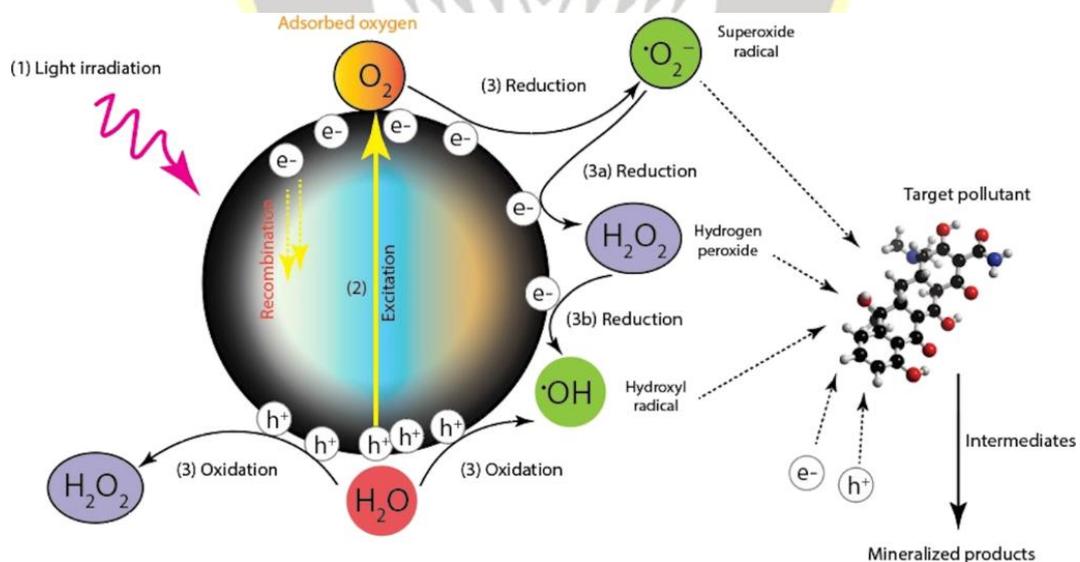


Figure 2.4 Photocatalyst reaction mechanism

Source : (Oladipo and Mustafa 2023)

Recently, the development of photocatalyst materials has focused on the use of solar radiation including simple oxides such as Bi_2O_3 , CuO , ZnO , WO_3 and some

oxides such as Bi_2MoO_6 , BiVO_4 dan Bi_2WO_6 . Bi_2O_3 potential to be developed as a photocatalyst material in the remediation of contaminated water due to its economical, effective and environmentally friendly (Sánchez-Martínez et al. 2016) Bismuth-based materials such as bismuth oxide have an energy band gap of less than 3 eV so they can be used in visible light. In addition, its distinctive structure of producing valence bands with O2p and Bi 6s orbitals can improve the mobility of photogeneration payload-carriers. Due to its distinctive structure, bismuth oxide exhibits better absorption in the visible light spectrum (Oladipo and Mustafa 2023)

2.7 Bismuth Oxide Thin Films

Bismuth oxide (Bi_2O_3) is the simplest bismuth-based semiconductor photocatalyst (Chen et al. 2018) The relatively low energy band gap allows Bi_2O_3 to be active in visible light (Hidayanto et al. 2017) This suggests that Bi_2O_3 has a greater potential to be developed given the presence of UV in only about 5% of the total sunlight received by the earth (Sutanto et al. 2017) However, photon-induced electron-hole pairs have poor light utilization and a fast recombination rate, thus limiting their photocatalytic activity. (Zhou et al. 2019)

Based on their mobility, there are two types of photocatalysts: mobilized photocatalysts (powder form) and immobilized photocatalysts (thin films). Based on previous research, it is stated that Bi_2O_3 powder is efficient for use in photocatalyst applications because it has a high surface area. This high surface area can increase light absorption, adsorption of reactant molecules and even the distribution of their particles so that it can increase their photocatalytic activity (Ilsatoham et al. 2023) However, the reuse of the powder is very difficult because of its very small size and the particles are evenly distributed. The use of immobilized photocatalysts in the form of thin films is an alternative to overcome this problem. The thin films of bismuth oxide offer the advantage of reusing materials that are more effective and durable. Another advantage of thin coatings is that they are relatively cost-effective due to reduced use of materials, avoid recycling and offer long-term performance and are easy to substitute into various substrates (Pedanekar et al. 2020)

The properties of the thin layer are dominated by the structure of the film in the form of mechanical and thermal properties of the film related to the reaction conditions and the method of its synthesis (Kasap and Capper 2007) Thin layer synthesis can be carried out by various methods including chemical methods, spray pyrolysis technique (SPT), chemical bath deposition (CBD), hydrothermal, Chemical Vapor Deposition (CVD), sputtering and sol-gel (Pedanekar et al. 2020) Among all these methods, the sol-gel method is one of the most effective methods used.

2.8 Sol-Gel Spray Coating

The sol-gel technique offers a straightforward and clean way to make thin films. When researchers apply sol-gel chemistry, they benefit from low material cost, minimal need for high-vacuum equipment, uniform composition, adjustable thickness, and surprisingly good microstructure (Surono and Sutanto 2014). Because the process starts from powder reagents, only a few inexpensive items are needed, which sharply reduces overall expense. In essence, a chosen solvent mixes with the precursors, and through polycondensation and polymerization, nanoparticles aggregate and eventually form the solid film.

In a modified sol-gel route, catalysts like acetic acid are mixed with the precursor solutions. These additives act as binding ligands and promote esterification, allowing the growth of complex polynuclear metal species. The altered method is thus attractive, saving energy, simplifying processing steps, and yielding films with high reproducibility. Because of these benefits, researchers increasingly regard the approach as a viable route for materials synthesis (Sahoo and Panigrahi 2022)

The formation of a thin films by the gel sole method generally proceeds in three steps: (a) mixing the precursor solution, (b) applying the sol to the substrate by an appropriate deposition method, and (c) heating the deposited layer to drive off solvent and cure the film (Pedanekar et al. 2020). The second stage can employ either spin coating or spray coating for attachment. In spin coating, a small puddle of sol is placed on the substrate, which then spins rapidly, spreading the liquid

outward by centrifugal force and leaving behind a uniform, thin layer. This method allows researchers to adjust thin-film thickness by varying rotational speed, sol-gel concentration, and solution viscosity (Pedanekar et al. 2020)

The *spray coating* technique is a coating technique by adding or stacking a material to the surface of another material. In this technique, the coating material (*feedstock*) as individual particles is pushed by a flow of pressurized gas to a surface (*substrate*). The particles will hit the substrate, stick to it and form a suitable thin surface (Baig et al. 2019).

2.9 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of statistical and mathematical methods that are useful for developing, improving and optimizing processes. RSM is applied to determine several input variables that have the potential to affect some measure of performance or quality characteristics of a process called a response (Widayat et al. 2012) RSM can be used to determine the minimum, maximum point and optimum point as well as parameters that affect the response using independent variables (Fonseca et al. 2024)

RSM can be used to investigate and select the most optimal process conditions. Process optimization in the manufacture of a product is important because it can affect production costs. This RSM method is expected to help to know and help researchers to determine the optimal operating conditions so that they can save costs, time and effort. In addition, the advantages of this RSM method include that it does not require a large amount of experimental data and does not take a long time (Prabudi et al. 2018)

The techniques used in RSM include Central Composite Design (CCD), Box Behnken Design, One Factor Design, 3-Level Factorial Design, Optimal Design, User-Defined Design and Historical Data Design. CCD is one of the techniques to design an experimental design for 2-level factorial. A suitable model will be obtained using variance analysis (ANOVA). With this CCD technique, the optimal value of several parameters will be known (Sarabia and Ortiz 2009)

CCD can determine the number of experiments during the study by determining the main point, star point and midpoint. Software Design Expert is a statistical tool used for regression analysis and graphical analysis of data obtained on CCD. This CCD technique is able to determine the influence of variables and the interaction of independent variables on the response so that it is possible to depict multidimensional and non-linear graphs. In general, polynomial multiple regression equations are used to determine the relationship between responses and independent variables. The quadratic equation corresponding to the interaction effect of independent variables with the response is illustrated through the following equation

$$Y = \beta_0 + \sum_{i=1}^K \beta_i X_i + \sum_{i=1}^K \beta_{ii} X_i^2 + \sum_{1 \leq i < j} \beta_{ij} X_i X_j \quad (2.1)$$

With Y is the predicted response variable, β_0 is the cut-off point, β_i , β_{ij} and β_{ii} and the coefficient of linear and double cut-off point influence and X_i , X_j is an independent variable or factor (Leili et al. 2020).

SEKOLAH PASCASARJANA