# Evaluation of veterinary antibiotics in a swine slaughterhouse wastewater and their removal using advanced oxidation processes

A thesis submitted in fulfilment of the academic requirements for the degree of

**Doctor of Engineering**

**Durban University of Technology**

**Faculty of Engineering and the Built Environment,**

**Department of Chemical Engineering,**

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# Declaration

I Martha Noro Chollom, student number (21144183), hereby declare that unless indicated, this thesis titled "*Evaluation of veterinary antibiotics in a swine slaughterhouse wastewaters and their removal using advanced oxidation processes"* is the result of my own investigation and has not been submitted in part or in full for any other degree at another University or Institution, and that its only prior publication was in the form of conference papers, or journal articles. I further declare that all the sources cited or quoted are acknowledged and indicated by means of a comprehensive list of references.

Martha Noro Chollom

[redacted] 21/10/2020

Signature Date

Prof S. Rathilal (Supervisor)

…… ……

Date

# Abstract

Antibiotics are found in low concentrations in water sources and surrounding environments. Despite their presence in low concentrations in the environment, they are associated with antibiotic resistant bacteria (ARBs) in water sources thus necessitating stringent legislations worldwide. The burden of ARBs has drawn worldwide attention into investigating this rising phenomena to better understand the seriousness of the effects of these contaminants. Studies conducted, however, have mostly been in the developed nations, and the focus has been on human pharmaceuticals. Information on veterinary pharmaceuticals is very limited, even though, the veterinary pharmaceuticals are known to cause as much havoc as human pharmaceuticals. Given the considerable impact that the veterinary antibiotics can have on humans and the environment, there is need for thorough investigations to be done regarding their detection and removal from water sources using biological and other appropriate technologies such as advanced oxidation methods. However, there is very little that has been done to date in this area globally and in South Africa in particular which highlights the novelty of this work. The findings of this study will therefore inform future decision making by policy makers and governments in handling these veterinary antibiotics in water sources.

This study was divided into four phases covering the five objectives investigated. The first phase covered the first and second objectives, in which a suitable and sensitive analytical method was developed for the determination of veterinary antibiotics based upon solid phase extraction (SPE), ultrahigh liquid chromatography with photodiode array detectors (PDA) and mass spectrometry (MS) (UHPLC-PDA-MS). Four classes of antibiotics were selected: tetracycline, β-lactam, sulphonamides and fluoroquinolones. The studied antibiotics were extracted from slaughterhouse wastewater samples using strata-X cartridges. The extraction of antibiotics from water matrices was tested at several pH values. The best recoveries were obtained at pH 2. Depending on the nature of antibiotic, the limits of detection (LOD) and limits of quantification (LOQ) were in the range of 0.1–0.3 μg/L and 1.3–2.9 μg/L, respectively. The range of antibiotics detected in the wastewaters in effluents was 0.008 to 4.9 ng/L

while in the influent, the range was 1 to 21 ng/L; thus higher concentrations were found in the influents as compared to effluents. This therefore confirmed the presence of these contaminants in the South African slaughterhouse wastewaters.

The second phase entailed the investigation of the third objective which was to determine the possible mechanisms of removal of antibiotics from wastewaters using anaerobic digestion and to evaluate the biodegradation kinetics. A laboratory scale upflow anaerobic sludge blanket (UASB) reactor was employed to treat synthetic wastewater to explore the removal efficiencies of five veterinary antibiotics with an initial concentration of 50 µg/L. In a like manner, batch reactors were further used to evaluate the removal routes of the antibiotics. The UASB reactor was operated continuously under mesophilic conditions to evaluate its performance regarding the removal of organics; biogas production was also monitored. Organic loading rate (OLR) was varied from 8 to 9.2 kg.COD.$m^{-3}$.$d^{-1}$while keeping the hydraulic retention time (HRT) constant at 12 h. A chemical oxygen demand (COD) removal efficiency higher than 75% was achieved at an OLR of 9 kg.COD.$m^{-3}$.$d^{-1}$, with a HRT of 12 hours. About 80% of the antibiotics were removed during the continuous processes, however, a distinctive pattern of removal was not observed. The kinetic studies using a batch process showed that the removal route for the antibiotics was majorly adsorption to the sludge. Biodegradation occurred alongside adsorption but to a lesser degree. The kinetic data showed that the antibiotics degradation followed a first order kinetic model with half-lives that ranged from 6 to 77 days.

Given the ineffectiveness of the biological process against the antibiotics, there was need to explore alternative wastewater treatment technologies. In this case adsorption and photocatalysis were investigated. Phase three presents the preparation and characterization of the integrated photocatalyst (IPCA). The adsorption properties of the IPCA, titanium dioxide ($TiO_2$) and activated carbon (AC) were assessed using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD). These adsorbents were used to treat wastewater containing the target antibiotics. The effect of process variables such as adsorbent concentration, contaminant concentration and solution pH was investigated. The IPCA demonstrated

good adsorption ability attaining removal efficiencies of over 50% while AC efficiency was over 60%. However, $TiO_2$ demonstrated negligible adsorption performance.

In the fourth phase, objective five was evaluated to determine the effectiveness of advanced oxidation processes (in this case photocatalysis) using IPCA and $TiO_2$. The effect of process variables such as photocatalyst concentration and solution pH were investigated. It was found that photocatalysis attained almost 100% degradation of the target contaminants. Maximum removal efficiencies for both AC and IPCA were above 50% for an initial concentration of 100 mg/L. Adsorption using AC and IPCA followed the Langmuir and Freundlich isotherms, however, higher coefficients of correlation were obtained for the Langmuir isotherms for four of the antibiotics viz. AMO, CIP, ENRO and TET. The Freundlich model was the best fit for the SULFA in terms of the coefficient of correlation. With regards to the photodegradation, it was found that photocatalysis attained almost 100% degradation of the target contaminants. Complete degradation was achieved within half-lives of 60 to 102 minutes for all the compounds. Although both photocatalysts effectively degraded the contaminants, the IPCA had the unique advantages of possessing both adsorptive and photocatalytic properties. The activated carbon in the IPCA provided sites for the attachment of the antibiotics and $TiO_2$ thus enhancing the photocatalytic performance. Apart from this, the IPCA can be easily recovered for reuse by decantation unlike the slurry $TiO_2$. Therefore, the study demonstrated the effectiveness of the IPCA as a suitable photocatalytic material for the complete degradation of these antibiotics.

# Dedication

This research work is dedicated to GOD almighty, without your Grace, Mercy and Divine direction this project will not have been a reality. I also wish to dedicate this work to my late Mum (Mrs Lyop Chollom), thank you so much for your guidance and care, I love you.

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# Abbreviations

| | |
|---|---|
| AC | Activated carbon |
| AD | Anaerobic digestion |
| AMO | Amoxicillin |
| API | Active pharmaceutical ingredient |
| BOD | Biochemical oxygen demand |
| CIP | Ciprofloxacin |
| COD | Chemical oxygen demand |
| DI | Deionised (water) |
| DO | Dissolved oxygen |
| DOC | Dissolved organic carbon |
| EDCs | Endocrine disrupting chemicals |
| EDX | Energy dispersive X-ray |
| ENRO | Enrofloxacin |
| FTIR | Fourier transform infrared spectrometer |
| GAC | Granular activated carbon |
| HRT | Hydraulic retention time |
| HPLC | High performance liquid chromatography |
| IPCAs | Integrated photocatalytic adsorbents |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| LC-MS | Liquid chromatography mass spectrometry |
| Log Dow | Log distribution coefficient |
| Log Pow | Log partition coefficient octanol water |
| OLR | Organic loading rate |
| pKa | Logarithmic acid dissociation constant |
| P25 | AEROXIDE® P25 |

| | |
|---|---|
| PPCPs | Pharmaceutical and personal care products |
| PZC | Point of zero charge |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SE | Standard error |
| SEM | Scanning electron microscopy |
| SRT | Sludge Retention Time |
| SULFA | Sulfamethazine |
| TET | Tetracycline |
| TOC | Total organic carbon |
| UASB | Up-Flow Anaerobic Sludge Blanket |
| UV | Ultra violet |
| VFA | Volatile Fatty Acids |
| WWTP | Waste water treatment plant |
| XRD | X-ray diffraction |

# List of Notations

| | | |
|---|---|---|
| $K_L$ | Langmuir adsorption constant | 1/mg |
| $K_F$ | Freundlich constant related to adsorption capacity | (mg/ g) |
| k | Reaction rate constant | mg/L/min |
| $K_1$ | Rate constant of pseudo first-order adsorption model | (1/min) |
| $K_2$ | Pseudo-second-order rate constant | gm/g/min |
| $K_{in}$ | Intra particle rate constant | $mg/g.hr^{1/2}$ |
| $R_L$ | Separation factor | Dimensionless |
| $R_2$ | Correlation coefficient | Dimensionless |
| m | Mass of adsorbents/IPCA | mg |
| n | Adsorption intensity | Dimensionless |
| $C_0$ | Initial concentration of contaminant | mg/L |
| C | Final concentration of contaminant at time | mg/L |
| t | Reaction time | minutes, hours, days |
| $C_e$ | Equilibrium concentration of contaminant at time t | mg/L |
| $q_e$ | Mass of contaminant adsorbed at equilibrium | mg/g |
| $q_t$ | Adsorbate uptake at time t | mg/g |
| $q_{max}$ | Maximum contaminant adsorption at equilibrium | mg/g |

# Preface

PUBLICATIONS

This study has resulted in a number of publications such as: peer reviewed journal articles and peer reviewed conference papers. One is a manuscript that has been accepted for publication while two others are under review in selected peer-reviewed journals.

PUBLISHED JOURNAL ARTICLES

1. **Chollom, M.N**., Rathilal, S., Swalaha, FM., Bakare, BF., 2018. Fate, Transport, and Toxicity of Veterinary Antimicrobials with an Insight on Africa: A Review. *Ecology, Environment and Conservation Paper*. 24(3), 1201-1220. ISSN 0971–765X.
2. **Chollom, M.N**., Rathilal, S., Swalaha, F.M., Bakare, B.F. and Tetteh, E.K., Comparison of response surface methods for the optimization of an upflow anaerobic sludge blanket for the treatment of slaughterhouse wastewater. Submitted to Environmental Engineering Research Journal (EER). Manuscript number: **DOI:** https://doi.org/10.4491/eer.2018.366

MANUSCRIPTS ACCEPETED WAITING FOR PUBLICATION

1. **Chollom, M.N**., Rathilal, S., Swalaha, F.M., Bakare, B.F. Adsorptive removal of veterinary antibiotics from water using an integrated photocatalyst (IPCA). Submitted to International Journal of Environmental Studies. Manuscript ID: 199320429.

MANUSCRIPTS UNDER REVIEW

1. **Chollom, M.N**., Rathilal, S., Swalaha, F.M., Bakare, B.F. Development of a chromatographic method for the detection of selected veterinary antibiotics in slaughterhouse wastewater, submitted to International Journal of Environmental Analytical Chemistry, manuscript ID: GEAC-2019-0908

2. **Chollom, M.N.,** Rathilal, S., Swalaha, F.M., Bakare, B.F. and Tetteh, E.K., Removal of antibiotics during the anaerobic digestion of slaughterhouse wastewater. Submitted to International Journal of Sustainable Development & planning, Manuscript ID: SDP 50021

PEER-REVIEWED CONFERENCES AND PUBLICATION (Oral presentations)

1. **Chollom, M.N.,** Rathilal, S., Swalaha, F.M., Bakare, B.F. and Tetteh, E.K., 2019. Anaerobic treatment of slaughterhouse wastewater: evaluating operating conditions: 5th International Conference on Water and Society, 2-4 October, 2019. Valencia, Spain. *WIT Transactions on Ecology and the Environment, 239*, Pp.251-262. ISSN 1743-3541. Doi:10.2495/WS190221
2. **Chollom, M.N**., Rathilal, S., Swalaha, F.M., Bakare, B.F., 2018. Slaughterhouse wastewater treatment using photocatalytic systems: Optimization study. 3rd International Conference on Composites and Bio-composites and Nanoparticles. 7-9 November, 2018, Port Elizabeth, South Africa.
3. **Chollom, M.N.,** Rathilal, S., Swalaha, F.M., Bakare, B.F. and Tetteh, E.K., 2018. Lab Scale Study of HRT and OLR Optimization in an UASB Treating Slaughterhouse Wastewater. CBU International Conference on Innovations in Science and Education. UDC classification: 502/504. March 21-23, 2018, Prague, Czech Republic. **DOI:** https://doi.org/10.12955/cbup.v6.1290
4. **Chollom, MN,** Rathilal, S., Swalaha, FM., Bakare, B., 2018. Degradation of Veterinary Antibiotics from Slaughterhouse Wastewater using Titanium Dioxide as a Catalyst. 10th International Conference on Sustainable Development and Planning. 4-6 September, 2018. Siena, Italy. WIT Transactions on Ecology and the Environment, Volume 217, WIT Press, 2018, ISSN 1743-3541. Doi:10.2495/WS170111.
5. **Chollom, M.N.,** Rathilal, S., Swalaha, F.M., Bakare, B.F. And Tetteh, E.K., 2017. Study of the Start-Up of an Up-flow Laboratory-Scale Anaerobic Sludge Blanket for the Treatment of Slaughterhouse Wastewater. 4th International

Conference on Water & Society. 5-7th June, 2017. Seville, Spain *WIT Transactions on Ecology and the Environment, 216*, Pp.123-130. ISSN 1743-3541. Doi:10.2495/WS170111.

# Chapter 1 - Introduction

## 1.1 Overview of emerging contaminants

The past decades have seen the emergence of new chemical contaminants of several classes such as: pharmaceuticals, hormones, endocrine disrupting chemicals and toxins (Xagoraraki and Kuo 2008; Berninger *et al.* 2016; Alonso *et al.* 2017; Chollom *et al.* 2018a). Their detection has been due to new analytical technologies that are capable of quantifying very low concentrations of these contaminants. Similarly, advances in science and engineering have improved the understanding of the risk these contaminants pose on human health, aquatic life and the environment in general. Emerging contaminants have been defined in diverse ways by different researchers, but the ultimate aim is that each definition describes similar trends currently happening in the environment. For example, according to Xagoraraki and Kuo (2008), an emerging contaminant is any chemical or microorganism that is not normally monitored in water, but, which has through research been suspected to cause adverse effects on ecological and/or human health. The paramount causes of this emergence are attributed to: human behaviour, changes in demography, changes in climates and landscapes, increasing technology advancement and microbial adaptation amongst others (Xagoraraki and Kuo 2008).

Sauvé and Desrosiers (2014) revealed that Rachel Carson was one of the pioneers in raising the issue of emerging contaminants when she wrote the book “Silent Spring” in 1962. In that work, she strongly correlated the usage of Dichloro Diphenyl Trichloroethane (DDT), which was used for the elimination of mosquitoes and other pests, to the deaths of many birds and other aquatic organisms. She identified that the continuous usage of the DDT had a serious impact on the environment; one of which was the deaths of birds and aquatic organisms. At the time of that finding, she was criticised about the validity of her findings, however, years down the line, her findings were proven to be right which thereafter led to the ban on the use of DDT (Sauvé and Desrosiers 2014).

The use of DDT was later banned in 1970's in the USA and other countries followed suit later. Furthermore, the Stockholm convention on persistent organic pollutants was formed for the possible elimination and production of DDT (Smith 1999; Rogan and Chen 2005). Currently, pharmaceuticals, as emerging contaminants, are beginning to receive the attention that DDT had received some years back and the scientific world is beginning to research their effects on the environment. Studies carried out have investigated the fate, transport, and toxicity of these contaminants on receiving bodies.

Several classes of emerging contaminants exists such as newly synthesised substances and other substances that has long been in the environment. Examples of such include: a wide range of different compounds and their transformation products: pharmaceuticals, personal care products (PPCPs), pesticides, endocrine disrupting compounds (EDCs), veterinary products, industrial compounds/by-products, food additives, and engineered nano-materials amongst others (Daughton and Ternes 1999). Because a variety of these compounds exists, this study will focus on pharmaceuticals with more emphasis on pharmaceuticals used in livestock farming.

Pharmaceuticals are chemicals that are used for diagnosis, treatment or prevention of disease, health condition, or structure/function of the human body or animal body (Daughton and Ternes 1999). The metabolism of the active compounds in pharmaceuticals by both humans and animals has been identified to be less than 100%. A wide variation in the metabolism rate is said to exist; some compounds are metabolised by 90%, while others less. The remainder of the un-metabolised pharmaceuticals is expelled through urine and faeces which are discharged later into the environment through different means (Kummerer 2009b).

Sources of pharmaceuticals contamination of the environment include: pharmaceutical production plants, wastewater treatment plants (WWTPs), hospitals, agricultural activities, aquaculture, and landfills, among others. Amongst the aforementioned, the most investigated route of pharmaceuticals entry into the environment is the WWTPs (Akhtar, Amin and Shahzad 2015; Barra Caracciolo, Topp and Grenni 2015; Gavrilescu *et al.* 2015). Studies have reported the incomplete removal of these contaminants in most wastewater treatment plants. Effective biodegradation of some

pharmaceuticals occurs in the WWTPs while for some it is ineffective. This is due to the fact that some of them can be sorbed onto the sludge which is then used as fertiliser or disposed of into landfills. Those that are not sorbed remain in the aqueous phase and are released into the environment through the effluent water (Wang *et al.* 2014).

The continuous exposure of these contaminants could lead to bio-concentration (this is when the concentrations of the chemicals in the aquatic organisms surpasses those in water due to exposure to chemicals in the water) and bioaccumulation (the process through which these pollutants get into the food chain) thereby putting these organisms at risk (Baquero, Martínez and Cantón 2008; Kummerer 2009a; Thomaidi *et al.* 2015).

### 1.1.1 Prioritisation of the Pharmaceuticals

Several reasons have been outlined for the need to monitor pharmaceuticals in the environment. The main reason, however, is to understand the risk their presence poses to the environment, on the food chain and on human health (Zuccato *et al.* 2005). In addition, monitoring is important to enable the inclusion of the identified contaminants in developed analytical protocols for their quantification (Di Nica *et al.* 2015). Since a very large number of these contaminants exists, monitoring of each contaminant is difficult to achieve. This is worsened by the fact that these contaminants are made up of different chemical structures, have various physicochemical properties and produce diverse metabolites. Therefore there is the need to prioritise which contaminants to monitor (Di Nica *et al.* 2015).

Prioritisation has been studied using different approaches. However, the studied methods are based on the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH SC), where two phases are used; Phase I (VICH GL6) and Phase II (VICH GL 38). Phase I is based on the authorisation of the protocol on the decision tree for risk assessment of veterinary pharmaceuticals. The decision tree approach is used such that contaminants with low exposure are said to have a minimal hazard on the environment and thus are not expected to pose any significant risk. In Phase II, the assessment is based on a risk quotient (RQ) approach which is determined for every non-target test species

considered as representative of different ecosystems. It establishes the harmonized, two-tiered approach for conducting an environmental risk assessment at international level in the European Union (EU), Japan, United States of America (USA), Canada and Australia/New Zealand (Kim *et al.* 2008; Di Nica *et al.* 2015; Burns *et al.* 2018).

Different classes of pharmaceuticals have been prioritised from both human and veterinary pharmaceuticals, with the human pharmaceuticals receiving the most attention. However, interest in veterinary pharmaceuticals is increasing. The first study on the effects of veterinary antibiotics on the environment was carried out by Boxall *et al.* (2003) where use was made of a qualitative prioritisation method. This method considered the following aspects: the usage of veterinary microbial, the degree of metabolism in the animal, degradation during storage of manure before land application, and the toxicity of the substances to both terrestrial and aquatic organisms (Boxall *et al.* 2003). Other studies by Kim *et al.* (2008), Xagoraraki and Kuo (2008), Guo *et al.* (2016) and Burns *et al.* (2018) have all prioritised veterinary pharmaceuticals for the aquatic environment based on usage, potential to enter the environment and their toxicological hazard.

The overall objective of these studies was to categorise these classes of contaminants into groups for ease of identification as well as for risk analysis. Antibiotics as a class of pharmaceuticals have received the most attention. In addition, studies on antibiotics have focused on those from human sources; however, veterinary antibiotics are now receiving considerable attention. Based on the aforementioned, the focus of the present study is to i) detect the presence of veterinary antibiotics in a swine slaughterhouse in South Africa and surface impacted water receiving effluent from the treatment plant; and ii) evaluate the use of biological and advanced oxidation methods for their remediation. Though the study focuses on veterinary antibiotics, reference to human antibiotics is made when necessary especially for comparison purposes. Given the considerable impact that the veterinary antibiotics can have on humans and the environment, there is need for thorough investigations to be done regarding their detection and removal from water sources using biological and advanced oxidation methods. However, there is very little that has been done to date in this area in South

Africa and even globally and this highlights the novelty of this work. The findings of this study will therefore inform future decision making by policy makers and governments.

## 1.2 Antibiotics as emerging contaminants

The term antibiotics (ABs) or antimicrobials are used interchangeably in this study unless otherwise stated. Antibiotics are a class of pharmaceuticals used in human and veterinary medicine, as well as in aquaculture for the purpose of averting or treating microbial infections. Increased consumption of antibiotics for prophylaxis and therapy in humans and for agriculture has been observed (Gelband *et al.* 2015). This increase is attributed to: increase in income, thus providing more people with the ability to accesses antibiotics; and increase in the demand for foods containing animal proteins (Van Boeckel *et al.* 2014; Gelband *et al.* 2015; Puckowski *et al.* 2016).

Pharmaceuticals used for livestock and aquaculture are grouped into different categories. However, amongst all the classes, the antibiotics are most widely used (Sarmah, Meyer and Boxall 2006). Ever since their introduction in 1949 in the USA, antibiotics have been used both in human and livestock farming to treat diseases. In livestock farming they are sometimes used as growth promoters and to improve feed efficiency (Sarmah, Meyer and Boxall 2006). The data regarding the consumption of veterinary antibiotics varies globally. Where the data exists, it is mostly in the more developed economies (Song and Guo 2014; O'Neill 2015; Chollom *et al.* 2018a).

O'Neill (2015) studied antimicrobials used in agriculture and noted the global consumption to be around 63,000 tonnes a year and Van Boeckel *et al.* (2015) and Grace (2015) suggested 240,000 tonnes a year. Van Boeckel *et al.* (2015), estimated that global consumption will increase by 67% from 2010 to 2030 and further stated that the BRICS (Brazil, Russia, India, China, and South Africa) countries would experience a 99% increase in the same time period as their population increases by 13%.

It is estimated that more than 70% of the antibiotics produced are used for livestock production in the United States of America (USA). According to the Food and Drug Administration (FDA), other countries have more than 50% consumption (O'Neill 2015). The most startling fact is that similar classes of these antibiotics are used in both human and animal treatment. According to Gelband *et al.* (2015) and Boxall (2012), of the 27 antimicrobial classes used for both human and animal purposes, only 9 are used exclusively for livestock production.

The different prioritisation studies **(section 1.1.1)** have identified antibiotics as one of the predominant groups that call for urgent attention for monitoring and legislation (Di Nica *et al.* 2015). Boxall *et al.* (2003) and Kim *et al.* (2008) identified antibiotics as the top-ranked, due to the prevalence of antibacterial resistant genes (ARGs) and antibacterial resistant bacteria (ARBs) in the environment. Witte (1998), provided evidence linking the use of antibiotics in livestock feeds to the discovery of the resistance of *E. coli* in the guts of the pigs and in meat products. In the study, the pigs were exposed to antibiotics for a period of time. However, before exposing the pigs to the antibiotics, initial tests had shown there was no resistance selection for the veterinary antibiotics used, however, after exposure to the antibiotics, they tested positive to the resistance genes. To further buttress the seriousness of this impact, the gut flora of pig farmers, their families, and citizens of the community also tested positive to the resistance, thus signifying that it had spread to them (Witte 1998; Cooper, Siewicki and Phillips 2008; Naidu *et al.* 2016). Spellberg *et al.* (2016), observed that using veterinary antibiotics for livestock production for more than 40 years would result in an increase in the direct spread of antibiotic-resistant bacteria to humans. Further evidence has been provided in other studies by O'Brien and Dietrich (2004); O'Neill (2015); Rosi-Marshall *et al.* (2015). Amongst the groups of antibiotics, the commonly used are the tetracyclines, penicillins, and sulphonamides (Figure 1-1).

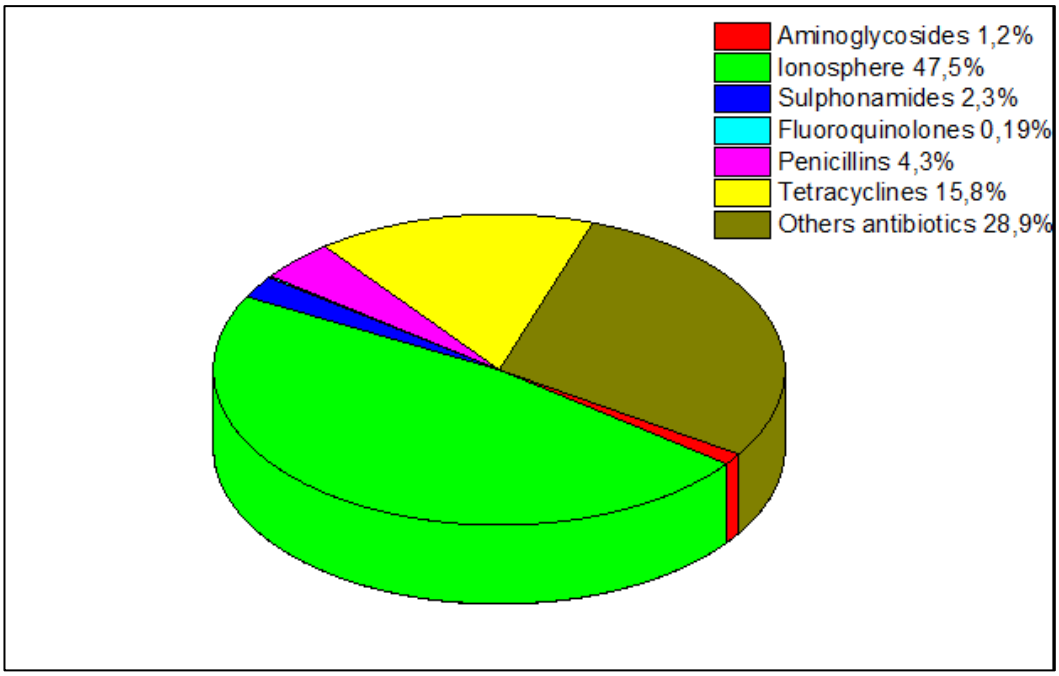



Figure 1-1: Percentage composition of antibiotics use as reported by Animal Health Institute (AHI) survey in 1999 (Sarmah, Meyer and Boxall 2006).

Similar findings have been detailed in more recent studies. For example, Eagar, Swan and van Vuuren (2012) found the tetracyclines were the most consumed antibiotics in South Africa. Again, Bhavsar and Thaker (2012) found a similar trend to the findings of Eagar, Swan and van Vuuren (2012). These same antibiotics from Figure 1.1 which are the most consumed, are thus detected in most studies on environmental monitoring (Karci and Balcioglu 2009; Saxena *et al.* 2018).

### 1.2.1 Pathway, Fate, and Transport

There are different entry ports of antibiotics into the environment such as through wastewater treatment plants, waste from agricultural food/livestock production and aquaculture, direct application to some plants, industrial effluents from pharmaceutical production, and agricultural run-off (Qiao *et al.* 2018).

The major sources of environmental contamination are classified as point sources and diffuse or non-point sources (Lapworth *et al.* 2012). Point sources release contamination from a specific location, and as such their contributions into the

environment are defined by a distinct means and usually, the loading from these sources are concentrated. Examples of point sources are WWTPs and pharmaceutical production plants. Non-point sources do not have a distinct pattern of pollution like those of the point sources; therefore pollution from these sources occurs over a wide area. Examples include runoff from agricultural land where fertilizers are applied and open grazing is practised. Despite the fact that diffuse sources are usually found in smaller concentrations, they are said to be more responsible to the offsetting of the natural environment than point sources. This is due to the fact that it is difficult to link the contamination to the original source (Lapworth *et al.* 2012; Chollom *et al.* 2018a).

The interaction of antibiotics with the environment is majorly affected by the physicochemical properties such as: molecular structure, size, shape, solubility, and hydrophobicity as well as the soil type, texture, pH, and organic matter content. The chemical properties could affect their behaviour in WWTPs (Lapworth et al. 2012; Wang and Wang 2015; Chollom et al. 2018a). The partition coefficient (KOW) describes the ability of the active components to remain either in aqueous solution or sorbed to the soil and thus describes the hydrophilicity of the substance. Because of the differences in the properties of these contaminants, their behaviour in the environment differ and as a result, a series of phase transformation processes such as biodegradation, dilution and photolysis, occurs (Caliman and Gavrilescu 2009; Chollom et al. 2018a).

Various classes of antibiotics have been detected in influents and effluents from wastewater treatment plants as well as in the aquatic environment, soil, and manure from livestock production. The most frequently detected antibiotics include sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, trimethoprim, tetracycline, oxytetracycline, ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, roxithromycin, and erythromycin-$H_2O$ (Qiao *et al.* 2018).

Fluoroquinolones (FQ), sulfonamides (SA) and tetracyclines (TET) are the most frequently detected veterinary antibiotics. The properties of tetracyclines and sulphonamides favour their persistence in the environment for a long period of time (Qiao *et al.* 2018). Concentrations as high as 225 mg/kg (norfloxacin) and 1421 mg/kg

for enrofloxacin, have been reported and these are some of the highest detected. For oxytetracycline and chlortetracycline, 417 mg/kg were reported from chicken manure and 764 mg/kg from swine manure. Sulphonamides detected concentrations were however lower at 10 mg/kg. These have been detected mainly in soil, manures, and runoffs from animal farms, with limited detection from the slaughterhouses.

Further discussion on the pathway, fate and transport of antibiotics can be obtained in our review on fate, transport, and toxicity of veterinary antimicrobials with an insight on Africa Chollom *et al.* (2018a).

## 1.3 Identification and detection of antibiotics

The advancement in the development of new analytical procedures and equipment for analysis and environmental monitoring has made it possible to assess the presence of emerging contaminants, identify them and measure their concentration (Saxena *et al.* 2018). These methods have better reproducibility and repeatability and lower detection limits. Identified antibiotics and other contaminants are usually found in small quantities, in ng/L or µg/L (Kim *et al*. 2018).

In the early 1990s, gas chromatography (GC) was commonly employed for the chromatographic separation of compounds; however, a further derivatisation step of the compounds was necessary to enable detection by the GC. Nowadays, the use of high performance liquid chromatography (HPLC) and liquid chromatography (LC) is being employed leading to the determination of a broader range of compounds, including pharmaceuticals, thus permitting a more comprehensive assessment of environmental contaminants (Hu *et al.* 2014; Gbylik-Sikorska *et al*. 2015).

The earlier analytical methods that were developed were aimed at detecting antibiotics that belonged to a single class, such as the sulphonamides, tetracyclines, or macrolides. However, since a variety of contaminants have been detected in trace-levels, researchers have recently started targeting the development of procedures which are optimised for the simultaneous determination of multiple classes of antibiotic residues. More so, it is evident that the frequent detection and quantification of the contaminants

from the environment has been observed from different classes of antibiotics, as a result, the development of a multiclass analytical method for their determination is the preferred choice. The single analytical method developed detects single compounds thus making the whole process tedious and more expensive since similar procedures are involved for both single and multiclass analyses (Hu *et al*. 2014; Gbylik-Sikorska *et al.* 2015).

The environmental samples from which the contaminants are detected and quantified are usually composed of matrixes which interfere with the separation processes. Apart from the presence of the matrixes, the contaminants are usually detected in very minute amounts which could be below the detection levels of the instruments and as such sample preparation and concentration steps are necessary before the chromatographic separations (Kim *et al.* 2018; Saxena *et al.* 2018). Solid phase extraction (SPE) is used for the sample preparation as well as for the pre-concentration. SPE is an alternative to liquid-liquid extraction (LLE) due to the fact that smaller volumes are required in SPE as compared to LLE, therefore making it less expensive and less time consuming. It also offers higher selectivity and reproducibility for the simultaneous extraction of multiple compounds from an aqueous matrix (Kim *et al.* 2018).

Thus this study is aimed at developing a multiclass analytical method for four different classes of veterinary antibiotics and validating the method. The method development considers the optimisation of both the chromatographic separation as well as the SPE optimisation. Further details on the chromatographic and preparation methods for the environmental samples is provided in the literature review.

## 1.4 Remediation of wastewater containing antibiotics

Conventional treatment methods such as biological systems are mostly employed for the treatment of wastewaters. These systems are designed for the removal of organics which are estimated as dissolved organic matter, solids and nutrients. The wastewater treatment systems are grouped into various categories viz. destructive and non-destructive methods. Destructive methods are processes whereby the contaminants are destroyed thus changing the contaminants initial form completely e.g. chemical,

biological and advanced oxidation processes. Non-destructive methods are processes whereby the contaminants are not destroyed but are transferred from the bulk solution to another phase e.g. adsorption, membrane filtration, liquid extraction techniques among others (Homem and Santos 2011).

Most WWTPs are made up of different units collectively coupled and operated with the common goal of reducing organic contaminants before discharging the effluents into the environment. Optimum removal is obtained in most cases, however, the fact that these systems have shown capabilities in the removal of organics and other contaminants, they are still limited by the fact that they are unable to remove antibiotics and other emerging contaminants due to their low concentrations and some of them being bio-recalcitrant (Semblante *et al*. 2015; K'Oreje K *et al*. 2016; Naidu *et al*. 2016).

The removal of antibiotics from wastewaters has been identified to be either through biotic and abiotic processes. The biotic process involves biodegradation by microorganisms while the abiotic processes are sorption, hydrolysis, oxidation-reduction, and photolysis. In some instances, both biotic and abiotic processes could occur at different stages during the treatment process. For example, during the wastewater treatment, two streams are generated; the solid and liquid (aqueous) phase. Therefore the antibiotics which are not removed by either of the mentioned processes could be found either in the aqueous or solid stream of the WWTPs. The behaviour of the antibiotics in the WWTPs is, however, determined by their physical and chemical characteristics. Some of these contaminants have moderate to large octanol-water partitioning coefficients and therefore they can be predicted to undergo hydrophobic partitioning into the organic-rich solids phase during the treatment processes (Renew and Huang 2004; Kinney *et al.* 2008; Haddad, Baginska and Kummerer 2015; Hernandez-Maldonado and Blaney 2015; Katsigiannis *et al*. 2015).

It is common practice that the aqueous (effluents) phase is discharged into the environment or are used for the purposes of irrigation while the solids are used for soil amendments or are discarded into landfills. These common practices have exposed the environment and aquatic systems to these contaminants, thus leading to the prevalence

of ARBs and ARGs due to the presence of these contaminants to organisms in the receiving bodies (de Jesus Gaffney *et al*. 2015). To provide alternatives that could further remove these types of contaminants from the WWTPs, the optimisation of the WWTPs and the incorporation of advanced wastewater treatment technologies is imperative. For example, advanced oxidation processes such as ozonation and Photo-Fenton processes have been applied (Kanakaraju *et al*. 2015; He *et al*. 2016). Adsorption, which is a physical separation, has been applied for the removal of antibiotics from wastewater streams (Yu *et al.* 2016). Degradation of antibiotics using advanced oxidation processes where Titanium dioxide are immobilised on surfaces such as activated carbon, quartz, sand are novel methods that are being employed and have been found to degrade these contaminants completely in most instances (He *et al*. 2016). Further discussion of these methods is provided in the literature review, **section 2.4.** Similarly, details regarding the type and nature of antibiotics that have been detected in wastewaters and the environment have also been elaborated in the literature review.

## 1.5 Problem statement and Justification

In livestock production, the huge consumption of antibiotics is linked to the fact that modern livestock farming is employed in large and densely populated management schemes. These animals are confined in large numbers, therefore antibiotics are used routinely in a quest to prevent and manage diseases (Qiao *et al*. 2018). The use of antibiotics in livestock helps to offset the effects associated with the crowdedness, poor hygiene and living conditions in intensive animal agriculture (Qiao *et al*. 2018). The administration of the required antibiotics could be through drinking waters, feeds, injections or oral implants. Antibiotics are administered or used at both therapeutic and sub-therapeutic levels in livestock to prevent diseases and promote animal growth. Animals are typically treated at therapeutic levels with antibiotics for a period of 3 to 7 days and a further 3 to 4 days at prophylactic dosages. The sub-therapeutic or non-therapeutic use contributes more to the consumption of animal antibiotics, in sub-therapeutic usage; the antibiotics are used as in-feeds (Boxall et al. 2002; Sarmah, Meyer and Boxall 2006; Qiao *et al*. 2018; Charuaud *et al*. 2019).

Some classes of antibiotics that are used in livestock production are similar to those used for human therapy. Daughton (2014) reported that cross-using these antibiotics in humans and animals could lead to a speedy development of resistant microbial strains toward these drugs.

The detected amounts of antibiotics from the environment and other sources are usually in small concentrations. Detection from aquatic systems, rivers, and other water sources have been in µg/L or ng/L while a higher concentration detected in mg/kg was found in manures. Although in small concentrations, their effects on the environment have raised concerns about human health. The presence of these contaminants in the environment has been linked to antibiotics resistant strains (Kummerer 2009b; Richardson 2012; Daughton 2014). The growing extent of the environmental pollution and the rise in the ARBs and ARGs has initiated complex legislations worldwide. This is due to the fact that there is a potential transfer of ARGs from the environmental bacteria to human pathogens, and as a result, the efficacy of antibiotic treatment is reduced, thus compromising public health (Qiao *et al*. 2018). Other contaminants which are not antibiotics like the estrogen have also shown to cause feminisation of fish living downstream of wastewater outlets (Richardson 2012). Similarly, it has been suggested that the release of hormones from birth-control tablets could lead to feminisation in some fish populations (Richardson 2012). The use of diclofenac has been associated to a decline in the vulture population in the Indian subcontinent (Gavrilescu *et al.*, 2015; Bártíková *et al*., 2016). Studies by Cooper, Siewicki and Phillips (2008) and Naidu *et al.* (2016) indicated that diclofenac had a harmful effect on the population of vultures, which caused kidney failure to them thereby leading to so many of their deaths.

It has also been shown that the use of antibiotics in livestock production is directly related to the incidences of resistant bacteria in food producing animals. It is important that strategies are developed to monitor this new development aggressively. Therefore the gathering of reliable and relevant data regarding the fate, transport, behaviour and distribution of these contaminants into the environment is deemed necessary. Sauvé and Desrosiers (2014) revealed that the environmental quality criteria should be

closely related to the presence of these emerging contaminants. In view of this, data accumulation on the environmental chemistry, ecotoxicology, human toxicity as well as its epidemiology needs to be investigated. The availability of the data aids in government action to establish environmental guidelines and criteria for the assurance of adequate protection (Sauvé and Desrosiers 2014).

The burden of the ARGs and ARBs has drawn worldwide attention into investigating this rising phenomena in order to better understand the seriousness of the effects of these contaminants. Studies conducted, however, have mostly been in the developed nations. Hughes, Kay and Brown (2013) showed that 80% of the data collected from rivers and aquatic systems on the research on emerging contaminants was carried out in developed nations while 16% was in Asia. Very limited information was found in Africa. This makes it necessary for more studies to be done in Africa.

The studies carried out so far in South Africa have been concerned with human pharmaceuticals; little or no information exists on veterinary pharmaceuticals. However, the veterinary pharmaceuticals are known to cause as much havoc as human pharmaceuticals (Swart and Pool 2007; Olujimi *et al.* 2012; Surujlal-Naicker and Bux 2013; Manickum and John 2014, 2015; Matongo *et al*. 2015b, 2015a; Segura *et al*. 2015; Wood, Duvenage and Rohwer 2015). Swart and Pool (2007) conducted one of the pioneer studies on emerging contaminants in South Africa. Their study was based on the detection of selected steroid hormones from the Kuils River water catchment area. Studies on the endocrine disrupting compounds (EDCs) were carried out by Manickum and John (2014) and Olujimi *et al.* (2012). Anti-retroviral compounds and human pharmaceuticals were detected by Matongo *et al.* (2015a) and Wood, Duvenage and Rohwer (2015), respectively.

Van Boeckel et al. (2015) reported that South Africa as one of the growing economies in Africa is experiencing an increase in population and consequently an increase in the demand for animal products. This increase will then mean an increase in the consumption of veterinary antibiotics used in livestock production as earlier indicated (Eagar, Swan and van Vuuren 2012; Chipangura et al. 2017). Although the South African government has set the limits for residual of some veterinary antibiotics in

animal foodstuff, there is currently no regulation that has been issued to control the discharge of antibiotics contained in livestock wastewaters.

This is one of the first studies to evaluate the presence of veterinary contaminants in a slaughterhouse wastewater treatment plant in South Africa. This study i) assesses the presence and transformation of these compounds; and ii) evaluates the treatment of the wastewater and efficiency of removal or degradation of the contaminants. Through the prioritisation studies regarding consumption from the literature on veterinary antibiotics, the following classes of antibiotics were considered for this study: fluoroquinolone, tetracyclines, penicillins, and sulphonamides. In addition to prioritisation on consumption, prioritisation from toxicity studies carried so far has identified the major resistant traits in Africa to be from tetracycline, penicillins, and sulphonamides with other traits such as plasmid-mediated quinolone resistance genes and extended spectrum B-lactamase (Alonso *et al.* 2017; Chollom *et al.* 2018a). The revelation of the above further informed the choice of the chosen antibiotics.

## 1.6 Aim and objectives

### 1.6.1 Aim

The aim of this study was to develop a suitable and sensitive analytical method for the determination of veterinary antibiotics based upon solid phase extraction (SPE) and ultrahigh liquid chromatography with photodiode array detectors (PDA) and mass spectrometry (MS) (UHPLC-PDA-MS) and to evaluate the performance of biological and advanced oxidation processes for remediation of wastewater containing veterinary antibiotics.

#### 1.6.1.1 Specific objectives

1. To develop and optimise an extraction method based on SPE UHPLC-PDA-MS method for veterinary antibiotics (VAs) from the slaughterhouse wastewater treatment plant.

2. To apply the optimised SPE and (UHPLC-PDA-MS) method for determining veterinary antibiotics from the slaughterhouse wastewater.
3. To determine possible mechanisms of removal of antibiotics from wastewaters using anaerobic digestion and study the biodegradation kinetics
4. To prepare and characterize the integrated photocatalyst (IPCA) and evaluate its adsorption performance against the target antibiotics
5. To evaluate the effectiveness of the IPCA as a photocatalyst for the degradation of the target veterinary antibiotics.

## 1.7 Project scope

The monitoring of influent and effluent discharge of emerging contaminants from a slaughterhouse wastewater treatment plant and surface impacted rivers represents a small but important portion with regards to micropollutants. The study presented here was limited to monitoring influents and effluents from a real slaughterhouse wastewater treatment plant. This slaughterhouse slaughters only swine. Furthermore, monitoring and removal using a laboratory biological treatment unit incorporated with advanced oxidation of five selected antibiotics belonging to four different groups was considered.

## Thesis layout

The thesis is divided into eight chapters as follows:

**Chapter 1─ Introduction**

The introduction gives an overview of emerging contaminants, the need for prioritisation of these emerging contaminants and processes used for remediation. It also presents the problem statement and the aim and objectives of the study.

**Chapter 2─Literature Review**

Chapter two provides a comprehensive overview of emerging contaminants, veterinary antibiotics, their fate, and transport into the environment. Treatment

efficiencies of various methods are highlighted. Finally, analytical method development for the detection of these contaminants is elaborated. A conclusion is provided giving the gaps in the literature and what the present study will focus on.

**Chapter 3─Materials and Methods**

The materials and methods section is divided into three sections incorporating the six objectives of the study. The first section describes the methods and materials used for the method development, while the analytical optimisation procedures are elaborated. The second section describes the materials and methods employed for the biodegradation of the antibiotics using an upflow anaerobic sludge blanket (UASB). The final section presents the photodegradation of the antibiotics, thus the materials, methods, and equipment used for characterisation of integrated photocatalysts (IPCAs) are described.

**Chapter 4─Results and Discussion on the development of SPE-HPLC/PDA-MS for the analysis of selected veterinary antibiotics in slaughterhouse wastewater**

Chapter four is dedicated to results obtained from the development of an analytical technique by SPE UHPLC-PDA-MS for the analysis of multiclass veterinary antibiotics. Results in this section include the data obtained from the optimisation of both the chromatographic separation and solid phase extraction and the validation of the developed method. Finally, the application of the method for the detection of the effluents from the slaughterhouse wastewaters is also presented.

**Chapter 5─Results and Discussion on the evaluation of the removal mechanisms of antibiotics from wastewaters using anaerobic digestion**

Chapter five presents the results obtained from the UASB systems used for the removal of the selected veterinary antibiotics from the wastewater. The degradation pathways for the selected antibiotics are discussed. Data obtained by virtue of the optimization is further elaborated and the biodegradation kinetics also described.

**Chapter 6─Results and Discussion on the development and evaluation of an efficient polishing step (advanced oxidation) for the removal of veterinary antibiotics**

Results obtained from the characterisation of the prepared catalyst are presented here. Adsorption studies are presented and the data obtained subjected to different kinetic studies: pseudo first order, pseudo second order and the intra-particle diffusion models are used. Furthermore, the Langmuir and Freundlich isotherms are used.

**Chapter 7─Results and Discussion on the photocatalytic degradation of the antibiotics as well as the optimisation of the process**

The results obtained from the photodegradation of the antibiotics using IPCA and $TiO_2$ are presented in this chapter.

**Chapter 8─Conclusion and Recommendation**

Conclusions drawn from the developed method for detection and the results from the monitoring program are presented. Findings and conclusions are also presented and finally, further research directions to widen our understanding of the contribution of slaughterhouse wastewaters to the environmental impact of antibiotics and treatment at source are recommended.

# Chapter 2 - Literature Review

## 2.1 Introduction

This chapter is divided into three main sections. The first section discusses pharmaceutical compounds as emerging contaminants and their main effects on the environment. The sources of veterinary contaminants such as wastewater treatment plants, livestock farming and others are elaborated. The second section presents the treatment methodologies currently employed by most treatment plants. It also presents the post-treatment methods employed for further reduction, and/or elimination of these contaminants from wastewaters prior to discharge into the environment. The third section reviews the theory and application of advances in analytical approaches that have allowed for the detection of trace levels of pharmaceutical residues from environmental samples. Furthermore, a discussion on the three main steps that are necessary for the determination of the pharmaceuticals from environmental samples is provided. These three main steps are: i) sample pre-treatment and pre-concentration which is carried out to increase the sensitivity of the method developed; ii) the use of liquid chromatography (LC) as an analytical separation technique; and iii) the detection using mass spectrometry (MS).

## 2.2 Pharmaceuticals

Pharmaceuticals are classes of emerging environmental contaminants which are extensively used for human and veterinary medicine. Their use is continuously increasing (Fent, Weston and Caminada 2006; Kümmerer 2010; Elliott, Kenny and Madan. 2017). Pharmaceuticals are classed into anatomical, therapeutic, and chemical (ATC) groups; target anatomical system; therapeutic properties of the drug; or chemical characteristics of the molecule (Di and Kerns 2015). In most instances, they are basic or acidic, thus having amino or carboxyl groups which could be basic, acidic, neutral or zwitterionic under environmental conditions (Kümmerer 2010).

Pharmaceuticals can be divided into the following groups: antibiotics, hormones, analgesics, and anti-inflammatory drugs, chemical compounds used for disinfection and cleaning, and endocrine-disrupting compounds (Kot-Wasik *et al.* 2007; Charuaud *et al.* 2019).

Of all the pharmaceuticals, the antibiotics are becoming more and more of a focus point of research due to their high detection frequency in the environment and the increasing bacterial resistance (Van Doorslaer *et al.* 2014b). It represents about 70% or more of all the consumed pharmaceuticals that are used as human and veterinary medicines (Charuaud *et al.* 2019). Further still, a higher percentage of the antibiotics consumed is used for veterinary purposes. Nearly, 2000 different veterinary pharmaceuticals are said to be manufactured from 400 different active chemical ingredients, produced for different purposes (Song and Guo 2014; Charuaud *et al.* 2019). The chemical ingredients are classified in such a way to suit the different therapeutic purposes for the treatment of animals, to prevent diseases, or to combat infections and relieve pains or injuries. Veterinary antibiotics are used in different animal sectors, such as poultry, swine, cattle, and aquaculture. The way of administration depends on the type of animal; it can be done orally through feed and water or by injection (Bhavsar and Thaker 2012).

The continuous usage of veterinary antibiotics is increasing. The human population is projected to increase from its current 7 billion to about 9 – 10 billion by 2050. This increase will be highest in Sub-Saharan Africa and some of the Asian countries. This increase in population will result in a higher demand for meat and other animal products (Gelband *et al.* 2015). This will place a higher demand for veterinary antibiotics. The increase in the consumption of antibiotics has been stated to be due to raising household incomes as well as the demand for foods with animal protein (Elliott, Kenny and Madan. 2017).

There is a limited with great variation regarding the data on the consumption of veterinary antibiotics globally, and where data exists, it is mostly for the more developed economies and even at that, it is not accurate. The reason could be due to the fact that most developing countries do not keep accurate records regarding the sales

and consumption of these pharmaceuticals and generally, there are higher rates of over-the-counter self-medications, thus resulting in poor surveillance and data collection (Song and Guo 2014; O'Neill 2015; Segura *et al.* 2015; Chollom *et al.* 2018a). Charuaud *et al.* (2019) argue that the variation in the consumption could be due to the different practices in each country. Each country has its preference that is given to the animals, for example, a higher demand could be placed on chicken in a certain country while another would prefer swine to chicken. Therefore the administration of antibiotics on chicken would be different from that of the swine. Again, other differences could be due to different animal farming methods and climate conditions (Charuaud *et al.* 2019). For example, Van Boeckel *et al.* (2015) estimated the global average annual consumption of antimicrobials for each kilogram of animal produced (cow, chicken, and pig) would be 45, 148 and 172 mg/kg respectively.

South Africa like other countries does not have an accurate way of quantifying the volume of antibiotics produced and consumed. Currently, even the data on the manufactured pharmaceuticals is not readily available, and this alone impedes obtaining the information needed. Therefore, it would be difficult to quantify the specific usage i.e. for agriculture or humans (Henton et al. 2011; Moyane, Jideani and Aiyegoro 2013).

Studies by Picard and Sinthumule (2002) and Eagar, Swan and van Vuuren (2012) on the antimicrobial database and survey of antimicrobial usage on animals in South Africa reported that the frequently used antibiotics by the measure of sales were for the treatment and prevention of diseases in poultry and pigs, and as growth promoters. Some of the commonly found were: tylosin, tetracyclines, sulphonamides, and penicillins. Estimation of the quantities of antibiotics sales from eight companies was about 1.5 million kilograms of active ingredients. About 42.2% of the antibiotics were related to macrolides, lincosamides, and pleuromutilins, thus representing the total quantities of sales (kg). Detailed information is shown in Table 2-1.

Table 2-1 Total quantities (kg) of antibiotics used from 2002 to 2004 as sourced from veterinary pharmaceutical companies, adapted from (Moyane, Jideani and Aiyegoro 2013).

| **Class of antibiotic** | **Amount (kg)** | | | **Total (kg) over 3 years** |
|---|---|---|---|---|
| | **2002** | **2003** | **2004** | |
| Penicillins | 49 465 | 55 677 | 59 688 | 165 717* |
| Cephalosporins | 5 470 | 3 321 | 3 316 | 12 107 |
| Tetracyclines | 58 342 | 71 842 | 58 974 | 257 755* |
| Aminoglycosides | 3 | 242 | 268 | 1 048* |
| Macrolides, lincosamides and pleuromutilins | 204 325 | 221 275 | 223 412 | 651 690* |
| Quinolones | 582 | 582 | 1 082 | 3 094* |
| Quinoxalines | 30 043 | 26 468 | 30 448 | 86 959 |
| Sulphonamides | 35 041 | 72 277 | 75 098 | 190 676* |
| Polipeptides | 27 011 | 26 985 | 42 191 | 69 820 |
| Ionophores | 14 736 | 5 582 | 43 674 | 69 820* |
| Glycolipids | 370 | 425 | 432 | 3 936* |
| **Total** | **425,388** | **484,676** | **538,583** | **1 538 443*** |

* Two of the eight veterinary pharmaceutical companies that provided data were only able to access their data for the three year period and not for each year individually.

Further, Eagar, Swan and van Vuuren (2012) revealed that a total of 234 registered antimicrobials were available for use as food in animals in South Africa. 72% were registered as stock remedies of the *Act 36 of 1947* and 28% were registered as antimicrobial of the *Act 101 of 1965*. Therefore most of the antimicrobials such as ionophores, macrolides, quinoxalines, polypeptides, streptogramins, glycolipids, oligosaccharides, phosphonic acids, and polymeric compounds were approved for usage in livestock production under both Acts. The annual increase in antibiotic consumption from 2008 to 2011 is shown in Table 2-2.

Table 2-2 Antibiotic utilisation in Units from 2008 to 2011 (Moyane, Jideani and Aiyegoro 2013).

| **Antibiotics** | **Year** | **Sum of MAT units,** | | | **Count of antibiotics in each class** |
|---|---|---|---|---|---|
| **ATC-Descriptor** | **2008** | **2009** | **2010** | **2011** | |
| J1A0 Tetracyclines + combs | 327 379 | 325 061 | 327 557 | 327 701 | 44 |
| J1B0Chloramphenicols + combs | 6 964 | 6 114 | 4 527 | 2 483 | 8 |
| J1C1 Broad-spect. penicill. oral | 10 683 704 | 11 441 888 | 11 962 722 | 12 305 433 | 277 |
| J1C2 Broad-spect. penicill. inj. | 551 335 | 1 251 442 | 1 133 503 | 1 463 327 | 45 |
| J1D1 Cephalosporins oral | 1 797 546 | 1 813 314 | 1 934 859 | 1 874 156 | 95 |
| J1D2 Cephalosporins inj. | 1 674 479 | 1 758 407 | 1 663 164 | 1 697 551 | 116 |
| J1E0 Trimethoprim combs | 3 261 544 | 4 021 542 | 3 300 302 | 3 316 420 | 124 |
| J1F0 Macrolides + similar type | 2 039 968 | 2 293 495 | 2 530 404 | 2 596 281 | 96 |
| J1G1 Oral fluoroquinolones | 3 242 849 | 3 617 302 | 3 635 646 | 3 832 065 | 95 |
| J1G2 Inj. fluoroquinolones | 479 409 | 554 631 | 565 952 | 584 255 | 21 |
| J1H1 Plain med.-/narrow-spect. penicillins | 419 243 | 386 095 | 485 923 | 435 640 | 42 |
| J1K0 Aminoglycosides | 80 624 | 87 089 | 83 880 | 80 349 | 41 |
| J1P1 Monobactams | 4 843 | 4 674 | 7 584 | 5 679 | 1 |
| J1P2 Penems and carbapenems | 679 147 | 809 668 | 916 184 | 1 019 767 | 8 |
| J1P3 Carbacephems | 7 652 | 15 512 | 23 191 | 69 908 | 3 |
| J1X1 Glycopeptideantibact. | 122 156 | 134 738 | 162 038 | 158 674 | 20 |
| J1X9 All other antibacterials | 15 132 | 14 361 | 15 849 | 16 229 | 10 |
| **Grand total** | **25 393974** | **28 535 333** | **28 753 285** | **29 785918** | **1 046** |

*MAT is the Moving Annual Turnover, *Number of the drug's formulation.

The grouping in Table 2-2 is based on the major categories of antimicrobials as per the World Health Organization (WHO) ATC classification system (Schellack et al. 2017). From the Table, there was an estimated sales unit from 25.3 to 29.8 million kilograms within the studied period. The broad-spectrum penicillins, fluoroquinolones, carbapenems and penems, carbacephems were the highest sold (Moyane, Jideani and Aiyegoro 2013).

Similarly, recently Charuaud *et al.* (2019) identified the most frequently used antibiotics worldwide for veterinary purposes to be tetracyclines sulphonamides, penicillins, and macrolides. These are also the dominant antibiotics found in South Africa.

Some of the antimicrobials such as tylosin that was still used in South Africa had been banned in Europe (Hao *et al.* 2014; Segura *et al.* 2015). Further still, 70% of the antimicrobials that were sold were found to be used in animal feeds. Unfortunately, South Africa has not aligned itself with the policies on the banning of the use of some of these antimicrobials with respect to the use of feed premixes for growth promotion (Eagar, Swan and van Vuuren 2012; Chollom *et al.* 2018a).

On a global scale, O'Neill (2015), studied the use of antimicrobials in agriculture and noted that consumption ranged from 63,000 and 240,000 tonnes per year in different countries. Van Boeckel *et al.* (2015), also estimated that global consumption will increase by 67% from 2010 to 2030. The BRICS countries would experience a 99% increase in the same time period as their population is projected to increase by 13%.

## 2.3 Selected veterinary antibiotics for the study

There are various classes of antibiotics that are administered for the treatment and prevention of diseases in livestock production as well as those that are administered to animals to improve their growth rate and feed efficiency (Sarmah, Meyer and Boxall 2006). There are two main origins of these antibiotics: (1) natural origin, some of which includes beta-lactam e.g. penicillins and cephalosporins; or protein synthesis inhibitors, such as aminoglycosides, macrolides, tetracyclines, polypeptides, etc. and

(2) synthetic origin which are produced in a way such that they are able to exert selective toxicity to target microorganisms (Renew and Huang 2004).

Over 3000 pharmaceuticals are currently in use worldwide. Analysing each of them would not be viable, therefore, prioritisation based on importance and usage is considered. The criteria for the selection of the antibiotics for the study were based on the following: (1) the relevance of the antibiotics to human usage, (2) usage amongst the different animal species, (3) their detection in wastewater treatment plants and environment. Table 2-3 highlights some of the important antimicrobials used by both humans and animals for the treatment and prevention of diseases.

Table 2-3: Major classes of antimicrobials shared by animals and humans (Leonard 2011).

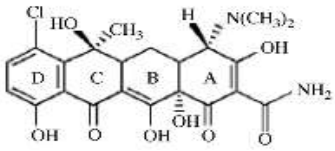
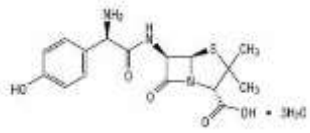
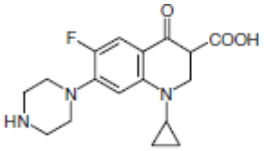
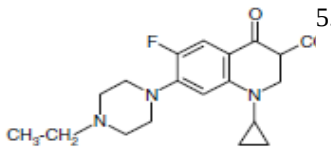
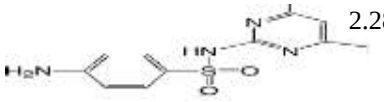
| Class | Antibiotics |
|---|---|
| β-lactams | Penicillin, amoxicillin; ceftiofur |
| Macrolides & lincosamides | Tylosin; tilmicosin; tulathromycin, lincomycin |
| Aminoglycosides | Gentamicin; neomycin |
| Fluoroquinolones | Enrofloxacin, danofloxacin |
| Tetracyclines | Tetracycline; oxytetracycline, chlortetracycline |
| Sulphonamides | Various |
| Streptogramins | Virginiamycin |
| Polypeptides | Bacitracin |
| Phenicols | Florfenicol |
| Pleuromutilin | Tiamulin |

Therefore, based on the criteria mentioned above and as shown in Table 2-3 the following classes of antibiotics were chosen for this study: β-lactams, fluoroquinolones, tetracyclines, and sulphonamides. In the β-lactams, amoxicillin was chosen while for the fluoroquinolones, ciprofloxacin and enrofloxacin were the chosen ones. In the class of the tetracycline and sulphonamides, chlortetracycline and sulfamethazine respectively, were selected.

Regarding their chemical nature, veterinary antibiotics are either amphiphilic or amphoteric, ionisable organic complexes that consist of a non-polar core and multiple polar functional groups (Sarmah, Meyer and Boxall 2006; Song and Guo 2014;

Bartikova, Podlipna and Skalova 2016). Table 2-4 shows the physical and chemical properties of the selected antibiotics.

Table 2-4: Physical and chemical properties of the selected antibiotics (Ahmed *et al.* 2015).

| **Antibiotics** | **MW** | **Class** | **Structure** | **pKa 1 and 2** | Log Kow |
|---|---|---|---|---|---|
| Chlortetracycline | 478.5 | Tetra-cycline |  | 3.3/4.5;7.3/9.33 | 0.09 |
| Amoxicillin | 365.4 | ßeta-Lactam |  | 3.2/11.7 | |
| Ciprofloxacin | 332.3 | Fluoroquin-olone |  | 6.68/8.63 | 0.28 |
| Enrofloxacin | 359.4 | Fluoroquin-olone |  | 5.86/8.24 | 0.70 |
| Sulfamethazine | 278.3 | Sulpho-namide |  | 2.28/7.42 | 0.14 |

### 2.3.1 Fluoroquinolone

The fluoroquinolones (FQs) are the third group of antibiotics that are commonly in use worldwide. They are reported to have about 17% of the global market share values.

Despite this high value, their usage in hospitals, households and as veterinary medicine is continually increasing (Van Doorslaer *et al.* 2014a; Ferreira *et al.* 2016). Fluoroquinolones are broad-spectrum antibiotics which have highly potent activity against pathogens that are of relevance in both human and veterinary medicine. They are used for prevention as well as therapy for diseases such as infections of the urinary, gastrointestinal, and respiratory tracts, sexually transmitted diseases, skin infections, chronic osteomyelitis and others (Anacleto *et al.* 2018). They are generally known as quinolones, however, some of the drugs contain a fluorine group and are therefore referred to as fluoroquinolones. From the structure of quinolones (Q's), they have carboxylic groups and as such are said to be acidic while FQs have an amino group in the heterocyclic ring (namely piperazinyl) (Riaz *et al.* 2018). Due to this distinction, the FQ's are sub-divided into two, namely; those with the acid-base properties and those with the heterocyclic group (Seifrtova *et al.* 2009).

#### 2.3.1.1 β-Lactam

This group of antibiotics forms one of the most important families used in veterinary medicines for the treatment of bacterial infections caused by susceptible, usually Gram-positive and Gram-negative organisms. The β-Lactam is named after the active components of the drugs compromising of the four membered β-Lactam rings and included is the seven-membered ring–structured cephalosporins and cephamycins. In addition to their chemical structure, the major difference between these two subclasses of β-lactams is their susceptibility to β-lactamase destruction, with the cephalosporins, in general, being more resistant (Samanidou, Giannakis and Papadaki 2009).

#### 2.3.1.2 Sulphonamide

The sulphonamides are synthetic bacteriostatic antibiotics, they have a wide spectrum against most gram-positive and many gram-negative organisms. They are characterised by a benzene ring, an amine moiety ($–NH_2$), and a sulphonamide group ($–SO_2NH_2$). The main veterinary compounds that are found in this group are sulfadiazine-trimethoprim, sulfadimethoxine, sulfamethazine, sulfathiazole and sulfadimethoxine-ormetoprim. They are said to be amphoteric and at a certain pH

range, are said to function as weak acids. Therefore they are seen as sodium salts with increased solubility as pH increases (Sarmah, Meyer and Boxall 2006). Most of the sulphonamides that are used for veterinary purposes have at least two nitrogen functions with the amide attached to the sulphur as shown in Table 2-4. They are usually deprotonated at pH 5.5-7. The amine that is attached to the aromatic cycle is said to be protonated at pH 2.5. Therefore under strong acidic conditions, they are positively charged while at alkaline conditions they are negatively charged and at pH 2.5-6, they are neutral (Sarmah, Meyer and Boxall 2006).

#### 2.3.1.3 Tetracyclines

Tetracyclines (TETs): chlortetracycline, oxytetracycline, and tetracyclines all belong in the same group and are used widely in livestock production in animal feeds to maintain health and improve growth efficiency in many countries (Sarmah, Meyer and Boxall 2006). The TETs are characterised to have positive properties such as a broad range of activities against Gram-positive and Gram-negative bacteria and the possibility of oral administration and side effects on organisms are limited. The other advantage of TETs to veterinarians is the fact that they are inexpensive and their cost in real terms is further reducing due to the improved manufacturing technology (Topal 2015). The TETs have a partially conjugated four-ring structure with a carboxyamide functional group, and the molecules are said to have several ionisable functional groups of which are said to be unusual with their charges dependent on the solution. Therefore, the multiple functional groups possessed by the TETs makes them exist either as cations, zwitterion or as a net negatively charged ion in the environment (Sarmah, Meyer and Boxall 2006; Seifrtova *et al.* 2009).

### 2.3.2 Regulations on antibiotics in the environment

It is becoming important for countries to develop regulations and monitoring schemes for anthropogenic activities of micro-organic pollutants in water sourses and the environment. There is need to establish threshold values (standards) for pollutants that put the water bodies at risk. The essence of this is to ensure the protection and improvement of the quality of water resources (Lapworth *et al.* 2012).

The contaminants are spatially distributed in the environment thus making it difficult to study and understand them. As a result, the insufficient knowledge on the toxicity, impact, behaviour, and limited monitoring data means that threshold values cannot be set as yet. This presents a great challenge in the development and implementation of policies. Progress has been made regarding the establishment of threshold limits for products of animal origin. For example, threshold values for antibiotics in food have been set at 4─1500 μg/kg for milk and 25─6000 μg/kg for the other foodstuff of animal origin (Homem and Santos 2011). Limits for drinking water as well as those of the environment are yet to be set. The fact that these limits do not exist presently does not mean that the negative impact of the presence of antibiotics is not felt (Homem and Santos 2011; Lapworth *et al.* 2012).

In recent years, significant efforts have been made in some advanced countries with regulations and policies, especially in protecting groundwater. In Europe, the regulatory framework Water Framework Directive (EC 2000) and Groundwater Daughter Directive (EC 2006) have requested that the threshold values (TVs) be set for all contaminants that affect groundwater bodies. Similarly, the USA has undertaken the monitoring of anthropogenic micro-organic pollutants (EPA 2002). The overall aim for the policies is the protection and improvement of water sources quality. The implementation of such policies was only possible due to the vast research relating to the importance of these contaminants in the environment. In most developing countries, little effort is being applied to curtail the contamination of water sources; this is mainly due to financial constraints among other factors.

### 2.3.3 Exposure pathways

The release of treated wastewaters into receiving water bodies is a common practice to all wastewater treatment plants (WWTPs). The WWTPs have been identified as hot spots through which emerging contaminants such as the antibiotics have been introduced into the environment. The reason is due to the inability of the WWTPs to completely remove these contaminants. Figure 2-1 describes the possible routes

through which these contaminants could get into the environment. Their behaviour in the environment has been of interest in recent years.

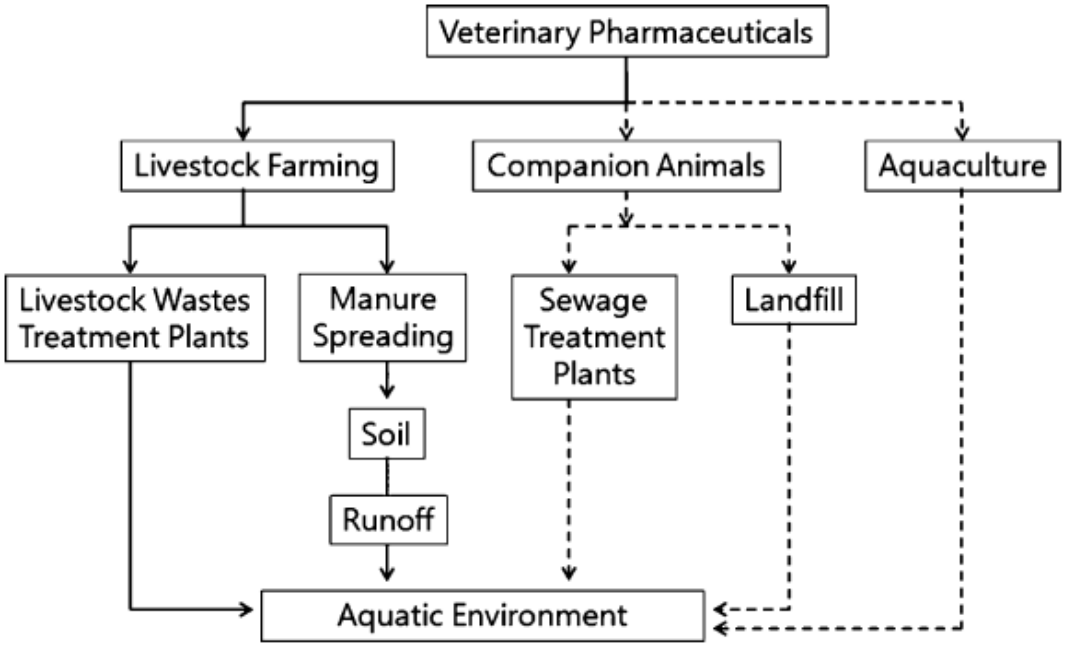



Figure 2-1: Routes of veterinary pharmaceuticals entering the aquatic environment *Solid line indicates major contribution pathways, while the dotted line represents relatively minor contribution pathways into the environment* (O'Neill 2015).

Antibiotics can enter the environment during the manufacturing process. This is a huge challenge in places like China, India and other developing countries where they are manufactured. For example in India, ciprofloxacin with concentrations as high as 2·5 mg/L were reported in river water downstream of a wastewater treatment plant receiving wastewater from 90 bulk drug manufacturers. Other antibiotics were also detected in the river, including enoxacin, enrofloxacin, lomefloxacin, ofloxacin, and trimethoprim (Wellington *et al.* 2013).

Veterinary antibiotic pathways to the environment are little different from those of human. The main pathway for the transport of human pharmaceuticals into the environment is predominantly through sewage treatment plants. However, various pathways exist for veterinary pharmaceutical. This can be through livestock waste

treatment plants but also through direct application in aquaculture, manure wash-off from topical treatments and through the application of sludge or animal manure (containing excreted products) to land (Boxall *et al.* 2003).

It is a common practice in most countries to intentionally use treated effluents for the supplementation of drinking water supplies as well as for agriculture. However, the treated effluent normally contains some amounts of contaminants in it. Different concentrations of antibiotics have been detected in the various environmental compartments. For example, in animal manure, higher concentrations of antibiotics in mg/kg can be detected. However, those measured in surface waters are lower. In general, concentrations are in the higher µg/L range in hospital effluent (sometimes, it could be ng/L or µg/L in municipal wastewater) and lower ng/L range in different surface waters, groundwater and sea water (Kümmerer 2009).

### 2.3.4 Physicochemical properties of pharmaceutical compounds

The structural and physicochemical properties of pharmaceuticals impacts on the interactions between the compounds and the targeted microorganisms within an environment. It is therefore important that synthesis of new molecules should correlate to the structural characteristics of the compounds with their physicochemical and biochemical characteristics (Di and Kerns 2015; Anacleto *et al.* 2018). The structural characteristics usually define the physical nature of the molecules some of which are: molecular weight, number of H-bonds, lipophilicity, acid dissociation constant (pKa) as well as the reactivity of the molecule. Molecules with similar structures frequently have the same modes of action and belong to the same chemical class (Kummerer 2009a; Kümmerer 2010; Di and Kerns 2015).

The solubility and chemical stability are some of the physicochemical properties which describe the interaction that would occur between the compound and its physical environment. Therefore, both the physicochemical properties and the structural characteristics that would influence the behaviour of the compounds within the target organism are also responsible for their interaction in the environment (Kümmerer 2010). The extent of microbial degradation and the partitioning between the aqueous phase and

the solid phase are factors to be considered. As a result of the differences in properties, the study of pharmaceuticals within the environment becomes difficult, and as a result researchers often have to evaluate how the compounds behave within the structural properties so as to ascertain the mechanistic performance of the biotic and abiotic removal processes (Charuaud *et al.* 2019).

### 2.3.5 The fate of Veterinary antibiotics in the environment

Antibiotics have been found in various water sources including ground waters, and in marine sediments. The understanding of the possible risks, fate and behaviours of these antimicrobials is very important. Different routes through which antibiotics could reach the environment exist as illustrated in Figure 2-1.

Due to the demand for animal products, farmers have adopted different practices to meet the demand. One of such is intensive farming whereby large numbers of animals are confined in a small space resulting in high stocking density. The confinement of these animals is carried out under extreme conditions and often, the animals are overcrowded in the little space, hence are susceptible to diseases. As a result, the use of antimicrobials to prevent the spread of diseases and to maintain the welfare of the animals becomes inevitable (Kumar *et al.* 2005; Song and Guo 2014).

The detection of antibiotics from the environment and WWTPs is often affected by their properties and chemistry. The properties and chemistry affect their mobility, persistence, and bioavailability in the environment. The physiochemical properties such as molecular structure, size, shape, solubility, and hydrophobicity greatly determine the amount and type of antibiotics that would be found. Others such as properties of the soil which includes texture, pH, and organic matter content play a major role too (Puckowski *et al.* 2016). Due to the variations in the properties of these antibiotics, they behave differently in the environment which is based on their interaction with solids and liquids. As a result, a series of phase transformation processes such as biodegradation, dilution, and photolysis occurs once the antibiotics are in the environment.

Antibiotics are said to be amphipathic (having both hydrophilic and hydrophobic properties) amphoteric, ionisable organic compounds which consist of polar or nonpolar cores as well as multiple polar functional groups (Wang and Wang, 2015). They are said to be made up of multiple ionic functional groups and acid dissociation constant (pKa). The pKa values are related to different functional groups thereby showing positive, neutral and negative valences with changes in pH values. Another property that affects the behaviour of the antibiotics is the partition coefficient (*Kow*), which is related to their solubility. The differences between *pKa* and *Kow* reflects differently on the way they react to water, their solubility, volatility, ultraviolet (UV) absorption and their reactiveness with other chemical oxidants (Qiang and Adams 2004; Hörsing *et al.* 2011; Wang and Wang 2015). The ability of antibiotics to either remain in an aqueous form or be adsorbed to the solid is defined by their hydrophobicity and hydrophilicity. The more hydrophilic pollutants have a greater tendency of remaining in the aqueous phase while the hydrophobic ones adsorbs onto the solids. As a result, the existence of these contaminants in the environment is defined by their hydrophilicity and hydrophobicity. Furthermore, their chemical partitioning behaviour (Kow) and the organic carbon normalized sorption coefficient (KOC) are important factors to be considered. Caliman and Gavrilescu (2009) reported that if Log $K_{OW}$ <2.5, then contaminants have low sorption, hence would be adsorbed onto the solids, whereas, if 2.5 < log Kow <4.0, it would mean a medium sorption potential. However, if the Log $K_{OW}$ > 4.0, then a high sorption ability would occur.

### 2.3.6 Effects of Veterinary antibiotics

The extensive use of antibiotics for veterinary purposes has been linked to the presence of antimicrobial resistant bacteria (ARBs) and anti-microbial resistant genes (ARGs) in the environment (Van Boeckel et al. 2015; O'Neill 2016; Spellberg et al. 2016). Even at trace levels, there is a high biological activity that is related to these contaminants and thus they have been proven to cause significant damage in the biosphere. The ARBs have been defined by O'Neill (2016) as microorganisms which causes disease infection survives exposure to medicines that would normally have killed or stopped their growth. The spreading of ARBs from breeding places to human

could take many different forms such as through soil and groundwater, direct contact with contaminated farm workers who then spread these bacteria through human communities and direct contact with contaminated objects, and finally through contaminated meat during the butchering process (Van Boeckel *et al.* 2015; O'Neill 2016; Spellberg *et al.* 2016). There are other factors that have been listed as the possible enhancers of the ARBs such as poverty, suboptimal control of the sale, quality and indiscriminate use of the antimicrobials. Other hotspots identified for the ARBs are WWTPs and sewages (Angulo *et al.* 2009; Chollom *et al.* 2018a).

Several attempts have been made to correlate antibacterial use and the prevalence of resistance in *Escherichia coli* (Kim *et al*. 2018). Chantziaras *et al.* (2014) correlated antimicrobial use and the prevalence of resistance in *Escherichia coli* which were isolated from pigs, poultry, and cattle, and demonstrated a strong linear relationship based on the data from seven European countries (Norway, Sweden, Denmark, Austria, Switzerland, Netherlands, and Belgium) (Chantziaras *et al.* 2014).

Most of the antibiotics that are administered to animals are not properly absorbed in the guts of the animals due to the fact that a fraction of the un-absorbed antibiotics finds its way into the environment (Kumar *et al.* 2005). Detection levels of these pharmaceuticals are usually in ng/L or µg/L. Even at these low concentrations, the rapid development of ARBs and ARGs has been linked to their presence. The following antibiotics have been identified to cause toxicity to freshwater microalgae (cyanobacteria), aquatic and soil organisms, and fish: trimethoprim, enrofloxacin, ciprofloxacin, sulfaquinoxaline, monensin, narasin, and salinomycin (Kim *et al.* 2018). Ciprofloxacin, a metabolite from enrofloxacin and also an important antibiotic in human medicine has been detected at concentrations around 10 µg/L and has been proven to exert a negative impact on amphibian larvae growth and development even at the low concentration mentioned above (Charuaud *et al.* 2019).

Cooper, Siewicki and Phillips (2008) reported that the presence of fluoroquinolone antibiotics exerts genotoxic effects on the genetically modified bacterial strain *Salmonella typhimurium* at concentrations as low as 5 µg/L for norfloxacin and 25 µg/L for ciprofloxacin. Most of the bacteria that cause infections in humans are said to

be found in livestock, for example, *Enterobacteriaceae* has been found in livestock and retail meat which contained pathogens such as *Escherichia coli* and *Klebsiella.* These pathogens have been identified to be the common causes of urinary tract infections and among the common causes of bloodstream infections in patients (Spellberg *et al.* 2016; Alonso *et al.* 2017). Similarly, *Staphylococcus aureus* which is the major cause of skin infections and the second common cause of bloodstream infections in patients has also been found on the skin of livestock and on retail meat (Spellberg *et al.* 2016). According to Spellberg *et al.* (2016), there is an ease in the movement of these pathogenic organisms between farm animals and humans and also from humans to other humans in the community and in health care settings. The gut and skin bacteria account for a significant proportion of the antibiotic-resistant infections and resulting deaths in the United States and throughout the world (Spellberg *et al.* 2016).

## 2.4 Treatment methods for wastewater containing antibiotics

Conventional wastewater treatment methods are used for the treatment of wastewaters. In most instances, conventional methods are divided into primary, secondary, tertiary and advanced methods of treatment. Table 2-5 gives a classification of common wastewater treatment processes. These processes are based on their level of advancement and are employed at different stages during wastewater treatment.

Table 2-5: Classification of common wastewater treatment process (Veenstra, Alaerts and Bijlsma 1997).

| Primary | Secondary | Tertiary | Advanced treatment |
|---|---|---|---|
| Bar or bow screen | Activated sludge | Nitrification | Chemical |
| Grit removal | Extended aeration | Denitrification | Membrane technology |
| Primary sedimentation | Aerated lagoons | Chemical precipitation | |
| Comminution | Trickling filter | Disinfection | Carbon adsorption |
| Oil/fat removal | Rotating bio-discs | Direct filtration | Selection ion exchange |
| Flow equalisation | Anaerobic treatment/UASB | Chemical oxidation | Hyper-filtration |
| pH neutralisation | Anaerobic filter | Biological phosphorus removal | Advanced oxidation |
| Imhoff tank | Stabilisation ponds | Constructed wetland | Detoxification |
| | Constructed wetlands | | |

The choice for the methods of treatment depends on the degree of pollution in the wastewater. The primary processes form the basis of the water treatment processes. A few examples include screening, flow equalization, sedimentation, flotation, and granular-medium filtration. The primary and secondary treatment produces sludge which is about 0.5% of the total wastewater flow. In most instances, micro-pollutants such as antibiotics and other emerging contaminants are sorbed onto the sludge.

Chemical processes which are the tertiary processes involve the use of chemicals such as disinfectants for disinfection and other chemically assisted primary treatment, for example using ferric salts or polyelectrolyte, to remove BOD and solids (coagulation and flocculation). They are always used in conjunction with physical unit operations

and biological processes. The major challenge is the generation of large amounts of sludge which needs to be discharged. The tertiary treatment processes are designed to remove nutrients such as ammonia, phosphorus, total nitrogen which is comprised of (Kjeldahl-N, nitrate and nitrite). The advanced processes are employed to wastewaters specifically from the industries for the removal of specific contaminants. This is important where high quality effluent is required and when reclamation is carried out for reuse purposes (Veenstra, Alaerts and Bijlsma 1997; Dan Eddy 2003).

Biological treatment processes are able to convert the organic matter in wastewaters using the microorganism such as bacteria (aerobically or anaerobically), algae, and fungi to form carbon dioxide and other compounds such as ammonia and phosphorous which are thereafter used as fertilizers. Biological systems are used in WWTPs which are engineered to boost biochemical degradation under carefully controlled conditions, thereby enhancing the removal of pollutants and the stabilization of sludge (aerobic or anaerobic) (Dan Eddy 2003). These systems have been grouped by Dan Eddy (2003) into: aerobic, anoxic, anaerobic, and pond processes. These processes can also be classified into suspended-growth and attached-growth system or a combination of both depending on the treatment objective.

Generally, the WWTPs are effective in the removal of organic pollutants in wastewaters, however, they are not as effective in the degradation of micropollutants such as antibiotics. Only partial degradation is achieved. As noted in **section 1.2.1,** the ability to remain either in the liquid form or be adsorbed onto the solids also plays a great role, hence the antibiotics would behave in the same way if they were in the environment. In the WWTPs, the antibiotics interact with solids contained in the wastewaters or with chemicals such as flocculants/coagulants which are usually added to the treatment process. The compounds that have low adsorption capacities tend to remain in the aqueous phase, and thus are discharged in the final effluent. On the other hand, compounds that have a great affinity for the sludge may be removed either by physical-chemical processes such as coagulation/flocculation, settling and floatation. It has been reported that the ability to be adsorbed onto activated sludge is a key factor

for the removal of organic micropollutants in conventional WWTPs (Gonzalez-Gil *et al.* 2016).

Sorption of the antibiotics occurs mostly in the primary treatment and in the activated sludge process. This is due to the fact that they are the first sections to receive the influent waters. In the secondary sludge, the sorption occurrence level is minimal, however, for sludge that is digested, the main path of removal is biodegradation rather than sorption (Alvarino *et al.* 2018).

An overestimation of the sorption of the antibiotics could be made during activated sludge processes; therefore, the monitoring of sorption efficiency is important. It can be used to predict the influences and fate of the antibiotics in WWTPs. The affinity of the antibiotics to the sludge is defined by the partitioning coefficient $Kd$. This is the ratio of the equilibrium concentrations of the antibiotics either in the sludge or aqueous phase, it is the most suitable to determine affinity or sorption of antibiotics to the solid phase. It is used to understand the partitioning behaviour of the antibiotics between the sludge and aqueous phases. It is therefore one of the important indicators of their fate in the WWTPs.

### 2.4.1 Slaughterhouse wastewater characteristics

The meat industry is one of the fastest growing industries globally. A study by Wang, Jena and Das (2018) on bio-methane production potential from slaughterhouse waste in the United States (US), observed that about 41.5 million metric tonnes (MT) of meat (chicken, pork and beef) is produced annually. They observed that almost an equal amount of wastewater is produced from the meat processing, thus indicating the huge amounts of wastewater consumed by such industries. Different stages are involved during the meat processing and as such the consumption of fresh water differs from one operation to another, however, the slaughtering line is said to contain the highest amount of contaminants.

Usually, the wastewaters contain high amounts of biodegradable organic matter, with soluble and insoluble fractions (Bustillo-Lecompte and Mehrvar 2015). Therefore, the

wastewater is characterised by fats, proteins and fibres thus leading to high organics some of which contribute to the chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS) and so on (Bustillo-Lecompte and Mehrvar 2015; Chollom et al. 2017). Table 2-6 shows the general characteristics of a slaughterhouse wastewater.

Table 2-6: General characteristics of a slaughterhouse wastewater (Bustillo-Lecompte and Mehrvar 2015).

| Parameter | Units (mg/L) | Range | Mean |
|---|---|---|---|
| TOC | mg/L | 700-1200 | 546 |
| BOD | mg/L | 150-4635 | 1209 |
| COD | mg/L | 500-15,900 | 4221 |
| Total nitrites | mg/L | 50-841 | 427 |
| Total suspended solids (TSS) | mg/L | 270-6400 | 1164 |
| pH | | 4.90-8.10 | 6.95 |
| Total phosphorus (TP) | mg/L | 25-200 | 50 |
| Orto-$PO_4$ | mg/L | 20-100 | 25 |
| Orto-$P_2O_5$ | mg/L | 10-80 | 20 |
| K | mg/L | 0.01-100 | 90 |
| Colour | FAU* | 175-400 | 290 |
| Turbidity | NTU* | 200-300 | 275 |

*FAU is formazine attenuation units (FAU) while NTU is nephelometric turbidity units.

From Table 2-6, it is observed that most of the measured parameters which are measured during treatment are much higher than domestic wastewaters and even higher than those from other industries. Because of the nature of contaminants from

the slaughterhouses, their treatment poses some challenges when compared to other agricultural processing industries. Problems encountered during treatment are the high suspended solid fats and protein contents. Another issue is the fact that some of the fats are insoluble thus lowering the rate of degradation and increasing the tendency to form scums. Therefore anaerobic digestion (AD) is commonly used. The AD processes have been the most used for slaughterhouse wastewater treatment due to the advantages they offer which are due to benefits of bio-methane and low environmental footprints e.g. greenhouse gas reduction, nutrient recovery, etc. (Cao and Mehrvar 2011; Bustillo-Lecompte and Mehrvar 2015; Wang, Jena and Das 2018).

Apart from the organic contaminants that are found in these wastewaters, other emerging contaminants such as veterinary pharmaceuticals have been detected (Boxall 2012; Carvalho *et al.* 2013a). Studies have indicated that the wastewater treatment plants from the animal wastewaters have been identified to be the hotspot for pharmaceutical contaminants. The discharge of pharmaceuticals from these treatment plants into receiving water bodies has increased the concerns of antibiotics and resistant bacteria been eventually introduced into the drinking water systems (Barra Caracciolo, Topp and Grenni 2015). Even though some studies have been carried out regarding veterinary contaminants from slaughterhouses, it has not received the attention that those from the domestic wastewaters have received.

### 2.4.2 Anaerobic Digestion

Anaerobic digestion is a multi-stage, biological process in which different groups of bacteria work cooperatively to break down complex biodegradable matter in the absence of oxygen (Khemkhao *et al.* 2012). It is one of the oldest biological process technologies and is used to treat industrial wastewaters. Recently, anaerobic digestion technology has enjoyed much attention as the biogas produced from the process is a source of renewable energy (Daud *et al.* 2018; Granada *et al.* 2018). The biogas produced consists of methane, carbon dioxide and traces of other contaminant gases. The biogas can be used as fuel for cooking or in heat and power gas engines. The use of biogas as a fuel helps to replace fossil fuels while the nutrient-rich digestate can be

used as fertilizer. Many medium- scale anaerobic plants have sprung up for the purpose of generating power from domestic or industrial organic wastes (Appels *et al.* 2008; Ware and Power 2016; Daud *et al.* 2018).

Different classes of microorganisms can be found both in the aerobic and anaerobic processes; the vast majority of them are bacteria (Wang *et al.* 2018). Advantages of the anaerobic digestion (AD) over aerobic processes are: the AD uses the readily available carbon dioxide ($CO_2$) as an electron acceptor, requiring no oxygen (Daud *et al.* 2018). Lower quantities of sludge are produced, about 3-20 times less than aerobic processes. This is due to the fact that the energy yield of anaerobic bacteria is low. The highest energy is derived from the breaking down of the substrate and it is found in the final product which is methane ($CH_4$) (Speece 1983). Finally, AD is suitable for high- strength industrial wastes, like those from the slaughterhouse wastewaters.

Despite the abovementioned advantages of the AD system, it has some disadvantages such as: it is a slower process than aerobic digestion; it is more sensitive to upsets by toxicants; and the start-up of the process requires longer time periods (Xie *et al.* 2016).

#### 2.4.2.1 Mechanism of Anaerobic Digestion

A variety of microorganisms is required to break down complex macromolecules present in sewage to produce biogas. Four different phases (hydrolysis, acidogenesis, acetogenesis and methanogenesis) can be distinguished in the overall conversion process of proteins, carbohydrates, and lipids (Figure 2-2).

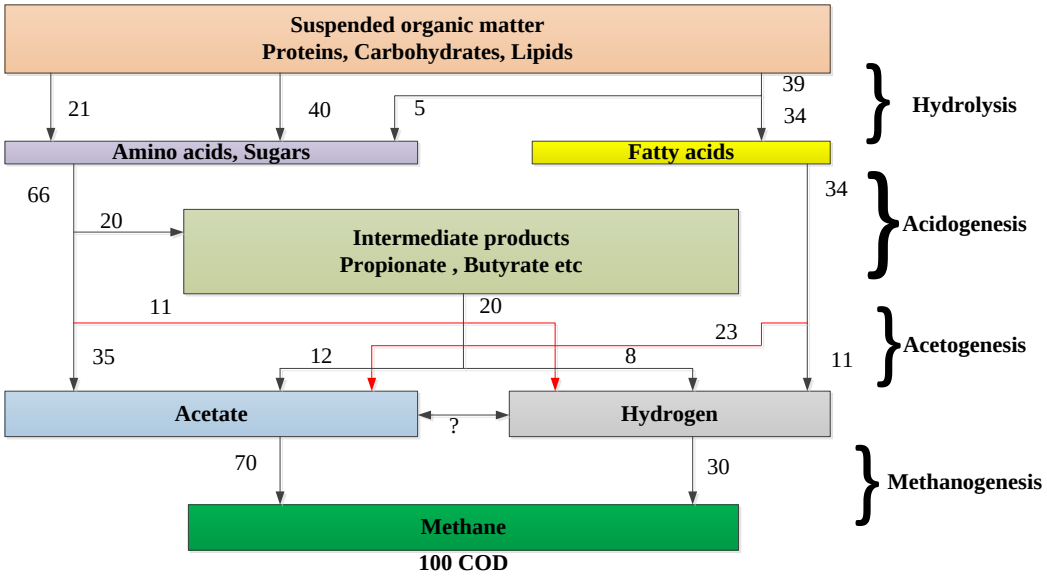



Figure 2-2: Reaction sequence for the anaerobic digestion of complex molecules; the numbers refer to percentages of conversion (Appels *et al.* 2008)

***Hydrolysis***

This is the first stage of digestion where the complex macromolecules are broken into simpler substances by the facultative and obligate bacteria. The proteins are degraded through the (poly) peptides to amino acids, while carbohydrates are transformed into soluble sugars (mono and disaccharides). The lipids are converted to long chain fatty acids and glycerine. The hydrolysis step is the limiting step for the overall rate of anaerobic digestion in most cases (Appels *et al.* 2008; Venkiteshwaran *et al.* 2015).

***Acidogenesis***

The bacteria utilized in this process make up 90% of the total bacteria in the reactor. The processes here can be divided into hydrogenation and dehydrogenation. The conversion of simpler organic compounds such as the volatile fatty acids, alcohols, formic, acetic, propionic, butyric, lactic acids and mineral compounds to carbon dioxide ($CO_2$), hydrogen, ketones and alcohols. This can cause a buildup of electrons which respond to a small rise to the concentration of hydrogen in the solution. Therefore, the new products that are formed are probably not used up in a direct way by the methanogenic bacteria and thus be converted to obligate anaerobes producing hydrogen (Venkiteshwaran *et al.* 2015).

***Acetogenesis***

The products of acidogenesis are converted into the final products for methane production: acetate, hydrogen and carbon dioxide. Approximately 70% of the COD that is usually in the influent is converted into acetic acid and the remainder of the electron donor capacity is concentrated in the hydrogen that is formed as illustrated in Figure 2-2. The acetogens are obligate hydrogen-producing bacteria that can only survive at very low $H_2$ concentrations. Acetogenesis occurs at low $H_2$ partial pressure, thus for the acetogenic bacteria to maintain the low pressure, the bacteria live in symbiosis with the hydrogen-utilizing methanogens. This is especially possible only when the reactor is operated at an optimal temperature and pH levels (Venkiteshwaran *et al.* 2015).

***Methanogenesis***

Methanogenesis is frequently said to be the rate-limiting step generally during the digestion process. Hydrolysis could however be the rate-limiting step at lower temperatures. Methane which is one of the main gases is formed from acetate or the reduction of carbon dioxide ($CO_2$) by hydrogen which uses the acetotrophic and hydrogenotrophic bacteria respectively as shown in equations 2-1 and 2-2 (Krzysztof 2012).

acetotrophic methanogenesis:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad 2\text{-}1$$

hydrogenotrophic methanogenesis:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad 2\text{-}2$$

The bacteria that produce methane from $H_2$ and $CO_2$ grow faster than those that utilise acetate and as such the acetotrophic methanogens are normally the rate limiting. This is with regards the transforming of the complex macromolecules in sewage to biogas. A number of different groups of bacteria are involved in the conversion of influent organic matter and anabolic and catabolic activities occur here. Therefore, as the release of several fermentation products occurs, the production of new biomass occurs

and this is associated with the four processes described above. The first three processes could be lumped together; however, the acid fermentation would be denominated, whereas the last stage would be the methanogenic fermentation.

There are two points which describe the different stages which are worth noting (Krzysztof 2012; Xie et al. 2016): (1) the removal of organic matter (COD), which occurs during the acid fermentation stage and is limited to the releasing of $H_2$. Figure 2-2 shows that only 30% of the organic matter is converted into methane through the hydrogenotrophic process. Therefore, it is important that there is a sufficient amount of acetotrophic methanogens that is developed; (2) the acid fermentation causes a reduction in the pH of the produced volatile fatty acids with other intermediates that dissociate to produce protons. This may cause instability in the system because the methanogenes are only produced at neutral pH. Once there is an imbalance between the acid removal by methane production and the acid production rate, the system could begin to sour leading to failure of the system. This usually is not good for the operation of the AD process. To avert this, it is important that a balance between the acidic and methanogenic fermentation is maintained (Appels et al. 2008; Krzysztof 2012; Xie et al. 2016).

### 2.4.3 Anaerobic Digesters

There are a variety of digesters available which have different characteristics and operational philosophies. The most prevalent types are shown in Figure 2-3.

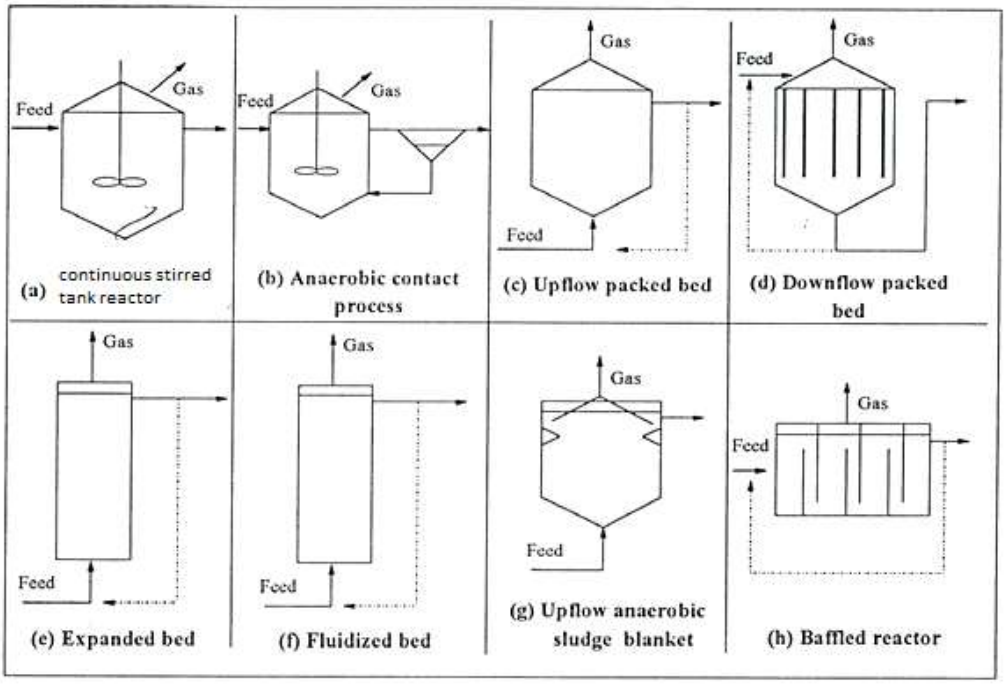



Figure 2-3: Anaerobic digester configurations (Appels *et al.* 2008).

The continuous stirred tank reactor (CSTR) contains a vertical shaft with a number of impellers and baffles for effective agitation. Mixing in anaerobic digestion is beneficial as it gets rids of scum and thermal stratification as well as promotes contact between the bacteria and the degradable organic substrate (Chernicharo 2007). The major disadvantages of completely mixing the digester is the fact that it is expensive and as well it is essential for the facility to separate the digested solids from the liquid phase. This reactor requires a significant hydraulic retention time (HRT) and solids retention time (SRT) for the biological conversions to occur. CSTR systems are susceptible to failure if shock loading occurs.

The anaerobic contact process is comparable to the CSTR except that there is a separator on the effluent line to separate the solids from the exiting liquid. These solids (biomass) are separated through settling and are recycled back to the reactor. The system has a better contact between the biomass and the substrate and it also has a high SRT. The unit is capable of handling 1-8 kg COD/$m^3$/ day and can obtain 85- 95 % removal efficiency. The settled solids or flocs are recycled at a moderate rate back to

the reactor to prevent shear forces from disrupting the floc structure (Rajeshwari *et al.* 2000).

The up-flow anaerobic sludge blanket (UASB) is designed to treat high volumetric wastewater with medium to high strengths. The most distinguishable feature of UASB is the gas-liquid- solids (GLS) separator which is located at the top of the reactor. It divides the reactor into the lower digestion zone and the upper settling zone. There is no supporting medium and the process is based on the immobilization of the biomass in the form of sludge granules. The highly flocculated granular biomass is retained in the reactor and this leads to good conversions. The wastewater enters through the bottom of the digester and flows upwards through the blanket of granules. The sludge of the blanket converts organic compounds to biogas (Lettinga and Pol 1991; Van Haandel and Lettinga 1994; Rajeshwari *et al.* 2000).

In the fluidized bed (FB), a granular material is kept fluidized as a result of the frictional resistance of the waste flow. There are a variety of granular media available such as sand, anthracite, and plastic. There were considerable difficulties in controlling the particle size and density of the flocs to such an extent that it is impossible to guarantee a stable process performance (Van Haandel and Lettinga 1994; Rajeshwari *et al.* 2000). The anaerobic attached- film expanded bed (AAFEB) is similar to the FB but only differs by the lower inflow velocity which only expands the bed by 10-20% (Switzenbaum and Jewell 1980; Appels *et al.* 2008).

The anaerobic packed bed reactor was first proposed by Young and McCarty (1969). Biomass is attached on an inert support material in biofilm form. The material/medium can be made out of different substances (plastics, granular activated carbon (GAC), sand reticulated foam polymers, granite, quartz, and stone) and can be packed in either a loose or granular form (Rajeshwari *et al.* 2000). The material that is not attached remains in the interstices between the medium; some by settling and others through the effect of the physical contact with the medium. Low velocities are upheld to avoid the washing out of the biomass that is not attached which is significant in the treatment activity. A disadvantage of the system is the possibility of blockages as a result of the buildup of non-attached biomass (Rajeshwari *et al.* 2000).

The anaerobic baffled reactor (ABR) consists of a series of baffles under which the wastewater flows. This results in increased contact time with the active biomass (sludge) and improved treatment performance. The majority of settleable solids are removed in the sedimentation chamber and the upflow chambers provide additional removal and digestion of organic matter. As sludge is accumulated, desludging is required every 2 to 3 years. Critical design parameters include a hydraulic retention time (HRT) of between 48 to 72 hours, up-flow velocity of the wastewater less than 0.6 m/h and the number of up-flow chambers (Rajeshwari *et al.* 2000).

### 2.4.4 Biodegradability, Activity, Inhibition, and Toxicity

Acclimation or acclimatisation is a process in which organisms adapt to gradual changes in their environment. The acclimation of anaerobic biomass to unconventional substrates and inherent toxicity has been demonstrated. However, its success is dependent upon having an acclimation period of 30-60 days prior to assaying. Assessment of anaerobic treatability before the attainment of the acclimation period may result in wrong conclusions and this may lead to misjudgment of the anaerobic treatability (Speece 1983; Speece 1996).

**Biodegradability**

Biodegradability refers to the susceptibility of a substance to undergo a biologically mediated degradation and the extent to which the degradation is achieved. It is known as ultimate biodegradability if the organic substances are transformed into products that cannot be degraded further; or primarily, if the chemical structure of the parent compound is changed to another such that a loss in the specific property of the original and as such produces products that may also be biodegradable. It is inherently biodegradable when the biodegradation is only possible if specific steps are taken; such as pre-exposure of the inoculum to a substrate, increased test duration and/or higher food-to-microorganisms ratios (McDonough *et al.* 2017). Biodegradability is expressed as the mass of the test substance transformed within a given period of time, as compared to the theoretical mass that could be converted stoichiometrically, i.e. based on its elemental analysis or the COD of the test material. The anaerobic

biodegradation of substances can be described by the following equation 2-3 (Krzysztof 2012):

$$COD_O + X_O \rightarrow COD_F + X_F + Gas \qquad 2\text{-}3$$

Where, COD = Chemical Oxygen Demand (mg/L), $X$ = Biomass Concentration (mg/L), $F$ = Final state, $O$ = Initial state, $Gas$ = Net biogas produced (mL).

If neither the chemical formula nor the carbon content of the test material is available, then the theoretical biogas production can be calculated by measuring the COD.

**Activity and inhibition**

Activity indicates the inherent ability of a microbial population to undertake degradation of the test material. It is measured as the specific rate of substrate consumption, referred to as either the total biomass (e.g. volatile suspended solids) or the targeted microbial population. Bioassays have been established since the 1970s, that employ serum bottles and measures the pressure increase or the volume of the liquid displaced by gas production and/or determine the composition of the gaseous phase by a gas chromatographic technique. Activity can also be assessed under non-limiting or limiting substrate concentrations. The two approaches are equivalent if anabolism and catabolism (i.e. growth and energy generation, respectively) are assumed to be coupled by a proportionality factor, as occurs in steady-state conditions (Chen, Cheng and Creamer 2008).

### 2.4.5 Factors affecting anaerobic treatment

#### 2.4.5.1 Solids

Solids refer to the suspended or dissolved matter in water and wastewater and may affect water or effluent quality. The solid analysis is vital in the control of biological wastewater treatment process like anaerobic digestion. There are many types of solid analysis that is critical and must be monitored. These are:

**Total Solids**: is the material residue left in the vessel after evaporation of a sample and its subsequent drying in the oven (Chernicharo 2007).

**Total suspended solids**: are solids in water that can be trapped by a filter. They include a wide variety of materials, such as silt, decaying plant and animal matter, industrial wastes, and sewage.

**Volatile solids**: is the weight loss of total solids upon ignition at 550℃.

**Volatile suspended solids**: is the weight loss of total suspended solids upon ignition at 550 $^{O}$C. The suspended solids are retained on a 2 µm glass filter (Chernicharo 2007).

#### 2.4.5.2 Volatile Fatty Acids (VFA's) and Bicarbonate Concentrations

The alkalinity of the system is a very good indicator when measuring the stability of an AD process. When dealing with alkalinity, the bicarbonates and volatile fatty acids (VFAs) are important to be considered. The bicarbonates are responsible for the buffering of the system at the optimum pH range. This is possible when the bicarbonates are maintained by the production of $CO_2$ (Padilla-Gasca, Lopez-Lpoez and Gallardo-Valdez 2011). The AD process consists of a train of biological activities and $CO_2$ and VFA are produced first, in addition to $H_2$, by acidogenesis and acetogenesis. Successively, methanogenesis occurs in two forms. It has been established that the Acetotrophic methanogenesis makes use of the VFA which is the acetate form only to produce methane and $CO_2$. The Hydrogenotrophic methanogenesis uses the $CO_2$ and $H_2$ to produce methane. The challenge, however, is the fact that methanogenic bacteria are more delicate as compared to acidogenesis and acetogenesis. Therefore, in the case of any toxicity, they are easily inhibited (Chen, Cheng and Creamer 2008).

As a result, the VFA starts to accumulate while $CO_2$ continues to be stripped to the gas phase thereby lowering the pH which causes the biological activities to be inhibited. An additional effect is a mutual relationship between both forms of methanogenesis. For instance, if the hydrogenotrophic form is inhibited, $H_2$ will start to build up to a level that inhibits acetogenesis and therefore less acetate becomes available for the

other form. VFA's, as well as lactate, will start to build up in the reactor (Xie *et al.* 2016).

#### 2.4.5.3 Chemical Oxygen Demand

The chemical oxygen demand (COD) is a commonly used testing method for determining organic content. It is based on the principle that all organics are oxidisable. A strong oxidizing agent, such as a mixture of dichromate and sulphuric acid, and silver sulphate (catalyst) are added to the test sample. The concentration of organic material can be calculated from the decrease in the dichromate concentration, which can be determined titrimetrically or spectrophotometrically (Van Haandel and Lettinga 1994). COD measurement of the feed (influent), biomass and effluent material will answer many questions related to the type of reactor to be used and the operational methodology. A high COD feed should result in higher biogas production as there are more organics available for degradation. However, the biogas production is also dependent on the biodegradability of the substrate (Abyar *et al.* 2017).

#### 2.4.5.4 Carbon/Nitrogen (C/N) Ratio

This is the ratio of the total elemental carbon to the total elemental nitrogen present in the wastewater or the digester. The nitrogen is used by the bacteria for growth and metabolism and the carbon is converted to methane and carbon dioxide. A high C/N ratio is favourable whereas a low ratio may result in system failure as there may be an accumulation of ammonia (Abyar *et al.* 2017).

#### 2.4.5.5 pH

Speece (1996) recommends a pH of 6.5 to 8.2 within the reactor. As the microbes can alter the pH of the feedstock, he observed that it was wasteful and possibly detrimental to try and neutralise the wastewater. The value and stability of the pH are extremely important as methanogenesis can only proceed at a high rate when the pH is maintained in the neutral range. At pH less than 6.3 and higher than 7.8 the methanogenic bacteria are adversely affected, hence methanogenesis is reduced significantly. Since acidogenic bacteria are not affected by high or low pH, the acid formation will take

place, as usual, however, due to the reduced methanogenic activity the reactor will start to "sour" due to the accumulation of acid (Van Haandel and Lettinga 1994; Daud *et al.* 2018). The pH will affect the solubility and reaction behaviour of other potentially influencing substances.

#### 2.4.5.6 Temperature

Anaerobic digestion strongly depends on temperature. Maximum temperature required for mesophilic is 35- 40°C and 55°C for the thermophilic range. For sewage treatment, only the mesophilic temperature is of important. The optimum temperature range is from 30 to 40°C. For temperatures below the optimum range, the digestion rate decreases by about 11% for each °C temperature decrease (Van Haandel and Lettinga 1994). Methanogenesis has been shown to be dependent on temperature strongly with the reacting rates increasing up to 60°C. When complex microbial consortia such as sulphate and nitrate reducers are present, temperature influences may be more significantly advantageous to certain species and to the detriment of others. Methanogenesis is reported to occur from temperatures of 4°C to 100°C. Anaerobic digesters are operated at mesophilic temperatures of 30°C to 37°C but it may be necessary to compromise and operate at lesser temperatures but with long contact times (Speece 1996; Daud *et al.* 2018).

#### 2.4.5.7 Toxic Compounds

Several compounds affect the rate of digestion such as the hydrogen ion concentration and heavy metals and chloro-organic compounds even at very low concentrations. However, the concentration of these compounds in sewage is too low to cause an inhibitory effect on the anaerobes. Other potentially toxic compounds may be oxygen and sulphide. Some oxygen may be introduced by the influent distribution system but this will be taken up by the acidogenic bacteria for oxidative metabolism. Sulphide can be formed by the reduction of sulphate, however, the sulphide concentration will normally be too low for it to cause any problem in the system (Van Haandel and Lettinga 1994).

The anaerobic process can accommodate a variety of toxicants of various industrial wastewaters and even biodegrade them. Many toxicants including formaldehyde, acrylate, chloroform, trichloroethylene, and cyanide are biodegradable and will lose their toxicity with anaerobic digestion (AD). Toxicity or inhibition can be caused by a variety of circumstances, including the generation of intermediary products such as volatile fatty acids which has adverse effect on pH. Much of the information concerning inhibition in terms of cause and effect, differences in culturing circumstances, or system configurations and operations have led to contradictions and possible misinterpretations of results from different investigators. The effects of VFAs are manifested in other environmental conditions, particularly pH and buffering capacity.

The overall inhibitory effect of VFAs is related to the pH established and may involve elevation of the unionized or disassociated species. VFAs may act as weak acid buffers to lower the pH but can also exert an inhibitory effect with pH on the microbial consortia. The detrimental effects of the accumulation of VFAs are manifested on the populations of the methanogens. The order of decreasing toxicity of the heavy metals have been recorded as Ni > Ca > Pb > Cr > Zn, with iron considered more beneficial than detrimental because of its mediating effects on sulfide toxicity (Chen, Cheng and Creamer 2008).

Inhibitory substances studied for anaerobic digestion have been majorly focused on those mentioned above. The effect of emerging contaminants such as antibiotics has not been studied and are beginning to receive some attention. This is crucial due to the fact that several studies have indicated the inability of the AD systems to remove this contaminant.

#### 2.4.5.8 Metabolism time

Two factors to be considered with regards to time are the hydraulic retention time (HRT) and solids retention time (SRT). HRT is the time afforded to the microorganisms to complete their tasks while SRT governs the rate at which the microorganisms can multiply and become predominated in the system as well as the

biomass inventory. HRT is responsible for contact time for microbial treatment and it relates greatly to the nature of the substrate; this is reflected in the degree of the difficulties encountered by the biomass in metabolising it. Adequate SRT must be sustained to permit the regeneration of the biomass and the accumulation a concentration that will be sufficient for the efficient performance of the system (Daud *et al.* 2018).

### 2.4.6 Conventional methods for removing antibiotics

The performances of anaerobic processes for the removal of emerging contaminants such as antibiotics has been considered by various researches both at the laboratory and industrial scale (Wang *et al.* 2017; Cheng *et al.* 2018; Huang *et al.* 2018). Vidal *et al.* (2018) reported on the removal of Nafcillin which belongs to the β-lactams class, using anaerobic digestion (AD) and the Photoelectro-Fenton process. They observed that the biodegradation of the Nafcillin AD process was not complete hence they had to use the Photoelectro-Fenton process for the complete removal. Göbel *et al.* (2007), studied the elimination of two sulphonamides, four macrolides and trimethoprim from a WWTP which had a conventional activated sludge (CAS) coupled with a fixed-bed reactor (FBR) and membrane bioreactor (MBR). In both the CAS systems and FBR, the sulphonamides showed inconsistent removal with either positive or negative elimination values, suggesting a possible retransformation between their main metabolites. Trimethoprim showed only a slight elimination of up to 20%, and varying results, including negative values, were obtained for the studied macrolides (-20 to 20%). This showed a disparity to the results of the CAS system coupled with the MBR, which did not only showed no increase in the load of any antibiotic but also a higher tendency of elimination. Despite this, full removal was not obtained for the studied compounds (Göbel *et al.* 2007).

Arikan, Mulbry and Rice (2009) applied anaerobic processes in the removal of macrolides and tetracyclines, respectively. In the two cases, a reduction of 90% for macrolides and 75% for tetracyclines was achieved. Zheng *et al.* (2018) studied the removal of veterinary antibiotics using AD as a pre-treatment and an intermittently

aerated sequencing batch reactor to further treat the effluent from the AD process. Removal from the AD process varied due to the nature of the antibiotics as well as variations in the animal feeds. Removal rates were therefore from 21 to 67%. However, further treatment with the intermittent aerated sequencing batch rector revealed a removal of up to 90%, thus leading to an improvement on the final discharge effluent (Zheng *et al.* 2018).

Dong *et al.* (2016) evaluated the removal of 19 pharmaceuticals including antibiotics from the constructed wetland (CW) and stabilization pond (SP) and two conventional wastewater treatment processes (activated sludge (AS) and micro-power biofilm (MP)) in a county of eastern China. They were able to detect the 19 selected antibiotics in both the influent and effluent samples. Detection frequency varied with the type of antibiotics. Their findings generally showed an incomplete removal.

Carvalho *et al.* (2013b) studied the removal of three veterinary antibiotics (ENRO, TET and ceftiofur) using activated sludge at a laboratory stage. Sludge reactors were doped with 100 μg/L of the initial compounds; they observed an incomplete removal of 68% and 77% for ENRO and TET.

The rate at which each of these antibiotics is removed during conventional treatment differs. This is due to the fact that the removal could be through biodegradation or sorption to the sludge. Some antibiotics undergo both biodegradation as well as sorption, while others have one of the two predominating. Those chemicals with a higher hydrophobicity tends to be adsorbed to sediments, thereby favouring their mobility through the WWTPs (Carvalho *et al.* 2013b).

Le-Minh *et al.* (2010) reviewed some crucial factors that affect the varying efficiencies in conventional treatment methods for the different classes of antibiotics and found that β-lactam antibiotics are highly susceptible to chemical and biochemical hydrolysis of the β-lactam ring during biological treatments. They noted that the removal efficiencies may even vary between antibiotics belonging to the same class, i.e., presenting similar molecular structure and physico-chemical properties (Le-Minh *et al.* 2010).

Miège *et al.* (2009) published a database on the fate of pharmaceuticals and other personal health care products in WWTPs. They found that the mean removal efficiencies ranged from 18% for Trimethoprim and up to 80% for fluoroquinolones especially norfloxacin. In summary, most of the studies using conventional treatment including anaerobic digestion did not give 100% removal of the studied compounds.

## 2.5 Advanced oxidation processes

Alternative methods of treatment that are used together with biological systems to enable the production of effluents that comply with the stringent regulations regarding emerging contaminants such antibiotics are the advanced oxidation processes (AOPs) (Adamek, Baran and Sobczak 2016; Mecha *et al.* 2016). AOPs have been applied in industrial processes for the degradation of resistant compounds that persist in wastewaters; some of these wastewaters are from petroleum by-products, textile dyeing wastewaters, pulp and paper, explosives production and landfill leachate, slaughterhouse waste and hospital waste. In a like manner, removal of pathogens and persistent endocrine disrupting chemicals, metal plating wastes, pesticides, metalloids such as chromium, and arsenic have been degraded using AOPs. Recently, quite a number of emerging contaminants that are found in the environment have been degraded by these processes (Vasconcelos *et al.* 2009; Elmolla and Chaudhuri 2010b; Mohammadi and Sabbaghi 2014; Safari *et al.* 2014; Sacco *et al.* 2018).

Advanced water treatment technologies have been identified to effectively eliminate recalcitrant contaminants. Numerous studies have been carried out covering the application of AOPs for antibiotic degradation (Abellán, Giménez and Esplugas 2009; Vasconcelos *et al.* 2009; Elmolla and Chaudhuri 2010a; Keane *et al.* 2010; Pereira *et al.* 2011; Dimitrakopoulou *et al.* 2012; Zhu *et al.* 2013). Other studies have focused on the use of AOPs as polishing steps to further treat effluents from conventional systems (Homem and Santos 2011; He *et al.* 2016; Balbayeva *et al.* 2018; Chollom *et al.* 2018b).

The observation made in most of the studies is that AOPs are effective for the complete removal of the antibiotics. For example, Elmolla and Chaudhuri (2010a) studied the

application of AOPs to β-lactam antibiotics and observed a complete removal of the β-lactam with a high removal of dissolved organic carbon of over 80%. For sulphonamides, Batista, Pires and Teixeira (2014); Fan *et al.* (2015) and Adamek, Baran and Sobczak (2016) observed more than 80% degradation and were often accompanied by important mineralisation levels of 40-70% total organic carbon removal. Further still, they observed that the produced intermediate compounds were less toxic and more biodegradable than the parent compounds. Similarly, tetracycline degradation was also carried out by Pereira *et al.* (2011) and its degradation was over 98% and mineralisation was above 50%.

Worthy of mentioning is the fact that the initial concentrations used in those studies were much higher than the amounts that are usually found in environmental samples and those of the WWTPs. In most instances, concentrations from 10 mg/L to over 250 mg/L are used for some of the studies. This quantity is hardly found in most of the WWTPs or environmental samples. The fact that the removal efficiencies reported are high for almost all of the studied compounds implies that the AOPs will also be effective in the treatment of water sources with low loadings such as river water, groundwater and drinking water (Homem and Santos 2011).

### 2.5.1 Heterogeneous photocatalysis

Heterogeneous photocatalytic oxidation is receiving much attention for the degradation of recalcitrant contaminants both in drinking water and wastewater treatment. The process of degrading the contaminants involves the use of semiconductors as catalysts. For the reaction to begin, an initial step of exciting the semiconductors by the means of an irradiation source (i.e. UV lamp, sunlight) has to occur. This initial step thus causes the production of several highly reactive radical-species such as the hydroxyl radicals and others ($OH^{\bullet}$, $O_2^{\bullet-}$, $O_3$), which produces redox reactions that contribute to the degradation of organic contaminants (Chong *et al.* 2010). The produced radicals have strong oxidizing and disrupting properties which react with the complex organic molecules at the right conditions, and therefore, the

conversion of the complex compounds into simpler compounds, such as carbon dioxide and water, occurs (Grote 2012; Chollom *et al.* 2018b).

Heterogeneous photocatalysis using semi-conductors such as titanium dioxide ($TiO_2$), zinc oxide (ZnO), iron (II) oxide ($Fe_2O_3$), cadmium sulphide (CdS), gallium phosphide (GaP) and zinc sulphide (ZnS) has been widely applied in the degradation of recalcitrant substances into readily biodegradable substances (Grote 2012; Suzuki, Araki and Yamamoto 2015; Chollom *et al.* 2018b). $TiO_2$, as a semiconductor has gained a great interest of most research work due to the fact that it is one of the most active photocatalysts under the photon energy of 300 nm. It has great stability even after the various catalytic cycles. In addition, $TiO_2$ has been promoted for use due to its ability to be biologically and chemically inert and thermally stable. Further still, it is not toxic and relatively affordable (Chong *et al.* 2010; He *et al.* 2016).

The performance of the $TiO_2$ is said to be dependent on parameters such as: crystallinity, phase, particle size, and surface area. Regarding crystallinity, $TiO_2$ exists in three forms: rutile, brookite, and anatase. The anatase phase has a band gap of 3.2 eV while that of rutile is 3.0 eV (Chong *et al.* 2010).

### 2.5.2 Theory of photocatalytic degradation

The illumination of anatase $TiO_2$ by UV light that exceeds that of its band gap energy (3.2 eV) causes the formation of electron-hole pairs. The formed electron-holes degrade the organic compound by creating hydroxyl radicals. Figure 2-4 illustrates the energy diagram of the electron hole creation. The following steps can be observed: (1) the first step charge carriers are formed (these are electron-hole pairs) by the photons; (2) the second step is the recombining of the electron-hole pairs; (3) if re-combination does not follow, then the oxidation pathway by a valence-band hole will follow; (3) the third step is the reduction by the conduction-band; (4) at the fourth step, more of the degradation by-products are formed; (5) there is the possibility of trapping the conduction-band electron in a surficial molecule which yields Ti(III); and finally; (6) trapping of the valence-band hole at a surficial titanol group occurs (Dong *et al.* 2015).

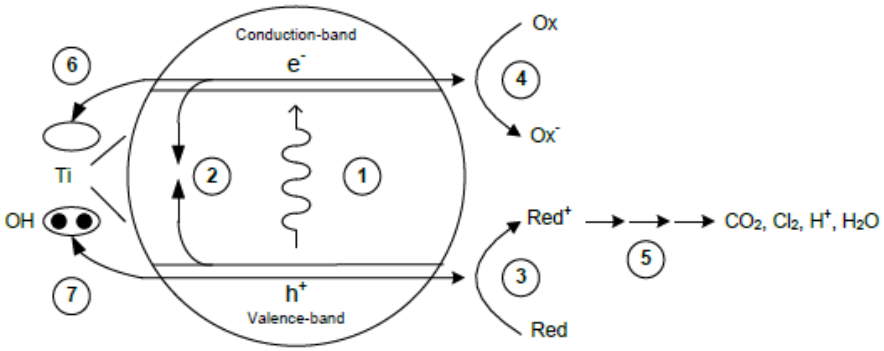



Figure 2-4: The main steps during a photo-electrochemical process (Dong *et al.* 2015)

Equation 2-14 shows the chemical reactions that occur at the surface of the reaction from steps 3 and 4. The electrons are extracted from the valence bands (VB) to the conduction bands (CB). The outcome is a positive region formation in the VB holes (h+) and as such free electrons (e-) in the CB as shown in equation 2-4 (Dong *et al.* 2015; Suzuki, Araki and Yamamoto 2015).

$$TiO_2 \overset{hv}{\rightarrow} TiO_2 + e^-(CB) + h^+(VB) \qquad 2\text{-}4$$

The holes at surface of catalyst will then react with the hydroxyl ions (OH-) thereby adsorbing water which leads to the formation of hydroxyl free radicals (•OH) (Equations 2-5 and 2-6) (Dong *et al.* 2015; Suzuki, Araki and Yamamoto 2015; Mecha *et al.* 2016).

$$TiO_2(h^+) + OH^- \rightarrow TiO_2 + \bullet OH \qquad 2\text{-}5$$

$$TiO_2(h^+) + H_2O \rightarrow TiO_2 + \bullet OH + H^+ \qquad 2\text{-}6$$

The CB electron reduces oxygen to the superoxide ion: $O_2^{\bullet-}$ as shown in Equation 2-7. This reaction prevents the e-/h+ from recombining, this happens when other electron acceptors such as pollutants are absent.

$$O_2 + e^- \rightarrow O_2^{\bullet-} \qquad 2\text{-}7$$

$H_2O_2$ is produced by the further reduction of $O_2^{\bullet-}$ as shown in Equation (2-8)

$$O_2^{\bullet-} + e^- 2H^+ \rightarrow H_2O_2 \qquad 2\text{-}8$$

The superoxide ion and which is in its protonated form thereafter compete to produce hydrogen peroxide or a peroxide anion as shown in Equation (2-9 to 2-11)

$$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet} \qquad 2\text{-}9$$

$$O_2^{\bullet-} 4HO_2^{\bullet} \rightarrow 2 \bullet OH + 3O_2 + H_2O_2 \qquad 2\text{-}10$$

$$2HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \qquad 2\text{-}11$$

The addition of $H_2O_2$ is said to increase the photodegradation rate under certain conditions either through the formation of $^{\bullet}$OH radicals (equation 2-12) or the reduction of $H_2O_2$ by the CB $e^-$ (equation 2-13)

$$O_2^{\bullet-} + H_2O_2 \rightarrow \bullet \mathrm{OH} + OH^- + O_2 \qquad 2\text{-}12$$

$$H_2O_2 + e^- \rightarrow \bullet OH + OH^- \qquad 2\text{-}13$$

The recombination of the $^{\bullet}$OH could lead to the formation of hydrogen peroxide as shown in Equation 2-14

$$\bullet OH + \bullet OH \rightarrow H_2O_2 \qquad 2\text{-}14$$

### 2.5.3 Operational parameters

The integration of the semiconductor catalyst with a photo-reactor, the oxidation rates and efficiency of the photocatalytic system are all highly dependent on a number of operational parameters that govern the kinetics. The most important parameters that are considered during a photocatalytic reaction are: pH, catalyst loading, amount of oxygen, contaminant loading, light intensity and temperature (Chong *et al.* 2010). Briefly described in the sections that follow are few of the factors.

#### 2.5.3.1 $TiO_2$ loading ($TiO_2$ concentration)

The $TiO_2$ loaded into the reacting system has an effect on the overall photocatalytic performance during the degradation of organic contaminants. An increase in the $TiO_2$ loaded increases the rate of the reaction. However, if the amount of $TiO_2$ is increased above a value which exceeds the saturation level, the $TiO_2$ particles in excess will form a light screening effect thereby reducing the surface area of $TiO_2$ which is exposed to illumination by the light, consequently reducing the photocatalytic efficiency (Suzuki, Araki and Yamamoto 2015; Chollom *et al.* 2018b). Therefore, operating the photoreactor below the saturation level of the $TiO_2$ photocatalyst avoids the excess usage of the catalyst and ensures the efficient adsorption of the photons. The catalyst loading and the light scattering effect influence the optical path length in the reacting system.

Various studies have investigated the effect of $TiO_2$ loading on the process efficiency; however, a direct comparison of the findings is difficult due to differences in the reactor geometries, radiation fluxes, intensity, and wavelengths employed (Chong *et al.* 2010; Suzuki, Araki and Yamamoto 2015). Therefore, the optimum catalyst loading for photo-mineralization and photo-disinfection may vary depending on the dimensions of the photoreactor and process dynamics. For instance, the diameter of the photoreactor has an effect on the water flow hydrodynamics and distribution of photons. A steady-state residence time is observed when the flow in the system is uniform, while on the contrary, with turbulent flow, the removal of the catalyst deposition or reaction dead zone occurs. With small reactor diameters between 20 - 25 mm, it is not possible to observe turbulent flow and diameters greater than 50-60 mm seems impractical (Chong *et al.* 2010; Suzuki, Araki and Yamamoto 2015).

#### 2.5.3.2 Solution pH

The pH of the solution is an important parameter during a photocatalytic reaction. The pH affects the surface charge or isoelectric point of the photocatalyst directly as well as the catalyst particles, size of catalyst aggregates and the positions of conductance and valence bands (Chong et al. 2010). The point of zero charge (PZC) of $TiO_2$ is the

main parameter used to study the effect of pH in photocatalyst reactions. The PZC is a condition where the surface charge of $TiO_2$ is zero or neutral and it is in the range of 4.5 - 7.0. This, however, depends on the catalysts used.

There are minimal interactions between photocatalyst particles and the contaminants at the PZC of the $TiO_2$ due to the absence of an electrostatic force. It has been reported that when the pH is less than the PZC of $TiO_2$, the surface charge of the catalyst becomes positively charged and it will gradually exert an electrostatic attraction force towards the negatively charged compounds. This type of polar attractions between the $TiO_2$ and the charged anionic compounds intensifies the adsorption onto the photon $TiO_2$ surface for other photocatalytic reactions. However, when the pH of the solution is greater than the PZC of the $TiO_2$, the catalyst surface will be negatively charged and repulse the anionic compounds in water. Equations 2-15 and 2-16 show how pH affects the surface charge density of the $TiO_2$ (Chong *et al.* 2010).

$$At\ p^H < PZC: TiOH + H^+ \leftrightarrow TiOH_2{}^+ \qquad 2\text{-}15$$

$$At\ p^H < PZC: TiOH + OH- \leftrightarrow TiO- \ + H2O \qquad 2\text{-}16$$

At pH = PZC, the neutral surface charge of the catalyst particles will not be able to produce the interactive rejection necessary for the separation of the solid-liquid. Therefore, this can induce the aggregation of the catalyst. When this happens, the catalyst becomes larger, thereby leading to the sedimentation of the catalyst.

Therefore, the pH affects the photocatalyst surface properties and the chemical form of the compound to be degraded. This is usually seen in the changes in the reaction rates and the tendency for the photocatalyst to aggregate. Therefore controlling the pH of the solution ensures better treatment effectiveness (Chong *et al.* 2010).

#### 2.5.3.3 Contaminant loading

The initial concentration of the organic contaminants to be degraded affects the efficiency of the photocatalytic reactions. Different concentrations of the initial contaminants will result in different irradiation times to achieve complete

mineralisation of the organic contaminant or to meet the set standards of treatment. It has been reported that an excess amount of the contaminants will simultaneously saturate the $TiO_2$ surface and thus reduce the photonic efficiency leading to the deactivation of the photocatalyst. Not all organic substrates will have such a profound effect on the irradiation time, and this also depends on the corresponding chemical nature of the targeted compounds for $TiO_2$ photocatalysis (Chong *et al.* 2010; Shahadat *et al.* 2015; Suzuki, Araki and Yamamoto 2015).

For instance, 4-chlorophenol will undergo a degradation pathway with the constant evolution of intermediate products such as hydroquinone and benzoquinone. In another instance, oxalic acid will undergo direct mineralisation to form carbon dioxide and water. In the first instance of 4-chlorophenol, the development of the intermediates will lead to prolonging of the irradiation time which is needed for total mineralisation due to the direct competition over unselective $TiO_2$ surfaces (Chong *et al.* 2010).

Consequently, it is paramount that at the stage of developing mathematical models which represent the kinetics of mineralisation especially when considering catalyst loading that water quality parameters such as chemical oxygen demand (COD), total organic carbon (TOC) or dissolved organic carbon (DOC) are included. This is to account for competition between the intermediate and these parameters (Chong *et al.* 2010; Suzuki, Araki and Yamamoto 2015).

### 2.5.4 Integrated photocatalytic adsorbents

The most common form in which the $TiO_2$ is used in water treatment is in its slurry form. In its slurry form, the generation rate of ROS is very high and it is proportional to the number of active surface sites thus increasing the efficiency of the catalyst. The disadvantage, however, is that with the slurry $TiO_2$, a post-separation step is needed afterwards to recover the $TiO_2$ from solution. Table 2-7 shows the advantages and disadvantages of using the slurry $TiO_2$ and immobilised $TiO_2$. The separation process is important for the avoidance of the loss of catalyst particles and as well introduction of the new pollutants which would contaminant the $TiO_2$ in the water been treated (Chong *et al.* 2010; Lim *et al.* 2011; Ibhadon and Fitzpatrick 2013). Different methods

have been suggested for the recovery of the $TiO_2$ from the solution such as conventional sedimentation and membrane filtration. One of the methods to overcome the problem encountered in the slurry type $TiO_2$ is to immobilise the $TiO_2$ on a supporting surface. Different support materials exist: activated carbon (AC), optical fibre, fibreglass, glass beads, glass wool, membranes, quartz sand, zeolites, silica gel and others (Ibhadon and Fitzpatrick 2013; Srikanth *et al.* 2017). Among these support materials, activated carbon has received the most attention due to the advantages it offers.

Table 2-7: A comparison of the slurry $TiO_2$ and immobilised $TiO_2$ photocatalytic systems (Srikanth *et al.* 2017).

| **Slurry type $TiO_2$** | **Immobilised $TiO_2$** |
|---|---|
| **Advantages** | **Advantages** |
| There is a uniform distribution of the catalyst | It is more convenient to use in the continuous reactor process |
| A higher ratio of illuminated photocatalytic surface area to reactor volume | There is an improvement in the removal of organic material from the aqueous phase while using immobilising agents especially those with adsorptive properties |
| There is the minimisation of fouling due to the fact that the catalyst is continuously added and removed simultaneously | Separation of the integrated photocatalytic adsorbents (IPCA) from the wastewater stream is easy and as such less expensive |
| Better mixing of the suspension is achieved | |
| There is a decrease in pressure drop in the reacting system | |
| There are almost no mass transfer hindrances | |
| **Disadvantages** | **Disadvantages** |
| It requires expensive and tedious methods of recovery of the $TiO_2$ from suspension | There is a possibility for the catalyst to be washed out and deactivated |
| When the concentration of the catalyst is too high, the suspended catalyst in solution would tend to cause the scattering of the light thereby reducing the rate of reaction | The accessibility of the photons to the catalyst is limited |
| It is common to experience aggregation of the $TiO_2$ particles especially at higher loading rates | High external mass transfer limitations at low flow rates of the pollutant to be treated occurs. This is because of an increase in the diffusion path length of the reacting species from the bulk to the catalyst surface. The internal mass transfer play the main role in limiting the use of the supported catalyst especially when there is an increase in the catalyst film thickness |

Srikanth et al. (2017) identified the properties that are essential for a support material which were; i) the photocatalyst must be strong and permanently immobilised on the medium it would be trapped in. ii) upon immobilisation, the catalyst must not show a high decrease in its activity iii) after immobilisation, the catalyst must possess a high surface area iv) pollutants should be able to be adsorbed on the immobilising agent surface for an effective photocatalytic degradation process v) the material serving as support must have excellent stability against degradation by strong oxidative radicals which are generated during the photocatalytic degradation process and finally (vi) it must provide a significantly large surface area

The use of integrated photocatalyst adsorbent (IPCA) was first explored in the early 1990s and has been studied ever since. The IPCA incorporates the adsorptive potential of activated carbon with the photocatalytic properties of $TiO_2$. The use of activated carbon (AC) provides a synergistic effect to enhance adsorption of the targeted pollutants onto the activated carbon phase, the transfer through an interphase to the $TiO_2$ is followed closely thereby improving the photodegradation process (Gao *et al.* 2011; Lim *et al.* 2011; Srikanth *et al.* 2017).

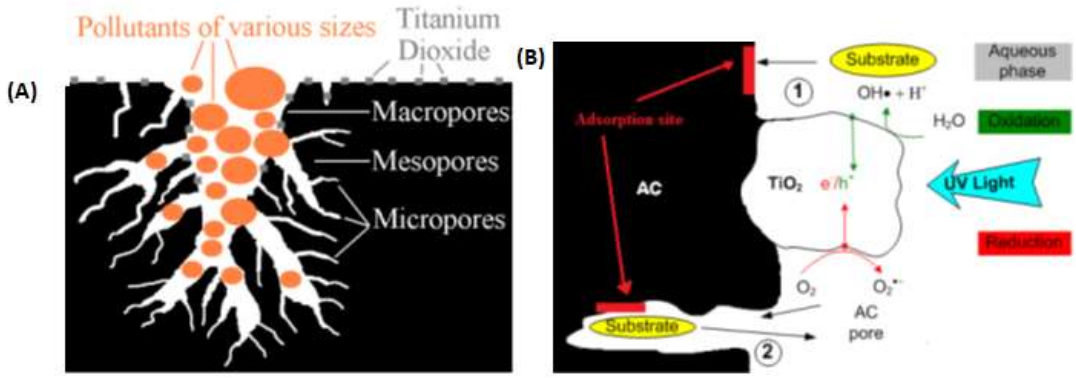



Figure 2-5: (A) Activated carbon modified with $TiO_2$ (B) the mechanisms of IPCA photodegradation (Devipriya and Yesodharan 2005; Gao *et al.* 2011; Lim *et al.* 2011).

Figure 2-5 (A) shows a simple illustration of $TiO_2$ embedded on activated carbon while Figure 2-5 (B) shows the mechanisms of IPCA photodegradation. In Figure 2-5A, the

transfer of the substrate to be degraded is said to be from the solution to the adsorption sites which are in close proximity of the $TiO_2$. Srikanth et al. (2017), Keane et al. (2010) and Gao et al. (2011) summarised the mechanism that occurs in an IPCA. They proposed that the pollutant to be degraded is concentrated by the AC close to the surface of photocatalyst therefore allowing for the efficient use of the electron-hole pairs which are formed through illuminating the $TiO_2$, illustrated in Figure 2-5 (B). However, this process is still not fully understood because, there could be transfer of the pollutant from the solution to the adsorption sites which are in proximity to the $TiO_2$ only or it could be by diffusion of the pollutant from the pores of the AC to the $TiO_2$ surface, or even still, a combination of both (Keane et al. 2010). For the regeneration of the adsorption capacity of IPCA, it is important that the substrate diffuses out of the AC pores. Again, it is important that the pollutant diffuses out of the pores of the AC due to the fact that it could cause the clogging of the pores, bonding which could be covalent with the functional groups of the AC (Keane *et al.* 2010; Gao *et al.* 2011).

### 2.5.5 Preparation of the IPCA

IPCAs are composed of a mixture of anatase and rutile $TiO_2$. The mixture is usually in the ratio of 70-90 % anatase and 10-30% rutile. Sometimes, the mixtures are the results from the calcination step which occurs at 700˚C during the preparation of the IPCA. Heating at higher temperatures of 700-900˚C causes more of the anatase to turn to rutile. Further increasing the calcination temperatures will result in the $TiO_2$ particles to become bigger and the bigger particles will not be able to block the pores of the AC, therefore this will cause an increase in the adsorption capacity of the IPCA (Xu *et al.* 2008; Ibhadon and Fitzpatrick 2013).

There are two major methods to prepare the IPCA: wet and dry. Dry methods use a physical, gaseous or in a vapour form. While the wet processes use an aqueous phase such that the $TiO_2$ is applied onto the adsorbent. Wet methods include the following: sol-gel, boiling impregnation, liquid phase deposition, and electrophoretic deposition, they present the most common that is usually used. This is due to the fact that preparing

the IPCA using the wet methods does not require the use of any special equipment (Li *et al.* 2010).

Amongst the wet methods, the sol-gel method has received wide application. In the sol-gel method, the majority of the IPCAs are prepared by applying the $TiO_2$ onto the AC while a smaller number involves the impregnation of the $TiO_2$ onto some other carbonaceous materials, which are then converted into the AC. In most instances, the $TiO_2$ is prepared using a Titanium precursor such as tetrabutyl orthotitanate which is dissolved in absolute ethanol. An acid could then be added to control the pH. Also, a hygroscopic compound such as diethanolamine is usually added to enhance the optimisation of the solution (Li *et al.* 2007; Li *et al.* 2010; Srikanth *et al.* 2017). After that, the $TiO_2$ is added following: i) dip coating onto the sol-gel solution ii) dipping the solution onto the AC iii) immersing the AC in the solution and finally iv) AC could be ultra-sonicated with the $TiO_2$ solution (Li *et al.* 2007; Xu *et al.* 2008; Li *et al.* 2010).

After successful impregnation of the $TiO_2$, the resulting IPCA is treated with heat at about 200-250˚C. Treatment at these temperatures is usually preferred due to the fact that the surface area of the $TiO_2$ particles is not reduced due to the heat treatment.

## 2.6 Analytical approaches used for the determination of veterinary antibiotics in environmental samples

Analysis of antibiotics from wastewaters and environmental water sources is a challenge due to the following reasons: i) the great variability of antibiotics as well as other pharmaceutical compounds; ii) the need to identify not only the pollutants of interest but also their derivatives and metabolites; iii) the variety of matrices (e.g., sediment, sludge, surface water, wastewater and biological samples) and variations in pollution-load levels; iv) the possibility of interference between sample components of similar physicochemical characteristics that are present in the same sample at different concentrations; and, v) the lack of suitable standards and certified reference materials (Kot-Wasik, Dębska and Namieśnik 2007; Tong *et al.* 2009). Therefore, for detection and quantification, it is important that the samples undergo preparation to

reduce matrixes and pre-concentration. Solid phase extraction (SPE) is the commonly reported method that is used for pre-concentration and sample preparation. After the SPE, separation is carried out with the use of high-performance liquid chromatography (HPLC) and a detector. There are five steps involved: i) sampling, ii) sample preparation, iii) chromatographic separation, iv) detection, and v) data analysis. Of these, 80% of the time is spent on sample preparation (Seifrtova *et al.* 2009; Gros, Rodríguez-Mozaz and Barceló 2013).

It has been indicated in **section 2.3** that the conventional treatment process employed in WWTPs does not completely remove antibiotics before discharging the effluents into the environment. Furthermore, there is a vast difference in the treatment plants designs and operations and as such impacting on the performance of each treatment plant. This, therefore, influences the removal of the contaminants. For the detection of antibiotics, it is a necessity to set up fast, sensitive and reliable analytical methods that enable the determination of a wide range of antibiotic. It is important because correlations could be made between the detected antibiotics residues and the antibiotic resistance genes.

### 2.6.1 Sample preparation techniques

Sample preparation is a crucial step in analysis. The analysis is impacted by two main factors: the physical and chemical properties of the analytes of interest, as well as their matrixes. The main goals of sample preparation are: concentration of the analytes, removal of interferences from the matrixes, and to prepare analytes to a suitable form for subsequent chromatographic analysis. Further still, during sample preparation, it is important that the following are considered: minimal loss of analytes, efficient removal of coexisting components, quick to conduct, economical and not cause any problems in the chromatography process (Seifrtova *et al.* 2009). Sample preparation has a direct impact on the accuracy precision and quantification limits and is often a limiting step for many analytical methods.

There are currently many methods that are used for sample preparation. The preparation method is chosen mainly based on the nature of the sample to be prepared.

Table 2-8 shows the various sample preparation methods. Samples to be prepared could be in a solid form e.g. sludge or liquid form such as wastewater.

Table 2-8: Sample preparation methods

| **Liquid samples** | **Solid samples** |
|---|---|
| Liquid-liquid extraction (LLE) | Soxhlet extraction (SE) |
| Solid phase extraction (SPE), | Microwave assisted extraction (MAE) |
| Solid phase micro extraction (SPME), | Pressurized liquid extraction (PLE) |
| Membrane extractions: | Supercritical fluid extraction (SFE) |
| Supported liquid membrane (SLM) extraction | Matrix solid phase dispersion (MSPD) |
| Hollow-fiber liquid phase micro extraction (HF-LPME) | Ultrasonic extraction (UE) |
| | Ultrasonication |
| Single drop micro extraction (SDME), | |
| Homogeneous liquid-liquid extraction (HLLE) | |
| Dispersive liquid-liquid micro extraction (DLLME) | |
| Stir bar sorptive extraction (SBSE) | |
| Cloud point extraction (CPE) | |

The most commonly used preparation method for liquid samples from different water sources especially for the detection of pharmaceuticals is the solid phase extraction (SPE). This method has shown some major advantages against the other preparation methods and is reviewed further in this study. Further information on other methods could be found in Kim *et al.* (2018).

#### 2.6.1.1 Solid phase extraction

Solid phase extraction (SPE) is used for the cleaning and concentration of water samples. It is an attractive alternative to liquid-liquid extraction (LLE) due to the advantages it offers. The use of SPE consumes less time as compared to LLE. It is also

less labour intensive and consumes smaller volumes of solvent. It also offers a higher selectivity and reproducibility for the simultaneous extraction of multiple compounds from an aqueous matrix (Kim et al. 2018). The principle of SPE is similar to that of LLE, for both, the partitioning of solutes between two phases occurs. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase (Żwir-Ferenc and Biziuk 2006).

The main steps involved in SPE are: sample preparation, conditioning, loading, washing and eluting. SPE is achieved through the interaction of three components: the sorbent, the analyte and the solvent (Żwir-Ferenc and Biziuk 2006). The analyte must be attracted more strongly to the sorbent than to the matrix. Sorbent selection is dependent on the characteristics of the analyte, similarly, column selection is dependent on the impurities that would be separated from the analyte. Żwir-Ferenc and Biziuk (2006) stated that the properties of sample impurities can often be exploited in analyte purification. It is important to understand the mechanism of the interaction between the sorbent and analyte of interest. To gain this, having good knowledge of the hydrophobic, polar and ionogenic properties of both the solute and the sorbent is important. The most common retention mechanisms in SPE are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interactions) (Żwir-Ferenc and Biziuk 2006). Each sorbent has unique properties which can be applied to a wide variety of extraction problems.

Therefore, the most important properties of the analyte to be considered before choosing the sorbent of interest are: nature of the sample, number of compounds/analytes present, chemical structure, molecular weight of the compounds, the pKa values, the log P and/or log D values (hydrophilicity), the compounds concentration, sample matrix and finally, the solubility of the compounds (Kim *et al.* 2018).

The interaction in the sorbent could be either in the following theories that have been identified in literature; reversed-phase, ion exchange, and normal phase. For the

application of environmental samples especially when trying to detect for pharmaceuticals, the reversed phase is used.

Separating the analyte from the matrixes (interferents) in SPE is achieved in three ways (Seifrtova *et al.* 2009; Kim *et al.* 2018):

**Selective extraction**- this occurs on the sorbent bed where only selected components are retained while the remaining compounds are not.

**Selective washing**- this occurs when both the compounds of interest and impurities are retained on the sorbent bed when the sample is passed through the sorbent. The impurities are therefore washed off with a selective solvent that is strong enough to remove them but weak enough to leave the compound of interest behind.

**Selective elution**- this occurs when the adsorbed compound of interest is eluted in a solvent but the strongly retained impurities are left behind.

## 2.7 Separation techniques

Separation of various mixtures of organic compounds is based on their distribution between a stationary phase and a mobile phase present in a chromatographic column. Different types of chromatographic separation techniques exist. These include liquid chromatography (HPLC) and gas chromatography (GC) (Petrović *et al.* 2005). GC had been previously employed for the separation of various types of pharmaceuticals, however, due to the fact that the derivatization of compounds is required in order to make the non-polar compounds volatile, the process of derivatisation presented a great challenge and therefore an alternative means was considered.

The derivatisation of the compounds of interest is not favourable due to the fact that it increases the level of variability of the method and as well the procedures are not specific, thus there is a high chance for multiple peaks to be reported for a single analyte as its functional groups increases as such making the quantification complicated. With the use of the HPLC, the derivatisation of the compounds of interest is not necessary, thereby making HPLC the preferred separation technique. Most of

the pharmaceuticals are non-polar in nature. Their detection and separation with the HPLC do not require a further derivatisation stage.

### 2.7.1 Liquid chromatography

Chromatography is defined as the distribution of analytes (compound being identified) between two phases: a stationary phase and a mobile phase. The difference in migration rate of the analytes through the column is based on their affinity for either the stationary or mobile phase. This makes the separation possible (Seifrtova *et al.* 2009; Kim *et al.* 2018). The mobile phase, which is the liquid phase, consists of a solvent or a mixture of solvent while the stationary phase is a solid which could be chemically modified or not. The stationary phase could be a normal phase; whereby it is polar in nature and as such the mobile phase would be non-polar. It could be a reversed phase chromatography (RPC). In this case, the stationary phase would be non-polar while the mobile phase would be polar. The RPC is the commonly used chromatography. It contains silica which has been treated with $RMe_2SiCl$, where *R* is a straight chain alkyl group such as $C_{18}H_{37}$ or $C_8H_{17}$ (Kim et al. 2018).

### 2.7.2 High-Performance Liquid Chromatography (HPLC)

The High-performance liquid chromatography or high-pressure liquid chromatography, (HPLC) is a chromatographic technique that separates a mixture of compounds for the purpose of identification, quantification and sometimes purification of the mixed components. Different stationary phases, like reversed or normal could be used. A pump is necessary to enable the movement of the mobile phase and the analyte through the column. Of necessity, is the detector that would provide the retention times for the analytes. Retention of analytes is based on factors such as: its interaction strength with the stationary phase, composition, and ration of the mobile phase as well as its flow rate.

#### 2.7.2.1 HPLC operating principle

In the HPLC, a small amount of the sample to be analysed is introduced in very small quantities into the stream of the mobile phase. Both the sample and the mobile phase then move along the column by certain physical or chemical interactions with the

stationary phase. The movement along the column is dependent on the velocity and the nature of the sample as well as that of the stationary phase. The time taken for a specific analyte to be eluted is referred to as the retention time. Retention of the analyte is considered under certain favourable conditions. One of which is the column packing whereby a smaller particle sized packing column increases the linear velocity thereby giving the analytes lesser time to diffuse within the column thus increasing the resolution of the chromatograms. The retention is a factor that helps in the identification of the analyte under certain optimised conditions.

#### 2.7.2.2 Detectors in HPLC

A number of detectors are used in liquid chromatography for the detection of analytes e.g. ultraviolet (UV), diode array detectors (DAD). The detectors exploit some of the properties of the analytes. Basically, the detectors are considered based on certain characteristics: sensitivity, linearity, stability and reproducibility, rapid response, selectivity and its contribution to the broadening of bands should be minimal (Żwir-Ferenc and Biziuk 2006; Kim *et al.* 2018). Again, the detector must also match the conditions set for the separation, for example, the electrochemical detectors alongside gradient elution should be avoided. The use of volatile mobile phase modifiers could be considered alongside with mass spectrometry (Seifrtova *et al.* 2009). The UV detector has most of the properties mentioned above and as such, almost 95% of the separations carried out in the pharmaceuticals industries use the UV for detection. Most of the analytes are tagged with a chromophore which aids with their detection. UV detectors are fairly sensitive and their detection limit is in the range of 100 pg – 1 ng.

Similarly, the photodiode array detectors (PDA) or DAD detectors have the advantage over the UV detectors due to their extra selectivity to the analysis with the ability to record a complete UV spectrum during the elution of the peak. This, therefore, provides quality information due to the fact that the obtained spectral could be matched. Another advantage of the PDA is the fact that it is able to assess the peak purity and deconvolution of unresolved peaks (Kim *et al.* 2018).

Mass Spectrophotometer (MS) is another detector that is used for detecting analytes. The use of the MS has been used for most pharmaceuticals due to the fact that it is highly selective and sensitive and it can provide the structural information of the target compounds. The principle of the MS consists of ionizing chemical compounds to generate charged molecules or molecular fragments and the measurement of their mass to charge ratios, therefore the aim is to separate ionised molecules according to their mass to charge ratio. The main parts of MS are an ion source, a mass separator, and an ion detector. Precursor ions (parent ions) and their product ions (fragment or daughter ions) are used for quantification of the compounds in MS/MS (Petrović *et al.* 2005).

For LC-MS and LC-MS/MS analysis, two interfaces are commonly used for the detection of analytes; Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), due to their sensitivity and robustness (Seifrtova *et al.* 2009). Both interfaces produce protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecules. The ESI is preferred over APCI for pharmaceutical analysis due to the fact that it has a higher sensitivity and better reproducibility, especially when dealing with polar and non-polar compounds like antibiotics. In ESI, the ionisation could be carried out in positive or negative modes. For most veterinary antibiotics, the positive mode is preferred. Many antibiotic compounds are non-volatile and have high molecular weights and therefore respond well in a positive ESI (Seifrtova *et al.* 2009; Kim *et al.* 2018).

### 2.7.3 Matrix analysis

LC-MS and LC-MS/MS are considered one of the most efficient techniques for analysing pharmaceuticals from environmental samples. The MS is susceptible to a matrix effect and this needs to be accounted for especially when dealing with samples such as wastewaters. The presence of the matrix in the sample influences the ionisation of the target compounds and may, therefore, cause the response to increase or decrease and thus leading to inaccuracy and imprecision of the analysis. Apart from the main analyte (s) of interest, all other compounds in the samples are known as matrices. Some of the common causes of the matrix are the co-elution of metabolites, impurities and degradation products. A common occurrence in ESI MS analysis is the matrix effect.

It is the main disadvantage of ESI MS, because ESI source is highly susceptible to other components present in the matrix, thus resulting in signal suppression or enhancement (Seifrtova et al. 2009).

Three main factors were mentioned by Seifrtova et al. (2009) to be the major causes of reduction in the method sensitivity. Firstly, the sorption of antibiotics to organic matter in the samples which causes the preparation step to be less effective especially with regards to concentrating of antibiotics. The second factor is the fact that the contaminants in the sample matrix interferes with the analyte peaks by raising the chromatogram baseline. Finally, there is a tendency for the contaminants to reduce the ionisation efficiency of the analytes by taking up some of the limited numbers of excess charged sites on the surfaces of electrospray droplets (Seifrtova et al. 2009).

To account for the matrix effect in MS, Chambers *et al.* (2007) reported two methods to be used: the post-column infusion method and the post-extraction spike method. The first method is time-consuming and does not provide a quantitative understanding of the effect. Therefore the second is preferred over the first. The post-extraction spike method assesses the matrix effect by comparing between the response of the target compound in pure solution (e.g. mobile phase) and response of the target compound spiked in the blank matrix after sample preparation. This method provides a quantitative assessment of the matrix effect (Chambers *et al.* 2007).

### 2.7.4 Optimisation of HPLC conditions

#### 2.7.4.1 Mobile phase

Two types of gradient conditions exist viz. isocratic and gradient elution. When the composition of the mobile phase does not change during the analysis, the method is said to be isocratic. This means that the composition of the mobile phase remains unchanged. This method is associated with some disadvantages which include:

i. When the range of analytes polarities in the composition is broad, some of them will be poorly retained and resolution will be lost with peaks eluting at or near the void volume

ii. Some of the components in the analyte might be more hydrophobic and will, therefore, show longer retention times
iii. Due to the fact that these analytes have different band-broadening processes, the late eluting peaks will be broad and show reduced sensitivity due to the reduced peak height.
iv. There is a possibility that some of the components will be adsorbed onto the column irreversibly thereby causing contamination.

Gradient elution occurs when the composition of the mobile phase is changed. This is done by modifying the composition of the organic modifier. This method is best used during method development.

Some of the steps that are considered in gradient elution include: i) resolving the issues with early elution of the analytes thus indicating that the strength of the initial composition should be chosen appropriately ii) the elution strength should then be increased steadily to elute compounds with an optimum iii) the final mobile phase composition will then be chosen to ensure elution of all compounds of interest from the column within a reasonable time and finally, iv) the organic modifier concentration can be increased to wash strongly retained and other possible contaminants from the column.

The advantages of gradient elution are:

i. Improved resolution
ii. Increased detection
iii. Ability to separate complex samples
iv. Shorter analysis time
v. Decrease in column deterioration due to strongly retained components

Setting the right gradient should be a priority. This can be developed by scouting gradient analysis. Literature has recommended a scouting gradient linear gradient from 5-10% B to 100% B (20 minutes standard) (Seifrtova *et al.* 2009).

#### 2.7.4.2 Limit of detection (LOD)

The limit of detection (LOD) is defined as the minimum detectable concentration of the analyte of interest. It is related to both the signal and noise ratio of the system and is defined as a peak whose signal-to-noise (S/N) ratio is at least 3:1 (Shrivastava and Gupta 2011).

#### 2.7.4.3 Limit of quantification (LOQ)

The limit of quantification (LOQ) is defined as the minimum quantifiable concentration of the analyte of interest. It is related to both the signal and noise ratio of the system and is defined as a peak whose signal-to-noise (S/N) ratio is at least 10:1 (Shrivastava and Gupta 2011).

## 2.8 Conclusions

The past one to two decades has seen the emergence of new chemical contaminants of several classes such as: pharmaceuticals, hormones, endocrine disrupting chemicals, viruses, and toxins. These contaminants have been detected in the environment. Their detection has been due to the development of new analytical technologies that are capable of quantifying very low concentrations of these contaminants.

The main sources of these contaminants have been linked to the wastewater treatment plants (WWTPs). This is due to the fact most of the WWTPs employ conventional methods to treat wastewater. These systems are designed for the removal of dissolved organic matter, solids and nutrients; however, they are not effective against bio-recalcitrant compounds like the emerging contaminants. Other sources of the contaminants are aquatic systems, rivers and other sources. They have been detected at low concentrations in the range of µg/L to ng/L in water sources; however, higher concentrations have been found in manures. Although present in small concentrations, their effects on the environment have raised concerns about human health and therefore made studies on their detection and removal a major priority.

The present study focused on veterinary antibiotics as one of the emerging contaminants that have not been well studied. The presence of these contaminants in the environment has resulted in antibiotic resistant genes and antibiotic resistant bacteria. The burden of the ARGs and ARBs has drawn worldwide attention into investigating this rising phenomenon in order to better understand the seriousness of the effects of these contaminants.

Most studies carried out, however, have tended to focus on human antibiotics. The few studies on veterinary antibiotics have also focused on their presence in animal manure and wastewaters from livestock farming. However, it has been noted that slaughterhouses present possible sources of antibiotics and hence the need for further research in this area, since it is lacking. In addition, most studies have been done in developed nations; but, with the recent developments in the developing countries such as South Africa, there is need for more studies to be done to provide the necessary information for future planning and policy formulation.

Based on this background, the aim of the present study was to develop a suitable and sensitive analytical method for the determination of veterinary antibiotics from a slaughterhouse wastewater based upon solid phase extraction (SPE), ultrahigh liquid chromatography, photodiode array detectors (PDA) and mass spectrometry (MS) (UHPLC-PDA-MS); and to assess the removal of the target antibiotics using biological method and advanced oxidation processes for remediation of wastewater.

# Chapter 3 - Materials and Methods

This chapter presents the materials and methods used to address the objectives of this study.

**Sections 3.1** to **3.5** present the materials and methods used for the development and optimisation of an extraction method based on SPE UHPLC-PDA-MS techniques for veterinary antibiotics (VAs) from the slaughterhouse wastewater treatment plant. Similarly, the method used for the application of the optimised SPE and UHPLC-PDA-MS method is elaborated. These cover objectives one and two of the study.

The third objective entails the evaluation of the removal mechanisms of antibiotics from simulated wastewaters using anaerobic digestion as well as the assessment of biodegradation kinetics to determine the reaction rates. The materials and methods employed for these are presented in **sections 3.6** to **3.8.** The fourth and fifth objectives of the study involves the preparation and characterisation of the integrated photocatalyst (IPCA) and evaluates its adsorption performance against the target antibiotics and also the evaluation of the effectiveness of the IPCA as a photocatalyst for the degradation of the target veterinary antibiotics. The materials and methods employed are presented in **sections 3.9** to **3.11.** The catalyst preparation is presented in **section 3.9.2** and the characterisation of the catalyst is presented in **section 3.9.3. Sections 3.9.4** and **3.9.5** describe how the adsorption studies were conducted as well as the kinetic models employed. Further still, the isotherm models are presented in **section 3.9.6.** Lastly, **sections 3.10** and **3.11** describe the photocatalytic degradation of the antibiotics as well as the optimisation of the process.

## 3.1 Development and validation of the analytical method for the detection of five veterinary antibiotics from slaughterhouse wastewater

The study aimed at developing a combined analytical method for monitoring the levels of five antibiotics from slaughterhouse wastewater from the influent and effluent

streams as well as from the surface impacted water receiving the effluents. Three main steps involved in the method development are: pre-concentration, chromatographic separation, and the identification and quantification steps. The pre-concentration step requires the use of the solid phase extraction (SPE) and optimisation of the process is necessary to obtain accurate results.

The chromatographic separation of the antibiotics was carried out using ultra-high-performance liquid chromatography (UHPLC) and the identification step was carried out with the use of mass spectrometry (MS). Each of the parameters required in each step was optimised separately and thereafter combined into a single method. The method was first developed with the antibiotics in Millipore water and thereafter was validated with the actual wastewater. Therefore, this section describes the method development for the analytes of interest. The methodology adopted here was not only used for the analysis of antibiotics in WWTPs but also to analyse samples obtained from the biodegradation, adsorption and the photodegradation tests. Therefore, to achieve the first and second objectives of the study, the following aspects were investigated:

- Development and optimisation of the High performance liquid chromatography (HPLC) method
- Development and optimisation of the solid phase extraction (SPE) method
- Development and optimisation of the mass spectrophotometry (MS) method
- Integration of the developed methods into a single method suitable for the analysis of the veterinary antibiotics in slaughter house wastewater (SHWW)

## 3.2 Materials

### 3.2.1 Chemicals

The antibiotics used include: amoxicillin (AMO), ciprofloxacin (CIP), enrofloxacin (ENRO), sulfamethazine (SMZ) and chlortetracycline (TET); they were purchased from Sigma Aldrich. The mobile phases: Methanol, Acetonitrile (CHROMASOLV®, HPLC grade) were purchased from Merck. Ultrapure water (>18 MΩ $cm^{-1}$) was

obtained from the Millipore system. Formic acid (>99%) and Ethylene diamine tetra acetic acid disodium ($Na_2EDTA$) (>98%) were purchased from Sigma Aldrich. Hydrochloric acid (HCl) and Sodium Hydroxide (NaOH) for pH adjustment were purchased from Associated Chemical Enterprise (ACE), South Africa. For decontamination purposes, all plastics and glassware were cleaned with soap water, soaked in 20 % (v/v) nitric acid also from ACE, rinsed with water again and dried before use.

### 3.2.2 Other consumables

Pall nylon filters with pore sizes of 0.22 and 0.45 µm were purchased from United Scientific. The 0.2 µm syringe filters were used to filter the samples before injection. Membrane filters of 0.45 µm pore size and 47 mm diameter were used for sample pre-treatment before the SPE procedure. Syringes of volume 2 and 5 ml were used with the syringe filters.

### 3.2.3 Equipment

Table 3-1 shows the equipment used for this study.

Table 3-1: List of the major equipment used

| Equipment | Model | Purpose |
|---|---|---|
| Portable pH Meter | Orion Star™ A121 | pH measurement |
| Dry Heated Nitrogen Evaporator | No: 41104803 | To dry eluted samples |
| Grant ultra-sonicator | XUBA3 | For mixing |
| Shimadzu UHPLC | SPD-M20A | Analytes separation |
| SPE Cartridges | | Sample purification |
| SPE vacuum manifold | | Concentration of analytes |

#### 3.2.3.1 Ultra-high-performance liquid chromatography

An autosampler Shimadzu UHPLC system (SPD-M20A) equipped with a photodiode array detector (PDA) connected to a mass spectrophotometer (MS) and operated by Lab solution software was used for the optimisation of the chromatographic separation. Incorporated to the UHPLC was a Gemini C18 column (150 x 4.6 mm x 5 µm) from Phenomenex (Torrance, CA, USA) which was used for the separation of the antibiotics. The pH of the solutions was measured using the Orion pH meter.

#### 3.2.3.2 Vacuum Manifold

The SPE equipment was a 24-fold vacuum extraction manifold purchased from Phenomenex (Torrance, CA, the USA). The manifold consisted of a clear glass chamber and a polypropylene lid, to which vacuum was applied thereby drawing the sample through the SPE cartridge. Filtered samples were passed through polypropylene needles and collected into vials placed on the collection racks.

#### 3.2.3.3 SPE cartridges and pH meter

The selection of the cartridge suitable for the analytes targeted in this study done was based on the literature. The suppliers provided advice on the choice of the cartridge and after intensive review, the Strata X cartridge was chosen. Strata X cartridges (500 mg 6 mL) were purchased from Phenomenex (Torrance, CA, the USA). The Strata-X cartridge has a surface-modified styrene skeleton with a pyrrolidone group, whose retention mechanisms are hydrophobic, hydrogen-bonding, and aromatic. This sorbent is used for reverse-phase extraction of acidic, basic, and neutral compounds (Moreno-Bondi *et al.* 2009). The properties of this cartridge are similar to those of the hydrophilic-lipophilic-balanced (HLB) cartridges which have found vast application in similar studies. The pH of the solutions was measured using the Orion pH meter.

## 3.3 Methods

### 3.3.1 Sample collection and description of WWTP

Wastewater samples from the slaughterhouse were collected in 2 L amber bottles. These were transported immediately to the laboratory for preparation within 24 hours. Samples for method development were collected over a period of three months. The samples were collected at the influent and effluent points of the wastewater treatment plant. Samples collected were typically at pH 6-7 and contained particulate matter. They were analysed for total suspended solids (TSS) and chemical oxygen demand (COD). They were afterwards filtered through Whatman glass fibre filters to remove particulates and were further filtered using 0.45 µm nylon filters before SPE. The pH of the filtered samples was adjusted to 2 using HCl or NaOH.

The slaughterhouse operation consisted of the following: slaughtering operations and wash waters from the intestines and stomach. Collected samples, therefore, contained a lot of blood. The slaughterhouse capacity is 700 swine a day. The onsite wastewater treatment processes employed are: solid-liquid separation and biological treatment, aeration and clarification (two stages) followed by chlorination. Grab samples were collected at the inlet and exit (just before chlorination) and finally in the receiving river. Samples were collected in 5 litres amber glass bottles and immediately transported to the laboratory in cooler boxes for antibiotic analysis. Wastewater samples were collected twice monthly and were characterised within 24 hours of collection.

### 3.3.2 Preparation of standards

Antibiotic standards were prepared by dissolving 0.1 g of each into 100 mL of the required solvents to give a concentration of 1000 µg/mL. The stock solution was stored in well cleaned amber bottles to prevent photodegradation and thereafter stored for up to 1 month at 4°C. A 100 µg/mL mixed working solution was prepared in the mobile phase. For the SPE-LC-PDA/MS method, further dilution of the working standard

solution was carried out to the desired range of standards to be used for the investigation. The mobile phase used was acetonitrile (ACN) and Millipore water. Formic acid (FA) was added to both the ACN and Millipore water to give concentrations of 0.1% in both ACN and water. The acid was used to enhance the ionisation.

### 3.3.3 Sample pre-treatment

The pre-treatment of the sample started with the filtration of both the effluent and influent samples collected from the WWTP. The reason for the filtration was to reduce the clogging of the SPE cartridges. Prior to the filtration of the wastewater samples, 0.1 g disodium ethylenediaminetetraacetate ($Na_2EDTA$) was added to the wastewater samples. This was to prevent the antibiotics, especially the chlortetracycline, from chelating with metal ions. The samples were first filtered with Whatman glass-microfibre filters (GF/A) with a pore size of 1.6 μm because the smaller pore size filter got clogged easily. The samples were further filtered with the 47 mm diameter nylon membrane filters with pore size of 0.45 μm. Filtration was carried out with a Büchner funnel under vacuum. After filtration, the pH was adjusted with 0.1 M HCl or 0.1M NaOH and then the filtered samples were subjected to SPE.

### 3.3.4 Sample preparation using Solid phase extraction (SPE)

The aim of preparing the sample is to transfer the analyte of interest from its current matrix into a suitable form for injection into the instruments; the other reason is the pre-concentration of the analytes. The main parameters considered during SPE are: conditioning, equilibration, loading, washing, and elution. Each of these parameters needed to be optimised to enhance the extraction efficiency. The goal for optimisation of SPE parameters is to obtain a high analyte recovery and enrichment. To obtain the extraction efficiencies, the factors were first investigated using Millipore water samples spiked with 1 μg/L of the chosen antibiotics.

The SPE cartridges were conditioned with 5 mL of methanol and then equilibrated with 5 mL of deionised water. Care was taken so that they were not left to dry before

loading the samples. After the conditioning and equilibration, 100 mL of the prepared spiked solution was then loaded onto the cartridges. The loading was carried out at a flowrate not greater than 4 mL/min. The vacuum pressure was maintained below 10 mmHg at all times. This was conveniently done by controlling the stop-cock valves on the manifold. After sample loading, the cartridges were left to dry under vacuum for 30 minutes and thereafter, the analytes retained were eluted with 10 mL of 0.1% FA in methanol. The eluent was then dried under a gentle stream of nitrogen gas at 40˚C. The dried samples were reconstituted in 1 ml of the mobile phase. Prior to analysis, the samples were filtered using 0.22 µm nylon filters. The percentage of the recovered standard was determined by the result obtained from the HPLC analysis. Pre and post-extraction spiked samples were compared to determine the percentage recovery. Validation was later carried out on the actual wastewater samples. The recovery of analytes was calculated using Equation 3-1

$$Recovery = \frac{C_{measured}}{C_{theoretical}} \times 100 \qquad 3\text{-}1$$

The recoveries of the extracted analytes were determined by the use of spiked ultra-pure water samples with the analytes of interest at a pre-determined concentration. Recoveries were determined by comparing the concentrations obtained with the initial spiking levels. The response for each of the target analyte was detected with the UHPLC method. In each case, samples were analysed in triplicate.

#### 3.3.4.1 pH optimisation of sample

Before loading, the samples were adjusted to a pH of 2, 3, 4, 6 and 8. After the pH adjustment of each sample, the samples were run on the SPE using the method described in **section 3.3.4.**

#### 3.3.4.2 Selection of the eluting solvent

The elution of the analyte was carried out by optimising solvent type and volume of solvent used. The solvents used were ACN and Methanol (MeOH). Acidification of both solvents was considered as well to evaluate the effect of a change in the elution

pH. Finally, the elution volume was varied from 5 to 10 mL for each solvent. Elution was carried out after the conditioning, equilibrating, loading and drying as described in **section 3.3.4.**

## 3.4 Optimization of HPLC-PDA-MS

Factors optimised were: mobile phase composition and analyte detection wavelengths.

### 3.4.1 Chromatographic separation

The optimisation of the HPLC parameters was carried out using the Shimadzu UHPLC incorporated with an autosampler. Separation of analytes was carried out with the use of a Gemini C18 column (150 x 4.6 mm x 5 µm) coupled with a C18 Gemini C18 guard cartridge (4.0 × 3.0 mm) from Phenomenex (Torrance, CA, the USA). The injection volume was 10 µL, and an ambient temperature was used in the column compartment. The composition of the mobile phase was 15% ACN and 85% acidified Millipore water. A simple multistep gradient method was employed as shown in Table 3-2. The wavelengths optimised were from 230 to 280 nm.

Table 3-2 UHPLC gradient conditions

| Time | (min) | 0.1 | 25 | 26 | 32 | 33 | 43 |
|---|---|---|---|---|---|---|---|
| Solvent A | (%) | 85 | 85 | 0 | 0 | 85 | 85 |
| Solvent B | (%) | 15 | 15 | 100 | 100 | 15 | 15 |
| Flowrate | (mL/min) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

#### 3.4.1.1 Mobile Phase Optimisation

At the initial stage of the experiment, non-acidified ACN and Millipore water were used, however, good responses were not achieved. Therefore, both the ACN and Millipore water were acidified with 1% FA to give a concentration of 0.1% in each mobile phase. An isocratic method was initially developed for the separation of analytes. This method gave good responses and peak separation, however, it was later

modified to a gradient method. The advantages of the gradient method over the isocratic method were discussed in **section 2.6.**1 and the gradient composition has been described in Table 3-2.

### 3.4.2 Mass spectrophotometer (MS)

The MS was incorporated into the UHPLC. It was equipped with an electron spray ionisation (ESI) interface. All the targeted antibiotics were analysed in the positive ion mode. Samples were analysed in the selected ion monitoring (SIM) mode. The analytes were also scanned in the range 200-600 amu. The following conditions were thereafter set on the MS for the detection of analytes: capillary voltage of 4.0 kV; drying temperature of 400℃; drying gas flow of 1.5 L/min and nebulizer pressure of 35 psi. Nitrogen gas was used as the nebulizing gas. The flowrate was 0.5 mL/min. Table 3-3 shows the mass to charge ratio of the selected antibiotics, used in the SIM mode.

Table 3-3: Mass-to-charge ratio of the selected antibiotics

| **Compound** | **Product ion (m/z)** |
|---|---|
| Amoxicillin | 420 |
| Ciprofloxacin | 332 |
| Enrofloxacin | 360 |
| Sulfamethazine | 279 |
| Chlortetracycline | 479 |

#### 3.4.2.1 Wavelengths Optimisation

The wavelengths of the five analytes were optimised from 220 to 280 nm on a Photodiode Array Detector (PDA) with a path length of 10 mm. The optimum wavelength (264 nm) was chosen to give good signal for most of the analytes. For amoxicillin, 230 nm was observed as its optimum wavelength.

#### 3.4.2.2 Limit of Detection and Quantification

Limit of detection (LOD) and limit of quantification (LOQ) were determined on the instrument. LOD and LOQ defined as the minimum detectable and measured amounts of the analyte, respectively, were determined by the analysis of the spiked samples at the concentrations that would give a signal to noise ratio (S/N) of 3 and 10, respectively. The method detection limit (MDL) and method quantification limit (MQL) were determined by spiking the wastewater sample with the lowest expected concentration and thereafter was run through the SPE and then analysed.

### 3.4.3 Matrix effect

The matrix effect as defined earlier is the change in HPLC-MS response to an analyte, either positively or negatively that is caused by the co-elution of matrix compounds, relative to an injection of a pure standard, thus affecting the response of the MS. A reduced response is referred to as ion suppression and an increased response was referred to as ion enhancement. Therefore to account for both, Equation 3-2 was used to calculate for the effect of matrix on the recovery of the analytes (Arsenault 2012).

$$\% ME = \left(\frac{P}{E} - 1\right) \qquad 3\text{-}2$$

Where, $ME$ is the matrix effect (%) $P$ the response of post extracted spiked sample (with standard) and $E$ is the response of the standards (Neat standard)

### 3.4.4 Relative standard deviation (RSD)

To determine the closeness between the data, the relative standard deviation (RSD) was determined using statistical parameters as shown in Equation 3-3 and Equation 3-4 (Arsenault 2012).

$$S = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}} \qquad 3\text{-}3$$

$$RSD(\%) = \left(\frac{SD}{\bar{x}}\right) \times 100 \qquad 3\text{-}4$$

Where S = Standard deviation, RSD (%) = Relative standard deviation, $\bar{x}$ = Average of a set of N measurements, $x_i$= the individual measurement, N = Number of samples.

## 3.5 Method Validation

The developed SPE-UHPLC-PDA/MS method was validated for linearity, repeatability, LOD, LOQ, MDL, and MQL as well as recovery. The linearity of each analyte was determined in the standard mixture using calibration standards ranging from 0.01 to 10 µg/L. The calibration curve for each analyte was constructed by plotting the peak area against the analyte concentration. The results obtained were analysed by the linear regression method. The repeatability (precision) was determined by analysing the same sample using the same procedure. In a like manner, the intra-day analysis was performed on the same day while the inter-day precision was performed on different days. The results were expressed as relative standard deviation (RSD). For the analysis of the wastewater samples, matrix matched standards were prepared by spiking the wastewater samples. The concentration of the analytes was calculated by subtracting the concentration of the spiked wastewater samples from the un-spiked wastewater samples. This was because it was assumed that un-spiked wastewater samples already contained some of the targeted analytes.

## 3.6 Anaerobic digestion for the removal of antibiotics

This section describes the materials and methods that were considered during the anaerobic degradation of the antibiotics and are elaborated in **sections 3.6** to **3.8**. Studies were carried out in continuous operation of upflow anaerobic sludge blanket reactors and batch reactors. Batch experiments were carried out in serum bottles. Both experiments were aimed at evaluating the removal of the antibiotics and also to understand the elimination route of the antibiotics during anaerobic digestion. Synthetic wastewater was used for the experiments. The experimental design for the batch processes was such that some of the experiments were carried out with synthetic

wastewater while others were with the actual wastewater from the slaughterhouse. Therefore, to investigate the third objective of the study, the following were undertaken

- Monitoring the performance efficiency of the UASB reactor treating slaughterhouse wastewater (synthetic) with respect to contaminants removal (organics)
- Investigation of the elimination routes of the antibiotics using biodegradation, adsorption
- Evaluation of the biodegradation kinetics for the target antibiotics

## 3.7 Materials and chemicals

### 3.7.1 List of chemicals

The list of chemicals used for preparing the synthetic feed is shown in Table 3-4. The synthetic feed was used as a control for the study of the biological degradation of the selected antibiotics. The names of the antibiotics were listed in **section 3.2.1**. Other chemicals that were used were 1 M NaOH and 1M HCl for pH adjustment.

Table 3-4: List of chemicals used for preparing the synthetic feed (Cao and Mehrvar 2011).

| Chemical | Formula | Amount (g/L) |
|---|---|---|
| Peptone | | 4 |
| Meat extract (Oxoid Lab Lemco L0029, Oxoid Ltd.) | | 2.75 |
| Ammonium Chloride | $NH_4Cl$ | 0.2 |
| Urea | $CH_4N_2O$ | 0.75 |
| Sodium Chloride | NaCl | 0.175 |
| Calcium Chloride | $CaCl_2.2H_2O$ | 0.1 |
| Magnesium sulphate) | $MgSO4.7H_2O$ | 0.05 |
| Potassium dihydrogen orthophosphate | ($K_2HPO_4$) | 0.7 |
| | $CuCl_2.2H_2O$ | 0.002 |
| Glucose | $C_6H_{12}O_6$ | 6.25 |
| Sodium hydrogen carbonate | $NaHCO_3$ | 27.5 |

The synthetic wastewater was prepared to simulate a slaughterhouse wastewater before each experiment. The pH of the synthetic wastewater was adjusted to values between 6.8 and 7.2. For the granules formation, OLR was varied from 2.8 to 9 $kg.m^{-3}.d^{-1}$. The ratio of calculated biological chemical demand to chemical oxygen demand was 0.40/0.53, thus emulating that of a real slaughterhouse wastewater (Cao and Mehrvar 2011).

### 3.7.2 Equipment

Table 3-5 shows a list of the equipment, the model and purpose for which it was used during the anaerobic digestion process. The following parameters: pH, chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VSS), were measured.

Table 3-5: List of equipment, the purpose for use and models

| Name of equipment | Purpose | Model |
|---|---|---|
| Water bath | Cooling the UASB | Grant instrument, model: GD120 |
| pH Portable Meter | pH measurement | Orion Star™ A121 |
| Convectional oven | Drying sludge for TSS | Digital incubator, model: 295 |
| muffle Furnace | For VSS test | Nabertherm, LE 2/11 |
| Spectrophotometer | For COD, $NH_3$, TOC and other tests | DR 3900 Hach |
| COD/TOC Hach digester | For digesting COD vial (reagents) | DRB 200, digital reactor block |
| Master flex peristaltic pump | Feeding reactor | EW-77925-10 |
| Freeze dryer | Preparing sludge for adsorption | Lyophilizer - ALPHA 1-2 LDPlus |
| New Brunswick Programmable Incubator shaker | Anaerobic tests | Innova 44/44R |
| Thermo Fisher Scientific Sorvall T1 Centrifuge | Centrifuge samples | Sorvall T1 |

### 3.7.3 Continuous experiment (UASB)

This section describes the experiment carried out on the UASB when it was fed continuously with the wastewater.

### 3.7.4 Description of the UASB reactor set-up

The reactor was constructed from Plexiglas glass with an effective working volume of 4.5 L. It was composed of three zones: the feed entrance zone, the sludge and blanket zone, and the settling zone. An inverse cone which served as the gas-liquid-solid separator (GLS) was installed at the top portion of the sludge bed to enhance the separation of the biogas from the liquids and solids. To reduce variation in temperature, continuous recycling of cooling water through the water jacket on the reactor was carried out using a water bath. The reactor had four sampling points used for the monitoring of reactor performance regarding effluent quality, sludge production, desludging of the reactor; there was no recirculation of the effluent.

### 3.7.5 Methods

#### 3.7.5.1 Sludge collection for reactor start-up

Digested sludge was collected from the anaerobic digester treating similar wastewater, in Durban, South Africa. This wastewater treatment plant (WWTP) received its waters from a slaughterhouse treatment plant. Collected sludge samples were immediately transported to the laboratory and the physical and chemical properties where analysed. The following parameters were analysed: VSS, TSS, methanogenic activity and COD.

Table 3-6: Operational phases of the reactor

| **Operational Phase (days)** | **COD (mg/L)** | **OLR ($kg.m^{-3}.d^{-1}$)** | **HRT (Hrs)** |
|---|---|---|---|
| I (1-15) | 1000-1500 | 2.8-3 | First three days. 24 hours and 12 hours thereafter |
| II (16-48) | 1500-2500 | 3.7-4.4 | 12 |
| III (49-71) | 3000-4000 | 5.1-6 | 12 |
| IV (72-150) | 4000-5000 | 6.1-9.2 | 12 |

Phases I and II indicate the start-up of the reactors. Phases III and IV indicate the period of reactor stabilization. The influent concentration was gradually increased from 20% after two weeks of start-up and gradually to 40% until eventually 100% of the prepared synthetic feed was introduced into the reactor. This method was adopted from Cao and Mehrvar (2011).

### 3.7.5.2 Analytical methods

The following physicochemical properties were measured: chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS), total organic carbon (TOC) and pH. The mentioned parameters were evaluated by following the procedures for standard methods of the examination of water and wastewater (APHA–AWWA–WPCF 1998). TSS was determined gravimetrically by drying well-homogenised samples respectively at 103 $^{o}$C for 24 h. The VSS fractions were determined gravimetrically by incineration in a muffle furnace at 550$^{o}$C for 1 h. COD was determined using close refluxing according to the standard method 5220D. The pH and temperature were measured using a pH meter (Orion) and thermometer respectively. VFAs were quantified by distillation of the sample and titration of the distillate with 0.1N sodium hydroxide to pH 8.3. Total alkalinity was determined by titration of the sample with sulfuric acid (0.1N) to pH 4.0 (Rajesh Banu *et al.* 2008). The pH, temperature and COD were analysed every day during the start-up of the reactor. Once the reactors had stabilised, TSS and VSS were analysed weekly for the duration of the experiment. The parameters were evaluated following the procedure for standard methods for the examination of water and wastewater and detailed description on procedures can be found in (APHA–AWWA–WPCF 1998).

### 3.7.5.3 Start-up of the reactor and its operation

The reactor was started with a digested sludge obtained from a wastewater treatment plant treating slaughterhouse wastewater from an abattoir in South Africa. The reactor was inoculated with 3 L of the activated sludge which was about two-thirds of its

working volume. Sludge was characterised to have a TSS of 19.4 g/L and VSS of 13.8 g/L. The digester was fed continuously from the bottom of the reactor by the use of a peristaltic pump at the rate defined by the HRT (12 h). However, for the first three days of the start-up, the reactor was operated in a batch mode at an HRT of 24 hours and thereafter, the continuous mode was used. An OLR of 1.54 $kg.m^{-3}.\ d^{-1}$ was used for the first 3 days to enable stabilisation of the reactor, and thereafter, the OLR was increased stepwise as the treatment efficiency of the reactor improved with time as shown in Table 3-6.

The biogas produced was channelled through the gas outline (Figure 3-1) and was passed through 3M NaOH solution so as to capture the $CO_2$ present in the biogas (Padilla-Gasca, Lopez-Lpoez and Gallardo-Valdez 2011). The biogas present was measured volumetrically by the displacement of water (Figure 3-1). Gas produced was measured daily to allow the kinetics of the process to be followed and to provide direction as to the stability of the process. To achieve stable operation of the reactor, the temperature of the reactor was maintained at 35˚C with the use of a water bath. Reactor efficiency was evaluated by monitoring the removal of organics using Equation 3-5

$$Reactor\ efficiency\ = \left(\frac{C_i - C_f}{C_i}\right) X\ 100 \qquad 3\text{-}5$$

Where, $c_i$ = Substrate influent and $c_f$ = Substrate effluent

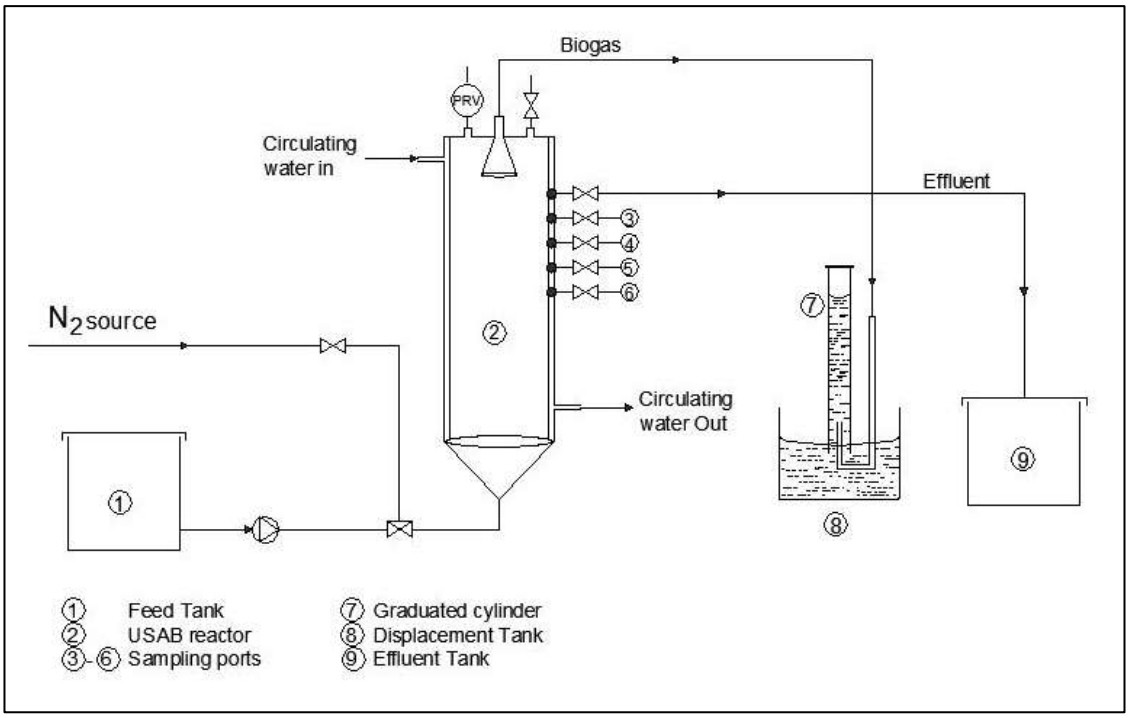



Figure 3-1: Schematic diagram of the experimental set-up (Chollom *et al.* 2017)

#### 3.7.5.4 Antibiotic removal studies using continuous reactor operation

After the start-up period of the anaerobic reactor, the reactor was then run according to the design in Table 3-6. The COD, TOC, TSS, alkalinity, VFAs, biogas production and antibiotics degradation were monitored. Therefore, the antibiotics mixture was added to the reactors, at an initial concentration of 50 µg/L, and effluent was collected and analysed using the method developed for antibiotic analysis that is discussed in Chapter 4, **section 4.4**. Effluent samples were collected after every 24 hours and analysed for the mentioned parameters.

## 3.8 Batch experiments using serum bottles

Batch experiments were carried out to give an insight into the removal pathway for the antibiotics. The following were considered: adsorption, hydrolysis, volatisation and biodegradation. Experiments were therefore carried out in serum bottles. The following were used: sludge (un-treated), freeze dried (treated), actual wastewater and

synthetic wastewater. Finally, mixed solutions of the antibiotics were used for the studies.

### 3.8.1 Activated sludge

Collected digested sludge as previously described in the start-up **section 3.7.31** was used in this section. 30 mL of the sludge was weighed into 50 mL centrifuge vials and were then centrifuged at 5000 g for 5 mins and the supernatant was discarded. The solid pellets were then shaken and thoroughly rinsed with deionised water repeatedly. The samples were further centrifuged for another 5 min and the process of rinsing was repeated. The sludge was thereafter suspended in the synthetic feed and allowed to acclimatise.

### 3.8.2 Biodegradation and adsorption studies using wastewaters and sludge

To elucidate the degradation pathway of the antibiotics, batch experiments were carried using 250 mL serum bottles, and the working volume was 150 mL. The bottles were dosed with the standard solution of the mixed compounds and the pre-determined amounts of sludge and wastewater was added to it where necessary. To ensure that there was no interference with oxygen, the bottles were purged with nitrogen before loading and after loading. The headspace was again flushed with nitrogen. After flushing, the bottles were sealed with gas-tight silicone septa and crimping tool with aluminium rings. The sealed bottles were incubated at 35˚C throughout the batch process time (30 days). They were shaken at a low speed of 100 rpm. The initial antibiotics concentration was 100 ppm. Table3-7 shows the experimental design for the batch experiments. Samples were collected at the following times 0, 0.5, 1, 2, 4, 8, 10, 15, 20, 28 days. The chosen time of 28 days was adapted from a related study by Wang *et al.* (2017). Collected samples were analysed based on the developed method discussed in Chapter 4, **section 4.4** for the antibiotics analysis. The initial and final COD was measured as an indicator of the biological activity. Other authors have used caffeine which is a biodegradable compound to evaluate for bioactivity (Li and Zhang 2010; Wang *et al.* 2017). For the inhibition of biological activities, 0.1% Sodium azide ($NaN_3$) was added to selected serum bottle. All the bottles used for the

studies were wrapped with aluminium foil to avoid possible photolysis of the compounds.

Table 3-7: Experiment design for the batch experiments.

| Treatment | Treated sludge | wastewater | antibiotics | 0.1% $NaN_3$ | Removal routes |
|---|---|---|---|---|---|
| R1: R1' | Yes | Yes | Yes | No | B+A+H |
| R2 | No | Yes | Yes | No | H |
| R3: R3′ | Yes | Yes | yes | yes | A |
| R4 (control) | Yes | Yes | No | No | B |

Reaction pathways are indicated as shown below.

- B: biodegradation
- A: Adsorption
- H: Hydrolysis
- R (Symbol for reactor number) and R' (duplicate)
- Chemical oxygen demand (COD)

Sorption efficiency as well as COD reduction were calculated according to Equation 3-6 (Ferreira *et al.* 2016).

$$\%\ COD\ reduction = \frac{(C_o - C_t)100}{C_o} \qquad 3\text{-}6$$

### 3.8.2.1 Biodegradation Kinetics Models

Three biodegradation kinetic models (first order models) were applied for the biodegradation data as represented in Equation 3-7. First order models were applied because the concentration of the antibiotics studied for the biodegradation tests were low, even lower than the those in sludge (Li and Zhang 2010; Wang *et al.* 2017).

$$\frac{dC}{dt}\frac{dC}{dt} = -K_1.C \Leftrightarrow C_t = C_0.e^{-k.t} \qquad 3\text{-}7$$

Where, $C_0$ is initial concentration of the antibiotic; $C_t$ is concentration of the antibiotic at time t; $K_1$ is the first order rate constant. The half-lives of the compounds $t_{1/2}$ were calculated from equation the 3-7 to give $\frac{(\ln 2)}{K_1}$

## 3.9 Development and evaluation of an efficient polishing step (advanced oxidation) for the removal of veterinary antibiotics

This section on adsorption and photo-degradation describes the materials and the methods that were employed in achieving the degradation of the antibiotics. The section describes the method used to prepare the catalyst (IPCA) as well as the different methods used for the physiochemical properties characterisation. The methods on adsorption, kinetic models and photo-degradation are also presented. Therefore, to achieve the fourth and fifth objectives of the study, it was necessary to carry out the following;

- Characterise the IPCA using Scanning Electron Microscopy (SEM), Energy dispersive x-ray (EDX) and X-ray diffraction (XRD).
- Investigate the effects of operating parameters including initial antibiotics concentration, solution pH, adsorbent dosage and contact time on adsorption performance.

- Determine the adsorption kinetics, equilibrium isotherms on the experimental data on the AC and IPCA.
- Evaluate the application of photodegradation of the antibiotics on using integrated photocatalytic adsorbent (IPCA and $TiO_2$).

### 3.9.1 Materials and Methods

#### 3.9.1.1 Chemicals

The chemicals used for the adsorption and kinetic studies included HCl, $H_2SO_4$, and NaOH. Others such as the antibiotics have been listed in **section 3.2.1.** Synthetic wastewater used for the study in this section has previously been listed in Table 3-3.

### 3.9.2 Integrated photocatalyst (IPCA) preparation

The Integrated photocatalyst (IPCA) was prepared in the ratio of 1:10. Therefore, 6 g of activated carbon (AC) and 0.6 g of a mixture of anatase and rutile $TiO_2$ (composed of 75% anatase and 25% rutile) were added to 200 ml of deionised water. The mixture was then ultrasonicated for 1 hr. After the sonication, the water was decanted and the solid part was oven dried at 80˚C for 12 hours. The dried IPCA was then washed using 200 to 300 ml of deionised water and thereafter, the water was decanted. The reason for the washing was to get rid of the loosed $TiO_2$. This process was repeated five times. The washed IPCA was oven dried at 110˚C. The dried IPCA was collected and stored in sealed glass vials until required for usage.

### 3.9.3 Characterisation of the catalysts

The characterisation was carried out on the activated carbon, IPCA, and the $TiO_2$ as described in **sections 3.9.3.1 to 3.9.3.3.**

#### 3.9.3.1 Scanning Electron Microscopy (SEM)

The morphology of the catalyst was determined using a Scanning Electron Microscopy (SEM). One of the important features of the IPCA is its morphology. It is important that the $TiO_2$ is evenly distributed on the surface of the AC. The distribution on the surface of the AC would enhance the photocatalytic degradation of the contaminants. The SEM analysis was carried out with the SEM coupled with an energy dispersive x-ray analyser (EDX) Model: EVO 15 HD, Carl Zeiss, Germany. A thin layer of the sample was mounted on the aluminium specimen holder by double-sided tape. The samples were coated with a thin film of gold with a thickness of about 30 nm to avoid charging effects (sputter-coating) (Oyeyinka *et al.* 2015).

#### 3.9.3.2 Energy dispersive x-ray (EDX)

Elemental analysis together with the phase compositions of both the AC and IPCA was conducted using the same SEM analysis instrument. The Energy dispersive x-ray

(EDX) was used to analyse the distribution of $TiO_2$ across the surface of the IPCA and as well for the determination of its elemental composition.

#### 3.9.3.3 X-ray diffraction (XRD)

X-ray diffraction (XRD) was used to determine the crystallinity as well as the amorphous phases of the adsorbents. X-ray diffraction of the samples was carried out following a method by Oyeyinka *et al.* (2015). X-ray diffractometer (Empyrean, PANalytical Netherlands) operating at 40 kV with a target current of 40 mA was used for the analysis. A sample to be analysed was tightly packed in a rectangular glass cell and scanned over a region of 4 to 80 $(2\theta)^{\circ}$ at a scanning speed of 0.06°/min.

### 3.9.4 Adsorption experiment and Kinetic studies

Adsorption studies were carried out using AC, $TiO_2$, and IPCA in order to determine the optimum adsorbent mass and equilibrium time so that the kinetic and isotherm data could be generated. All the adsorption experiments were carried out in 250 mL conical flasks. The appropriate amounts of the catalysts were weighed and placed into the flask already containing 100 mL solution of the antibiotics. The top of the flask was sealed with parafilm. The flasks were then arranged on the orbital shaker and shaken at 150 rpm in the dark at 30˚C for the set time. Samples were collected at intervals using pasture pipettes. Collected samples were filtered using 0.22 µm syringe filters. The samples with the residual concentration were analysed with the HPLC using the method developed in **section 3.3**. The reduction in the volume during the adsorption tests was accounted for in the adsorption loading calculation. To account for adsorption loading, $q_e$ (mg/g), Equation 3-8 was used. The percentage sorption capacity was calculated using Equation 3-9.

$$q_e = \frac{(C_i - C_f)\mathrm{V}}{\mathrm{W}} \qquad 3\text{-}8$$

$$\%(A) = \frac{(C_i - C_f)}{C_i} \qquad 3\text{-}9$$

Where, $q_e$ is the amount of antibiotics adsorbed on the adsorbent at a time ($q_t$) at equilibrium, *V* is the volume of the substrate in the solution (L), $C_i$ and $C_f$ are the initial and final concentration of substrate in solution (mg/L) respectively, and *W* is the mass of adsorbent (g).

### 3.9.5 Kinetic studies

#### 3.9.5.1 Effect of contact time

The effect of contact time was determined for the studied antibiotics in order to evaluate the removal efficiency. The following parameters were kept constant: pH of 6, adsorbent loading of 1 g/L and 100 ppm initial concentration of the antibiotics. A fixed mass (0.1 g) of the adsorbent was weighed and added to 100 mL of the sample in the conical flasks and were placed in the orbital shaker which was set at 150 rpm and temperature of 30˚C.

#### 3.9.5.2 Effect of pH on contaminant removal

Similar methodology as followed for the adsorption test was carried out in evaluating for the effect of the pH except that in this case, the pH values 3, 6, 7, 8 and 10 were evaluated. 1M of NaOH and 1M HCl were used for pH adjustment and this was carried out on the sample solution. During the pH measurement, the sample was continuously stirred as the pH was adjusted until the desired pH was reached.

#### 3.9.5.3 Effect of antibiotic concentration

The methodology presented in **section 3.9.4** was followed, except, in this case, the contaminant concentration was varied from 10 to 100 mg/L while other parameters remained unchanged.

#### 3.9.5.4 Effect of catalyst loading on contaminant removal

The methodology presented in **section 3.9.4** was followed, except, in this case, the loading rate of the catalyst was varied from 0.4 to 2 g. Other parameters were kept constant while the catalyst loading was varied.

### 3.9.6 Kinetic models for kinetic studies

Data obtained from the adsorption studies were assessed to see which of the kinetic models would better describe the rate or the mechanism of the sorption processes. The studies provide important information on the various parameters such as time needed for reaching equilibrium, the rate of the adsorption process and finally, the adsorption potential rate-limiting steps. Three kinetic models were employed namely: the pseudo-first order, pseudo-second order, and the intra-particle diffusion model. After fitting the data into the various kinetic models, the results obtained were used to determine the kinetic models that would better describe the reaction rates.

#### 3.9.6.1 First order reaction

The pseudo-first-order kinetic model indicates that the rate of adsorption is directly correlated to the difference of uptake at equilibrium and time, *t*, shown as ($q_e$-$q_t$) in the Equation 3-10.

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \qquad 3\text{-}10$$

Where, $q_e$ is adsorption capacity at equilibrium (mg/g), $q_t$ is the adsorbate uptake at time *t* (mg/g) and $K_1$ $is$ rate constant of the pseudo first-order adsorption model (1/hr). Assuming the boundary conditions: $q_t = 0$, $q_t = q_t$ at the time $t$ = 0 and $t$ = $t$ respectively, then Equation 3-11 is produced from equation 3-10

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \qquad 3\text{-}11$$

#### 3.9.6.2 Second order reaction

The second order model is also for the sorption of the adsorbates onto the adsorbents and is based on differentiating the kinetics of the second-order rate expression with respect to the adsorption capacity of the adsorbents. This is highly dependent on the adsorbent loading and the adsorbates (solutes) concentration. It is expressed as shown in equation 3-12. The rate controlling step here according to (Choy, Porter and Mckay 2004) is chemisorption.

$$\frac{dq_t}{dt} K_2 = (q_e - q_t)^2 \qquad 3\text{-}12$$

Where, $K_2$ (g/mg.hr) is the rate constant of the pseudo second-order adsorption model. $q_e$ and $q_t$ are the amount of antibiotics adsorbed (mg/g) at equilibrium and time, *t*, respectively. Equation 3-13 is obtained when 3-12 is integrated using the boundary conditions of $q_t = 0$, $q_t = q_t$ at the time $t = 0$ and $t = t$

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + K_2 t \qquad 3\text{-}13$$

On re-arranging Equation 3-13, Equation 3-14 is obtained.

$$\frac{t}{q_t} = \frac{1}{K_2 {q_e}^2} + \frac{1}{q_e} \qquad 3\text{-}14$$

By plotting ($\frac{t}{q_t}$) versus *t*, the equilibrium concentration $q_e$ is determined.

#### 3.9.6.3 Intra-particle diffusion model

The intra-particle diffusion model is expressed by the Equation 3-15. During adsorption, the adsorbate molecules need to be transferred from the bulk phase to the solid surface and thereafter penetrate into the adsorbent pores, thus diffusion occurs slowly. In general, for the adsorption process to take place the adsorbate molecules need to be transferred from the bulk phase to the solid surface and then penetrate into the adsorbent pores. Typically the diffusion through the pores is a slow step. Whether diffusion within the pores is the rate-limiting step or not, it can be determined based on the intra particular diffusion model.

$$q_t = K_{int}\, t^{\frac{1}{2}} + C \qquad 3\text{-}15$$

Where $q_t$ is adsorption loading (mg/g) at a given time (days), $K_{int}$ is the intra-particle diffusion rate constant (mg/g $day^{-1/2}$) and *C* is the intercept and it indicates the presence of the boundary layer diffusion and *t* is time (days). The diffusion rate constant $K_{int}$ is

determined from the plot of the slope of the line modelling $q_t$ vs $t^{\frac{1}{2}}$. The value of *C* is determined through the intercept of the line (Alkaim *et al.* 2014). When the fitted line passes through the origin, then the intra particle diffusion is the rate limiting mechanism. If the contrary occurs, then other mechanisms along with intra particle diffusion can be responsible for the kinetics of the process and as such, the boundary layer thickness could be the determining factor in the adsorption (Gupta *et al.* 2013).

### 3.9.7 Models used for isotherm studies

Analysis of the isotherm data is important for the development of the equation that will accurately represent the results and also that would be used for design purposes as well. The most common isotherms applied in solid/liquid systems are the theoretical equilibrium isotherms, Langmuir and Freundlich. The reason for their wide usage is due to their simplicity and ease of interpreting data.

#### 3.9.7.1 Langmuir adsorption isotherm

The Langmuir adsorption isotherm assumes monolayer adsorption on adsorbents which have homogeneous energy distribution. According to Jang *et al.* (2018), the basic assumptions that are made with regards to the model are: (1) a monolayer adsorption occurs on the adsorbent surface which has a finite number of similar active sites, (2) interaction does not take place between adjacent adsorbed molecules, (3) the active sites have equal affinities towards the adsorbate molecules and finally (4) the adsorption process is reversible (Chen *et al.* 2015; Jang *et al.* 2018). However, Gao *et al.* (2012) described that in real cases, these assumptions might not be well satisfied. The Langmuir model is described by Equation 3-16 (Dada *et al.* 2012).

$$q_e = q_{max}\frac{K_L C_e}{1 + K_L C_e} \qquad 3\text{-}16$$

The linearised forms of the equation are written in different forms as shown in Equation 3-17 and Equation 3-18

$$\frac{1}{q_e} = \left(\frac{1}{K_L q_{max}}\right)\frac{1}{C_e} + \frac{1}{q_{max}} \qquad 3\text{-}17$$

$$\frac{C_e}{q_e} = \frac{1}{q_{max}} C_e + \frac{1}{K_L q_{max}} \quad \text{3-18}$$

Where, $q_e$ is the adsorption capacity at equilibrium (mg/g), $q_{max}$ is the theoretical maximum adsorption capacity of the adsorbent (mg/g), $K_L$ is the Langmuir affinity constant (l/mg) and $C_e$ is the supernatant equilibrium concentration of the system (mg/l).

The Langmuir model is said to be evaluated by using a dimensionless constant which is known as the separation factor, $R_L$ which is represented with the formula shown in Equation 3-19.

$$R_L = \frac{1}{1 + K_L C_o} \quad \text{3-19}$$

Where, $C_o$ is the initial adsorbate concentration (mg/l) and $K_L$ the affinity parameter (l/mg)

When the $R_L$ value is between zero and unity, it indicates favourable adsorption while a value above one is an indication of very low values of $K_L$, thus showing that the adsorption was not favourable (Dada *et al.* 2012; Chen *et al.* 2015).

#### 3.9.7.2 Freundlich adsorption isotherm

The Freundlich isotherm describes the adsorption of organic and inorganic compounds on a wide variety of adsorbents. It was originally of an empirical nature model but was later interpreted as sorption to heterogeneous surfaces or surfaces that support sites with different affinities. It is presumed that the stronger binding sites are the first to be occupied and that the binding strength reduces as the number of sites being occupied increases (Chen *et al.* 2015). The Freundlich model (Equation 3-20) describes the adsorbed mass per mass of adsorbent (Dada *et al.* 2012).

$$q_e = K_f \, C_f^{\frac{1}{n}} \quad \text{3-20}$$

Where, $q_e$ is the equilibrium value of sorbate uptake (mg/g), $K_f$ is the Freundlich isotherm constant, $C_f$ is the equilibrium (final) concentrations of the adsorbate (mg/L) and $\frac{1}{n}$ is the Freundlich constants related to sorption capacity and sorption intensity of the adsorbents, which represent the quantity of sorbate adsorbed. The Freundlich intensity *n* is an indication of an existing adsorption driving force and heterogeneity degree of the surface (Dada *et al.* 2012; Alkaim *et al.* 2014). The parameter *n* is dimensionless and falls in the rage of 0 to 10 ($0 < n < 10$). If the value of *n* is higher than 10, it means that an irreversible isotherm would be obtained (Dada *et al.* 2012; Alkaim *et al.* 2014). However, the values of *n* greater than unity is an indication of a preferential adsorption, and less than unity indicates a poor adsorption (Chen and Huang 2010). Further still, *n* is described as; If n = 1 then the partition between the two phases are independent of the concentration. If the value of 1/n is below one it indicates a normal adsorption. On the other hand, if 1/n is above one, it indicates a cooperative adsorption. $K_f$ is an indication of the adsorption capacity of the adsorbents in use.

## 3.10 Photocatalytic degradation of antibiotics

Photolysis and photocatalysis batch studies were carried out using a 600 ml beaker, a method that was modified from (Amalraj Appavoo *et al.* 2014). Figure 3-2 illustrates a simple methodology that was used for the batch degradation of the antibiotics. The catalyst loading rate, pH and initial concentration of the contaminants were evaluated. Direct photolysis using the UV light without a catalyst was carried out to determine the baseline degradation of the antibiotics as well as the other organics. Thereafter, the $TiO_2$ and IPCA were suspended in 300 ml of a pH adjusted solution which contained varying concentrations (10, 20 and 50 µg/L) of antibiotics. The solution was stirred in the dark for 30─60 minutes to ensure complete equilibration of adsorption/desorption of the antibiotics onto the $TiO_2$/IPCA surface. Aliquots of the solution were collected for analysis, using the method described in **section 3.4.** The remainder of the solution was then subjected to UV-A irradiation provided by 9 W lamps emitting predominantly at 350-400 nm each and the runtime for each experiment was 4 hours and thereafter aliquots were collected for analysis. The lamp was suspended above the

beakers to enable the photo-degradation of the contaminants. Samples were collected at the set intervals using a syringe and filtered through a 0.22 µm nylon syringe filter for determination of antibiotic degradation. The degradation efficiency of the organics was evaluated using equation 3-9.

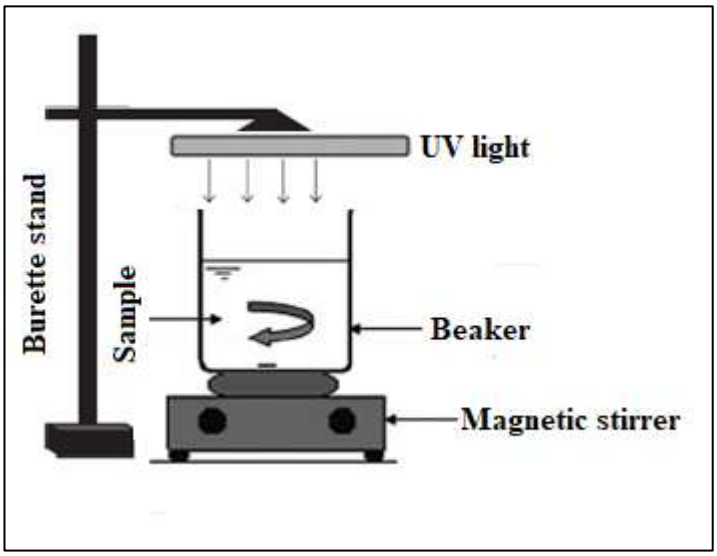



Figure 3-2: The schematic diagram of the photocatalytic experimental set-up for kinetic studies (Amalraj Appavoo *et al.* 2014).

For the kinetic studies, the effect of the operating parameters such as, catalyst dosage, initial antibiotic concentration and pH were analysed. A pseudo-first-order mathematical model was fitted to the experimental data obtained from the kinetic studies by a non-linear regression method. Equation 3-21 shows the pseudo first order model used.

$$\frac{dC}{dt} = K_1.C = r \qquad 3\text{-}21$$

Integrating equation (3-21) gives Equation 3-22

$$ln\left(\frac{C_o}{C_t}\right) = K_1.t \qquad 3\text{-}22$$

## 3.11 Optimisation

For the photocatalytic degradation, two parameters were evaluated viz. catalyst loading and pH. These two parameters were chosen based on the role they play during the photocatalytic reaction. Most photocatalytic reactions are said to be dependent on pH (Pereira et al. 2011; Zhu et al. 2013; Safari et al. 2014; Sacco et al. 2018). The pH values employed were 3, 6, 8 and 10 while catalyst loading was from 0.5 to 2 g/L.

# Chapter 4 - Development of SPE-HPLC/PDA-MS for the analysis of selected veterinary antibiotics in slaughterhouse wastewater

## 4.1 Introduction

This chapter discusses the results from the method developed for the detection and quantification of antibiotics from the slaughterhouse wastewater treatment plant. Due to the complex nature of the wastewater, the preparation and separation method is vital for routine analysis. UHPLC was chosen for the analysis over GC because it does not require the derivatisation of analytes. The analytes considered in this study were non-volatile, hence the use of GC would mean derivatisation of the compounds which would have increased the sample preparation time and further still, make the process more expensive. In this chapter, findings from the three main sections of the method development: i) the development of the SPE method which is the pre-concentration step; ii) HPLC method, which is the separation step; and iii) the MS method, which is the identification step, are elaborated. The parameters of the SPE and HPLC were optimised while the parameters of the MS method were not optimised. This is due to the fact that the operating parameters used obtained from the literature gave good enhanced signals for MS. After the optimisation processes, the methods were then combined and validated using the influent and effluent samples. Thereafter, the method was applied influent and effluent samples. The optimised chromatographic (HPLC) conditions are presented in **section 4.3.1**. Results on the SPE are presented in **section 4.2** and the validation of the developed method is discussed in **section 4.4.**

## 4.1 Method development steps

A method for the separation and quantification of the selected antibiotics based on UHPLC was developed. The UHPLC was coupled with a Photodiode Array (PDA) detector and a Mass spectrometer (MS). Standard procedures were employed and calibration curves were plotted using calibration standards for each analyte.

Furthermore, the determination of the equations for the linear regression lines were obtained from the calibration curves. Validation of the method was based on the instrument limit of detection (LOD) and limit of quantification (LOQ) for each analyte, as well as the method limit of detection and quantification (MDL, MQL). In addition to this, the evaluation of the linearity, precision, recovery (%) and finally, the matrix effects was performed. Figure 4-1 shows the method development steps.

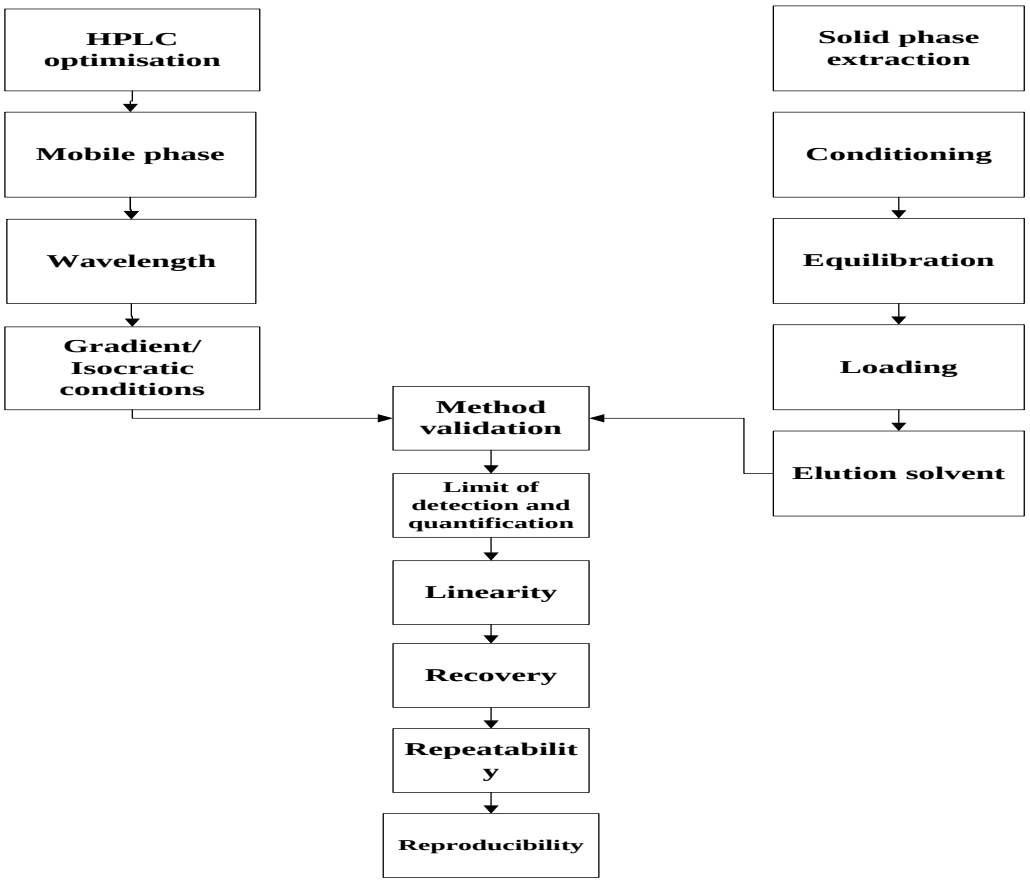



Figure 4-1: Method development steps for the SPE-HPLC/PDA-MS.

The criteria that were considered for the method developed include: a single method required for the efficient monitoring of the antibiotics in the wastewater treatment plant; the method should be simple, rapid, reproducible and not expensive; finally, the method should be validated to ensure the quality of the data obtained. The best conditions obtained from the optimisation were therefore used for the analysis. However, one disadvantage of the multi analysis methods for the analytes is the fact

that optimum conditions for each analyte are not met but a compromised one has to be agreed upon. This is due to the fact that it is not the best condition for each of the compounds of interest that is used for evaluation.

## 4.2 Optimisation of the Solid Phase Extraction (SPE) method

Pre-treatment of samples before SPE is crucial as it enables the removal of particulate matter that might impede on the SPE process. The use of glass micro-fibre filters was employed before SPE on the wastewater samples, and thereafter, the pH of the sample was adjusted to the predefined pH (2, 3, 4, 6, 7 and 8). Similarly, for the wastewater samples, a chelating agent, disodium ethylenediaminetetraacetate ($Na_2EDTA$) was added to the samples before the pre-treatment by filtration and SPE. The chelating agent complexes the metals or multivalent cations that are soluble in the sample. This increases the recovery of the analyte from the sample. Studies have indicated that recoveries of tetracyclines are improved with the use of $Na_2EDTA$ (Gros, Rodríguez-Mozaz and Barceló 2013; Gbylik-Sikorska *et al.* 2015).

The antibiotics that were used for the study had different chemical properties. As a result, the sorbent that was used was carefully selected based on literature (Opriş *et al.* 2013). Usually, the choice of the sorbent plays a critical role during SPE because it can affect the method performance especially with respect to selectivity, affinity and capacity. Strata-X made form Phenomenex was chosen. The cartridges used were 500 mg/6 mL. The Strata-X retention mechanism is based on hydrogen-bonding and is hydrophobic. Therefore, as a result, the sorbent was applied to the reversed-phase extraction of acidic, basic and neutral compounds. The SPE was evaluated using recovery performance data for a mixture of the five analytes. The SPE involved conditioning, equilibrating, loading and elution steps. The conditioning and loading steps were not optimised, however, pH, elution solvent and solvent volume were evaluated. Conditioning of the sorbent was carried out with 5 ml of methanol and was equilibrated with 5 ml of acidified millipore water. Both conditioning and equilibration were carried out at low flowrates (1 mL/min) to ensure that pores of the sorbents were properly wetted to allow the retention of the analytes during the loading steps. Again,

care was taken to ensure that cartridges did not dry up during both the conditioning and equilibration. Poor wettability of the sorbents would mean insufficient retention of the analytes (Arsenault 2012). Both the conditioning and equilibrating steps were carried out to prepare the sorbent for loading. The loading volume used was 100 mL.

#### 4.2.1.1 pH of the sample

The pH of the samples was optimised in order to enhance the analyte recovery. The studied pH ranged from 2 to 8 while the other parameters were kept constant. Figure 4-2 shows the recoveries at different pH values for the selected analytes. Four classes of antibiotics were considered for this study: tetracyclines, fluoroquinolones, sulphonamides and $\beta$-lactam.

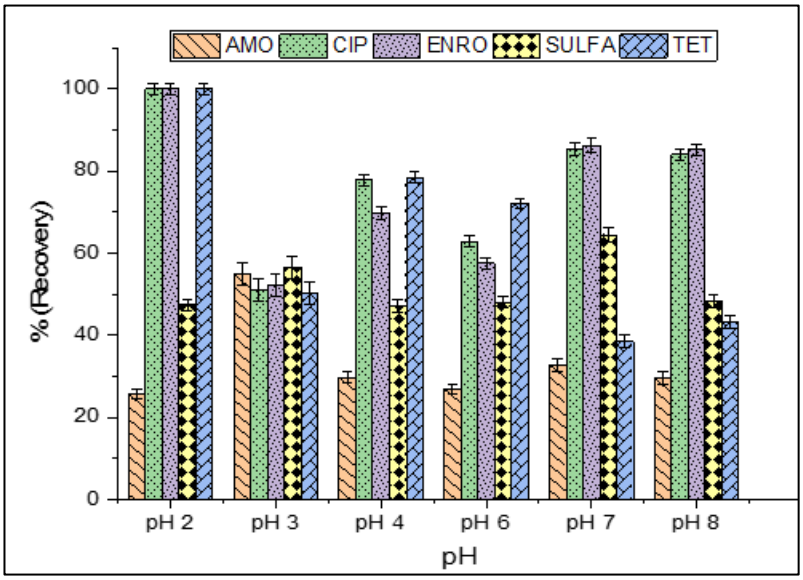



Figure 4-2: Recoveries of the antibiotics at different sample pH at 100 ml loading volume, initial spiking concentration is 1 µg/L and conditioning and elution volumes were 5 ml each.

From Figure 4-2, it was observed that for the fluoroquinolones and tetracyclines, high recoveries were obtained at a pH of 2. While for the sulphonamide, the recovery was highest at neutral pH. The $\beta$-Lactam (AMO) had a recovery of over 50% at pH of 3. With a recovery of 70% at neutral pH of 7 for SULFA, it was observed that its recovery was much higher than that of AMO for all the studied pH values. Recoveries of 100%

were obtained for CIP, ENRO and TET at pH of 2. The recovery of AMO for the studied pH was lower compared to other compounds, a similar observation was made by Opriș *et al.* (2013). At pH 3, lower recoveries were obtained for all the compounds. The reason for the low recovery could be attributed to factors such as the properties of the studied compounds as well as the SPE cartridges employed. Other factors such as: elution time, elution solvents amongst others have been reported for low recoveries (Bielicka-Daszkiewicz and Voelkel 2009; Philip *et al.* 2012). However, higher recoveries at pH of 3 have also been reported (Opriș *et al.* 2013).

Tlili *et al.* (2016) reported that pH influences the sensitivity of the detection and that the amphoteric properties of the compounds involved play a great role. For instance, it is said that the fluoroquinolones are made up of a carboxylic acid with a pKa value which is approximately 5 together with one or more amine functional groups with pKa ~ 8–9. On the other hand, the sulphonamides have characteristics of either weak alkali or weak acids due to anilinic nitrogen and the N─H bond of the sulfonamidic group. Therefore, the analytes properties play a major role on their behaviour in the chosen sorbent.

Based on the outcome of the pH optimisation, pH of 2 was selected for the subsequent studies. The pH selected for the analytes in this work is in agreement with previous studies carried out with similar compounds. Most studies have indicated that higher recoveries are made at lower pH (acidic) (Zhou, Maskaoui and Lufadeju 2012; Gros, Rodríguez-Mozaz and Barceló 2013; Gbylik-Sikorska *et al.* 2015). In fact for most multi-residue methods reported, the studies were carried out in acidic conditions ranging from pH 2 to 4. It is recommended that the acidification of at least 2 units under pKa values of target analytes in water samples should be used. This is to attain their neutral or acidic state of the samples, which may significantly improve their retention onto the SPE polymeric sorbent (Arsenault 2012; Gros, Rodríguez-Mozaz and Barceló 2013).

#### 4.2.1.2 Elution solvent

Elution of the analytes which are usually adsorbed onto the sorbent is necessary and it should be maximised to increase the sensitivity of analytical method. The SPE extraction is a surface phenomenon that involves the interaction of analytes and the adsorbent such that the recovery of analyte is highly dependent on the elution solvents. Therefore, optimising the elution solvents for the elution is important. Methanol and acetonitrile with and without acidification with formic acid were considered in this study. Results obtained showed that elution with acidified acetonitrile and the non-acidified acetonitrile gave lower recoveries as presented in Figure 4-3.

The use of methanol yielded higher recoveries. Furthermore, higher recoveries were obtained on acidifying the methanol. The recoveries presented in Figure 4-3 showed that the use of acidification for both solvents yielded a higher recovery, even though the difference with a very little margin. The recoveries on using acidified methanol were: 81% for CIP, 79% for SUL, 74% for ENRO, 67% for TET and 55% for AMO. While without acidification, they were: 77% for CIP, 76% for SULFA, 65% for TET, 72% for ENRO and 53% for AMO. From the results therefore, the use of acidified methanol gave higher recovery and was adopted for the elution of the analytes. The choice of methanol as an elution solvent is consistent with other studies (Zhou, Maskaoui and Lufadeju 2012).

Zhou, Maskaoui and Lufadeju (2012) optimised different solvents such as acetone, dichloromethane, ethyl acetate, hexane and methanol for the desorption of analytes and found that methanol gave the highest recoveries. The lowest recoveries were from elution with hexane, and they attributed the low recovery to the polar nature of the analytes.

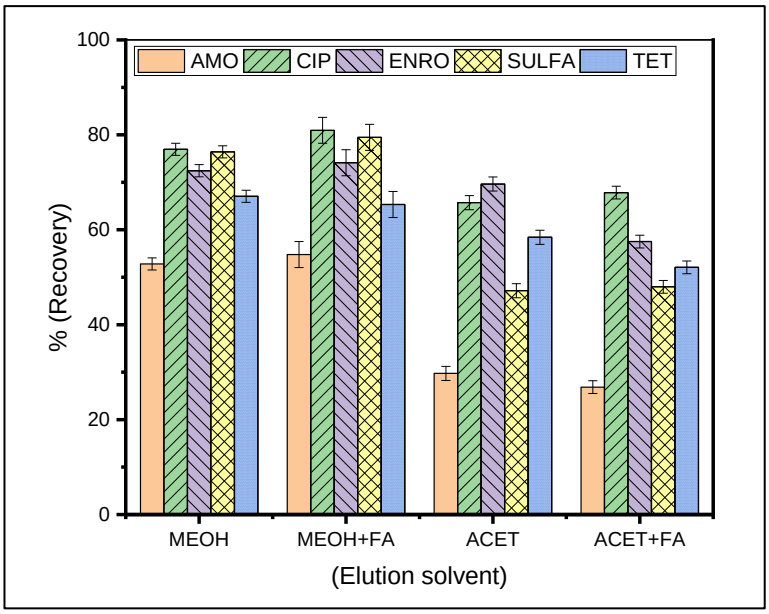



Figure 4-3: Recoveries from different solvents (MEOH; methanol, FA; formic acid and ACET (acetonitrile), at 100 ml loading volume, initial spiking concentration is 1 µg/L and conditioning and elution volumes were 5 ml each.

## 4.3 Optimization of Ultra High-Performance Liquid Chromatographic (UHPLC) Method

### 4.3.1 Mobile phase optimisation

The initial mobile phases were millipore water (A) and acetonitrile (B). At the early stage of the separation (method development), both mobile phases were not acidified which resulted in obtaining peaks that are not properly recovered and not enhanced as well. Therefore, at a later stage both mobile phases were acidified with formic acid (FA) to pH 3, thereby enhancing the peaks (Petrović *et al.* 2005). The use of acidic mobile phases has been recommended for the analysis of antibiotics and is, therefore, a common practice. It improves the ionisation efficiency and enhances the peak intensity (Tlili *et al.* 2016).

The initial experiment was started with a composition of 5% B and 95% A which was held at isocratic conditions for 15 minutes. However, the results showed that the initial

percentage of acetonitrile was too low and the analytes were not separated properly, as there was co-elution among the analytes. Therefore, the mobile phase composition of B was changed to 10% and held for 15 minutes at isocratic conditions. A similar occurrence was observed which then led to a further increase of B to 15%. At 15% B (isocratic), good separations were achieved as seen in Figure 4-2. The flow rate and injection volume used were 1 mL/min and 10 µL, respectively. However, when moving the method from HPLC-PDA to MS, the flow rate was changed to 0.5 mL/min.

Upon achieving the separation of the mixture of antibiotics at 15%, it was necessary to determine the retention sequence of each analyte. Therefore, individual analytes were injected separately and the retention times noted. The sequence therefore was amoxicillin (AMO), ciprofloxacin (CIP), enrofloxacin (ENRO), sulfamethazine (SULFA) and chlortetracycline (TET) as shown in Figure 4-4. The other parameter that was used to further confirm the sequence of the elution was the lambda max for each analyte. This is elaborated in **section 4.3.2.**

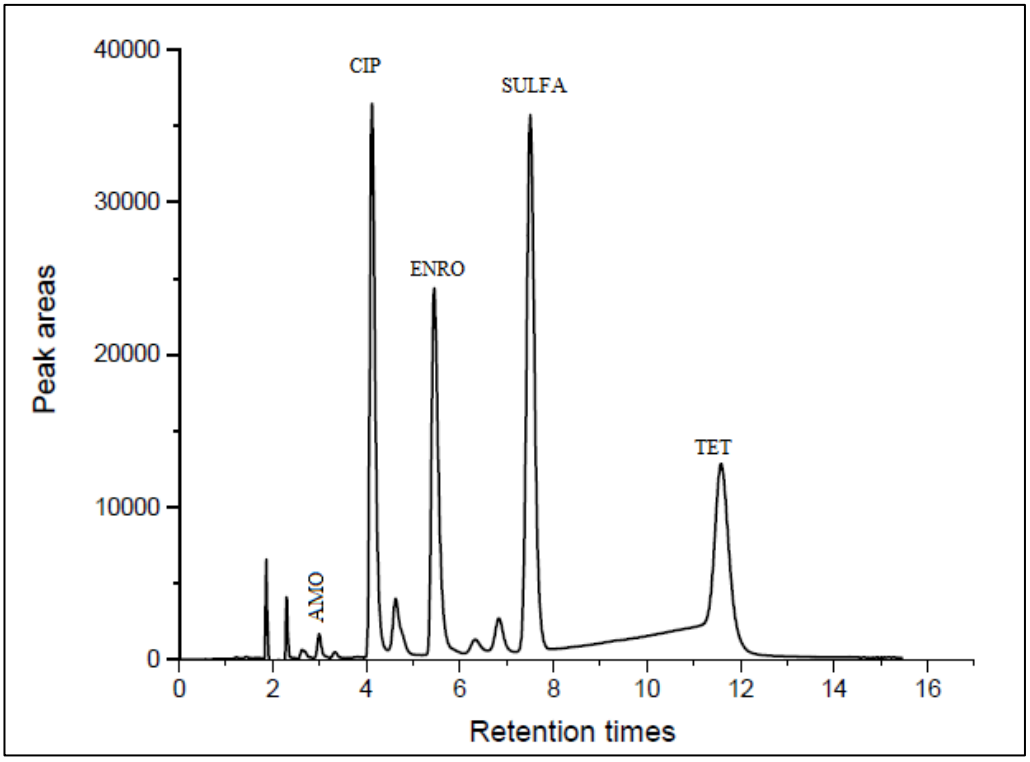



Figure 4-4: Chromatogram of the 10 µg/L mixture of the five analytes at a 15% ACN and 85% isocratic method (mobile phase was acidified with 1% formic acid)

Despite the separation of the analytes being achieved at 15% ACN using an isocratic method, it was necessary that a gradient method be developed and used for the analysis because of the complexity of the wastewater to be analysed. The advantages of the gradient method have been elaborated in the literature review **section 2.6.1.** A series of linear gradient methods were investigated until eventually the method shown in Table 4-1 was adopted. Table 4-1 shows the gradient conditions that were used; it is observed that the separation occurred in an isocratic mode for the first 25 minutes with 15% B. During this time, the analytes of interest had already been eluted and separated well. Just after that, the organic mobile phase concentration was increased from 15% to 100% and was held for 6 minutes. This was to enable the cleaning of the column and to avoid the carrying over of contaminants to the next samples. Further still, the last 10 minutes was used for equilibrating of the column before the next injection. Therefore, the method used for the analysis was a simple linear gradient which was more or less an isocratic method at the beginning. The total run time was 41 minutes.

Table 4-1: UHPLC gradient conditions

| Time | (min) | 0.1 | 25 | 26 | 32 | 33 | 43 |
|---|---|---|---|---|---|---|---|
| Solvent A | (%) | 85 | 85 | 0 | 0 | 85 | 85 |
| Solvent B | (%) | 15 | 15 | 100 | 100 | 15 | 15 |
| Flowrate | (mL/min) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Injection volume | (µL/min) | 10 | 10 | 10 | 10 | 10 | 10 |

### 4.3.2 Analyte wavelength optimisation

The optimum wavelength for each of the analytes was determined on the HPLC. A mixture of the standards at a concentration of 10 mg/L was used for this study. The wavelengths considered were 230 nm, 254 nm, 264 nm, and 270 nm. Appendix A (1-5) shows the spectra for each of the analytes. At the lambda max for each analyte, the peaks were enhanced; therefore, the lambda max was suitable for each analyte. Thus, if each of the lambda maxes is considered, the simplicity of the method would have been averted, hence the need to optimise wavelengths so that a common wavelength

would be chosen. The chosen wavelength must be based on the fact that the peaks are enhanced for each analyte. Therefore, a wavelength of 264 nm was chosen for the analysis/separation of analytes.

## 4.4 Method validation (SPE-HPLC/PDA-MS)

The developed method was validated so as to evaluate its performance especially relating to the monitoring of both the influents and effluents from the slaughterhouse. The results from method validation are usually used to judge the quality, reliability and consistency of the analytical results and is therefore an integral part of any good analytical practice. Validation was carried out using the standard method according to the International Conference on Harmonisation Guidelines (ICH). Mixed solutions of the analytes with concentrations from 0.01 to 10 µg/L for each were used to determine the characteristics of the developed method. Therefore the linearity range was from 0.01 to 10 µg/L. All the standards used for the validation of the method underwent SPE, whereby, 100 mL of the millipore water was spiked with the standards and was processed through the SPE. The standard curves were prepared by plotting the spiked concentrations against the peak areas as shown in Figures 4-5 and 4-6. A linear response was obtained for all the analytes. The correlation coefficient for each of the analytes was higher than 0.99, determined from at least five different concentrations and injected five times (Figure 4-5).

Table 4-2: Calibration ranges, correlation coefficients, limits of detection and quantification of the selected antibiotics (n = 5).

| Antibiotics | Calibration range mg/L | Correlation coefficient | LOD (µg/L) | LOQ (µg/L) | MDL (µg/L) | MQL (µg/L) |
|---|---|---|---|---|---|---|
| AMO | 0.01-10 | 0.9962 | 0.16 | 1.6 | 0.23 | 2.3 |
| CIP | 0.001-10 | 0.9985 | 0.29 | 2.9 | 0.12 | 2.4 |
| ENRO | 0.001-10 | 0.9992 | 0.19 | 1.9 | 0.14 | 2.8 |
| SULFA | 0.01-10 | 0.9989 | 0.13 | 1.3 | 0.93 | 9.3 |
| TET | 0.1-10 | 0.9976 | 0.23 | 2.4 | 2.5 | 2.5 |

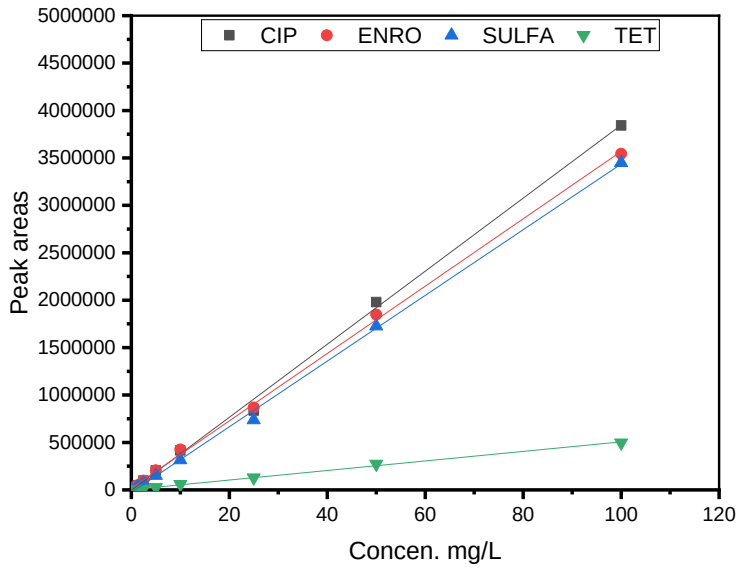



Figure 4-5: Standard calibration curves of the analytes determined with the developed method (CIP, ENRO, SULFA and TET).

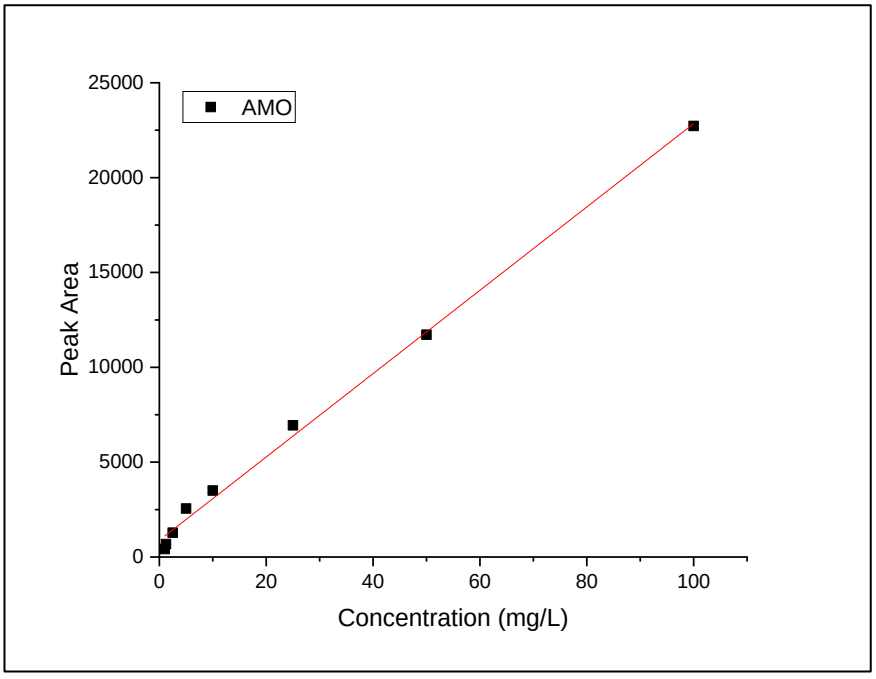



Figure 4-6: Standard calibration curves of the AMO determined with the developed method.

### 4.4.1 Linearity range, Limit of detection (LOD) and Limit of quantification (LOQ)

The linearity range used for the developed method was 0.01 to 10 mg/L for CIP and ENRO while for SULFA it was 0.1 to 10 mg/L. For AMO and TET, the linearity range was from 1 to 10 mg/L. The instrument LOD and LOQ were determined. The LOD refers to limit of detection which is the lowest concentration of an analyte in the sample that can be detected, but not necessarily quantitated, under the defined experimental conditions. On the other hand, the LOQ is the limit of quantification which is the lowest concentration of an analyte in the sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. In both cases, a mixture of the standards was spiked in millipore water and was serially diluted and ran through the chromatographic method. The LOD and LOQ for the analytes were shown in Table 4-2. The LOD and LOQ obtained were 0.1-0.3 µg/L and 1.3 to 2.9 µg/L, respectively. The values of LOD and LOQ obtained in the present study were lower compared with other studies. For instance, Cavenati *et al.* (2012) obtained LOD and LOQ of 0.8 to 3 µg/L and 2-10 µg/L. The authors stated that the LOD and LOQ are affected by the volume of sample loaded onto the SPE and also the sample matrix. The lower LOD and LOQ obtained in the present study indicated the sensitivity of the instrument and its ability to be applied for the detection of the targeted antibiotics at the low concentrations found in complex environmental samples.

### 4.4.2 Validation of SPE method using Method detection limit (MDL) and method quantification limit (MQL)

The method detection limit (MDL) and method quantification limit (MQL) were determined in a similar way that was used for the instrument LOD and LOQ using the signal-to-noise ratio. The MDL and MQL are basically related to the SPE method. For the MDL and MQL, the wastewater samples were spiked with a low concentration (concentrations obtained from the LOD) and a signal-to-noise ratio of 3 for MDL and 10 for MQL was determined. The MDL and MQL for the SPE method were shown in Table 4-2. The MDL ranged from 0.12 to 2.5 µg/L while MQL ranged from 2.3 to 9.3

µg/L. The MDL and MQL values were higher than the LOD and LOQ values. Since the MDL and MQL were determined from the wastewater samples, the matrixes from the wastewater could have resulted in this difference.

### 4.4.3 Precision

Precision refers to the closeness of two or more measurements to each other. Both repeatability (within a day precision) and reproducibility (between days precision) were determined for all analytes. Concentrations of 1-10 mg/L of a mixture of the analytes were used. From the experimental runs, the mean, standard deviation and relative standard deviation (RSD) was evaluated. Table 4-3 presents the intra-day while Table 4-4 presents the inter-day. Both the intra-day and inter-day gave RSD which were below 10%.

#### 4.4.3.1 Intra-day precision

The intra-day precision variation of the method was determined by using five replicate injections of the same concentration and analysed the same day. From Table 4-3, it was observed that the precision for the chosen concentration was relatively low, thus indicating the accuracy of the method. The same was applicable for the inter-day (reproducibility). The RSD values obtained were from 0.2 to 2.8%, with amoxicillin having the highest RSD.

Table 4-3: Intra-day precision in relative standard deviation (RSD) of the method at 5 standard concentrations (n = 5)

| Concentration mg/L | 1 | 1.25 | 2.5 | 5 | 10 |
|---|---|---|---|---|---|
| Amo | 2.8 | 1.7 | 1.0 | 0.5 | 0.4 |
| Cip | 0.2 | 0.3 | 0.3 | 0.4 | 0.4 |
| Enro | 0.4 | 0.4 | 0.2 | 0.3 | 0.2 |
| Sulfa | 0.4 | 0.7 | 0.4 | 0.7 | 0.5 |
| Tetra | 0.4 | 0.4 | 0.5 | 1.5 | 2.6 |

#### 4.4.3.2 Inter-day precision

Similarly as in the intra-day, the inter-day precision variation of the method was determined by using five replicate injections of the same concentration and analysed, however, this was carried out on different days. Table 4-4 shows the inter-day precision. It is observed that the precision for the chosen concentration were acceptable similar to those obtained from the intra-day even though higher values were obtained, the RSD for the intra-day were 0.2 to 2.8%.

Table 4-4: Repeatability in relative standard deviation (RSD) of the method at 5 standard concentrations (n = 6)

| Antibiotics | Concentration (mg/L) | | | | |
|---|---|---|---|---|---|
| | 1 | 1.25 | 2.5 | 5 | 10 |
| | Repeatability (RSD %) | | | | |
| AMO | 2.7 | 1.9 | 0.8 | 0.6 | 2.8 |
| CIP | 0.4 | 0.2 | 0.6 | 0.4 | 0.7 |
| ENRO | 0.4 | 0.4 | 0.5 | 0.4 | 0.6 |
| SULFA | 0.4 | 0.3 | 0.3 | 1.0 | 1.6 |
| TET | 0.38 | 0.35 | 0.53 | 1.4 | 1.62 |

### 4.4.4 Recovery (%) and Matrix effects

The recovery describes the yield obtained for the developed method. It involves both the pre-treatment given to the sample as well as the instrumental analysis. For the analysis of environmental samples, the recovery is determined from the extraction efficiency which is usually affected by a number of factors such as the sorption of antibiotics to organic matter in the samples which causes the preparation step to be less effective especially with regards to concentrating of antibiotics. The second factor is the fact that the contaminants in the sample matrix interferes with the analyte peaks by raising the chromatogram baseline. Finally, there is a tendency for the contaminants

to reduce the ionisation efficiency of the analytes by taking up some of the limited numbers of excess charged sites on the surfaces of electrospray droplets (Seifrtova *et al.* 2009). The recoveries were determined by spiking the wastewater with the standard. The spiking concentration was the concentration of the lowest limit of detection due to the fact that some of the analytes were found in low concentrations. The recoveries were determined by comparing the concentration obtained after SPE on a neat sample and the spiked wastewater samples. It was noted that the wastewater samples already contained some of the analytes, therefore, to account for that, a blank sample (non-spiked) was analysed and the concentrations found from the blank samples were subtracted from those of the spiked samples.

Table 4-5 presents the recoveries of the analytes. The recoveries were 58 to 90% for most of the studied analytes. However, a lower recovery was obtained for AMO, 44.5%. The reason for the poor recovery was not immediately identified; however, it was observed that other studies had also reported low recoveries of amoxicillin. For instance, Gros, Rodríguez-Mozaz and Barceló (2013) reported lower recoveries from the penicillins of which amoxicillin was amongst them. Similarly, Opriş *et al.* (2013) in their study on the determination of frequently used antibiotics in wastewater observed lower recovery of amoxicillin of 42%, which is lower than the one obtained in the present study. The antibiotics studied have different properties. Factors such as their chemical structure and polarity have a great role to play especially when it comes to the interaction with the sorbents. Usually, the analytes compete for spaces on the cartridge sorbent.

According to Babić *et al.* (2010), during the adsorption of the analytes onto the sorbent, the less polar compounds usually adsorbed more strongly by the sorbents. Therefore, for amoxicillin, it was assumed that its properties could have greatly affected its retention during the SPE process and therefore, the low recovery. Better recoveries were obtained for SULFA, TET and the fluoroquinolones. The highest recovery was for CIP at 90%. The recoveries obtained were similar to those reported by Babić *et al.* (2010); Opriş *et al.* (2013); Tlili *et al.* (2016).

Table 4-5: Sample recovery of antibiotics in wastewater (n = 8), (Average ± standard deviation)

| Compound | Effluent (%) | Ion enhancement (%) | Ion suppression (%) |
|---|---|---|---|
| AMO | 44.5 ± 18.3 | _ | 90 ± 17.5 |
| CIP | 90.5 ± 5.1 | 10.5 ± 6.1 | – |
| ENRO | 79.4 ± 3.2 | 25.8 ± 7.2 | – |
| SULFA | 74.4 ± 8.5 | – | 34.4 ± 18.5 |
| TET | 58.3 ± 7.9 | – | 71.5 ± 14.5 |

## 4.5 Matrix effect

One of the limitations of the use of the HPLC-MS is its susceptibility to matrix effects. The matrix effects could be observed as ion suppression or ion enhancement of the analytes. The effect of both suppression and enhancement is the fact that it could lead to a major difference in the response of the analyte in a sample as compared to a pure standard solution (García *et al.* 2008). Therefore, evaluation for the signal suppression was carried out to account for the effect of matrices on the quantification of the analytes since the wastewater used in this study was of a complex nature. Slaughterhouse wastewaters comprise of complex organic matter (Wang, Jena and Das 2018).

In most cases, suppression is usually more pronounced than the ion enhancement. Table 4-5 showed ion suppression and enhancement observed on the analytes. Suppression of AMO SULFA and TET were in the range of 34 to 90%, thus indicating the prominent influence of the matrices on the analytes. Furthermore, ion enhancement occurred for CIP and ENRO. It was 10.5% for CIP and 25.8% for ENRO. The enhancement of CIP and ENRO is similar to a study by Gros, Rodríguez-Mozaz and Barceló (2013) where signals for fluoroquinolones were enhanced. Suppression was more pronounced in AMO and TET. AMO is the first compound that eluted during the

chromatographic separation, while TET is the last to elute. Both compounds' signal intensities were not clearly defined as the others. It has been reported that the matrix effect could depend on the chromatographic retention time, such that analytes eluting at the beginning of the LC gradient are affected mostly by the matrix effect and it is the same for those that elute lastly (Hernando *et al.* 2004; Petrović *et al.* 2005).

While the suppression observed for the three analytes was high, higher values have been reported in other studies. For instance, Petrović *et al.* (2005) obtained high matrix effects such as ion suppression above 90% even though the compounds they studied were different from the ones considered in the present study. Other studies by Jelic *et al.* (2011) and Gros, Rodríguez-Mozaz and Barceló (2013) also obtained over 90% ion suppression especially for the antibiotics.

The presence of endogenous substances such as inorganic molecules, naturally occurring organic compounds which are usually present in the samples have been identified to contribute to ion suppression. These substances are often retrieved in the final extract (Babić *et al.* 2010). The effect of ion suppression leads to reduction in the detection capability, poor repeatability due to the variable degrees of ion suppression for the different samples. It also affects the linearity, ion ratio and quantification (Babić *et al.* 2010). According to Petrović *et al.* (2005), several phenomena could cause the signal suppression: i) adsorption of the pharmaceuticals to the organic matter in the samples which can cause the concentrations of dissolved pharmaceuticals to be detected in lower concentrations; ii) the fact that contaminants contained in the sample could cause a masking of the analyte peak, thus raising the chromatogram baseline which results in the area under the chromatographic curve to be underestimated; iii) the presence of the contaminants could reduce the ionisation efficiency of the analytes such that the excess charged sites surfaces of electro-sprayed droplets are taken up by the contaminants, thus reducing the signal (Petrović *et al.* 2005).

To reduce the effect of signal suppression due to matrix effects, the use of an internal standard is recommended, but due to the difficulty in obtaining internal standards, other options such as the introduction of additives such as propionic acid and

ammonium formate are added onto the mobile phase. Another option is to employ the standard addition method; however, this method is laborious.

## 4.6 Detection of veterinary antibiotics in livestock wastewater and-impacted surface water

The developed and validated method was applied to the influent and effluent of a slaughterhouse wastewater treatment plant. When it comes to the quantification of analytes from samples, different approaches could be used: internal standard, external standard or standard addition. Amongst the three, the external standard has proven to be the simplest method, but it does not account for the effect of matrices in the sample. The addition of the known analytes of interest to the sample is referred to as standard addition. This is usually the preferred method, due to the fact that it is easy for quantification. In internal standard, the addition of analytes that are similar to the compound of interest is applied. However, the problem with that is the difficulties in getting the standards that are closely related to the analytes of interest (García *et al.* 2008). For the standards to be considered, it is advisable that the retention times are similar and should be distinguishable by the instrument.

For the analysis done in the present study, samples were collected from a slaughterhouse wastewater at the influent and effluent points. Monitoring was carried out for a period of 8 weeks. During this period, samples were collected twice a week. Figures 4-7 and 4-8 show the mean recoveries for the detected compounds of interest that were found in both the influents and effluents of the wastewaters. The SPE method described was applied to the wastewaters. Five antibiotics that are commonly used as veterinary medicines in South Africa were detected. All the antibiotics studied in this work were detected in the wastewaters. The range of antibiotics detected in the wastewaters in effluents was 0.008 to 4.9 ng/L while in the influent, the range was 1 to 21 ng/L. Higher concentrations were found in the influents as compared to effluents, even though this was expected. The fluoroquinolones were determined both in the influent and effluent. The maximum amount of CIP and ENRO detected was 9.1 and 9.6 ng/L in the influent, respectively. As expected, lower amounts were detected in the

effluents within the range of 0.9 to 4.5 ng/L. Even though lower amounts were quantitated in the influents, these amounts detected were still capable of impacting the environment negatively. He and Blaney (2015) reported that for fluoroquinolones, as low as 1-3 ng/L is capable of inhibiting microbial species. Therefore they represent an important class of antibiotics in wastewaters and even in surface waters.

The detection of the fluoroquinolones in the wastewaters was not surprising due to the fact that they have been detected in several water bodies in other studies. Cavenati *et al.* (2012) reported residual concentration of ENRO from a wastewater treatment that receives it wastewaters from a slaughterhouse at 0.6 to 2 ng/L. These amounts are lower than the value reported in the current study. However, studies by Tagiri-Endo *et al.* (2009) and Ben *et al.* (2008) reported higher amounts of 0.28–160 µg/L and 6–25 µg/L, respectively. Both studies considered swine wastewaters. Similarly, Tong *et al.* (2009) detected amounts within a range of 8.5–21692.7 ng/L in the influents and in the effluent it was 1.6-11.7 ng/L.

Sulfamethazine which is a sulphonamide was not detected on four different days during the monitoring period. On days it was detected, the amount ranged from 0.01 to 3.3 ng/L. On the other hand, chlortetracycline was not detected on one of the days only; the determined range was from 0.1 to 3.9 ng/L. Though these compounds were not detected on certain days, other factors could have been responsible for this. From Figures 4-7 and 4-8, it is observed that the effect of matrix was significant on these compounds. The compounds were significantly suppressed by matrices. Furthermore, the slaughterhouse wastewater comprised of very high organic substances, including blood. Therefore, their presence could lead to signal suppression which would have affected the quantification of the compounds from the wastewater samples.

Chlortetracycline, which is a class of the tetracycline, has been identified to be one of the veterinary antibiotics consumed in high volumes in South Africa. Tetracyclines are used both for human and animal purposes, therefore their residual concentrations in the wastewater can be used as an indicator of its use. The concentrations of TET detected were lower than those of the fluoroquinolones and the SULFA. The highest concentration of TET found was 3.9 ng/L. The detected levels of both SULFA and

TET have also been reported in various wastewater treatment plants from their influents and effluents as well as surface and ground waters, especially those close to animal breeding places.

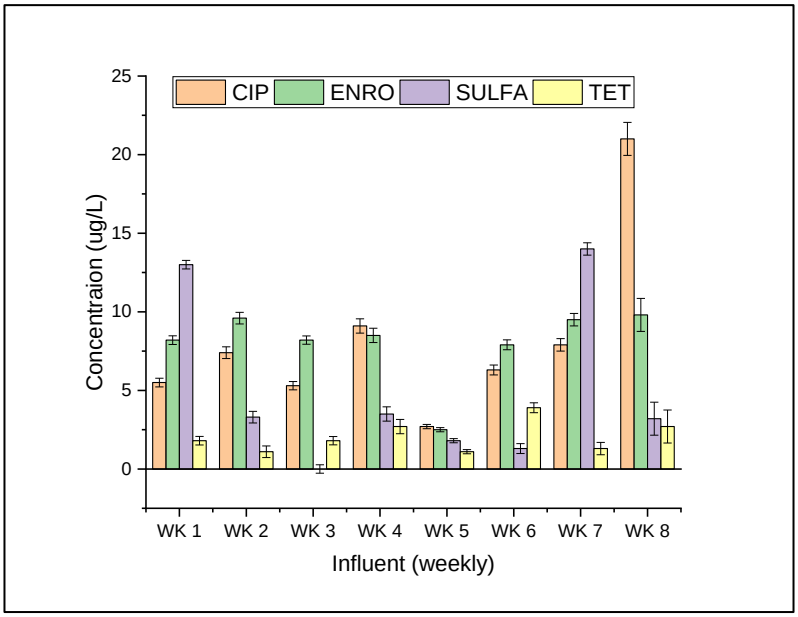



Figure 4-7: SPE-HPLC-PDA-MS method characteristics determined in influent samples (analysis was carried out in triplicates, hence average values are reported)

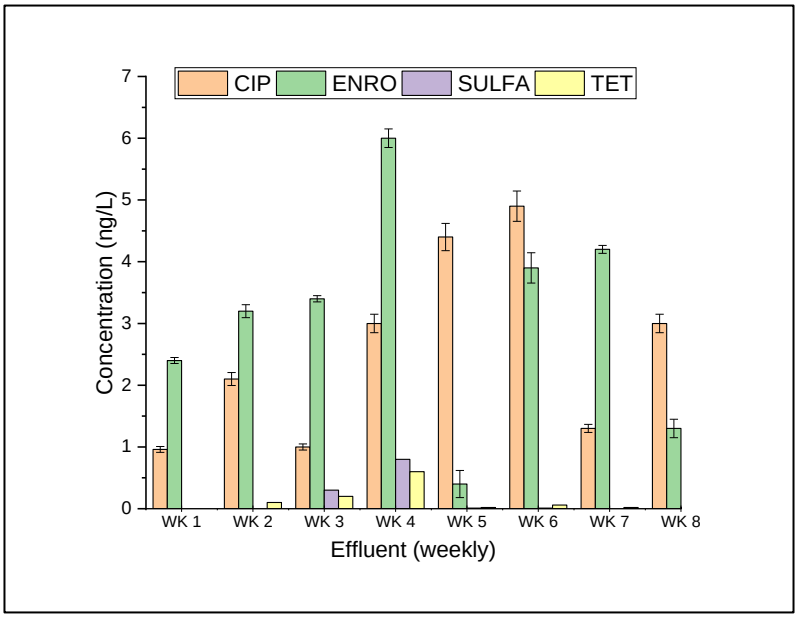



Figure 4-8: SPE-HPLC-PDA-MS method characteristics determined in effluent samples (analysis was carried out in triplicates, hence average values are reported)

Moreover, from Figures 4-7 and 4-8, traces of the antibiotics were detected in most of the effluents thus indicating that the treatment processes were unable to completely get rid of them. Apart from the animal wastewaters, veterinary antibiotics have been detected in various types of manures: swine, cows and poultry. The amounts of detected antibiotics from several studies show considerable variations. A number of factors could be responsible for this: the multi residue method developed that are used for analysing these contaminants and the wastewater characteristics. The high consumption of antibiotics has been equated to their behaviour in the environment, which is to say, the more the antibiotics are consumed, the higher the chances that they would find their way into the environment through various means.

## 4.7 Summary

This chapter described the method development of an optimised method based on solid-phase extraction (SPE) followed by ultra-high-performance liquid chromatography (UHPLC). The identification step was carried out with the use of mass spectrometry (MS) for the analysis of the five antibiotics. The developed method was validated accordingly. A sensitive, simple and reliable method was thus developed for the determination of five veterinary antibiotics: 2 fluoroquinolones, 1 sulphonamide, 1 β-lactam and 1 tetracycline. Coefficients of correlation for each analyte were >0.995 confirming the linearity of the method. From the SPE optimisation, a pH of 2 and elution with 0.1% FA in methanol was used. The method development limit (MDL) and method quantification limit were; MDL ranged from 0.12 to 2.5 µg/L while MQL ranged from 2.3 to 9.3 µg/L. The linearity range values were within 0.1 to 10 mg/L while the limit of detection (LOD) and limit of quantification LOQ were 0.1-0.3 µg/L and 1.3 to 2.9 µg/L, respectively. The recoveries of the target compounds were 40 to 90% for most of the studied analytes except for AMO which had a lower recovery of 44.5%. The validated method was applied to slaughterhouse wastewater samples from South Africa containing antibiotics (0.008 to 4.9 ng/L in the effluents and 1 to 21 ng/L in the influents). To the best of our knowledge, this is the first study to report the detection of veterinary antibiotics in South African slaughterhouse wastewaters.

# Chapter 5 - Removal of the antibiotics using anaerobic digestion

## 5.1 Introduction

As shown in **section 2.4.6**, conventional methods of wastewater treatment do no effectively remove antibiotics and other pharmaceuticals from wastewaters. The behaviour of antibiotics under anaerobic conditions is of particular concern due to the fact that they are not completely removed during the process; hence the potential for their entry into the environment as effluents is high. Inhibition of the methanogens by xenobiotic compounds has been demonstrated (Mitchell *et al.* 2013). Biogas production is one of the parameters for the evaluation of sludge activity, and can be affected due to inhibition.

One of the major factors influencing the removal of antibiotics from the wastewater is their ability to interact with solid particles in the treatment system or with other natural organic matter. Antibiotics with low adsorption coefficients tend to remain in the liquid (aqueous) form while those with high adsorption coefficients find themselves adsorbed to the solids in the reacting system.

This section explores the use of anaerobic digestion (AD) which is a biological treatment to pre-treat the effluent before applying the post treatment step which is the advanced oxidation (reported in **sections 2.5**). The results obtained during the batch processes are enumerated to give an understanding on the behaviour of the antibiotics to the sludge/effluent. Once that is established, the wastewater is subjected to a continuous treatment process. Therefore, results from the continuous process are also presented. All the effluent samples from the batch and continuous processes were analysed using the method developed in Chapter four, **section 4.4.**

## 5.2 Continuous experiment using Up-flow Anaerobic Sludge Blanket

### 5.2.1 Reactor start-up

The reactor start-up is the period taken for stable operation of the reactor to occur and is an important step for UASB systems. During this stage, the microorganisms are acclimatising to the new environmental conditions and substrate. An equilibrium is also slowly established between the various microorganisms present in the system, until the biomass is stable and efficient to degrade the substrate at the targeted OLR (Masse and Masse 2000). Reactor start-up is a complicated process, and as such the monitoring of both environmental and operating conditions is important (Rizvi *et al.* 2015; Chollom *et al.* 2017).

The VSS/TSS indicates the amount of biomass in the total sludge measured as suspended solids. Therefore, a high ratio of VSS/TSS in the inoculum to be used for the reactor start-up is important (Vlyssides, Barampouti and Mai 2009). For the start-up, the VSS/TSS ratio was about 0.71. This ratio continued to increase, which is an indication of microorganisms' adaptation and multiplication. Feed concentration of less than 3000 mg/L of COD (2.8 $kg.m^{-3}.d^{-1}$) was used for the start-up to help in preventing and controlling the excessive generation of VFA since at this stage the microorganisms were still adapting to the new environment.

### 5.2.2 Performance of the UASB reactor

Following the successful start-up of the reactor with the low initial OLR (2.8 $kg.m^{-3}.d^{-1}$), the reactor was gradually loaded with a higher OLR with the slaughterhouse wastewater as substrate by increasing the COD concentration and keeping HRT constant. Each loading rate was allowed a time of stability as was shown in Table 3-6. To evaluate the optimum loading rate, OLR was increased to 8 kg $COD.m^{-3}.d^{-1}$. The performance of the reactor is presented in the following sections.

### 5.2.2.1 Effect of OLR on pH

Figure 5-1 shows the daily pH and OLR in the reactor. In the AD system, the different microorganisms have different optimum pH values for maximum performance. The methanogenic bacteria which are responsible for the production of methane are more sensitive to pH. Optimally pH is between 6.5 and 7.5 (Ward *et al.* 2008; Bustillo-Lecompte and Mehrvar 2016). The OLR rate describes the liquid flowrate and contaminant concentration and can be defined as the mass of the pollutant introduced in a unit volume of the reactor per unit time (Daud *et al.* 2018). As such, this parameter integrates reactor characteristics, operational characteristics, and bacterial mass and activity into the volume of media (Ruiz *et al.* 1997; Bustillo-Lecompte and Mehrvar 2016).

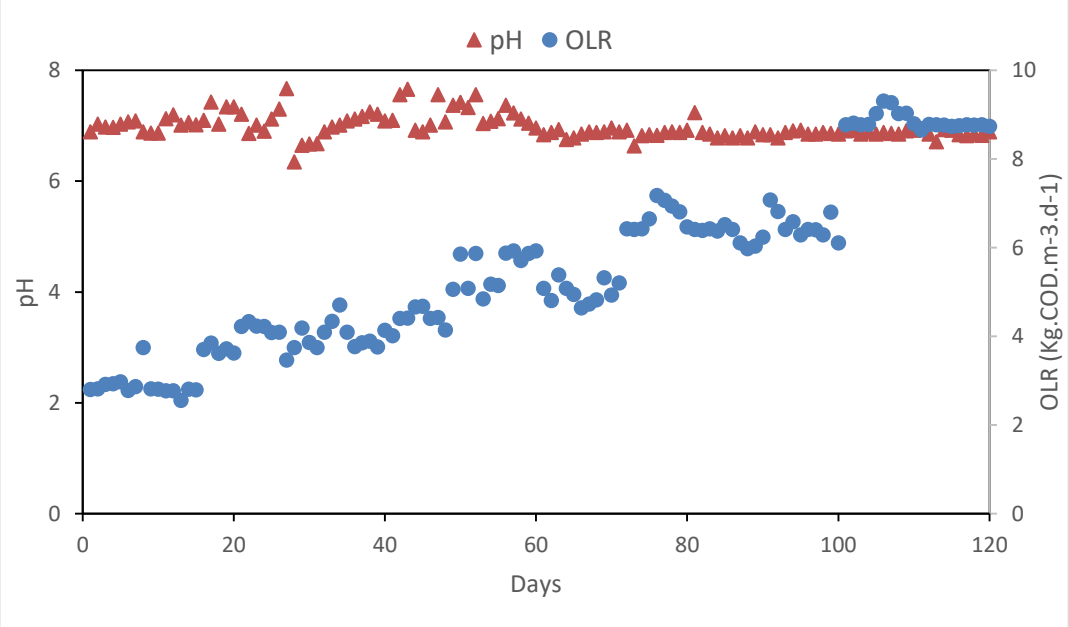



Figure 5-1: Daily pH and OLR of the UASB reactor at a HRT of 12 hours.

The influent pH for the wastewater samples was relatively stable within 6.8−7.1. For the effluent pH, it was between 6.64 on the low and 7.67 for the high as illustrated in Figure 5-1. The lowest pH was recorded on the 18$^{th}$ day as 6.64 while the maximum pH was 7.67 which was on the 27$^{th}$ day. Buffering of the system was not carried out because the reactor's pH was at most times within the required pH for the organisms. The pH levels during the experimental period were favourable to the methanogens

bacteria. Several authors have reported that methanogenesis in anaerobic digestion occurred efficiently at pH 6.5-8.2 while hydrolysis and acidogenesis occurred between pH 5.5 and 6.5. The ability of the system to perform to capacity is much dependant on the pH such that reactor failure or underperformance could occur (Khalid *et al.* 2011; Chollom *et al.* 2017; Ren *et al.* 2018).

Figure 5-2 shows the average weekly pH with respect to alkalinity. It was observed that the alkalinity of the reactor was above 1000 mg/L $CaCO_3$; however, this was below 1000 mg/L $CaCO_3$ at the beginning of the experiment. At this stage, the microorganisms were still adapting to the new conditions which they were exposed to. From Figure 5-2, it was again observed that from the 14$^{th}$ week, the pH of the reactor began to decline even though the alkalinity was relatively stable. The drop in pH could have been due to the increased OLR. The drop in the pH values was however, not detrimental to the reactor because the pH was still within the optimal limits suggested for the anaerobic processes.

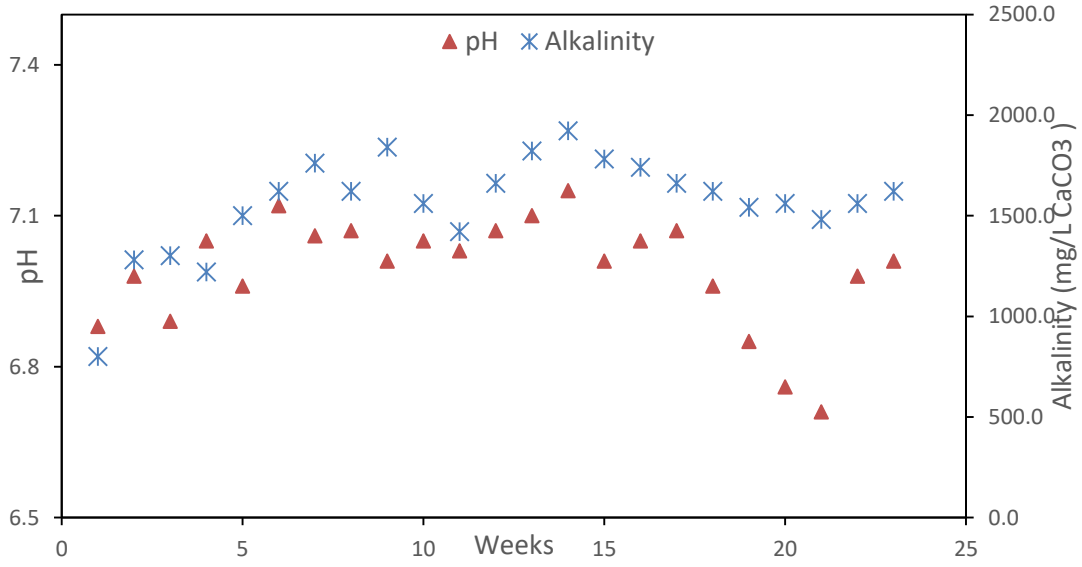



Figure 5-2: Weekly pH and alkalinity of the reactor at a HRT of 12 hours and different OLR.

### 5.2.2.2 Effect of OLR on COD reduction

Figure 5-3 shows the COD influent and effluent as well as the COD removal efficiency. The OLR was introduced in a stepwise manner from 2.28 kg COD.m$^{-3}$.d $^{-1}$ to 8 kg COD.m$^{-3}$.d $^{-1}$. During the start-up of the reactor, the removal efficiency was low, below 30% even at a low OLR rate of 2.28 kg COD.m$^{-3}$.d $^{-1}$.

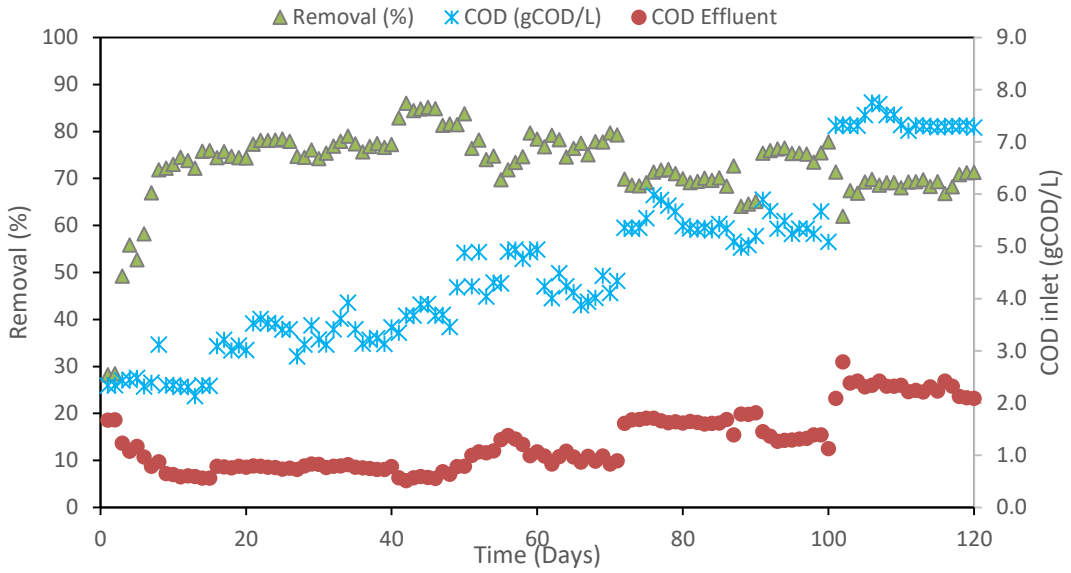



Figure 5-3: Influent and effluent COD and percent COD removal of the UASB reactor.

However, COD removal efficiencies showed an increasing trend from a low 37% to a maximum of 86%, (day 42 and OLR 4.2-5 kg COD.m$^{-3}$.d$^{-1}$). At the beginning of each phase of OLR, there was a corresponding decrease in removal efficiencies. Observable also was the fact that as OLR increased from 5 to 9 kg COD.m$^{-3}$.d$^{-1}$, an obvious reduction in the COD removal to 60-70% occurred. The reduction in the COD removal efficiency indicated that the microorganisms could not flourish at a higher level of OLR. Chollom et al (2017) and Torkian, Eqbali and Hashemian (2003) observed that, as OLR increased, the adaptation of the microbial community to the new condition is retarded thus leading to a decrease in the performance of the system. But the recovery of this is usually within a period of 24 hours in most cases as can be seen in Figure 5-3 for each new influent. OLR plays a significant role in the reactor efficiency. The

average removal for the experimental period was 73% and the maximum COD removal of 80-84% was achieved at an OLR rate of 4-5 kg COD.$m^{-3}$.$d^{-1}$. While removal in this study was said to be above the optimum of 5 kg COD.$m^{-3}$.$d^{-1}$ required for anaerobic treatment units, the values obtained were below those treating similar wastewaters. Borja, Banks and Wang (1994) reported COD removal efficiencies of 64-99% at OLR values of 12-17 kg COD. $m^{-3}$.$d^{-1}$. Ruiz et al. (1997) reported a 92% soluble COD (SCOD) removal at 5.2 kg SCOD $m^{-3}$ $d^{-1}$ and HRT of 1.2 day. The differences in COD removal efficiency as compared with other studies might be due to wastewater characteristics and operating conditions. From day 100, when the OLR was changed from 6 to 8 kg COD.m-$^{3}$.$d^{-1}$, a slight sludge washout was observed and hence a lower efficiency of 70-75% was achieved. Higher removal rates of 76.2% have been reported by Borja, Alba and Banks (1996) at an OLR of 17.8 kg COD.$m^{-3}$.$d^{-1}$ when treating wastewaters from virgin olive oil.

#### 5.2.2.3 Effect of OLR on biogas production

Figure 5-4 illustrates the relationship between OLR of the reactor and the biogas produced. Biogas production during the start-up period is usually the lowest due to the fact that the growth rate of the methane generating microorganisms is slow as compared to the acid forming microorganisms. In Figure 5-4, however, an increasing trend of biogas gas over time was observed in the system indicating that as the days progressed, the microorganisms were stabilised and were thriving to the new OLR, hence, contributing to the increase of biogas production. The results therefore indicated a strong relationship between OLR and biogas production. Biogas increase was observed to increase with the introduction of a new OLR indicating the availability of more substrate for digestion. However, just after the introduction of a new OLR, a decrease in biogas was observed, but picked up soon afterwards. The reason for that was the adaptation of the microorganisms to the new condition introduced, as earlier indicated for the COD removal. Once the microorganisms had adapted, a gradual increase was observed which tends toward stability until the next phase of introduction. A fluctuation in the biogas production was also observed. This was attributed to variability of biological degradation of the effluent and the possible

presence of various organic and inorganic materials that inhibited the treatment performance (Ruiz *et al.* 1997; Padilla-Gasca, Lopez-Lpoez and Gallardo-Valdez 2011; Loganath and Mazumder 2018).

On days 100 and 101 when the highest OLR of 8 kg COD.$m^{-3}$.$d^{-1}$ was introduced, 8.63 L/day of biogas was produced. This was the maximum obtained; however, this gradually began to reduce, as seen in the following days. The reason was attributed to the increase in the VFA content in the reactor and reduction in total alkalinity. The effect of which would have impacted on the production of the biogas. Ruiz *et al.* (1997); (Shi, Leong and Ng 2017) attributed this behaviour to the attachment of gas bubbles at higher OLR, thus leading to biomass suspension and cell washout as the biogas production rate increases.

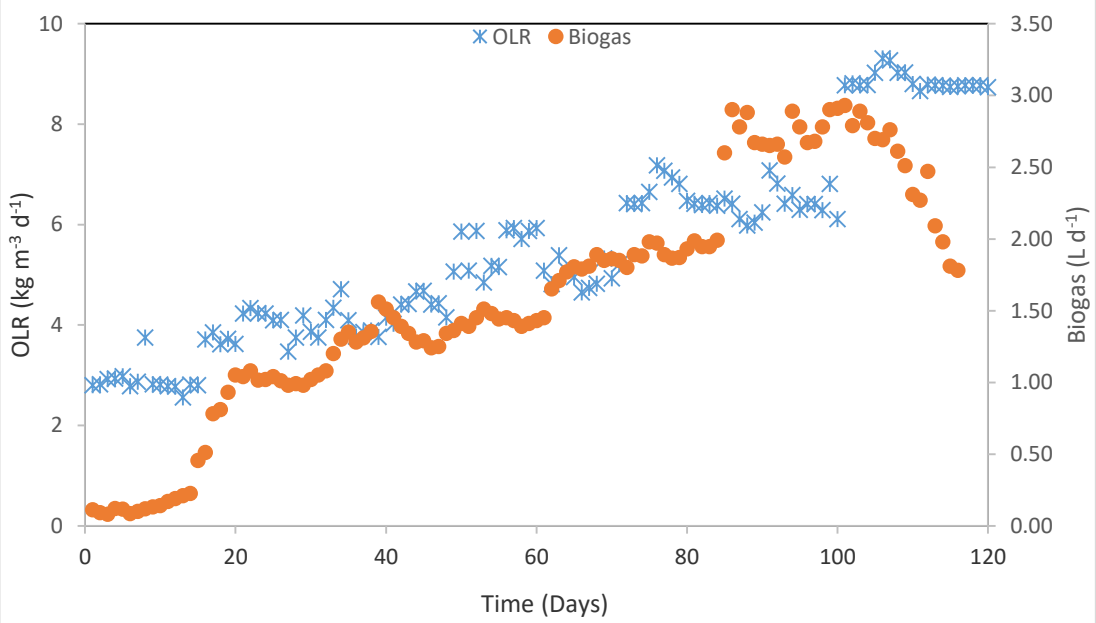



Figure 5-4: Applied OLR and the biogas production rate during the experimental period.

### 5.2.2.4 Biomass accumulation, sludge bed and its characteristics

The behaviour of the sludge bed in the reactor was analysed using the sampling points as shown in the schematic diagram used for the experiment (Figure 3-1). Results of the weekly TSS and VSS are shown in Figure 5-5. The rate of sludge production at the

start-up of the reactor was slow but it picked up with time. This indicated that most of the soluble as well as settled matter in the wastewater was degraded during the treatment in the reactor. There were four sampling points on the reactor, and each point in which samples were taken showed distinct characteristics. Ports 4 to 6 had more solids concentration while port 3 had aggregates which were suspended due to the mixing of flowing liquor and rising of gas bubbles. Similar findings were reported by Ruiz *et al.* (1997).

Sludge washout became noticeable as the biogas production rate increased with the OLR. The reason for the sludge washout was the expansion of the sludge blanket caused by the turbulence of gas produced at higher OLR which forced more sludge to come out with the effluent. Even though this was experienced, it did not cause sludge bulking in the system. Some studies have identified the factors responsible for the sludge washout: increase in gas production due to high OLR; and high relative content of coarse suspended solids in influent (Borja, Alba and Banks 1996; Chelliapan *et al.* 2011). Ruiz *et al.* (1997) reported sludge floatation and increased effluent solids concentration at OLR values higher than 5 kg $COD.m^{-3}.d^{-1}$.

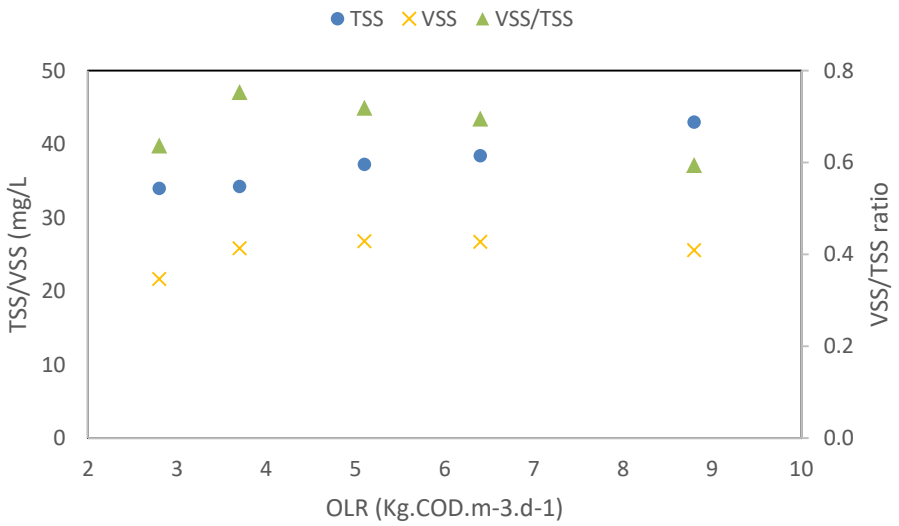



Figure 5-5: Weekly variation of TSS and VSS with OLR at a constant HRT of 12 hours.

### 5.2.2.5 Antibiotic degradation

Antibiotics were analysed by the developed method discussed in Chapter 4, **section 4.4.** The influent had antibiotics concentration of 50 µg/L. At the beginning when the dosing started, the removal rate for the antibiotics was low, however, it increased with time. Figure 5-6 shows the removal of the antibiotics at the three different OLRs (III, IV and V) from the experimental design in Table 3-6. In Figure 5-6, the results depict the averages of the weekly samples analysed over a period of 2 months.

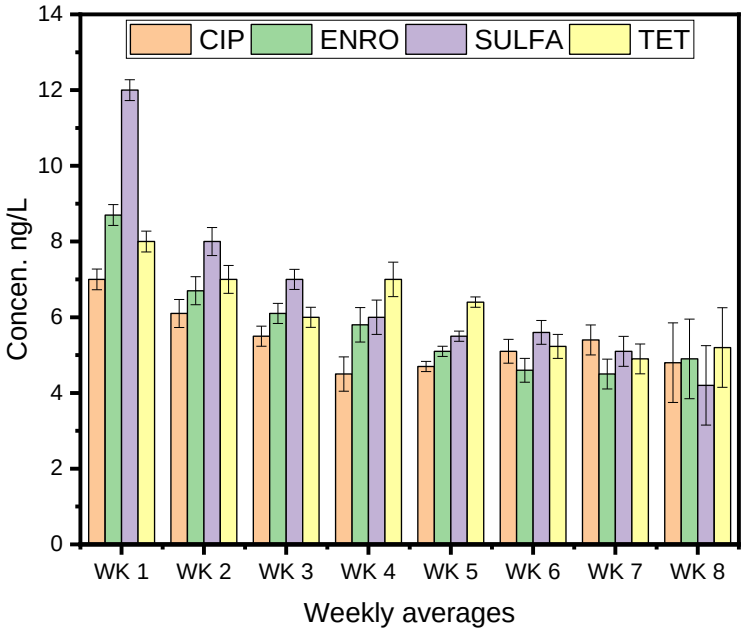



Figure 5-6: Concentration of antibiotics with time during operational periods of the antibiotics

The concentration of the influent containing the antibiotics was maintained at 50 µg/L, unlike in other studies where the concentration was varied. Some of those studies evaluated the point at which the biological system becomes inhibited by the presence of the antibiotics. For example, Chelliapan, Wilby and Sallis (2006) varied the concentration of Tylosin in the pharmaceutical wastewater from 20 to 200 mg/L and Meng *et al.* (2015) also varied the concentration of Amoxicillin in the pharmaceutical wastewater from 20 to 200 mg/L. The general findings from most of the studies indicate that the initial concentration of the contaminants (antibiotics) had a relatively

minor influence on the removal of COD and other organics from the treated wastewaters. Similarly, biogas production (methane and other gases) is not impacted on greatly even at high concentrations of the antibiotics (Chelliapan *et al.* 2011; Meng *et al.* 2015; Feng *et al.* 2017).

Therefore varying the concentration of the antibiotics does not really have a significant effect on the performances of the biological systems. The spiking of the reactor with the antibiotics did not show considerable effects on the performance of the reactor. However, a wide range of differences have been reported on the performance of anaerobic systems treating this kind of wastewaters in a review by Cheng *et al.* (2018). The main reasons stated for the variation was the different antibiotic concentrations and types as well as the combination of different antibiotics used for these studies. On the effect of antibiotics combination, Aydin, Ince and Ince (2015) observed that a combination of erythromycin-tetracycline-sulfamethoxazole (ETS), sulfamethoxazole-tetracycline (ST), erythromycin-sulfamethoxazole (ES) and erythromycin-tetracycline (ET)) had more serious inhibition than the individual antibiotics on the COD utilization and methane production. Similarly, they also found out that the inhibition of the anaerobic processes could be only at very high concentrations. With regards to the effect on the biogas (methane) produced, a different scenario is presented. Some reports have indicated a reduction in the gas produced. This was explained by the bactericidal characteristics that kill bacteria instead of inhibiting bacterial growth at high concentrations, which is very different to bacteriostatic characteristics at lower concentrations (Aydin, Ince and Ince 2015; Feng *et al.* 2017; Cheng *et al.* 2018).

One of the specific objectives of this study was to understand the removal pathway of the antibiotics from a biological treatment plant. Figure 5-6 shows that although removal rates were in most cases less than 5 ng/L representing over 80%, a distinctive pattern could not be established. This is expected due to the differences in the chemical structures as well as the characteristics of the various antibiotics would result in varied degradation and adsorption during treatment processes thereby resulting in different

removal mechanisms. The removal mechanisms are further explained in the batch digestion process in the sections that follow.

## 5.3 Kinetic studies

### 5.3.1 Removal of antibiotics using anaerobic batch reactors

The removal of antibiotics could follow different pathways during wastewater treatment such as: adsorption, hydrolysis, volatisation or biodegradation. Volatisation and hydrolysis were found to be negligible, which is in agreement with other studies (Li and Zhang 2010). Therefore, biodegradation and adsorption were the main pathways in which the antibiotics were removed, and are discussed in the sections below. The experimental design that was followed was elaborated in Table 3-7. A total of 6 serum bottles (anaerobic reactors) were used for the study, and are represented as R1 to R6. The constituents of the reactors were elaborated in Table 3-7. Figure 5-7 shows the changes in COD occurring during biodegradation in the control reactor.

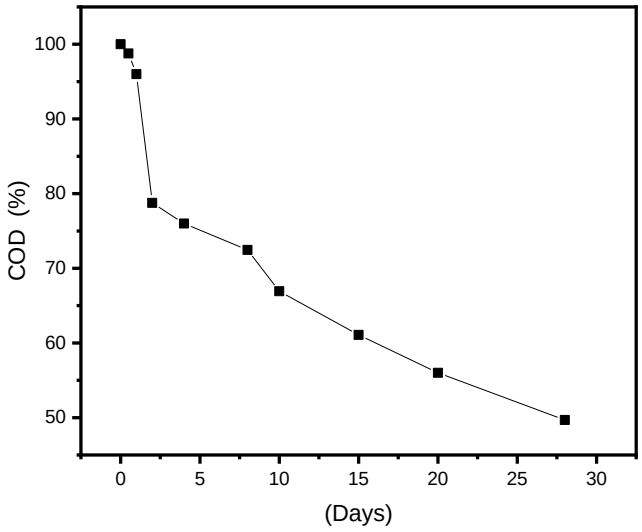



Figure 5-7: COD reduction in the control reactor during the biodegradation experiment at 100 rpm and 35℃.

Some studies have used sodium Azide ($NaN_3$) to inhibit microorganisms in biological reactors (Li and Zhang 2010). Comparisons between the reactors with the $NaN_3$ and

the control found that no change in the COD had occurred thus suggesting no microbial activity occurred. However, a reduction in COD with time was observed with the control reactor, thus indicating an occurrence of biodegradation in that reactor.

From batch experiments, it was observed that AMO was removed by both adsorption and biodegradation. By the end of 30 days, the adsorption rate was about 80% with only the sludge, while in the biodegradation bottle, degradation was about 60%. For fluoroquinolones, CIP and ENRO, the adsorption to sludge was much higher. This is noticeable in Figure 5-8 (B). Both adsorption and biodegradation were observed in both. However, adsorption was dominant. Adsorption was about 80% for the both; while biodegradation was 38%. The biodegradation of the compounds was much lower than adsorption. For SULFA, both adsorption and biodegradation values were lower than those of the other studied compounds, however, the biodegradation was observed to be higher than the adsorption. The adsorption capacity of SULFA to sludge was 49% and biodegradation was 37%. However, TET biodegradation was the lowest (29%) and adsorption was the highest (83.8%) amongst the studied compounds by the end of the 30 days.

Therefore, the removal routes observed for the studied compounds could be classified as: strong adsorption, strong biodegradation, both adsorption and biodegradation. Figures 5-8 to 5-10 illustrate the removal paths for each of the antibiotics.

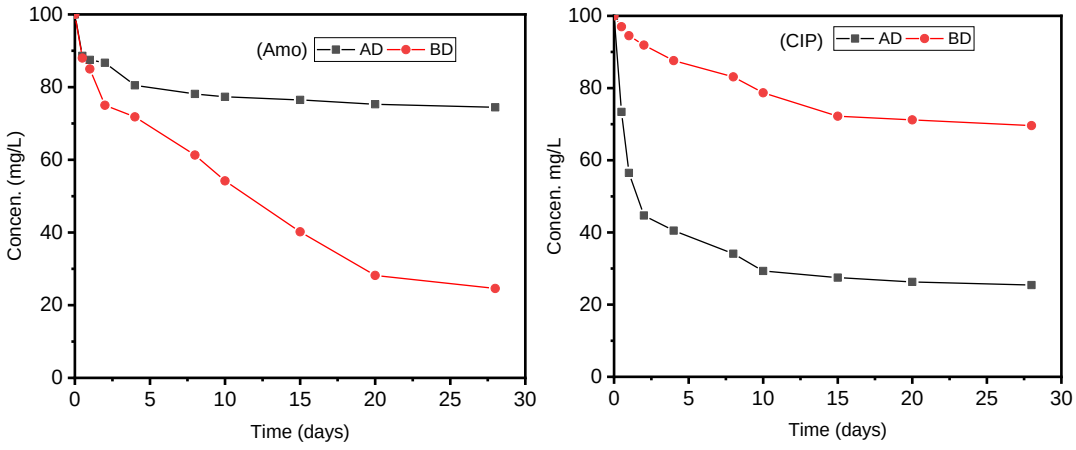



Figure 5-8: Biodegradation and Adsorption of (A) AMO and (B) CIP (AD refers to adsorption and BD refers to Biodegradation).

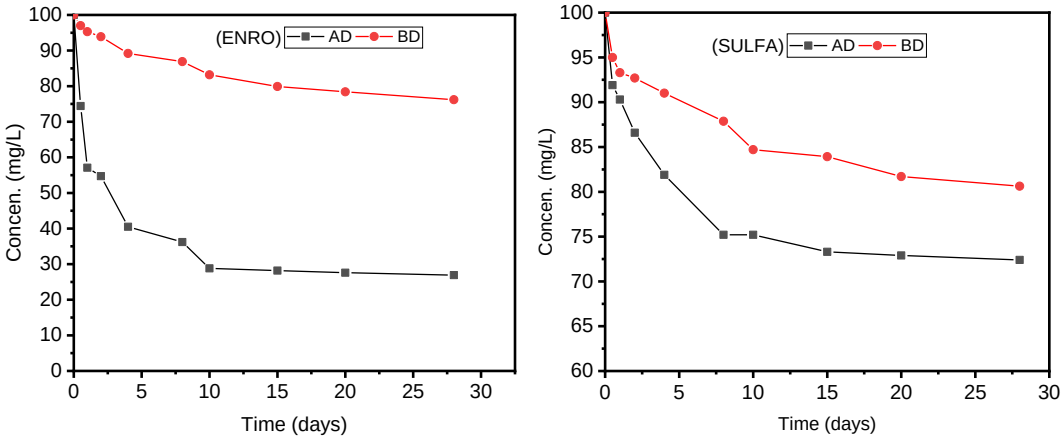



Figure 5-9: Biodegradation and Adsorption of (A) ENRO and (B) SULFA (AD refers to adsorption and BD refers to Biodegradation).

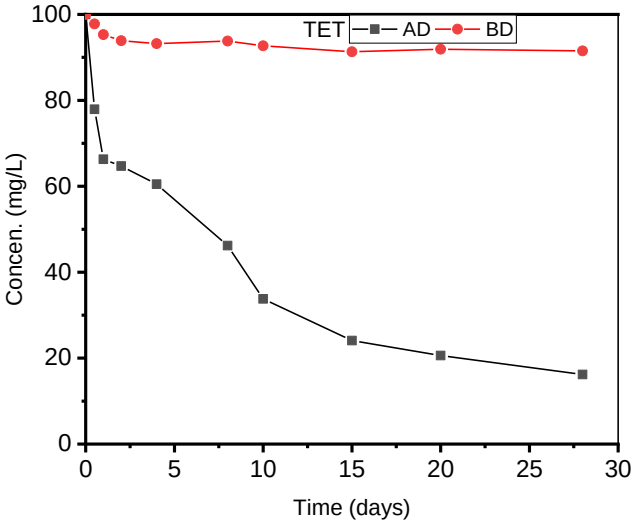



Figure 5-10: Biodegradation and Adsorption of Chlortetracycline (AD: adsorption and BD: Biodegradation).

Table 5-1 shows the kinetic parameters that were obtained from the plot of the natural logarithm of the ratio concentration at time *t* and the initial concentration (starting concentration).

Table 5-1: Slope (k) of the linear regression, regression coefficient ($R^2$), and half-life of the antibiotics from the batch biodegradation studies

| Antibiotics | k | $t_{1/2}$ (days) | $R^2$ |
|---|---|---|---|
| AMO | 0.11 | 6.33 | 0.982 |
| CIP | 0.065 | 10.66 | 0.969 |
| ENRO | 0.053 | 13.08 | 0.983 |
| SULFA | 0.022 | 34.65 | 0.964 |
| TET | 0.009 | 77.01 | 0.816 |

It is observed that almost all the antibiotics followed the first order degradation with coefficients of correlations ($R^2$) close to unity, except TET which was a little lower. AMO had the lowest half-life followed by the fluoroquinolones. SULFA and TET had the highest half-lives, thus signifying that biodegradation for these two compounds is low. This corresponds with the low biodegradation at 49% for SULFA and 29% for TET that was observed for both compounds. The half-lives that were obtained for all the antibiotics suggest that biodegradation was not very effective for them, even for AMO, which had the least half-life.

Different half-lives have been reported by various authors. Li and Zhang (2010) studied the adsorption and biodegradation of selected antibiotics in activated sludge treating fresh and saline wastewaters. Amongst the studied antibiotics were the fluoroquinolones and sulphonamides. Their experimental run time was 48 hours. The calculated half-lives were: 92.4 and 111.8 hours for the fluoroquinolones, SULFA was 64.2 and 133.3 hours. In another study by Hoang *et al.* (2012) on the fate of fluoroquinolones in the coastal wetlands ecosystem, they evaluated their half-lives during biodegradation to be 22.2 to 25.6 days. They observed that the biodegradation of the fluoroquinolones was poor. From both studies, it was observed that the adsorption of most of the studied compounds to sediments and other matrices was high, which could have prevented effective biodegradation.

## 5.4 Summary

The laboratory scale anaerobic reactor was employed to treat synthetic wastewater to explore the removal rates of five veterinary antibiotics. In a like manner, batch reactors were further used to evaluate the removal routes of the antibiotics.

The UASB reactor was operated continuously under mesophilic conditions to evaluate its performance with regards to the removal of organics and at the same time monitor biogas production. Organic loading rate (OLR) was varied between 2.8 to 9.2 kg.COD.$m^{-3}$.$d^{-1}$ while keeping the hydraulic retention time (HRT) constant at 12 h. Chemical oxygen demand (COD) removal efficiency higher than 75% was achieved at an OLR of 9 kg.COD.$m^{-3}$.$d^{-1}$, with a HRT of 12 hours. About 80% of the antibiotics were removed during the continuous processes, however, a distinctive pattern of removal was not observed.

With regards to the biodegradation, the current assumption that antibiotics do not degrade extensively under anaerobic conditions is correct, study on the biodegradation revealed that biodegradation occurred alongside adsorption but however, to a lesser degree. Adsorption was in the about 80% for AMO, CIP, ENRO, and TET while for SULFA, it was 49%. The compounds biodegradation was 29 to 38%. The kinetic data showed that the antibiotics followed a first order kinetic model with half-lives that ranged from 6 to 77 days.

# Chapter 6 - Advanced oxidation and adsorption as a post treatment of wastewaters containing antibiotics

## 6.1 Introduction

This chapter presents the findings from the characterisation of the AC, IPCA and the $TiO_2$ materials used for adsorption of antibiotics. **Section 6.2** discusses the findings from the characterisation of the AC, IPCA and $TiO_2$. **Section 6.3** discusses the results from adsorption of antibiotics using the three adsorbents. It was important that adsorption studies were carried out before the photodegradation. Therefore, the adsorption studies provided the preliminary information about the catalyst before the degradation.

## 6.2 Characterisation of adsorbents (AC, IPCA and $TiO_2$)

The AC and IPCA were characterised in order to evaluate their properties. Characterisation was carried out on all three: $TiO_2$, AC and IPCA using the scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and X-ray diffraction (XRD). Discussion of the findings is done in **sections 6.2.1 to 6.2.3.**

### 6.2.1 Scanning Electron microscopy (SEM) of AC, $TiO_2$ and IPCA

The scanning electron microscopy (SEM) was used to analyse the morphology and the texture of the catalysts. Figure 6-1 presents the results from the SEM on AC only while Figure 6-2 depicts the micrograph of the IPCA (AC with $TiO_2$).

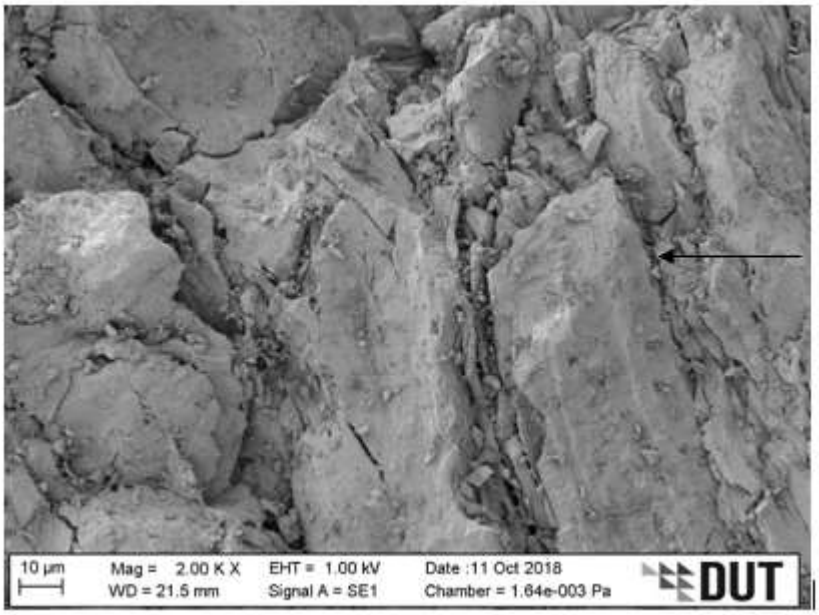



Figure 6-1: Scanning Electron microscopy micrograph of the AC without $TiO_2$

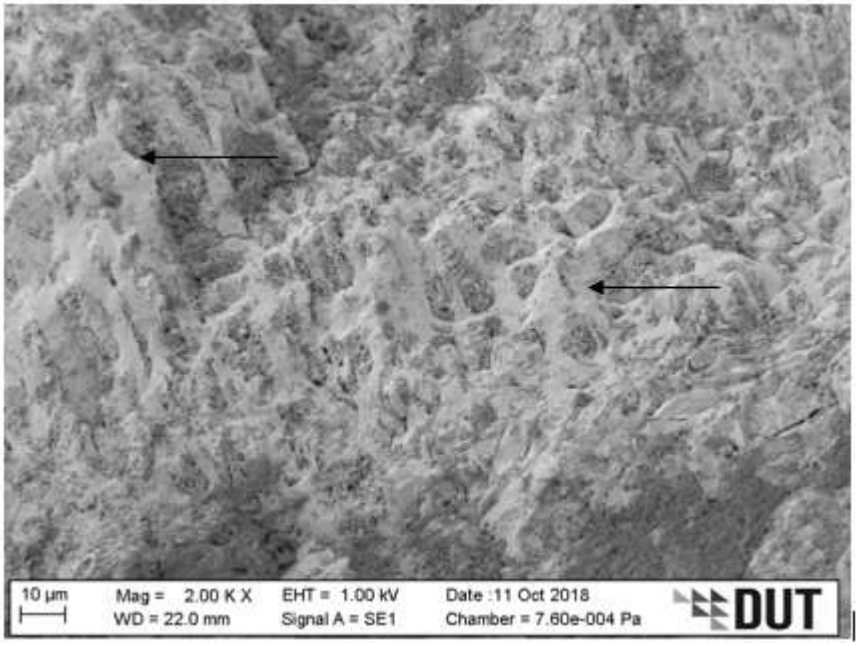



Figure 6-2: Scanning Electron microscopy micrograph of the IPCA (AC together with $TiO_2$).

From Figure 6-1, it was observed that the surface of the carbon was mostly rough, with a few smooth surfaces. The arrow on Figure 6-1 shows the roughened sites for the

possible attachment of the $TiO_2$. Figure 6-2, shows the dispersion of the $TiO_2$ on the AC surfaces as indicated by the arrows. The distribution/dispersion of the $TiO_2$ on the surface of the AC depended on the surface of the AC Keane *et al.* (2010). Surfaces that were roughened had more dispersion as compared to surfaces which were relatively smooth. The critical point to note, however, is the fact that the smooth surfaces were also well covered by the $TiO_2$. The good dispersion of $TiO_2$ across the surface of the AC was necessary to create an effective IPCA as large areas left without $TiO_2$ impacted on the photocatalytic ability of the IPCA by reducing its overall performance. A similar observation was described by Keane *et al.* (2010).

The texture and morphology of the catalyst are some of the important parameters that influence photocatalytic activity. Small aggregates of the $TiO_2$ that are uniformly dispersed on the AC are considered to be more advantageous due to the fact that they provide more active sites than the agglomerated particles (Keane *et al.* 2010).

### 6.2.2 Energy dispersive X-ray of AC, $TiO_2$ and IPCAs

The elemental analysis was carried out using the Scanning Electron microscopy-Energy dispersive X-ray (SEM-EDX) method. The results shown in Table 6-1, Figures 6-3 and 6-4 present the SEM-EDEX data of the AC without the $TiO_2$.

Table 6-1: Elemental composition of the AC only

| Elements | C | O | Fe | Al | Si | S | Total %) |
|---|---|---|---|---|---|---|---|
| Wt% | 27.07 | 72.54 | 0.10 | 0.19 | 0.06 | 0.06 | 100 |

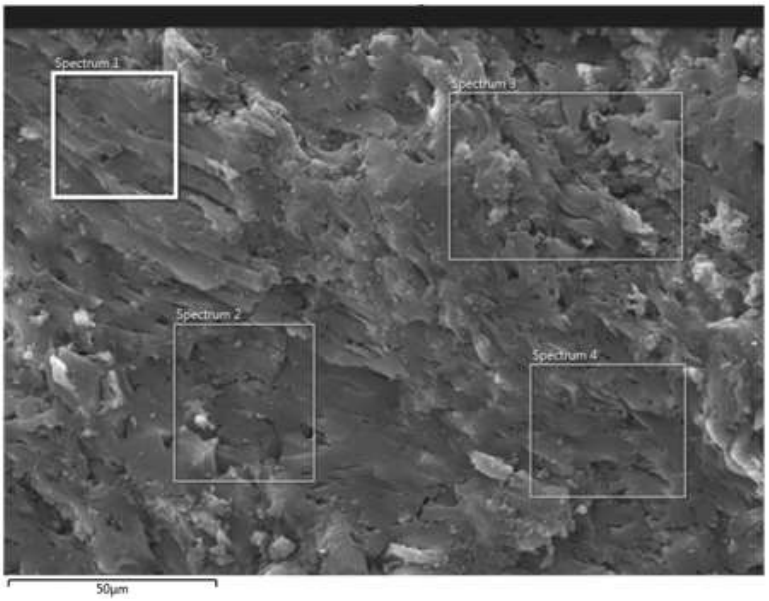



Figure 6-3: Spectrum of the scanning electron microscopy- Energy dispersive X-ray (SEM-EDX) of the AC without $TiO_2$

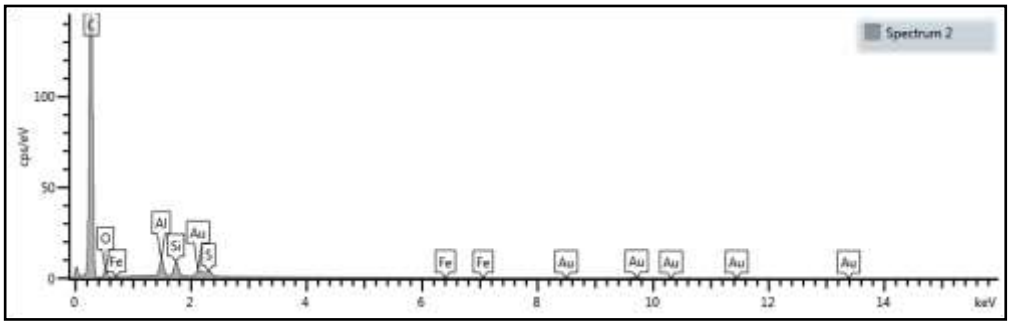



Figure 6-4: Energy dispersive X-ray (SEM-EDX) of the AC without $TiO_2$

Table 6-1 shows the elemental composition of the AC without the addition of the $TiO_2$. The elemental composition of the AC presented elements such as Fe, Mg, Au and others. The presence of the $TiO_2$ was not observed. This was also observed in the SEM-EDX in Figures 6-3 and 6-4.

Similarly, Figures 6-5 and 6-6 as well as Table 6-2 present the SEM-EDEX of the IPCA as well as the elemental composition. In the Table 6-2, the presence of $TiO_2$ was observed, thus indicating that the IPCA contained the $TiO_2$.

Table 6-2: Elemental composition of the IPCA

| Elements | C | O | Fe | Mg | Al | Si | S | K | Ca | Ti | Total %) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wt% | 22.88 | 66.21 | 0.06 | 0.01 | 0.08 | 0.10 | 0.09 | 0.0 | 0.02 | 10.46 | 100 |

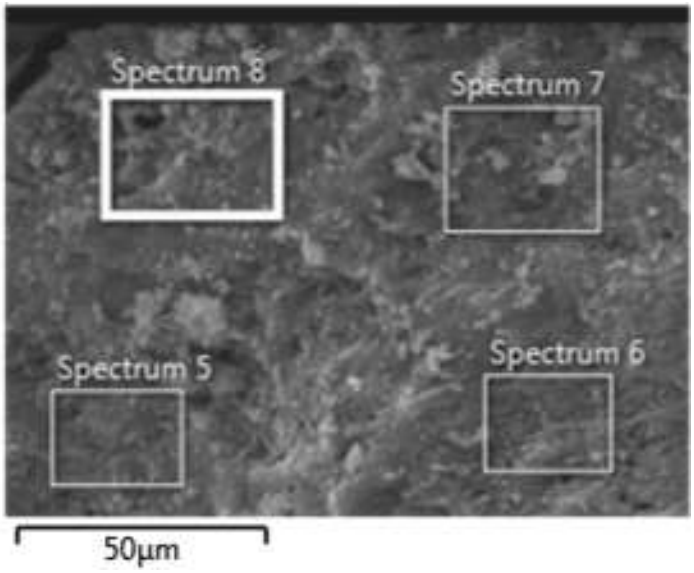



Figure 6-5: Spectrum scanning electron microscopy- Energy dispersive X-ray (SEM-EDX) of the AC with $TiO_2$ (IPCA)

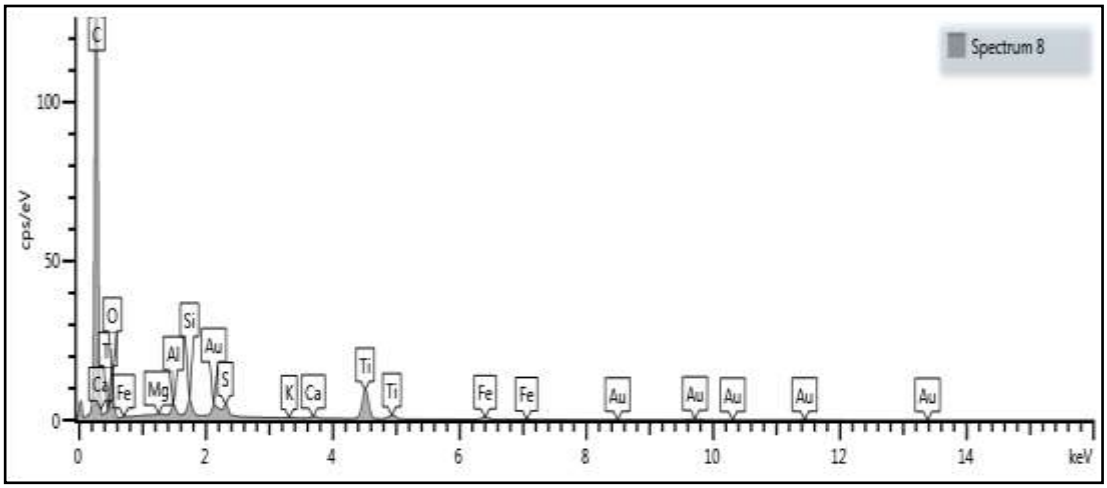



Figure 6-6: Energy dispersive X-ray (SEM-EDX) of the AC with$TiO_2$ (IPCA)

On comparing SEM-EDEX Figures with and without the addition of $TiO_2$, it is observed that the spectra without the $TiO_2$ had a smoother surface (Figure 6-3) while the presence of $TiO_2$ in Figure 6-5 made the surface to appear roughened. Different spots were identified which were indicated as spectrums and differentiated with the given numbers as shown in Figures 6-3 and 6-5. The distribution of the $TiO_2$ at each given spectrum varied. This was as a result of the surface of the AC as shown in Figures 6-3 and 6-5 and the distribution of the TiO2 as well as the emission of X-rays from surfaces of the IPCA that faced away from the detector and consequently could not be detected. The areas of the IPCA that had the highest $TiO_2$ composition were possibly those facing the detector (Keane *et al.* 2010). With regards to the spectra, spectrum 2 (AC only) and spectrum 8 (IPCA) were reported and the compositions are described in Tables 6-1 and 6-2 respectively. These findings corroborate those of Keane *et al.* (2010).

### 6.2.3 X-ray-diffraction (XRD) of AC, $TiO_2$ and IPCA

X-ray-diffraction (XRD) is an analytical technique which shows the information regarding the crystallographic structure as well as the chemical composition of powders and thin films (Basha *et al.* 2010). Adsorption and photo-activity of both the AC and IPCA could be influenced by the crystalline phase of the $TiO_2$ and the crystalline phase of the AC structure. The X-ray diffraction was used to evaluate the crystallinity of the catalysts as shown in Figures 6-7 and 6-8. In Figure 6-7, the peaks indicating A on the graphs depict that they are for anatase while R depicts peaks belonging to the rutile. Anatase peaks were observed at 29˚, 45˚, 57˚, 65˚, 75˚, and 83˚. The peaks for rutile were at 32˚, 42˚ and 68˚.(Basha *et al.* 2010) Peaks identification were matched with the bands as provided by the software as shown in Figures 6-7 and 6-8.

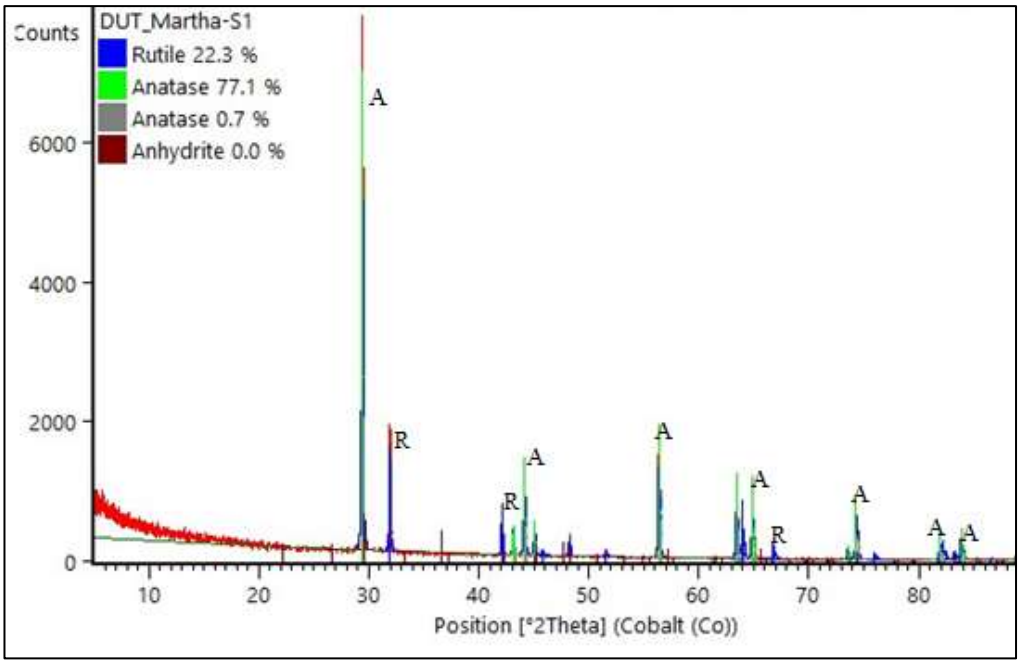



Figure 6-7: XRD of the $TiO_2$ alone (Peak marked A present the anatase peak while that marked R present the rutile)

The other peaks were not identified due to the fact that they were overlapping. Results obtained from Figure 6-7 showed that rutile had weaker signals compared to the anatase, however, higher signals were obtained with the anatase, thus indicating that the $TiO_2$ was mainly composed of anatase. Calculation which was based on the height indicated that the $TiO_2$ comprised of 77% anatase and the rest was rutile. This was corresponding to what is in literature (Chong *et al.* 2010). Most of the $TiO_2$ that are used in literature are said to contain 75%- 80% anatase and 20% -25% rutile. It has been reported that it is important that the percentage of the anatase be much higher than that of the rutile due to the fact that the rutile has a lower conduction band potential and may act as an electron sink thus reducing the recombination of the charge carriers (Zhao *et al.* 2010; Lim *et al.* 2011; Srikanth *et al.* 2017).

Figure 6-8 is the XRD of the IPCA; it was observed that the crystalline structure of the AC was prominent at a peak angle of 31$^o$ while that of anatase was more prominent at angle 29˚. Rutile had peaks that were not profound, hence not properly displayed. Again, the absence of the rutile peak could be due to the fact the graphite peak which

was properly enhanced at a position 31° was very close to the rutile (32°) and diminished that of the rutile. In Figure 6-7, one of the rutile peaks appeared at 32 °.

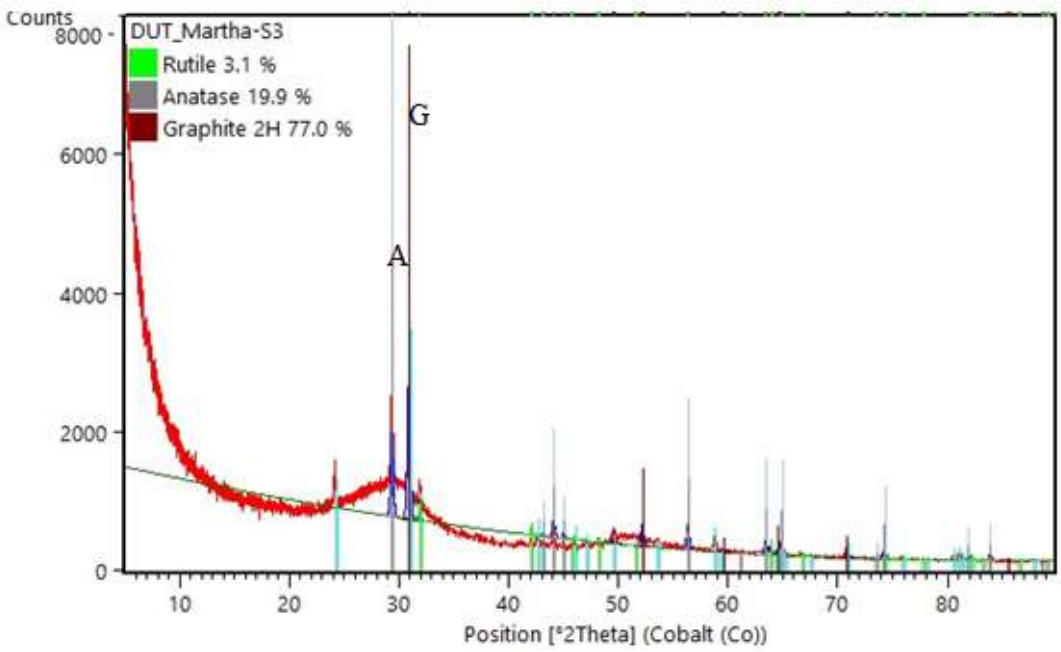



Figure 6-8: XRD of the IPCA (ration of $TiO_2$ to carbon 1:10) (Peak marked G present the graphite peak while that marked A present the anatase)

Therefore XRD results portraits the fact that the $TiO_2$ was a mixed phase and also further confirms the graphitic nature of the AC.

#### 6.2.3.1 Summary

The characterisation of the IPCA showed that the IPCA contained $TiO_2$ which were distributed on its surface, this was confirmed by the SEM as well as the EDEX. The XRD revealed that the AC alone was 77% graphitic while the $TiO_2$ was a mixture of anatase and rutile in a ratio of 77.1 and 22.3% and the rest brookite. Finally, the XRD further revealed IPCA was a mixture of 77% graphite, 19% anatase and 3.1% rutile. Therefore, characterisation for both the $TiO_2$ and IPCA indicated that the larger content of $TiO_2$ was anatase.

## 6.3 Adsorption studies

The adsorption studies describe the rate of the adsorbate uptake onto the adsorbent which usually controls the equilibrium time. The parameters from the kinetic studies are helpful for the prediction of the adsorption rate which provides crucial information for the designing and modelling of processes. Further still, it is useful in determining the adsorption mechanism and the potential rate-limiting steps (Ahmed 2017).

Adsorption studies were carried out on the $TiO_2$ and AC individually. Both the $TiO_2$ and AC gave the baseline properties for the IPCA. Therefore the adsorption capacities for the IPCA were also studied. This was to evaluate the effect of adding $TiO_2$ onto the AC surface. The antibiotics studied have been elaborated in Chapter 2 **section 2.3** and the method used for the analysis was based on the method which was developed and validated in Chapter 4 **section 4.4.**

## 6.4 Batch sorption experiments

#### 6.4.1.1 Effect of process parameters on adsorption equilibrium

Effects of various parameters on the adsorption of the antibiotics onto the AC, IPCA and the $TiO_2$ were evaluated. For the batch sorption experiments, a concentration of 100 mg/L of each antibiotic spiked in Millipore water was used for the $TiO_2$, AC and IPCA. Other sorption parameters were: contact time, adsorbent dosage and pH. These parameters were optimised to determine their effect on the adsorption efficiencies and capacities of the adsorbents for the antibiotics.

Where necessary a mass balance was carried out to determine the antibiotic adsorption capacity. The OriginPro software was used for the modelling as well as the data analysis. The responses from the optimisation studies were antibiotics reduction. For photodegradation, which is presented in Chapter 7, other parameters such as the total organic carbon (TOC) and chemical oxygen demand (COD) are also evaluated.

## 6.5 Kinetic studies (Adsorption kinetics of $TiO_2$, AC and IPCA)

**Sections 6.5 to 6.8** present the optimisation of the $TiO_2$, AC and IPCA. The main parameters studied were: Adsorbent loading, solution pH and, contact time. A concentration of 100 mg/L of a mixture of the antibiotics stock solution was used. The same procedure was followed for all the studied adsorbents. From literature, it has been reported that the percentage removal of most adsorbates increases proportionately to the adsorbent dosage (Alkaim *et al.* 2014). Therefore, for this study, the optimisation for the adsorbent dosage was not carried out and the adsorbent loading was 0.1 g/L for every 100 ml of the standard solutions of the different antibiotics (Alkaim *et al.* 2014).

### 6.5.1 Effect of contact time

#### 6.5.1.1 Effect of contact time on $TiO_2$

Figure 6-9 depicts the effect of contact time obtained when $TiO_2$ was used. It was observed that the adsorption capacity of the $TiO_2$ was low, therefore it was difficult to establish the equilibrium time for the $TiO_2$. This was due to the fact that the $TiO_2$ is not a good adsorbent. However, it was important that this study was carried out in order to determine the amount of the antibiotics that was adsorbed onto the $TiO_2$, to ascertain the base line.

Nonetheless, the equilibrium times obtained for the antibiotics were 300, 300, 300, 180 and 180 minutes for AMO, CIP, ENRO, SULFA and TET respectively, indicating that TET and SULFA was adsorbed in shorter times of 180 minutes while the remaining took longer times of 300 minutes. The respective sorption quantities were 7.4, 9.2, 11.4, 10 and 13.07 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively.

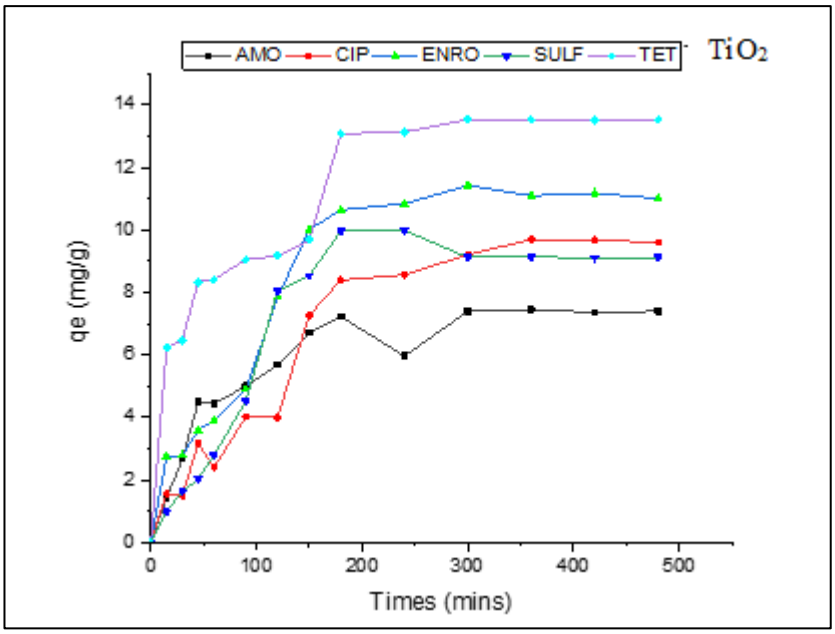



Figure 6-9: Concentration vs time for the studied compounds for the $TiO_2$ adsorption. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25℃. shaking speed 150 rpm and initial concentration 100 mg/L)

### 6.5.1.2 Effect of contact time on AC

Similarly, like with the $TiO_2$, the contact time needed to reach equilibrium for the AC adsorbent was evaluated. Figure 6-10 depicts the time frames that each compound took to reach equilibrium. The equilibrium times were 180, 300, 240, 360 and 300 minutes for AMO, CIP, ENRO, SULFA and TET respectively. The sorption quantities were 68.4, 89.6, 79.7, 59.9 and 94.3 mg/g respectively. Unlike the $TiO_2$, adsorption equilibrium was more stable with the AC and also, higher amounts of the adsorbates were adsorbed on the AC. The lowest adsorption time was again for AMO at 180 minutes, followed by CIP. ENRO and TET had the same equilibrium times of 300 minutes while SULFA had the longest at 360 minutes. The adsorption trend observed was similar to that obtained with the $TiO_2$, where, TET had the highest adsorbed quantity.

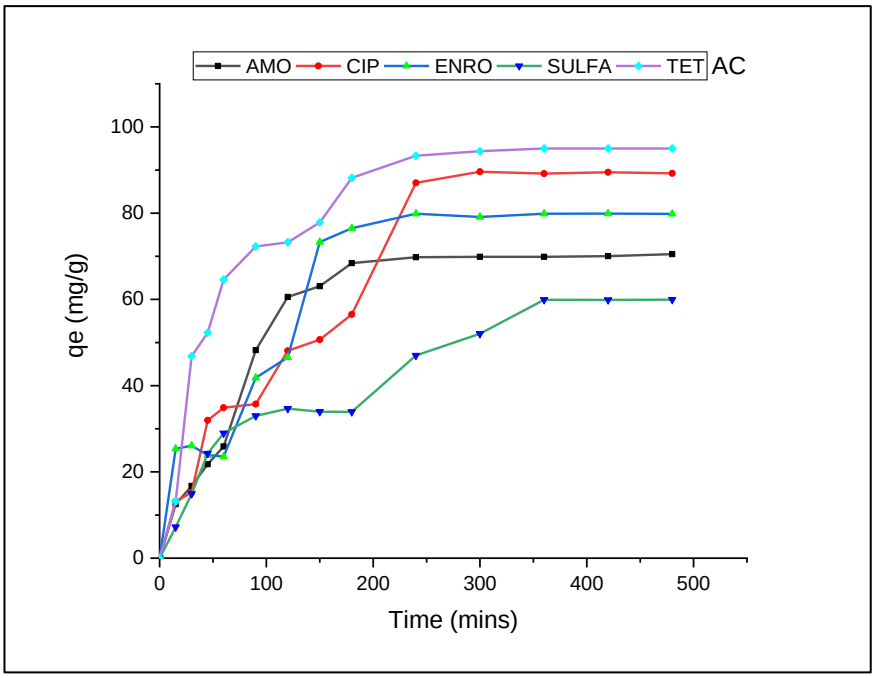



Figure 6-10: Concentration vs time for the studied compounds for the AC adsorption. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L)

### 6.5.1.3 Effect of contact time on IPCA

Figure 6-11 shows the effect of contact time on the IPCA adsorption. Again like the adsorption on $TiO_2$ and AC, different adsorption times were achieved. Equilibrium adsorption times were: 300, 240, 300, 360 and 240 minutes for the five different compounds respectively. Their sorption capacities were; 59.3, 79.7, 69.8, 54.7 and 84.7 mg/g respectively. Longer contact times were obtained with the IPCA. AMO which had a contact time of 180 minutes for the AC adsorption to reach equilibrium required a longer contact time of 300 minutes. A similar occurrence was observed with SULFA. However, for CIP a decrease from 300 to 240 minutes was observed while ENRO was at 300 minutes. TET was found to have different equilibrium times for the three adsorbents.

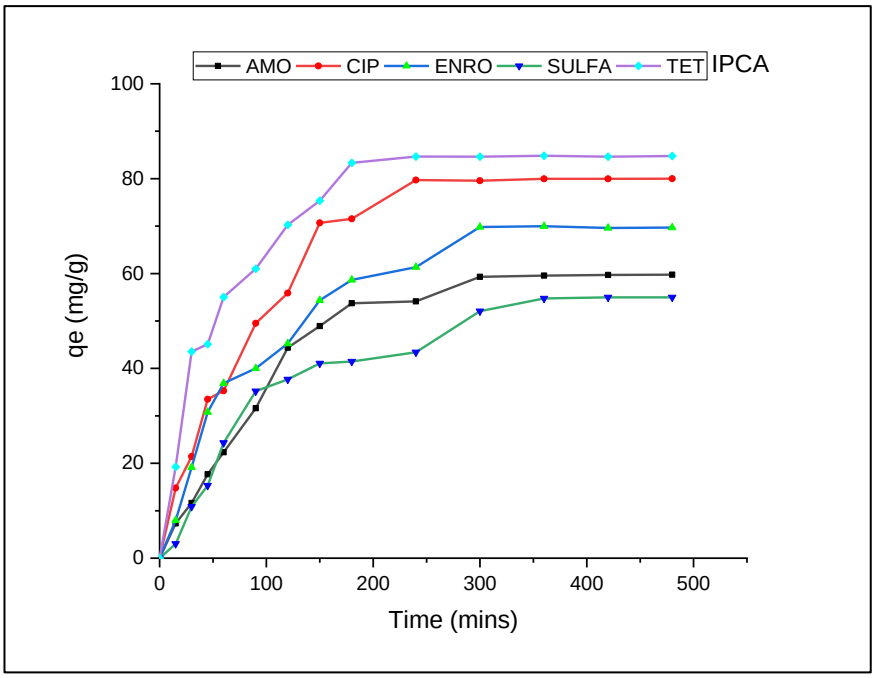



Figure 6-11: Concentration vs time for the studied compounds for the IPCA adsorption. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L)

#### 6.5.1.4 Summary on contact time

The contact times required to reach equilibrium for the adsorbent was found to differ for each of the adsorbents, even though similar times were observed for some of the adsorbents. From Figures 6-9 to 6-11, steep slopes were observed increasing for each of the studied compounds on the adsorbents, thus indicating the increase in the adsorption capacity of the adsorbates on each of the adsorbents. This may have probably been due to the availability of large numbers of sites for adsorption on the surface of the sorbent materials, however, over time, as the active surface sites became occupied a gradual decrease and thereafter the flattening of the curves was observed thus illustrating the attainment of equilibrium. Pouretedal and Sadegh (2014) observed that as the active surface sites became occupied, the intra-particle diffusibility (pore diffusion) of the adsorbate onto sorption sites of the adsorbents became slow as well. This occurrence could lead to an increase in the repulsive forces between the solid molecules and the bulk phase, thereby pushing the adsorption process towards

equilibrium. The sorption capacity of the adsorbents for all the antibiotics was not beyond the sorption equilibrium contact time, and as a result, any further increase in the contact time after equilibrium would not facilitate a significant difference on the sorption capacity of the adsorbents.

### 6.5.2 Sorption efficiency for the three adsorbents ($TiO_2$, AC and IPCA)

The rate of adsorption for molecules of the adsorbates onto the adsorbent surfaces is expressed using the kinetic process. Therefore, a comparative evaluation on the effectiveness of the use of the different adsorbents for the removal of the studied compounds from solution was conducted in order to understand the kinetics of the adsorption processes. Comparative sorption of AMO, CIP, ENRO, SULFA and TET onto the different adsorbents were evaluated and designated in Figures 6-12 to 6-16. These figures show the sorption capacity for the three adsorbents for each of the compounds. The sorption capacity differed for all the adsorbents, with the AC having the highest adsorption capacity followed by the IPCA, while $TiO_2$ gave the least amounts adsorbed.

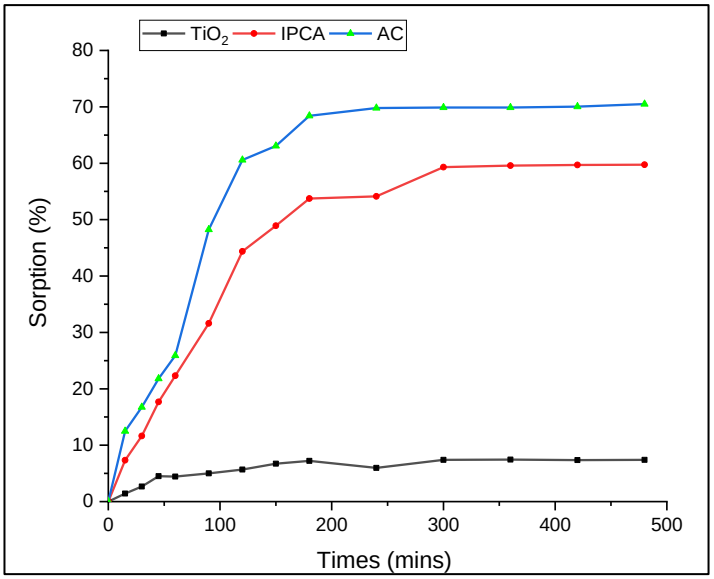



Figure 6-12: Sorption kinetics of AMO from solution using $TiO_2$, IPCA and AC. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L)

Figure 6-12 shows the sorption capacity of AMO on the three adsorbents. The sorption of AMO onto the adsorbents was rapid for the AC and IPCA, but slow for $TiO_2$. This is due to the fact the $TiO_2$ is a poor adsorbent. AC and IPCA thus signify the ability to be used as adsorbents for the removal of AMO from a solution with equilibrium times under 300 minutes. At equilibrium, the sorption efficiencies for both AC and IPCA were 70% and 59% respectively, thus implying the competitiveness of both adsorbents. With regards to $TiO_2$, sorption was less than 20%.

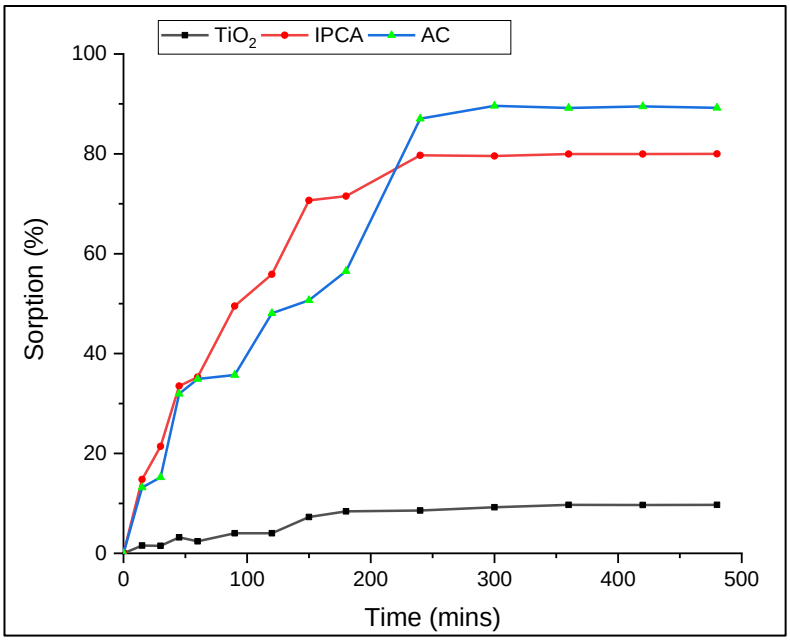



Figure 6-13: Sorption kinetics of CIP from solution using $TiO_2$, IPCA and AC. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25℃., shaking speed 150 rpm and initial concentration 100 mg/L)

Figure 6-13 shows the sorption capacity of CIP on the three adsorbents. Again, the sorption of CIP onto the adsorbents was rapid for the AC and IPCA. Like with the AMO, sorption was poor for $TiO_2$. AC and IPCA again displayed the ability to be used as adsorbents for the removal of CIP from a solution with equilibrium times under 300 minutes. At equilibrium, the sorption efficiencies for both AC and IPCA were 89% and 79% respectively, thus implying the competitiveness of both adsorbents, with values higher than those of AMO. With regards to $TiO_2$, sorption was less than 20%.

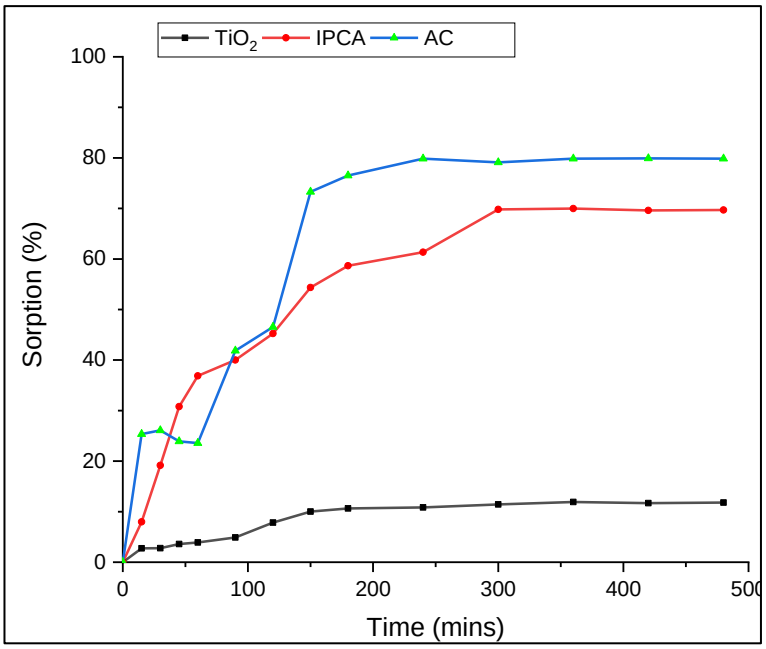



Figure 6-14: Sorption kinetics of ENRO from solution using $TiO_2$, IPCA and AC. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L)

Figure 6-14 shows the sorption capacity of ENRO on the three adsorbents. Like its counterpart CIP, ENRO's sorption onto the adsorbents was rapid for the AC and IPCA. Again, sorption was poor for $TiO_2$. AC and IPCA again displayed the ability to be used as adsorbents for the removal of ENRO from a solution with equilibrium times under 300 minutes. At equilibrium, the sorption efficiencies for both AC and IPCA were 79% and 69% respectively. The competitiveness of both adsorbents was again proven with the sorption efficiency of ENRO, however, $TiO_2$ sorption was less than 20%. Even though ENRO and CIP belonged to the same group, it was observed that sorption of CIP onto the adsorbents was higher than that of ENRO for all the adsorbents.

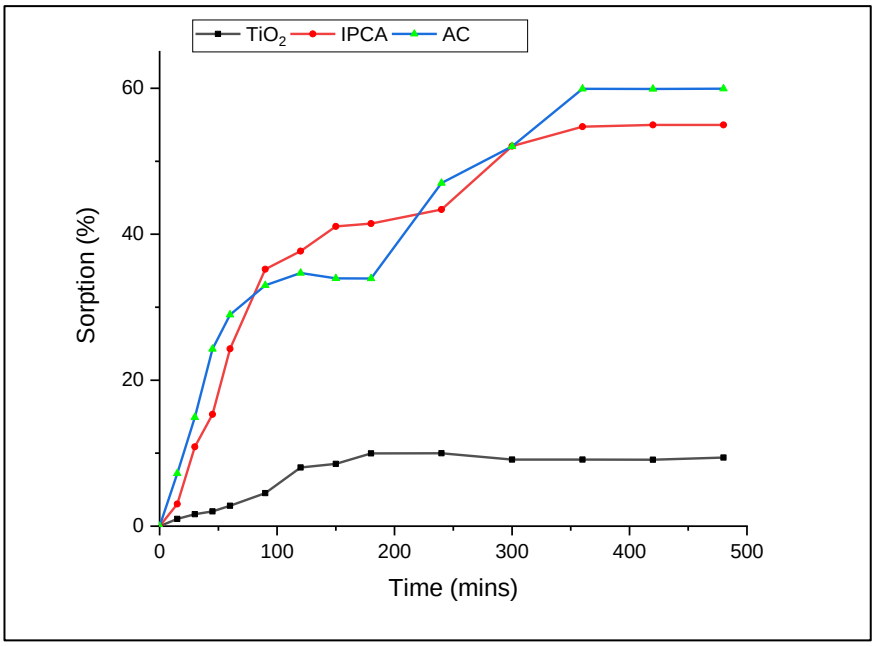



Figure 6-15: Sorption kinetics of SULFA from solution using $TiO_2$, IPCA and AC. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L)

Figure 6-15 shows the sorption capacity of SULFA on the three adsorbents. Even though a similar trend was followed for the AC and IPCA sorption where AC had a higher sorption capacity followed by the IPCA and $TiO_2$ with the lowest, sorption of SULFA showed the lowest percentage. Sorption capacity for the AC and IPCA were 59% and 54% respectively. $TiO_2$ sorption was again lower than 20%

Figure 6-16 shows the sorption capacity of TET on the three adsorbents. Like the others, TET's sorption onto the adsorbents was rapid for the AC and IPCA, however sorption was poor for $TiO_2$. AC and IPCA again displayed the ability to be used as adsorbents for the removal of TET from a solution with equilibrium times under 300 minutes. At equilibrium, the sorption efficiencies for both AC and IPCA were the highest for the studied compounds at 95% and 84% respectively. Less than 20% was obtained for the $TiO_2$.

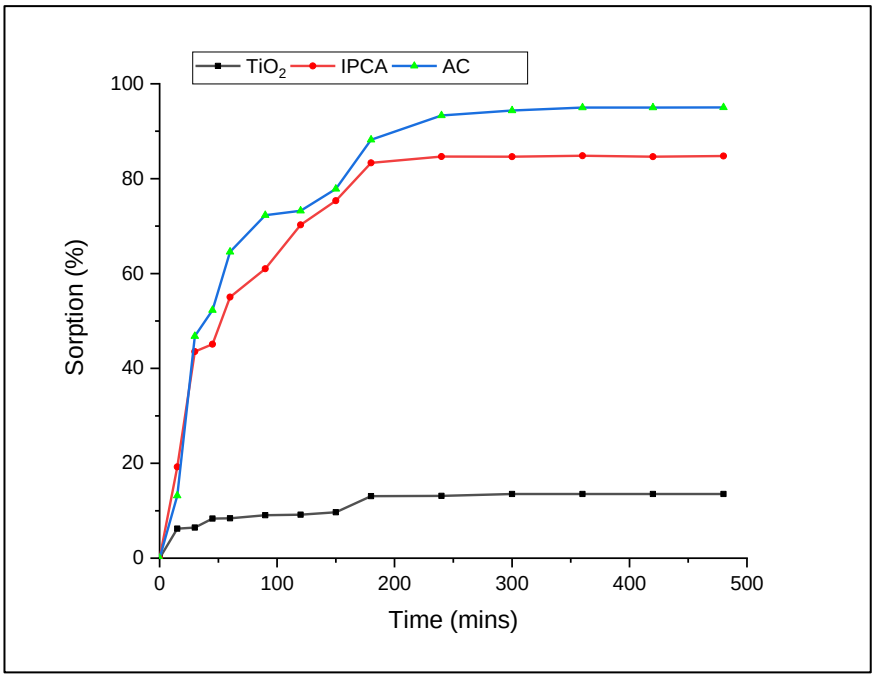



Figure 6-16: Sorption kinetics of TET from solution using $TiO_2$, IPCA and AC. (pH 6, contact time 0-480 minutes, adsorbent loading 1 g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L).

### 6.5.3 Summary on the sorption capacity of the adsorbents

Of the three adsorbents studied for the sorption of the five antibiotics compounds, AC and IPCA performed better than $TiO_2$. This is due to the fact that $TiO_2$ is not a good adsorbent. In one of the earliest study on AC and IPCA by Matos, Laine and Herrmann (1998), it was observed that $TiO_2$ was not a good adsorbent. Velasco, Parra and Ania (2010) reported that the BET surface area of P25 (a form of $TiO_2$) by $N_2$ adsorption is 44 – 53 $m^2/g$, thus signifying that $TiO_2$ would not be a good adsorbent due to its low surface area. The XRD analysis showed that the $TiO_2$ had a composition of about 75 anatase and 25% rutile which is similar to that of the P25 (Degussa), and as such performed poorly like the P25 with regards to the adsorption of the compounds.

On the other hand, AC has been proven to be an excellent adsorbent for the removal of contaminants from solution. Recently, the use of AC for the adsorption of antibiotics have gained much attention (Ahmed *et al.* 2015). According to Vona *et al.* (2015), the AC adsorption process has proved to be have excellent removal efficiency compared

to other technologies such membrane technology, biological and chemical treatment methods.

The contact time required for the adsorbates to reach equilibrium differed with the adsorbents in some cases, but was the same for others. For instance, AMO was observed to reach equilibrium in 180 minutes, thus indicating that equilibrium proceeded faster, while the remaining compounds took longer (from 240 to 300 minutes). The reason for that may have been due to the fact that AMO has a smaller molecular weight as compared to the other compounds. Therefore, there is the possibility of its particles to be adsorbed quicker and thus plugging the pores, and becoming saturated quickly. Adsorption capacity followed the trend: TET > CIP > ENRO > AMO > SULFA, noticeable from all the adsorbents. It has been reported that the adsorption ability of SULFA is limited due to its hydrophobic characteristics. Therefore, the findings in this study agrees with the literature (Rivera-Utrilla *et al.* 2013).

## 6.6 Kinetics modeling

The adsorption of the adsorbates on $TiO_2$ were observed to be poor, therefore as a result of the sections below focus on the kinetics of AC and IPCA only.

The sorption of sorbates onto the different adsorbents can be preceeded by different mechanisms which are governed by the physico-chemical conditions under which the adsorption processes take place, as well as the heterogeneity of the reactive sites. It is therefore crucial to understand the sorption mechanism that would be aligned with each adsorbate, in order to be able to describe and design effectively and efficiently future feasibilities of the adsorption at larger scales (Ahmed 2017). To evaluate and understand the mechanism controlling the adsorption processes, the following models: pseudo-first order, pseudo-second order, and the intra-particle diffusion model were used for the data on the studied compounds.

The calculated constant parameters for the pseudo-first-order, pseudo-second-order and the Intra-particle models obtained by non-linear regression for the adsorbates

using AC and IPCA are summarized in Tables 6-3 and 6-4. According to Kumar, Kumar and Kumar (2003) and Zhang *et al.* (2016) adsorption could proceed in the following main stages: (1) bulk phase transportation of the adsorbates to the external surface, (2) transportation could be across the boundary layer, also referred to as the external mass transfer, (3) transportation could be through the AC by surface diffusion or pore diffusion also referred to as intra particle diffusion and finally, (4) the adsorption could be on the surface of the adsorbents This step is usually considered to be instantaneous.

Table 6-3: Kinetic parameters for the sorption of AMO, CIP, ENRO, SULFA and TET from solution by AC

| AC | Adsorbate | $K_1$(min-$^1$) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
|---|---|---|---|---|---|
| Pseudo-first Order | AMO | 0.0118 | 0.8948 | 0.947 | 0.942 |
| | CIP | 0.0085 | 1.1722 | 0.886 | 0.876 |
| | ENRO | 0.0103 | 0.8712 | 0.916 | 0.909 |
| | SULFA | 0.0140 | 1.4971 | 0.921 | 0.915 |
| | TET | 0.0083 | 1.9717 | 0.893 | 0.878 |
| | | $K_2$(gmg$^{-1}$min$^{-1}$) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
| Pseudo-Second Order | AMO | 0.00563 | 1.84601 | 0.971 | 0.966 |
| | CIP | 0.00564 | 1.93438 | 0.961 | 0.956 |
| | ENRO | 0.00392 | 1.81235 | 0.979 | 0.976 |
| | SULFA | 0.00383 | 1.74037 | 0.930 | 0.923 |
| | TET | 0.00951 | 0.41935 | 0.981 | 0.979 |
| Intra-particle diffusion | | $K_{in}$(mg/gmin) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
| | AMO | 9.86083 | 11.6321 | 0.959 | 0.952 |
| | CIP | 3.71018 | 14.9207 | 0.951 | 0.942 |
| | ENRO | 10.8302 | 12.8377 | 0.911 | 0.894 |
| | SULFA | 5.69314 | 9.0126 | 0.958 | 0.950 |
| | TET | 24.9457 | 13.4472 | 0.973 | 0.968 |

Table 6-3 shows the kinetic parameters for the sorption of AMO, CIP, ENRO, SULFA and TET from solution by AC. It is observed that the correlation coefficients ($R^2$) for the pseudo first order was in the range of 0.886 to 0.947 while the pseudo second order correlation coefficients ($R^2$) was in the range of 0.930 to 0.981. Intra-particle diffusion model was in the range of 0.911 to 0.973.

Table 6-4: Kinetic parameters for the sorption of AMO, CIP, ENRO, SULFA and TET from solution by IPCA

| IPCA | Adsorbate | K ($min^{-1}$) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
|---|---|---|---|---|---|
| Pseudo-first Order | AMO | 0.00519 | 0.81988 | 0.928 | 0.913 |
| | CIP | 0.00417 | 1.04198 | 0.905 | 0.893 |
| | ENRO | 0.00483 | 1.11709 | 0.937 | 0.929 |
| | SULFA | 0.00667 | 1.0720 | 0.787 | 0.744 |
| | TET | 0.00307 | 0.93162 | 0.742 | 0.699 |
| | | $K_2$($gmg^{-1}min^{-1}$) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
| Pseudo-Second Order | AMO | 0.01313 | 1.40975 | 0.934 | 0.929 |
| | CIP | 0.01065 | 0.72027 | 0.973 | 0.971 |
| | ENRO | 0.01199 | 0.97679 | 0.964 | 0.961 |
| | SULFA | 0.01333 | 1.99331 | 0.817 | 0.801 |
| | TET | 0.01084 | 0.36827 | 0.992 | 0.992 |
| | | $K_{in}$(mg/gmin) | qe (mg/g) | $R^2$ Exp | $R^2$ Pre |
| Intra-particle diffusion | AMO | 0.12137 | 16.27476 | 0.974 | 0.969 |
| | CIP | 0.15121 | 26.80792 | 0.967 | 0.961 |
| | ENRO | 0.13193 | 21.78867 | 0.959 | 0.951 |
| | SULFA | 0.10950 | 14.03037 | 0.951 | 0.942 |
| | TET | 0.13456 | 38.66528 | 0.894 | 0.874 |

Similarly, Table 6-4 shows the kinetic parameters for the sorption of AMO, CIP, ENRO, SULFA and TET from solution by IPCA. The correlation coefficients ($R^2$) for the pseudo first order (PFO) were in the range of 0.886 to 0.947 while the pseudo

second order (PSO) correlation coefficients ($R^2$) was in the range of 0.817 to 0.992. Intra-particle diffusion model was in the range of 0.894 to 0.974.

The diagrams depicting the PSO, PFO and intra-particle diffusion models are illustrated in Appendix C. According to Table 6-3 and 6-4, the uptake of the adsorbates by both the AC and IPCA for the calculated and experimental $q_e$ were more consistent for the PSO than the PFO. This was also noticeable in the correlation coefficients ($R^2$). Even though the correlation coefficients for both the PSO and PFO were both closer to unity, they were higher for the PSO. Again, the experimental $q_e$ values for the PSO were closer to the predicted $q_e$ values in addition to the higher correlation coefficients obtained thus making the PSO to have a higher possibility to predict the adsorption process of the adsorbates onto the AC and IPCA than PFO. The inability of the antibiotics to follow the PFO suggests that the external diffusion was not the determining step in the adsorption process as explained by Zhang *et al.* (2016).

According to Sikarwar and Jain (2016) the PSO is observed to be more suitable for the prediction of the adsorption, then chemical adsorption (chemisorption) is said to occur. Chemisorption is facilitated by chemical forces of attraction (chemical bond) between the adsorbate and the adsorbent which results in the formation of a unilayer of adsorbate on the adsorbent and the enthalpy of adsorption process is usually high (Le-Minh *et al.* 2010). Also, Sikarwar and Jain (2016) noted that the mechanism for the removal of an adsorbate by an adsorbent is a complex if the linear portion of the curves do not pass through the origin. Both the AC and the IPCA graphs for the PSO did not pass through the origin, again assuming that chemisorption is dominant and that it controls the adsorption (Sikarwar and Jain 2016). These findings are further in agreement with those of Fu *et al.* (2017) and Yu *et al.* (2016).

To determine the rate controlling step affecting the kinetics of adsorption, the intra-particle diffusion model was evaluated. As earlier mentioned, generally adsorption is usually preceded mainly by the external surface adsorption; the gradual equilibrium stage with intra-particle diffusion dominating; and a final equilibrium stage with the intra-particle diffusion starting to slow down. It is reported that the rate limiting step is either the intra-particle diffusion step or transfer across the boundary layer. Kumar,

Kumar and Kumar (2003) identified the intra-particle diffusion step as the rate limiting step. Choy, Porter and Mckay (2004) observed that the differential equations that contains the diffusion terms have revealed that the fractional uptake of the adsorbates are a function of the square root of time ($t^{0.5}$) rather than time *t*. Therefore, in determining qualitatively the rate controlling step, this relationship is usually employed.

According to Tables 6-3 and 6-4, high correlation coefficients for both the AC and IPCA were obtained for the intra-particle diffusion model. *K* was determined from the slope of the linear plots while the intercept (*C*) revealed the information about the boundary layer thickness. A large intercept depicts the effects of a great boundary layer (Choy, Porter and Mckay 2004). High values of *C* ranging from 14 to 38 for the IPCA and 9 to 13 for the AC were observed, thus indicating that intra-particle diffusion was not the only rate controlling step, but other kinetic processes (e.g. external mass transfer) were simultaneously occurring thus contributing to the adsorption mechanism. According to the intra-particle diffusion model, if the plot of $q_t$ compared to $t^{1/2}$ has a zero intercept ($C = 0$), then the intra-particle diffusion model becomes a critical controlling factor in determining the kinetics of the process (Zhang *et al.* 2016). The Figure for both the AC and IPCA for the intra-particle diffusion model is shown in Appendix C.

### 6.6.1 Effect of solution pH on adsorption

The effect of pH on the adsorption depends on the characteristics of the adsorbents and speciation of antibiotics at different pH values in water (Ahmed 2017). The point of zero net charge (PZC) of the adsorbent indicates the condition at which the net charge density on the surface of adsorbents is zero. It is reported that at pH above the PZC of the adsorbent, the surface charge of the adsorbent is negative, while pH below the PZC, the surface charge of the adsorbent is positive (Matos, Laine and Herrmann 1998; Lim *et al.* 2011; Safari *et al.* 2014).

The effect of pH was studied to evaluate the adsorption of the antibiotics. The following pH values were used: 3, 6, 7, 8 and 10. The pH values were selected based

on the commonly used pH range from literature. A study on the fluoroquinolones was carried by Fu *et al.* (2017) where they observed that evaluating the effect of pH above 10 led to a decrease in the adsorption of the fluoroquinolones. Ahmed *et al.* (2017) studied the use of biochar for the removal of sulphonamides from water observed that increasing the pH of the solution above 10 during the kinetic studies led to a reduction in the adsorption capacity of the biochar. Therefore based on some of these findings from literature, the experiments were not run above pH 10 and below pH 3.

Figures 6-17 and 6-18 show the influence of changing pH on the adsorption capacity of AC and IPCA, respectively, on the studied compounds. Different observations were made with respect to the adsorbates response to changes in pH. Similar behaviour of the adsorbates on the AC and IPCA were observed, therefore a common discussion is presented for both AC and IPCA. It is observed at lower pH, the fluoroquinolones (CIP and ENRO) had a higher adsorption capacity in the region close to neutral (pH of 6) for both the AC and IPCA.

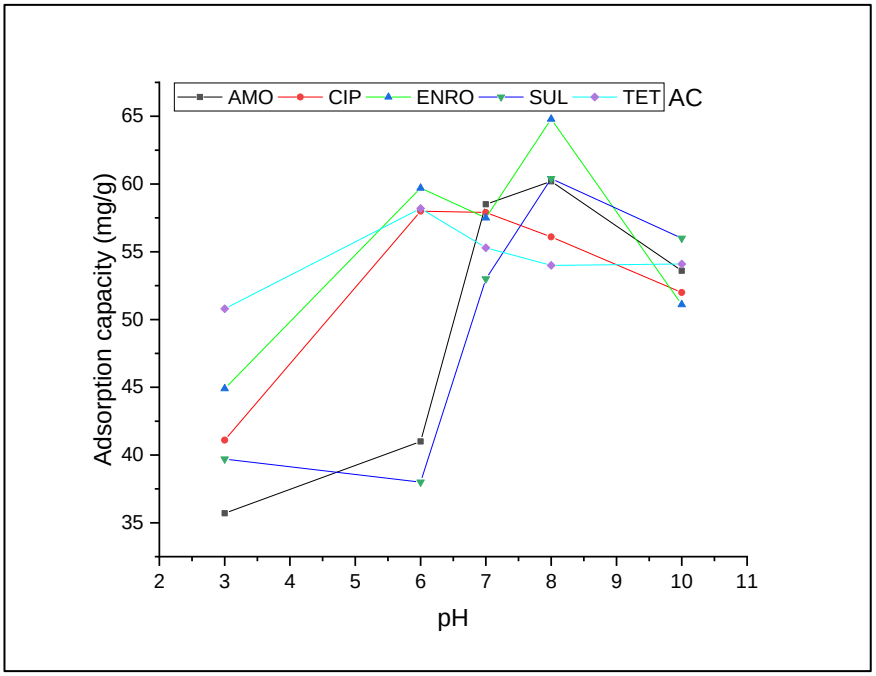



Figure 6-17: Effects of pH on the adsorption of the adsorbates onto the AC (pH 3-10, contact time 300 minutes, adsorbent loading 1 g/L, temperature 25 ℃., shaking speed 150 rpm and initial concentration 100 mg/L.

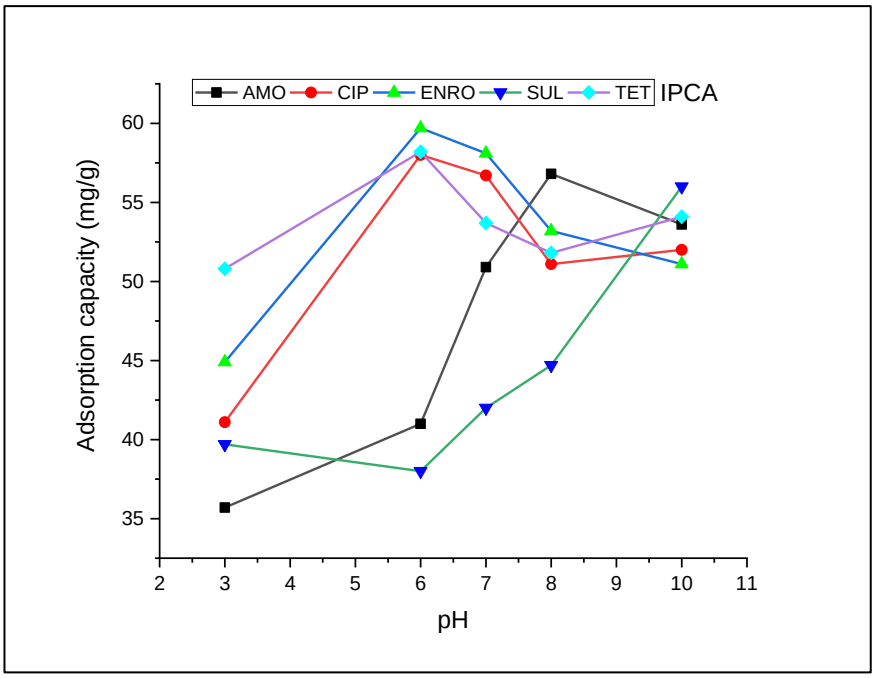



Figure 6-18: Effects of pH on the adsorption of the adsorbates onto the IPCA (pH 3-10, contact time 300 minutes, adsorbent loading 1 g/L, temperature 25℃., shaking speed 150 rpm and initial concentration 100 mg/L.

Increasing the pH from 3 to 10 saw an increase in the adsorption capacity for both adsorbents. pH 3 gave the lowest adsorption capacity of 48.6 and 49.4 mg/g, respectively for both adsorbents, while pH 6 gave the highest adsorption capacity at 62 and 64 mg/g for both adsorbents. At pH > 6, the adsorption capacity reduced. The weak sorption at low and high pH ranges was inferred to be due to the great electrostatic repulsive force between the fluoroquinolones species and the adsorbent surface. For effective adsorption to occur, it is important that adsorbate molecules and the adsorbent surface have dissimilar charges. When this happens, electrostatic attraction forces tend to exist which can benefit the adsorption process. However, occurrence of same sign charges of adsorbent surface and adsorbate molecules leads to electrostatic repulsion, suppressing the adsorption (Yu *et al.* 2016).

These findings are in agreement with the previous studies on the behaviour of the fluoroquinolones (Peng *et al.* 2015; Fu *et al.* 2017). It is observed that under acidic conditions (pH <6), the molecules of the fluoroquinolones exists as cationic ions due

to the protonation of the secondary amine on the piperazine ring. Under alkaline conditions (pH >9), the molecules of the fluoroquinolones have negative charges due to the deprotonation of a carboxyl group. Finally, at neutral conditions, the species of the fluoroquinolones are said to be in zwitterionic form, with a deprotonated carboxyl group ($-COO^-$) and a protonated pyrazine ring (Fu *et al.* 2017).

The TET, like the fluoroquinolones, had its highest adsorption capacity at pH <7. At pH >7, a decrease in the adsorption capacity was observed. However, compared with other compounds, a considerable amount of the TET was adsorbed at pH 3 (50.8 mg/g). At pH < 6 adsorption was highest (58.2 mg/g). TET is an amphoteric molecule with three values of pKa (3.3, 7.7 and 9.7) and its functional groups change at different pH ranges (Safari *et al.* 2014). Therefore as pH increases from 3-10, the dominant species of TET changed from being neutral or zwitterionic and to negatively charged. Electrostatic repulsion between similar charges of TET and AC surfaces was greater at either lower pH (positive–positive repulsion) or higher pH (negative–negative repulsion), thus creating a maximum electrostatic attraction at the intermediate pH range. Worthy of mentioning is that the point of zero charge of the AC used for this study is 5.5 to 6.8. Within the experimental pH of 3-10, the AC was positively charged when pH was below its PZC, and negatively charged when pH is above the PZC. These findings are in agreement with others found in literature (Chen and Huang 2010; Ahmed *et al.* 2015; Jang *et al.* 2018).

Again, like the other compounds, the sorption of sulphonamide (SULFA) onto the adsorbents was affected by the pH. The sorption was greatly governed by the electrostatic interactions between antibiotics and the functionalized AC surface. At an acidic pH of less than 3, it is said that SULFA possesses positive species. As a result, less adsorption is expected due to electrostatic repulsion of positively charged AC surface and SULFA, as seen in Figures 6-17 and 6-18. Adsorption in the acidic region was less (47.7 mg/g) by increasing pH from 6 to 8, the highest adsorption was observed at pH 8; 60.4 mg/g. However, on further increasing the pH from 8 to 10, a decrease in the adsorption capacity was observed. The reduction in adsorption capacity with pH could have been due to the negative surface zeta-potential value and the negative

surface electrostatic repulsion. The adsorption of SULFA at different pH values using various adsorbents has been reported. Some studies have even reported no significant change in the adsorption capacity when pH was changed (Nasuhoglu, Yargeau and Berk 2011). Another reported maximum adsorption capacity at various pH values starting from 2.5 to 7 (Yu *et al.* 2016; Ahmed *et al.* 2017).

Like SULFA, the adsorption capacity for AMO was highest in the medium alkaline region of pH = 8 (56.8 mg/g). At acidic pH, both the AMO and AC are positively charged and hence, the adsorption on the surface of AC is limited. Close to neutral pH, the AMO is negatively charged, while AC is positively charged, such that adsorption is favoured. At alkaline pH above 8, both amoxicillin and the AC would be negatively charged and repulsive forces exist between them thus adsorption capacity reduces. This is consistent with other findings reported by Elmolla and Chaudhuri (2010a). Based on the findings from the pH, the optimum pH that was used for the continuation of the study was 6. This is due to the fact that three out of the five studied compounds had an optimum pH of 6.

### 6.6.2 Influence of adsorbent dosage

The adsorption capacity with respect to adsorbent dosage differs for every adsorbent. The optimum adsorbent capacity is important because it influences the amount of adsorbate that is required to be adsorbed. It is therefore necessary to optimise this parameter. In the current study, adsorbent dosage was varied from 0.5 to 2.0 g/L for both AC and IPCA. The rotation speed, solution pH and contaminant concentration were kept constant. The final equilibrium adsorption capacities for each contaminant were determined and plotted against the adsorbent loading. From Figures 6-19 and 6-20, it was observed that there was asteady increase in the sorption capacities of AC and IPCA as the adsorbent concentration was increased from 0.5 to 1.5 g/L. However, at 2.0 g/L, no increase was observed. The continuous increase from 0.5 to 1.5 g/L may have been due to the availability of more active surface sites. Sorption capacities at 1.5 g/L for both the AC and IPCA were 51.1, 57.9, 57.5, 53 and 55.3 g/L for AMO, CIP, ENRO, SULFA and TET, respectively.

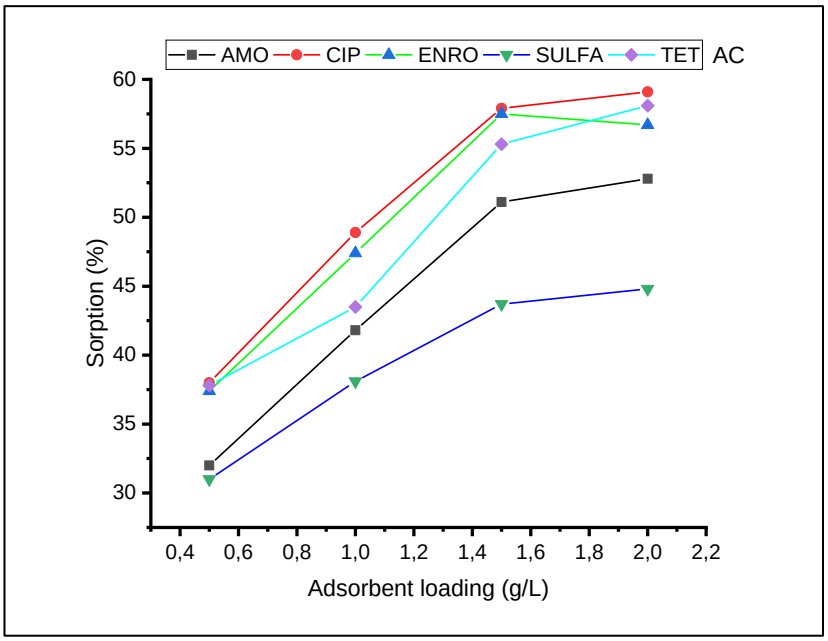



Figure 6-19: Effect of adsorbent mass (AC) on the sorption of the antibiotics, pH 6, contact time 300 minutes, adsorbent loading 0.4 to 2g/L, temperature 25°C., shaking speed 150 rpm and initial concentration is 100 mg/L.

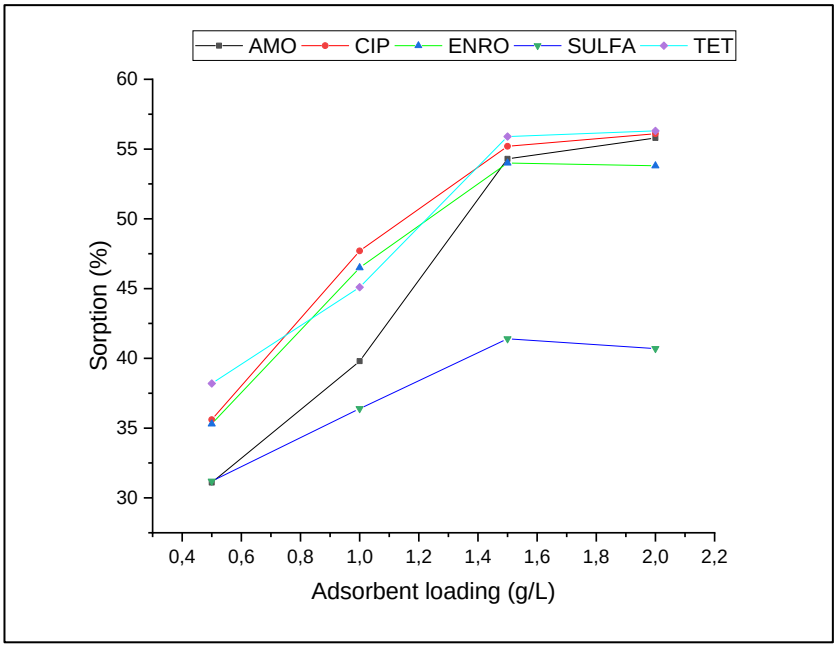



Figure 6-20: Effect of adsorbent mass (IPCA) on the sorption of the antibiotics, , pH 6, contact time 300 minutes, adsorbent loading 0.4 to 2g/L, temperature 25°C., shaking speed 150 rpm and initial concentration 100 mg/L.

At an adsorbent concentration of 2.0 g/L, the sorption capacities were: 52.8, 59.1, 56.7, 44.8 and 58.1 mg/g for the studied compounds. For IPCA, the adsorption capacities were: 55.8, 56.1, 53.8, 40.7 and 56.3 mg/g for AMO, CIP, ENRO, SULFA and TET respectively. The results indicated that at a higher loading of 2.0 g/L, the sorption capacity of the adsorbents were low. Even though increasing the adsorbent loading leads to an increase in the adsorption capacity, it was noted that the concentration of the adsorbates was not changing as the adsorbent dosage was changing. Zhang *et al.* (2016) suggested that a fixed dose of the adsorbent can only adsorb a certain amount of adsorbate, such that increasing the adsorbent dose would result in increasing the quantity of adsorbate that can be sorbed out of solution. Akhtar, Amin and Shahzad (2015) reviewed the adsorption of pharmaceuticals using AC and assessed the effect of adsorbent dosage; their findings corroborate the findings of the present study.

### 6.6.3 Influence of initial contaminant concentration

The influence of initial antibiotics concentration (10 to 100 mg/L) on adsorption was investigated and the results are shown in Figures 6-21 and 6-22.

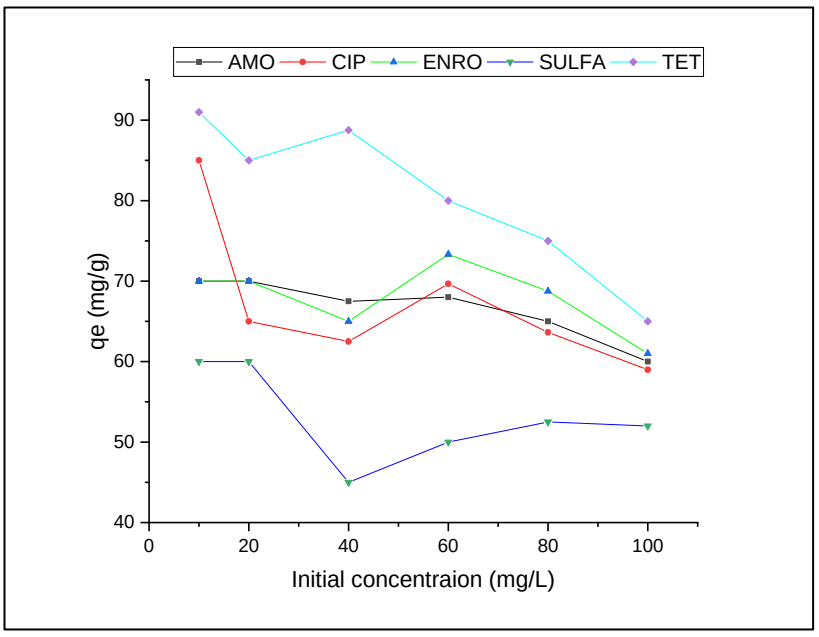



Figure 6-21: Effect of initial adsorbate concentration on the sorption using AC, at concentrations ranging from 10 to 100 mg/L at the pH of 6 and adsorbent loading of 1 g/L, temperature 25°C, shaking speed 150 rpm and varying concentration.

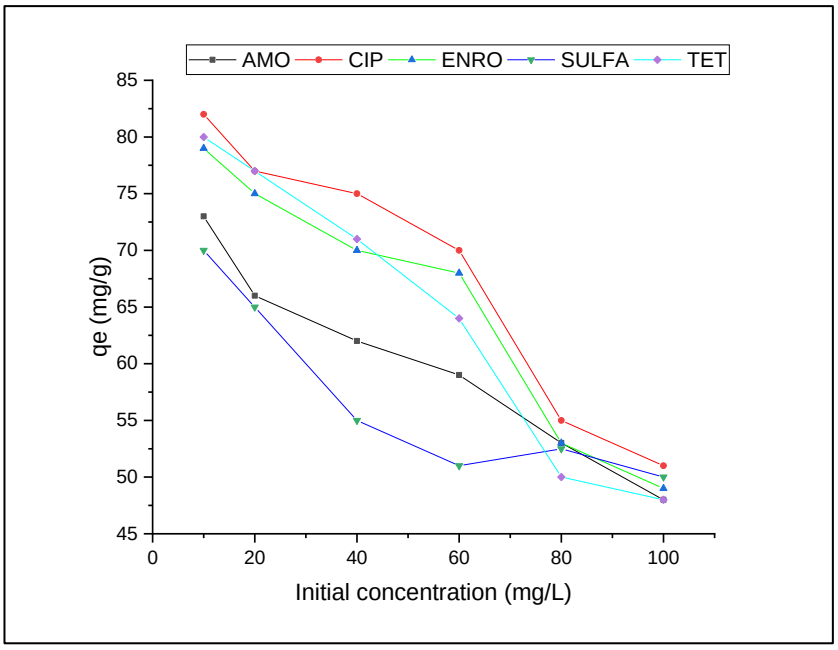



Figure 6-22: Effect of initial adsorbate concentration on the sorption using IPCA, at concentrations ranging from 10 to 100 mg/L at the pH of 6 and adsorbent loading of 1 g/L, temperature 25℃, shaking speed 150 rpm and varying concentration.

Figures 6-21 and 6-22 demonstrate that as the initial adsorbate concentration increases, the adsorption efficiency reduces gradually. This trend was prominent in the case of IPCA than AC. For the IPCA adsorbents, at the initial concentration of 10 mg/L, the sorption capacity was 73, 82, 79, 70 and 80 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively. However, at adsorbate concentration of 100 mg/L, the sorption efficiency dropped to 48, 51, 49, 50 and 48 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively. Similarly for AC, the sorption capacity was: 75, 85, 70, 60 and 91 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively at an initial concentration of 10 mg/L. However, at adsorbate concentration of 100 mg/L, the sorption capacity dropped to 60, 59, 61, 52 and 65 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively. The reason for the decrease in sorption capacity with increase in initial adsorbate concentration is the fact that all adsorbents have a limited number of active sites and at a certain concentration the active sites become saturated.

However, at equilibrium, adsorption capacity is said to increase with an increase in initial concentration. This is due to an increasing concentration gradient, which acts as

a driving force to overcome the resistances to mass transfer of antibiotics between the aqueous phase and the activated carbon (Pouretedal and Sadegh 2014). The observation from Figures 6-21 and 6-22 indicated that the AC presented more efficient adsorption capacities from 65 to 91 mg/g while IPCA was from 48 to 80 mg/g.

## 6.7 Isotherm modelling of adsorption onto IPCA and AC

Adsorption isotherms are important for the optimisation of adsorption systems. They are an indication of how the adsorption molecules distribute between the liquid and the solid phase until equilibrium. An important step to finding the right isotherm to use is to fit the data into the different isotherm models for evaluation and thereafter compare their correlation coefficients ($R^2$) values (Akhtar, Amin and Shahzad 2015). In lieu of this, Freundlich, and Langmuir isotherms were used to analyse the data. The data obtained from the effect of initial concentration on the efficiency of adsorption of the antibiotics were fitted to both isotherms and are presented in Tables 6-5 and 6-6. This section presents the results and the discussion on the adsorption kinetics of the two adsorbents (AC and IPCA).

### 6.7.1 Isotherm modelling of adsorption onto AC

The experimental data fitted for different concentrations of the antibiotics adsorbed on AC showed that the Langmuir model provided a better fit. However, this observation was not applicable for all the antibiotics. AMO had a correlation coefficient of 0.999, CIP 0.835, ENRO 0.988, SULFA 0.979 and TET 0.960. The correlation coefficients were all close to unity for each of the studied compounds except for CIP which was a little lower. However, the correlation coefficients for CIP from the Freundlich model was higher than that of Langmuir. This therefore implied that the Freundlich model explained the sorption mechanism better for CIP. For the antibiotics whose adsorption followed the Langmuir isotherm, it could be suggested that their sorption occurred on a monolayer heterogeneous surface having a maximum limited uptake of the adsorbate molecules corresponding to a saturated adsorbent surface. The essential characteristic of the Langmuir isotherm is expressed in the form of the dimensionless parameter $R_L$

which measures the efficiency of the adsorption process and as such is an indication of the preference of the adsorption process in the corresponding adsorbent/adsorbate system. It is observed from Table 6-5 that the $R_L$ values obtained for all the adsorbents were below unity, thus indicating that adsorption was favourable. The maximum quantities of the adsorbates removed from the solution were 21.93, 22.02, 21.93, 6.12, and 24.79 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively. From these data, AMO and ENRO had the highest values.

Table 6-5: Isotherm parameters for the sorption of the antibiotics from solution using AC

| | Adsorbate | qm (mg/g) | $R_L$ | $K_L$ | $R^2$ |
|---|---|---|---|---|---|
| Langmuir | AMO | 21.93 | 0.9001 | 0.0111 | 0.999 |
| | CIP | 22.02 | 0.3731 | 0.1680 | 0.835 |
| | ENRO | 21.93 | 0.9132 | 0.0095 | 0.988 |
| | SULFA | 6.122 | 0.9390 | 0.0066 | 0.979 |
| | TET | 24.79 | 0.3750 | 0.1667 | 0.960 |
| | Adsorbate | 1/n | $N_F$ | $K_F$ | $R^2$ |
| Freundlich | AMO | 0.852 | 1.173 | 3.394 | 0.989 |
| | CIP | 0.629 | 1.289 | 1.828 | 0.917 |
| | ENRO | 0.912 | 1.097 | 3.831 | 0.957 |
| | SULFA | 0.822 | 1.216 | 5.225 | 0.961 |
| | TET | 0.559 | 1.189 | 1.085 | 0.931 |

The use of the Freundlich isotherms also depicted high correlation coefficients for the adsorption of the studied antibiotics using AC. CIP which had a $R^2$ of 0.835 for the Langmuir isotherm was observed to have a higher $R^2$ of 0.917 for the Freundlich isotherm. This could imply that for this particular antibiotic, the sorption of its molecules occurred on a heterogeneous surface.

Again like in the Langmuir isotherm, $K_F$ is a constant associated with the adsorption capacity for the Freundlich model; and $n$ ($N_F$) is the concentration index associated with the adsorption intensity for the Freundlich model. Generally, adsorption occurs

readily when the value of *n* is between 1 and 10. Therefore from Tables 6-5 and 6-6, it was observed that the $N_F$ values were below 10, thus indicating favourable adsorption.

Dada *et al.* (2012) explained that the Freundlich parameter index $N_F$ is a measure of deviation from linearity of adsorption, which indicates that a linear adsorption process occurred at $N_F = 1$, while it is a chemical process when $N_F < 1$, and a physical process when $N_F > 1$. Finally, $N_F$ values above 10 indicate that the adsorption process was irreversible. With respect to the above description on the Freundlich isotherm, the $N_F$ values for all the antibiotics were above 1, thus suggesting that the sorption of the antibiotics was influenced majorly by physical adsorption.

### 6.7.2 Isotherm modelling of adsorption onto IPCA

Table 6-6: Isotherm parameters for the sorption of the antibiotics from solution using IPCA

| | Adsorbate | $q_{max}$ (mg/g) | $R_L$ | $K_L$ (L/mg) | $R^2$ |
|---|---|---|---|---|---|
| Langmuir | AMO | 20.35 | 0.8929 | 0.0120 | 0.988 |
| | CIP | 23.26 | 0.9166 | 0.0091 | 0.912 |
| | ENRO | 24.16 | 0.6250 | 0.0600 | 0.879 |
| | SULFA | 7.809 | 0.5917 | 0.0690 | 0.758 |
| | TET | 35.09 | 0.8937 | 0.0119 | 0.971 |
| | Adsorbate | 1/n | $N_F$ | $K_F$ (mg/g) | $R^2$ |
| Freundlich | AMO | 1.080 | 1.173 | 3.394 | 0.958 |
| | CIP | 1.040 | 1.551 | 11.08 | 0.835 |
| | ENRO | 0.813 | 2.632 | 6.500 | 0.869 |
| | SULFA | 0.795 | 1.540 | 6.235 | 0.889 |
| | TET | 0.749 | 4.446 | 5.745 | 0.849 |

Table 6-6 shows the model parameters obtained for the Langmuir and Freundlich isotherms for adsorption using IPCA. A similar trend was observed on the behaviour

of the adsorbates on the IPCA with that of AC. Higher correlation coefficients were obtained for the Langmuir isotherm compared to the Freundlich isotherm. The $R^2$ values for the Langmuir isotherm were: 0.988, 0.912, 0.879, 0.758 and 0.971 for AMO, CIP, ENRO, SULFA and TET, respectively. The corresponding $R^2$ values obtained from the Freundlich isotherm were: 0.958, 0.835, 0.869, 0.889 and 0.849 for AMO, CIP, ENRO, SULFA and TET, respectively. Based on the $R^2$ values, it was concluded that the data best fitted the Langmuir isotherm for all the antibiotics, with the exception of SULFA which had a lower $R^2$ of 0.758. The low $R^2$ of SULFA suggested that it did not fit into the Langmuir isotherm as also seen from a comparatively higher $R^2$ value for SULFA on the Freundlich isotherm of 0.889. This alludes to sorption on a heterogeneous surface. The values of the $N_F$ constant for SULFA adsorption were above 1; being 1.26 for AC and 1.54 for IPCA. This indicated a strong favourable physical adsorption process occurring on a heterogeneous surface. The $R_L$ values for the other four compounds AMO, CIP, ENRO and TET were below 1 just like in the case of AC, thereby indicating that adsorption was favourable. The $N_F$ for the Freundlich isotherms again were above 1 for all the antibiotics, again suggesting that the sorption of the antibiotics was influenced majorly by physical adsorption, thus influencing the adsorption.

On comparing the adsorbent capacity, it was observed that both adsorbents efficiently removed the antibiotics from the solution, although IPCA seemed to have a higher adsorption capacity. Adsorption capacities ($q_{max}$) for the IPCA were; 20.35, 23.26, 24.16, 7.81 and 35.09 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively. The $q_{max}$ for the AC were 21.3, 22.02, 21.93, 6.12, and 24.79 for AMO, CIP, ENRO, SULFA and TET, respectively. The predicted $q_{max}$ values were close to those obtained experimentally, however, higher values were obtained for the IPCA. This could be due to the fact that some of the antibiotics were adsorbed/attracted onto the $TiO_2$ as well. Regarding the individual contaminants, SULFA was the least adsorbed for both the AC and IPCA. In general, the adsorption of the antibiotics onto the AC and IPCA surfaces were observed to be more through physisorption than chemisorption, even though both processes occurred based on the calculated $R_L$ values.

Variations in the adsorption capacities as observed in this study, varied with those from other studies. The reason for this could be attributed to the fact that different parameters such as the pH values, initial contaminant concentration and adsorbent dosage could have differed from those in other studies. Therefore, due to the aforementioned factors, various adsorption capacities have been reported. However, the results from this study followed the general trend especially when AC adsorbent is considered, where a monolayer of the antibiotics is sorbed onto the AC, by the fact that activated carbon has a high surface area especially when present in nano-dimensions (Pouretedal and Sadegh 2014). According to Pouretedal and Sadegh (2014), the monolayer adsorption confirms that chemisorption for antibiotics removal could occur through the activated carbon sorbent. Both physisorption and chemisorption play important roles during the adsorption of antibiotics onto AC. The physisorption could have occurred due to the molecular sizes of the antibiotic molecules while the chemisorption could have occurred also due to the presence of functional groups on the AC surface (Putra *et al.* 2009). Furthermore, Ahmed *et al.* (2015) identified the main mechanisms that occur in carbon-based adsorbents such as AC to be electrostatic interaction (cation and anion attractions), hydrophobic effect (hydrophobic interaction), hydrogen bonds, partition into un-carbonized fractions, pore filling, and other processes (surface precipitation, π–π interactions).

Most studies have employed the Langmuir and/or Freundlich isotherms to fit adsorption data. For example, Chen *et al.* (2015) fitted the data on the removal of fluoroquinolones using Nano-Hydroxyapatite as the adsorbents and they observed that the adsorption isotherm of the fluoroquinolones fitted well for both Langmuir and Freundlich equations. The correlation coefficient and the isotherm constants both provided the needed information from which the conclusions were drawn, however, the adsorption capacities were not stated.

Putra *et al.* (2009) reported that both Langmuir and Freundlich models could equally well-fit the results of tests of adsorption of AMX onto a commercial AC with the maximum adsorption capacity of around 222 mg/g at an optimum acidic pH of 4.98.

## 6.8 Summary

The studies conducted demonstrated that $TiO_2$ is ineffective as an adsorbent on its own, however AC and IPCA had a higher efficiency in adsorbing the studied antibiotics from the solution. Both the Langmuir and Freundlich isotherm models gave some insights into the adsorption process on AC and IPCAs.

- The characterisation of the IPCA
- $TiO_2$ had low adsorption of about 20 mg/g.
- The adsorption capacity at 100 mg/L initial concentration for the AC for each of the antibiotics were: 60, 59, 61, 52 and 65 mg/g for AMO, CIP, ENRO, SULFA, and TET respectively. Thus indicating an efficiency of over 60% for the AC. The IPCA were: 48, 51, 49, 50 and 48 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively, with an efficiency of over 50%.
- Both the AC and IPCA followed the Langmuir and Freundlich isotherms, however, higher coefficients of correlation were obtained for the Langmuir isotherms for four of the antibiotics viz. AMO, CIP, ENRO and TET. The Freundlich model was the best fit for the SULFA in terms of the coefficient of correlation.
- This chapter therefore concludes the study on the evaluation of the $TiO_2$, AC and IPCA for the adsorption of the studied antibiotics. The information obtained is important for their use in photodegradation of the antibiotics studied.

# Chapter 7 - Photocatalytic degradation of the antibiotics using IPCA and $TiO_2$

## 7.1 Introduction

Chapter 6 evaluated the use of $TiO_2$, AC and IPCA as adsorbents. The findings indicated that $TiO_2$ attained negligible removal of antibiotics when used as an adsorbent; whereas considerable removal of antibiotics was achieved using AC and IPCA as adsorbents. However, $TiO_2$ is known to be an effective photocatalyst and therefore it was necessary to evaluate the performance of IPCA as a photocatalyst. To allow for comparative performance evaluation, the photocatalytic ability of $TiO_2$ and the modified AC (IPCA) was evaluated in this chapter. The combination of AC and $TiO_2$ was expected to produce a material that is effective as an adsorbent as well as a photocatalyst.

### 7.1.1 Effect of photocatalyst loading

For photocatalysis studies it is necessary to determine the optimum catalyst loading (concentration) that would give the highest degradation of the contaminants in solution. Therefore, the initial photo catalysts concentration was varied from 0.5 g/L to 2.0 g/L. This range was selected based on typical values employed in the literature. Figures 7-1 ($TiO_2$) and 7-2 (IPCA) depict the antibiotic removal efficiencies of the photocatalysts. For the $TiO_2$, at a low catalyst loading of 0.5 g/L, the removal of the contaminants attained was from 47 to 52 mg/g. However, on increasing the catalyst loading, the removal efficiency increased. At a loading of 1 g/L, removal was 50 to 54 mg/g, while at 1.5 g/L, the removal was 58 to 55 mg/L. Finally, at a loading of 2 g/L, the removal was 50 to 61 g/L. For the IPCA, at a catalyst loading of 0.5 g/L, the removal was 38 to 41 mg/g. At a loading of 1 g/L, the removal was 50 to 52 mg/g, while at 1.5 g/L, the removal was 51 to 56 mg/g. Finally, at 2 g/L, the removal rate was 52 to 59 mg/g.

Based on this performance, the catalyst loading of 1 g/L was selected since increasing the catalyst loading from 1.0 to 1.5 g/L increased the contaminants removal by 8%, which was considered minimal. Similarly, on increasing the catalyst concentration from 1.5 to 2.0 g/L, an increase in the contaminant removal of only 5% was observed. At low catalyst concentration, there is low photocatalytic activity since not all the photons are utilized due to limited photocatalyst surface thus leading to low contaminant degradation. . However, at high photocatalyst concentrations such as 1.5 and 2.0 g/L in this study, the removal efficiency is lowered due to the following reasons: i) a decrease in the light penetration due to blockage by the catalyst particles; ii) scattering of the light by the many catalyst particles, thus reducing the amount of photons absorbed; and iii) aggregation of $TiO_2$ particles causing a decrease in the number of active surface sites such that some parts of the catalyst surface become unavailable for photon absorption, and degradation rate decreases (Ghaly *et al.* 2011). These results are in agreement with previous studies (Ghaly *et al.* 2011; He *et al.* 2016; Srikanth *et al.* 2017). The performance of the two photocatalysts was comparable.

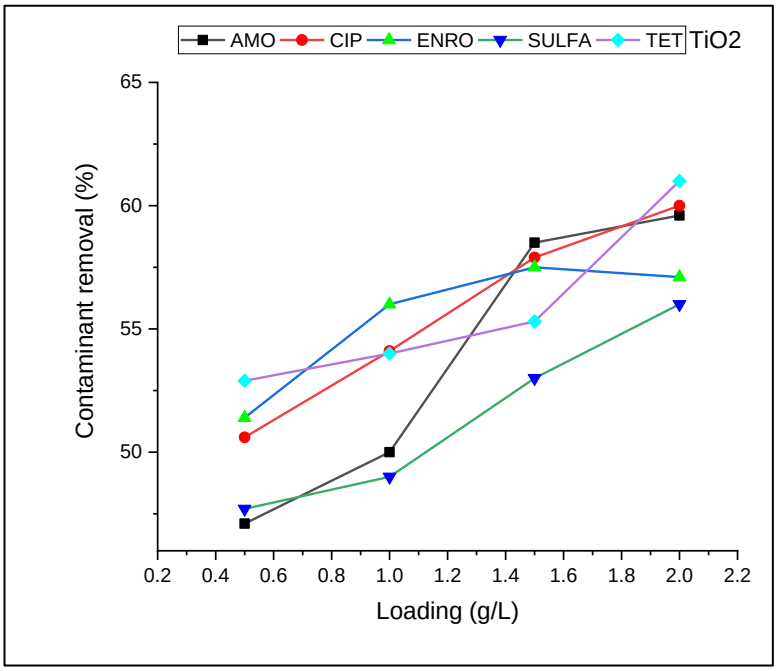



Figure 7-1: Effect of $TiO_2$ loading rate on antibiotics removal, at concentrations of 10 mg/L at the pH of 6 and adsorbent loading of 0.5 to 2 g/L.

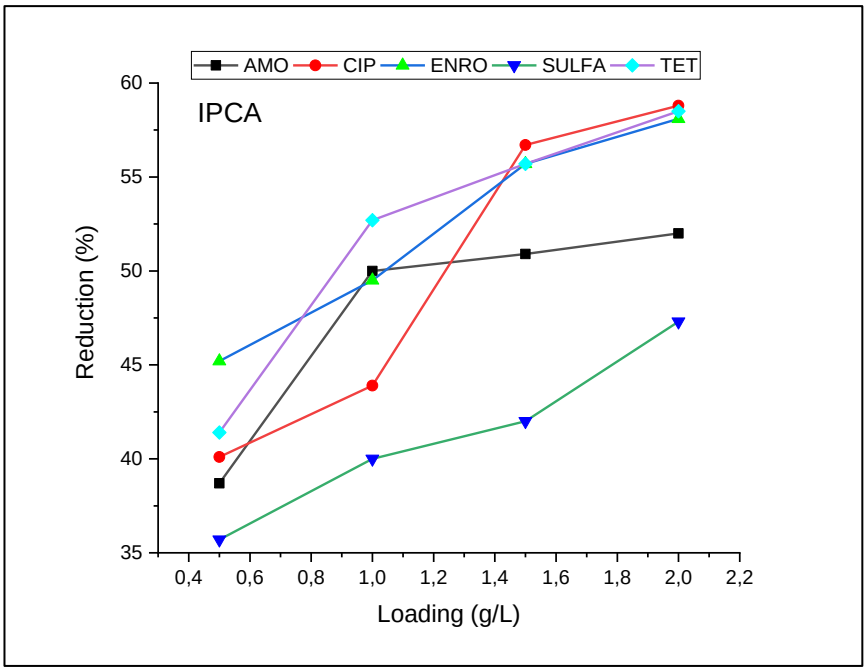



Figure 7-2: Effect of IPCA loading rate on antibiotics removal, at concentrations of 10 mg/L at the pH of 6 and adsorbent loading of 0.5 to 2 g/L.

### 7.1.2 Effect of solution pH on contaminants removal

The study on the effect of solution pH was conducted using an initial concentration of antibiotics of 10 mg/L and catalyst dosage of 1 g/L. Figure 7-3 shows the effect of changing pH on the contaminants removal. The experiments were conducted at pH 3, 6, 8 and 10. The chosen pH represented acidic, mildly acidic, mildly basic and basic conditions. The effect of pH on the degradation can be explained by taking into consideration the properties of both the catalyst and the antibiotics at different pH values just as it was during the adsorption process. The PZC for $TiO_2$ (Degussa 25), similar to the type used in this study is 6.25 (Pereira *et al.* 2011; Safari *et al.* 2014). It was reported by Pereira *et al.* (2011) that $TiO_2$ has amphoteric characteristics. It presents a zero-point charge between pH 5.6 and 6.4, thus resulting in repulsive or attractive effects when the antibiotics and the catalysts show equal or different charges, respectively. Safari *et al.* (2014) reported that it is difficult to interpret the influence of pH during photodegradation due to the multiple roles it plays. They further stated that as the pH of the solution increases, the overall surface charge of the $TiO_2$ changes from positive to negative.

The AMO displays different ionization stages owing to its various ionisable functional groups, carboxyl ($pK_{a1}$ = 2.68), amine ($pKa_2$ = 7.49) and phenol ($pK_{a3}$ = 8.49) (Moosavi and Tavakoli 2016). It is observed that at the acidic region, the degradation of AMO was limited. This is due to the fact that at acidic conditions, both the $TiO_2$ and AMO are positively charged (pH < PZC); and as a result, they both have a repulsive effect such that degradation by the $TiO_2$ is limited. On increasing the pH from the neutral state to the alkaline region, it was observed that the degradation increased from 55% to 66%. The high degradation at the alkaline conditions were highlighted by Moosavi and Tavakoli (2016) to be due to the enhancement of the hydroxyl radical formation at high pH due to the availability of hydroxyl ions on the $TiO_2$ surface that can be oxidized easily to form hydroxyl radials. Another reason is the hydrolysis of AMX due to instability of the β-lactam ring at high pH. These findings are in agreement with those reported by Chollom *et al.* (2018b) and Elmolla and Chaudhuri (2010b).

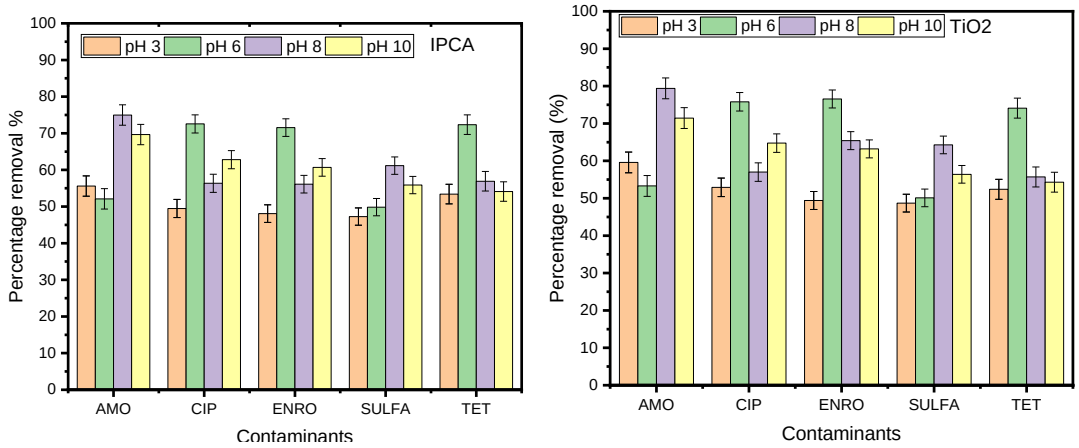



Figure 7-3: Effect of changing pH on antibiotics removal, at concentrations of 10 mg/L and adsorbent loading of 1 g/L.

For TET, reduction at pH values of 3, 6, 8 and 10 were 52, 54, 72 and 56%, respectively. Higher reduction was observed at pH 8 followed closely by pH 10, therefore showing a higher reduction in the alkaline region. This agrees with Safari *et al.* (2014) and Zhu *et al.* (2013). Safari *et al.* (2014) reported that at acidic pH, both the $TiO_2$ surface and TET molecule are positively charged, and so, the adsorption of

TET on the surface of $TiO_2$ decreased at acidic pH. In alkaline pH, both the $TiO_2$ surface and TET molecule are negatively charged, and thus, repulsive forces between the $TiO_2$ and TET are developed. Higher removal of TET in alkaline conditions may be due to the fact that at alkaline pH and OH• radicals were easier to be formed by oxidizing more hydroxide ions available on the $TiO_2$ surface (Safari *et al.* 2014).

The fluoroquinolones showed a higher degradation at lower pH. The removal efficiencies of the CIP were 51, 75, 57 and 64% for pH 3, 6, 8 and 10. The corresponding values for ENRO were 49, 76, 64 and 63% for pH 3, 6, 8 and 10, respectively. Fluoroquinolones have been noted to show faster degradation when their molecules carry an overall positive charge. De Bel *et al.* (2009) reported that positively charged molecules will usually accumulate at the negatively charged interface. The removals for SULFA were 47, 50, 61 and 56% for pH 3, 6, 8 and 10, respectively.

Table 7-1: The half-lives of the antibiotics calculated from the effect of pH on the degradation of the individual antibiotics on IPCA.

| Contaminant | pH | K ($min^{-1}$) | $t_{1/2}$ ($mins^{-1}$) | $R^2$ |
|---|---|---|---|---|
| AMO | 3 | 0.00580 | 119.51 | 0.9224 |
| | 6 | 0.00507 | 136.72 | 0.9418 |
| | 8 | 0.00867 | 79.94 | 0.9622 |
| | 10 | 0.00789 | 87.85 | 0.9432 |
| CIP | 3 | 0.00454 | 152.68 | 0.9655 |
| | 6 | 0.00882 | 78.59 | 0.9803 |
| | 8 | 0.00506 | 136.99 | 0.9648 |
| | 10 | 0.00606 | 114.38 | 0.9649 |
| ENRO | 3 | 0.00423 | 163.86 | 0.9051 |
| | 6 | 0.00879 | 78.86 | 0.9839 |
| | 8 | 0.00583 | 118.89 | 0.9830 |
| | 10 | 0.00681 | 101.78 | 0.9624 |
| SULFA | 3 | 0.00427 | 162.33 | 0.9691 |
| | 6 | 0.00456 | 152.00 | 0.9375 |
| | 8 | 0.00674 | 102.84 | 0.9559 |
| | 10 | 0.00056 | 131.77 | 0.9749 |
| TET | 3 | 0.00509 | 138.18 | 0.9211 |
| | 6 | 0.00829 | 83.61 | 0.9912 |
| | 8 | 0.00600 | 115.52 | 0.9819 |
| | 10 | 0.00542 | 127.89 | 0.9754 |

The pseudo first order model was used to obtain the half-lives and the coefficients of correlation. The half-lives and other parameters such as the rate constant, and the coefficient of correlation were calculated from the rate constants as shown in Tables 7-1 and 7-2. These tables show the half-lives of the antibiotics at various pH values using IPCA and $TiO_2$. For all the studied pH values, high coefficients of correlation ($R^2$) were obtained, however, at optimum pH, the $R^2$ values were comparatively higher. This indicated that the degradation of the antibiotics depended on the initial solution pH. From Table 7-1, the fluoroquinolones were the fastest to be degraded at half-lives of 78.59 and 78.86 minutes at a pH of 6 which was their optimum pH. The $t_{1/2}$ for the other compounds AMO, SULFA and TET were 79.94, 102.84 and 83.61 minutes respectively. Similarly, for the $TiO_2$, the half-lives at the optimum pH were 63.59, 73.58, 72.73, 93.92 and 80.32 minutes for AMO, CIP, ENRO, SULFA and TET respectively. Again, these half-lives were obtained at the optimum pH of each of the compounds. From the calculated half-lives, it was observed that the fluoroquinolones had the shortest half-life, followed by AMO. SULFA had the highest, with a half-life of 102.84 minutes at its optimum pH. The half-lives obtained with the photodegradation were much lower than those obtained during biodegradation for all the compounds. The half-lives observed for $TiO_2$ were smaller than those of the IPCA. This difference was however with a few minute intervals for each of the compounds. The findings therefore elucidate further the advantage of photodegradation over biodegradation.

Table 7-2: The half-lives of the antibiotics calculated from the effect of pH on the degradation of the individual antibiotics on $TiO_2$.

| Contaminant | pH | K ($min^{-1}$) | $t_{1/2}$ ($mins^{-1}$) | $R^2$ |
|---|---|---|---|---|
| AMO | 3 | 0.0063 | 109.68 | 0.936 |
| | 6 | 0.0050 | 138.63 | 0.943 |
| | 8 | 0.0109 | 63.59 | 0.969 |
| | 10 | 0.0080 | 86.32 | 0.979 |
| CIP | 3 | 0.0048 | 145.62 | 0.967 |
| | 6 | 0.0094 | 73.58 | 0.975 |
| | 8 | 0.0052 | 132.53 | 0.970 |
| | 10 | 0.0062 | 111.62 | 0.924 |
| ENRO | 3 | 0.0043 | 161.95 | 0.909 |
| | 6 | 0.0095 | 72.73 | 0.994 |
| | 8 | 0.0071 | 97.48 | 0.961 |
| | 10 | 0.0071 | 97.48 | 0.971 |
| SULFA | 3 | 0.0043 | 161.95 | 0.969 |
| | 6 | 0.0045 | 153.35 | 0.938 |
| | 8 | 0.0074 | 93.92 | 0.956 |
| | 10 | 0.0053 | 130.29 | 0.975 |
| TET | 3 | 0.0050 | 138.91 | 0.910 |
| | 6 | 0.0086 | 80.32 | 0.983 |
| | 8 | 0.0058 | 118.69 | 0.946 |
| | 10 | 0.0053 | 130.78 | 0.967 |

The half-lives obtained in this study are fairly close to those reported in the literature. Conde-Cid *et al.* (2018) proposed that some of the factors responsible for the different half-lives reported by various studies are: differences in the process parameters such as the light intensity, the type of light source, initial concentration of the antibiotics employed and temperature. Similar reports were made by Batista, Pires and Teixeira (2014).

### 7.1.3 Photocatalytic degradation of the antibiotics

The adsorption capacities of the AC, IPCA and $TiO_2$ were determined in chapter 6. This section describes the use of IPCA and $TiO_2$ for the photo degradation of the antibiotics. Experiments were carried out in the absence of light for a period of 30 minutes. This time was not enough achieve adsorption equilibrium (**section 6.5.**1); the minimum equilibrium time for all the compounds was 250 minutes. Therefore, the time was increased to 1 hour for dark adsorption as shown in other studies using IPCA's (Fernández *et al.* 1995; Basha *et al.* 2010; Keane *et al.* 2010; Lim *et al.* 2011). Figure 7-4 depicts the dark adsorption period for both the IPCA and $TiO_2$.

Figure 7-4 shows the removal efficiency of the antibiotics during photocatalysis. For all the studied compounds, a decrease in the initial concentration was observed during photodegradation using both IPCA and $TiO_2$. For the $TiO_2$ alone, the degradation rates were: 98, 98, 99, 97 and 100%. For the IPCA, there was a dark adsorption period and adsorption of the contaminants had started occurring. During the dark adsorption for the IPCA, reduction for the first 30 minutes was 15% and by the 60$^{th}$ minute, it was 31% for AMO. Afterwards on exposure to the UV light, photodegradation occurred and within 180 minutes, over 95% reduction was achieved for all the compounds.

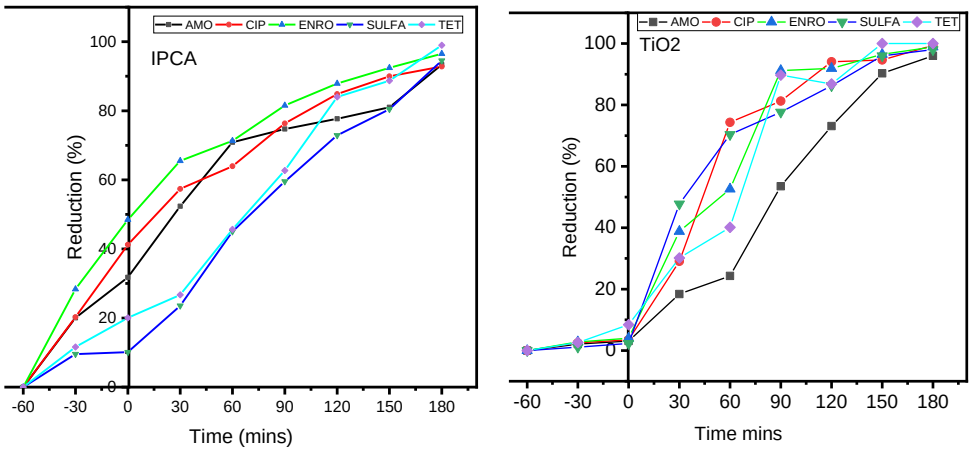



Figure 7-4: Percentage reduction of contaminants during photocatalytic reactions using both the IPCA and $TiO_2$ at a pH of 6 and a catalyst loading of 1 g/L and initial concentration of 100 mg/L.

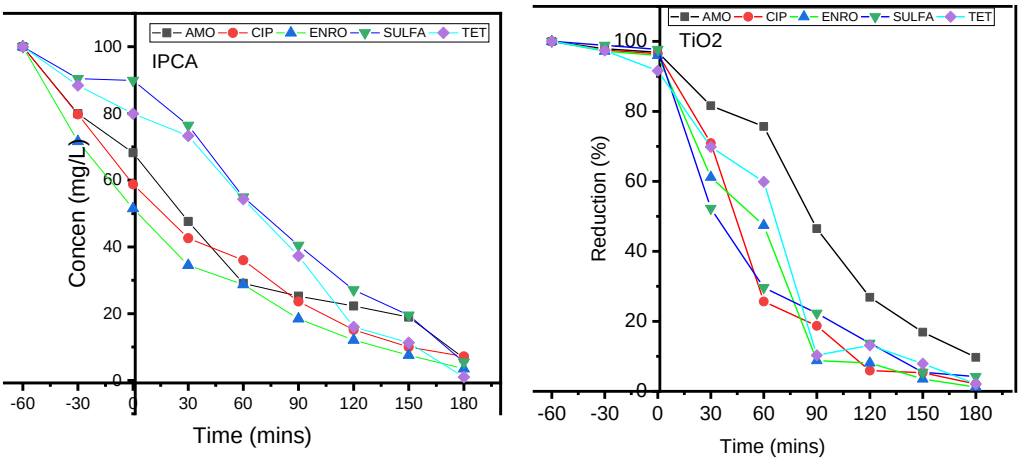



Figure 7-5: Contaminant removal during photocatalytic reactions using both the IPCA and $TiO_2$ at a pH of 6 and a catalyst loading of 1 g/L. and initial concentration of 100 mg/L.

Both Figures 7-4 and 7-5 indicated that almost a complete degradation of the contaminants from the solution was achieved using both the IPCA and $TiO_2$. From Figure 7-3 (IPCA), it was observed that in the 60 minutes of the adsorption in the dark, about 31% of the contaminants had been removed even before the photocatalytic degradation had started. Thus this confirmed that adsorption of the contaminants on the IPCA. After 60 minutes of operation, the IPCA was exposed to UV light, and the photocatalytic reaction continued. After 30 minutes, the reduction had reached 48% for the different contaminants. Subsequent reductions above 95% for all the compounds were observed as the experiment continued until 180 minutes when almost complete degradation had occurred. The behaviour observed therefore, was the case of adsorption as well as photocatalytic degradation occurring. For the case of the $TiO_2$, the dark adsorption was not comparable to that of the IPCA, less than 10% adsorption occurred after 60 minutes for all the antibiotics. However, on exposure to the UV light, a reduction of 96 to complete degradation of 100% was observed by the end of the 180 minutes for the $TiO_2$.

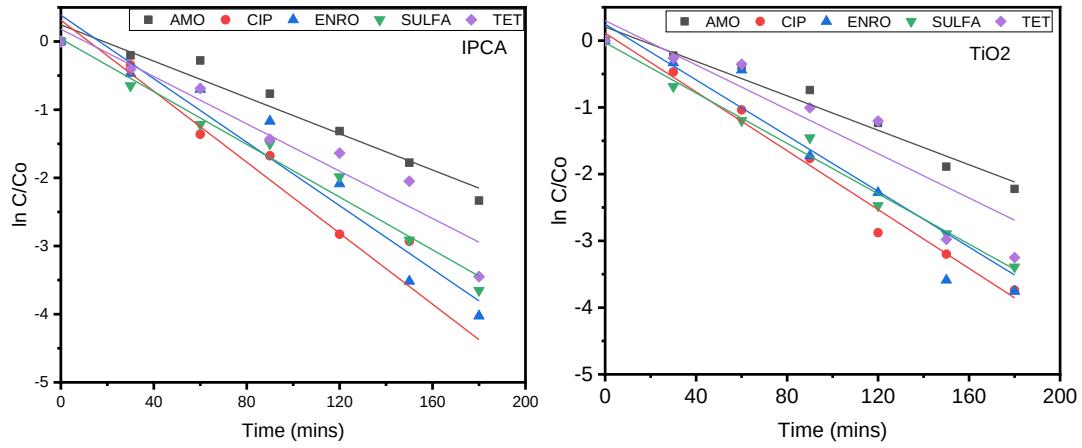



Figure 7-6: Kinetic analysis of the antibiotics at a pH of 6 and a catalyst loading of 1 g/L. The total run time for each experiment was 180 minutes

The rate constants were evaluated for each compound on both $TiO_2$ and IPCA. Figure 7-6 shows the linear plots (the natural logarithm of the final concentration to the initial concentration against time). The linearity of the plots suggests that the photocatalytic reactions followed the pseudo first-order approximately for both the $TiO_2$ and IPCA. This means that an exponential decrease in the initial concentration of the contaminants with time occurred. The rate constants for $TiO_2$ were 0.0129, 0.0220, 0.0208, 0.0189 and 0.0166 $min^{-1}$ for AMO, CIP, ENRO, SULFA and TET, respectively. For IPCA, the rate constants were: 0.0133, 0.0261, 0.0233, 0.0193 and 0.0174 $min^{-1}$ for AMO, CIP, ENRO, SULFA and TET, respectively. The rate constants for both the $TiO_2$ and IPCA were within the same order of magnitude. Similar studies of this nature have been carried out by other authors. For example, Basha *et al.* (2010) observed a 10% improvement on the use of the IPCA than on $TiO_2$ alone.

Similarly, other studies have observed a synergetic effect on the use of $TiO_2$ supported on AC for the removal of various contaminants from different wastewater streams. For example, Kanakaraju *et al.* (2015) integrated $TiO_2$ on Zeolites (IPA) for the degradation of AMO and they found that the overall performance had a synergetic effect between the $TiO_2$ on the Zeolite which thus impacted on the performance of the IPCA. Other researchers have demonstrated similar findings, even though the material used for the support of the $TiO_2$ could be different as well as the contaminants being

considered (Li *et al.* 2007; Li *et al.* 2010; Lim *et al.* 2011; Mukherjee, Barghi and Ray 2013; Chong *et al.* 2015; He *et al.* 2016; Murgolo *et al.* 2017).

The findings from the use of IPCA and $TiO_2$ indicated that both photocatalysts reduced the concentration of the antibiotics from 100 ppm to less than 5 ppm. It is important to note that that the magnitude of this concentration is much higher than the detected amounts in the WWTPs and other water sources found in the environment. Various researchers have used similar and higher concentrations based on the following reasons; for the evaluation of the process efficiency within a given time and also for the accurate determination of the residual antibiotics that will not be degraded (Dimitrakopoulou *et al.* 2012).

The IPCA displayed a lower performance with less than 10%. The reason for this observation was probably due to the fact that the IPCA had a lower $TiO_2$ content (10% by weight) compared to that of the pure $TiO_2$ which may somewhat explain its poorer performance. In addition, the IPCA material could have influenced light scattering during irradiation, thus reducing its penetration into the reaction mixture. Despite this finding, an advantage of the IPCA over $TiO_2$ is its simple recovery from the solution by decantation after irradiation, which is not easily achieved for the finer $TiO_2$ particles. Further still, the IPCA possessed adsorption capabilities of the AC such that the antibiotics are attached on the surface of the AC and the photocatalytic activity of the $TiO_2$ and thereby degrading the antibiotics, this was observed by Gao *et al.* (2011). They proposed that the pollutant to be degraded is concentrated by the AC close to the photocatalyst surface thus allowing for more efficient usage of the electron-hole pairs which are created by illumination of the $TiO_2$. Therefore, together with the ease of catalyst recovery and the contaminants degraded, the IPCA has a distinct advantage over traditionally applied $TiO_2$ in suspension.

### 7.1.4 Studies of COD and TOC

Even though there was a degradation of the antibiotics using photocatalysis, it is usually necessary to evaluate for degradation of the compounds.

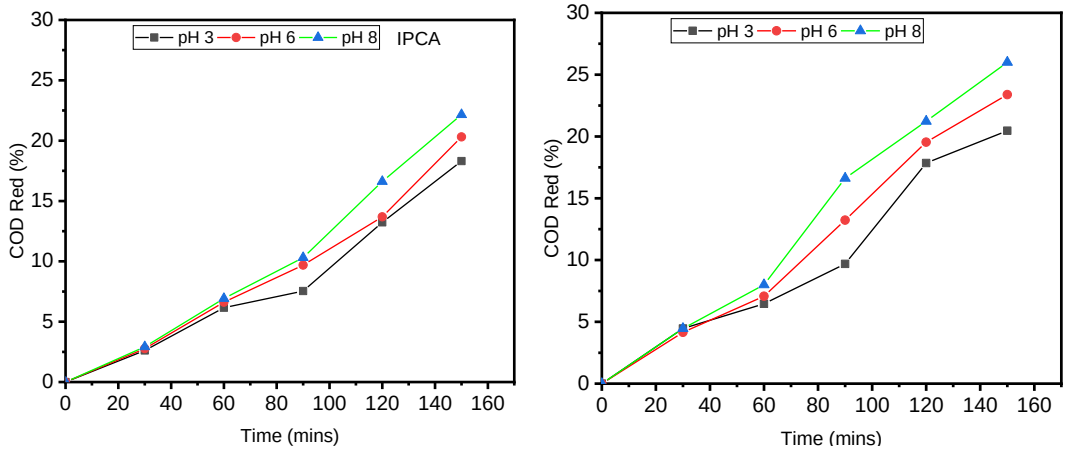



Figure 7-7: (A) $TiO_2$ (B) IPCA: Effect of pH on COD removal.

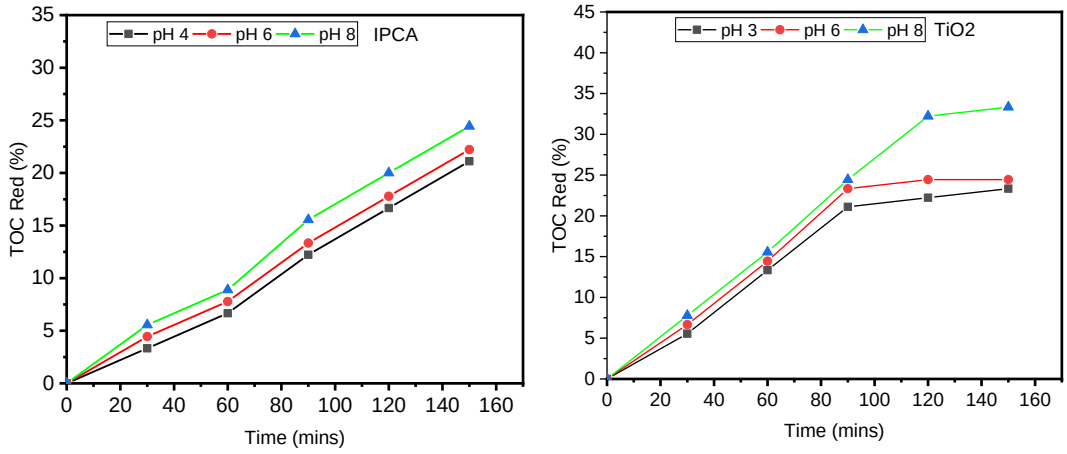



Figure 7-8: $TiO_2$ and IPCA: Effect of pH on TOC removal

For the COD and TOC studies, samples were collected at pH 3, 6 and 8 only; pH 10 was not included. Figure 7-6 illustrates the COD reduction using $TiO_2$ and IPCA, while Figure 7-7 shows the TOC reduction for both catalysts. Despite the higher degradation/removal efficiencies attained for the antibiotics, the mineralisation efficiencies were comparatively lower. Nonetheless, for both the $TiO_2$ and IPCA, the reduction of both the COD and TOC were in the range of 25 to 30%. With regards to the reduction based on pH, more reduction was observed at the basic pH of 8. Similar findings were observed by Kanakaraju *et al.* (2015) and Adamek, Baran and Sobczak (2016). It was observed that the TOC reduction attained in the present study is lower compared to that reported by Abellán, Giménez and Esplugas (2009). They had

reduction in TOC to about 51%, however, a longer reaction time of 15 hours was used for their study whereas in this study the reaction time was 3 hours. Other operating parameters differed as well apart from the reaction time.

The slow degradation of COD and TOC according to Kanakaraju *et al.* (2015) are as a result of intermediates formed during the photocatalytic degradation. These intermediates slow the degree of mineralisation as they compete for adsorption sites and subsequent photocatalytic degradation radicals. In fact, some of the intermediates could be more resistant to further oxidation. Adamek, Baran and Sobczak (2016) identified the intermediate compounds resulting from the photocatalytic degradation of veterinary antibiotics to be: carboxylic acids (1,4-benzenedicarboxylic acid, 4-propanedioic acid, and hydroxymalonic acid) which are resistant to further oxidation.

Since photodegradation leads to the transformation of the original compounds into smaller intermediate compounds, it is necessary to evaluate the ease of degradability of the resulting compounds. One of the ways used is to measure the biodegradability of the compounds before photocatalytic degradation and compare it to the biodegradability of the compounds after the photocatalytic degradation (Abellán, Giménez and Esplugas 2009). A reduction in the TOC after the photocatalytic treatment indicates a breaking down of the complex recalcitrant molecules. This was observed in the present study and could allude to an increase in the biodegradability of the compounds. Similar findings were observed by Elmolla and Chaudhuri (2010b) and Abellán, Giménez and Esplugas (2009).

## 7.2 Summary

The integrated photocatalytic adsorbent (IPCA) comprising of titanium dioxide ($TiO_2$) and activated carbon (AC) was prepared using an ultrasonic impregnation technique for the degradation of 5 types of antibiotics from solution. Photodegradation kinetics of the target contaminants followed the pseudo first-order rate law. The dark adsorption for $TiO_2$ was less than 10% within 60 minutes while its removal efficiency was almost 100% during photocatalysis.

Photodegradation using $TiO_2$ attained the following removal efficiencies: 98, 98, 99, 97 and 100% for AMO, CIP, ENRO, SULFA and TET respectively. Similarly, for the $TiO_2$, the half-lives at the optimum pH were: 63.59, 73.58, 72.73, 93.92 and 80.32 minutes for AMO, CIP, ENRO, SULFA and TET, respectively. For the IPCA, both adsorption and photodegradation occurred in significant measure. For the 60 minutes of dark adsorption using the IPCA, about 31% of the contaminants were removed. The removal efficiency increased to 95% when the solution was exposed to light (photocatalysis).

The half-lives for IPCA for AMO, CIP, ENRO, SULFA and TET were 79.94, 78.59 and 78.86, 79.94, 102.84 and 83.61 minutes respectively. For the biodegradation study in Chapter 5, section 5.3, it was observed that the kinetic data during biodegradation of the antibiotics followed a first order kinetic model with half-lives that ranged from 6 to 77 days. Looking at the half-lives of the photodegradation and biodegradation, a wide marginal magnitude was observed. The half-lives during photodegradation for both catalysts were in the range 60 to 100 minutes. This therefore reiterated the effectiveness of the photodegradation over the conventional biological systems for the treatment of bio-recalcitrant compounds.

The degradation of the antibiotics using both the $TiO_2$ and IPCA were found to be pH dependent. The optimum pH for CIP, ENRO and TET were 6 while SULFA and TET were 8. Thus, shorter half-lives were obtained for all the compounds at these pH. Both catalysts performed well in terms of degrading the contaminants. Despite the comparable performance of the two photocatalysts; the IPCA has the advantage of being easily recovered from the reaction mixture by a simple decantation process unlike the finer $TiO_2$ particles. Furthermore, IPCA demonstrated good adsorptive and photocatalytic properties while $TiO_2$ was only effective as a photocatalyst.

# Chapter 8 - Conclusions and Recommendation

## 8.1 Main findings and conclusions

This study entailed the investigation of the following specific objectives as identified in Chapter 1:

1. To develop and optimise an extraction method based on SPE UHPLC-PDA-MS method for veterinary antibiotics (VAs) from the slaughterhouse wastewater treatment plant.
2. To apply the optimised SPE and UHPLC-PDA-MS methods for the detection and quantification of veterinary antibiotics from the slaughterhouse wastewater.
3. To determine possible mechanisms of removal of antibiotics from wastewaters using anaerobic digestion and study the biodegradation kinetics.
4. To prepare and characterize the integrated photocatalyst (IPCA) and evaluate its adsorption performance against the target antibiotics.
5. To evaluate the effectiveness of the IPCA as a photocatalyst for the degradation of the target veterinary antibiotics.

The extent to which these objectives were met and the main findings are outlined below:

From the first and second objectives, a sensitive, simple and reliable SPE UHPLC-PDA-MS method was successfully developed for the determination of five veterinary antibiotics: 2 fluoroquinolones, 1 sulphonamide, 1 β-lactam and 1 tetracycline. Coefficients of correlation for each analyte were >0.995 confirming the linearity of the method. From the SPE optimisation, a pH of 2 and elution with 0.1% FA in methanol was used. The method development limit and method quantification limit were: MDL = 0.12 to 2.5 µg/L and MQL = 2.3 to 9.3 µg/L. The linearity range values were within 0.1 to 10 mg/L while the limit of detection (LOD) and limit of quantification LOQ were 0.1 to 0.3 µg/L and 1.3 to 2.9 µg/L, respectively. The recoveries of the target

compounds were 40 to 90% for most of the studied analytes except for AMO which had a lower recovery of 44.5%. The validated method was applied to slaughterhouse wastewater samples from South Africa. The range of antibiotics detected in the wastewaters in effluents was 0.008 to 4.9 ng/L while in the influent, the range was 1 to 21 ng/L. To the best of the author's knowledge, this is the first study to develop a method to detect and quantify veterinary antibiotics in South African slaughterhouse wastewaters.

With regards to the third objective, a laboratory scale anaerobic reactor was employed to treat synthetic wastewater to explore the removal efficiencies of five veterinary antibiotics with an initial concentration of 50 µg/L. In a like manner, batch reactors were further used to evaluate the removal routes of the antibiotics. The UASB reactor was operated continuously under mesophilic conditions to evaluate its performance with regards to the removal of organics and at the same time monitor biogas production. Organic loading rate (OLR) was varied from 8 to 9.2 kg.COD.$m^{-3}$.$d^{-1}$while keeping the hydraulic retention time (HRT) constant at 12 h. A COD removal efficiency higher than 75% was achieved at an OLR of 9 kg.COD.$m^{-3}$.$d^{-1}$, with a HRT of 12 hours. About 80% of the antibiotics were removed during the continuous processes, however, a distinctive pattern of removal was not observed. The kinetic studies using a batch process showed that the removal route for the antibiotics was majorly adsorption to the sludge. Biodegradation occurred alongside adsorption but to a lesser degree. The kinetic data showed that the antibiotics followed a first order kinetic model with half-lives that ranged from 6 to 77 days.

The fourth objective involved the preparation of the IPCA as a catalyst and evaluating its adsorption properties. The characterisation of the IPCA showed that the IPCA contained $TiO_2$ particles which were distributed on its surface. This was confirmed by the SEM as well as the EDX. The XRD revealed that the AC alone was 77% graphitic while the $TiO_2$ was a mixture of anatase and rutile in a ratio of 77.1 and 22.3% and the rest brookite. Finally, the XRD further revealed IPCA was a mixture of 77% graphite, 19% anatase and 3.1% rutile, the rest been brookite. The adsorption studies demonstrated that $TiO_2$ was ineffective as an adsorbent on its own, however, AC and

IPCA had a higher efficiency in adsorbing the studied antibiotics from the solution. The adsorption capacities at 100 mg/L initial concentration for the AC for each of the antibiotics were: 60, 59, 61, 52 and 65 mg/g for AMO, CIP, ENRO, SULFA, and TET respectively. Thus indicating an efficiency of over 60% for the AC. The corresponding removal efficiencies using IPCA were: 48, 51, 49, 50 and 48 mg/g for AMO, CIP, ENRO, SULFA and TET, respectively, with an efficiency of over 50%. Adsorption using AC and IPCA followed the Langmuir and Freundlich isotherms, however, higher coefficients of correlation were obtained for the Langmuir isotherms for four of the antibiotics viz. AMO, CIP, ENRO and TET. The Freundlich model was the best fit for the SULFA in terms of the coefficient of correlation.

The final objective entailed evaluating the effectiveness of the IPCA and $TiO_2$ as photocatalysts for the degradation of the target veterinary antibiotics. Photodegradation kinetics of the target contaminants followed the pseudo first-order rate law. The dark adsorption for $TiO_2$ was less than 10% within 60 minutes while its removal efficiency was almost 100% during photocatalysis. Photodegradation using $TiO_2$ attained the following removal efficiencies: 98, 98, 99, 97 and 100% for AMO, CIP, ENRO, SULFA and TET respectively. Similarly, for the $TiO_2$, the half-lives at the optimum pH were: 63.59, 73.58, 72.73, 93.92 and 80.32 minutes for AMO, CIP, ENRO, SULFA and TET, respectively. For the IPCA, both adsorption and photodegradation occurred in significant measure. For the 60 minutes of dark adsorption using the IPCA, about 31% of the contaminants were removed. The removal efficiency increased to 95% when the solution was exposed to light (photocatalysis). The half-lives for IPCA for AMO, CIP, ENRO, SULFA and TET were 79.94, 78.59 and 78.86, 79.94, 102.84 and 83.61 minutes respectively. It was observed that the kinetic data during biodegradation of the antibiotics followed a first order kinetic model with half-lives that ranged from 6 to 77 days. Looking at the half-lives of the photodegradation and biodegradation, a wide marginal magnitude was observed. The half-lives during photodegradation for both catalysts were in the range 60 to 100 minutes. This therefore reiterated the effectiveness of the photodegradation over the conventional biological systems for the treatment of bio-recalcitrant compounds. The degradation of the antibiotics using both the $TiO_2$ and IPCA were

found to be pH dependent. The optimum pH for CIP, ENRO and TET was 6 while for SULFA and TET it was 8. Thus, shorter half-lives were obtained for all the compounds at these pH values. Both catalysts performed well in terms of degrading the contaminants. Despite the comparable performance of the two photocatalysts; the IPCA has the advantage of being easily recovered from the reaction mixture by a simple decantation process unlike the finer $TiO_2$ particles. Furthermore, IPCA demonstrated good adsorptive and photocatalytic properties while $TiO_2$ was only effective as a photocatalyst.

## 8.2 Limitations of the project and recommendations

Despite the milestones that this study achieved, there is still room for further studies. For instance, this study focused on five priority antibiotics in South Africa. It is necessary to explore the removal of other antibiotics and emerging contaminants. The study focused on wastewater from slaughterhouses dealing with pigs; there is need to explore wastewaters from poultry, cattle, and other animals for comparison purposes. Furthermore, detection of contaminants from animal farming houses and runoffs from such should also be investigated since these areas have been identified to be hotspots for such contaminants.

Evaluation of the biotoxicity of the antibiotics under investigation in this study and the degradation products are beyond the scope of this project. The information from such studies is, however, important due to the fact that there is a possibility of compounds from the oxidation to be more toxic than the parent compounds. In addition, legislative investigations and risk assessments of these antibiotics to the environment are possible avenues for further investigation but were beyond the scope of this study.

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# Appendix A: Development of SPE-HPLC/PDA-MS for analysis of veterinary antibiotics

**Spectrums for the analytes**

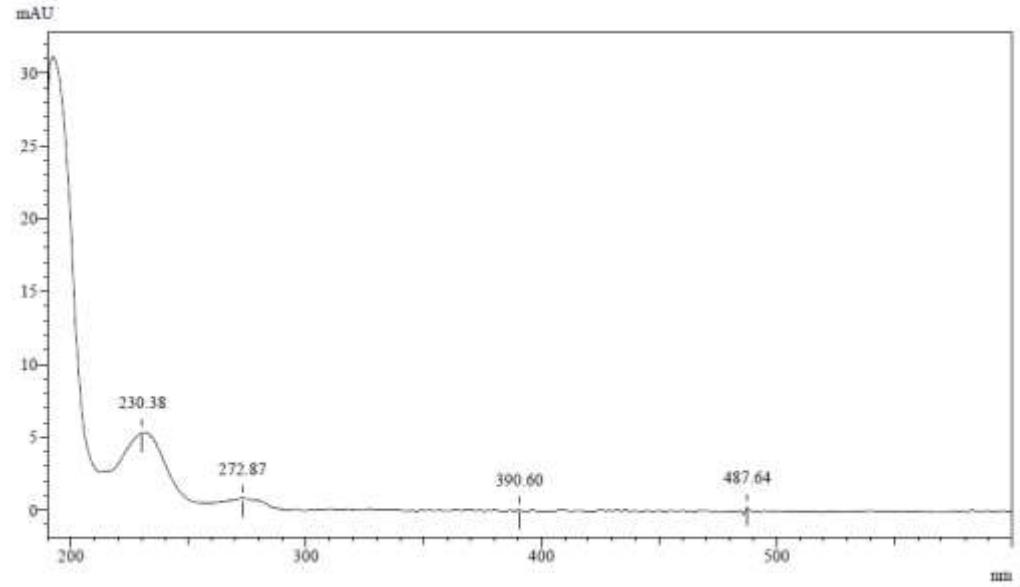



Figure A1: Spectrum of Amoxicillin, lambda max is 230 nm.

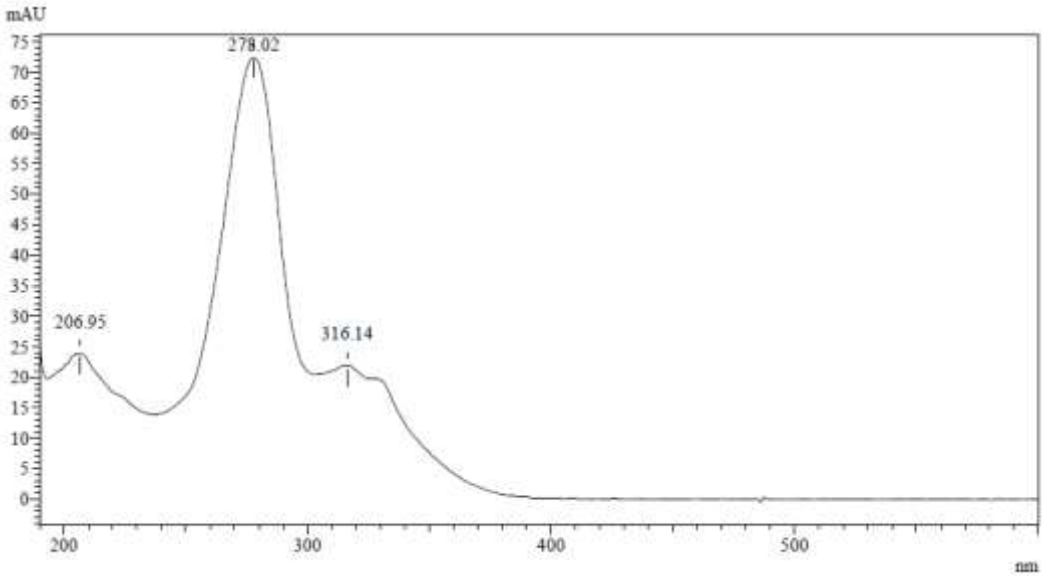



Figure A2: Spectrum of Ciprofloxacin, lambda max is 278 nm

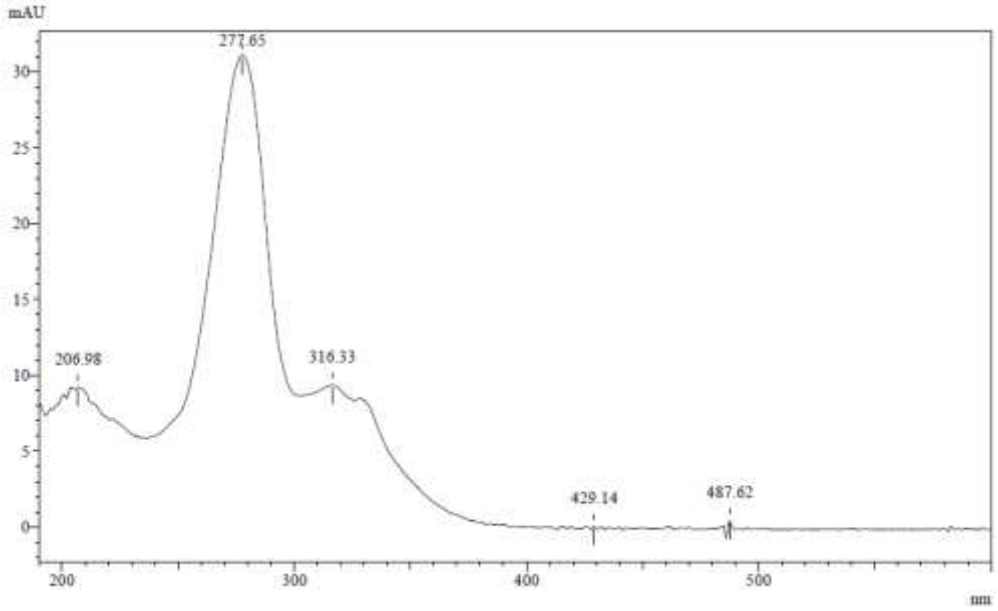



Figure A3: Spectrum of Enrofloxacin, lambda max is 278 nm

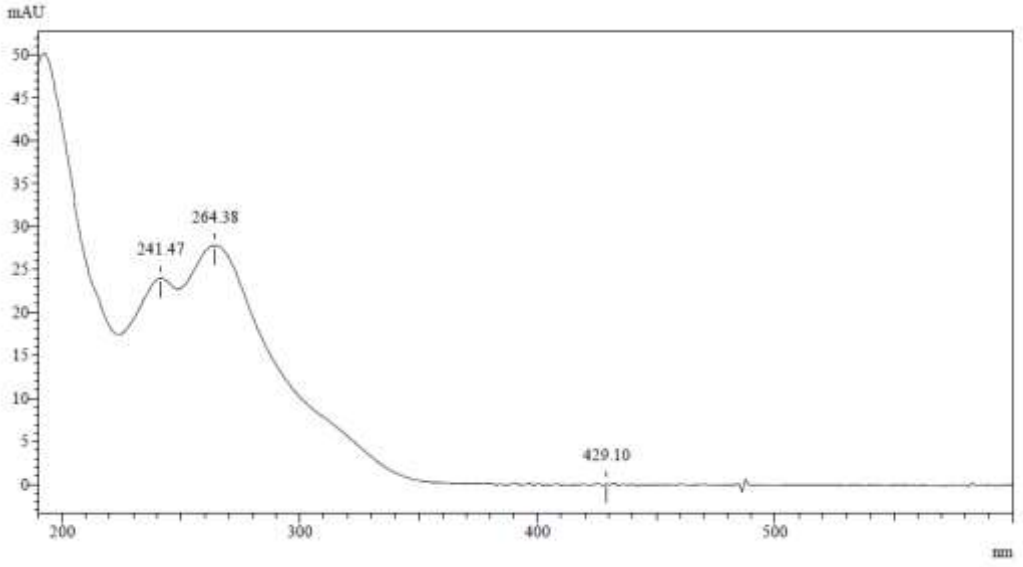



Figure A4: Spectrum of Sulfamethazine, lambda max is 264 nm

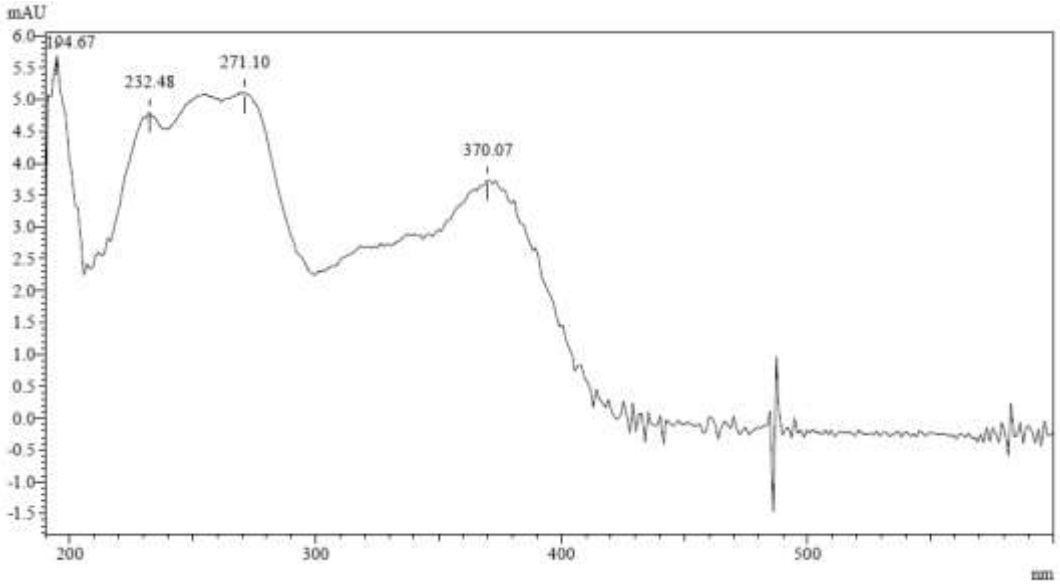



Figure A5: Spectrum of Chlortetracycline, lambda max is 370 nm

# Appendix B: Removal of the antibiotics during anaerobic digestion

## Kinetic models for biodegradation

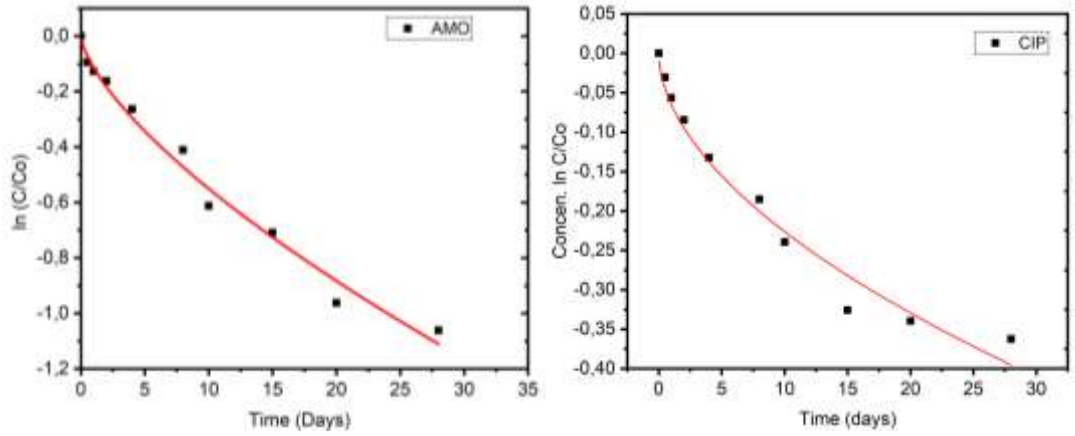



Figure B1: First order biodegradation for AMO and CIP

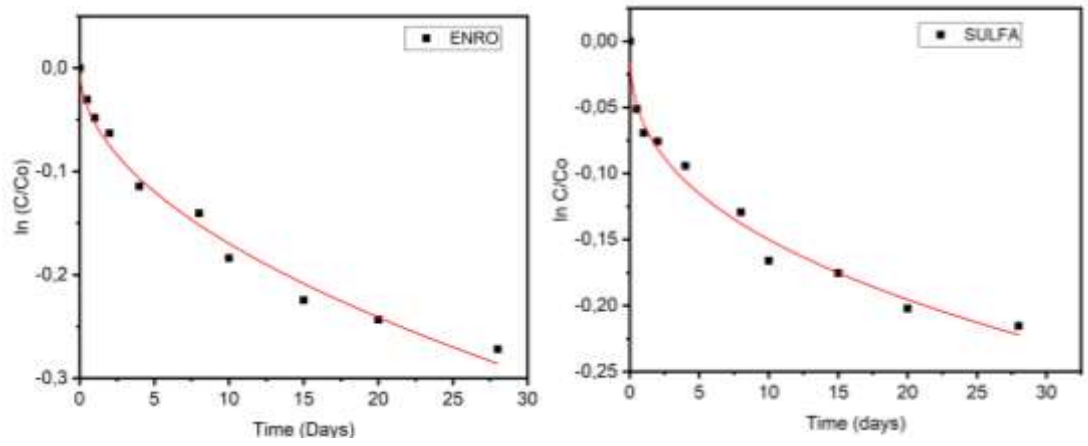



Figure B2: First order biodegradation for ENRO and SULFA

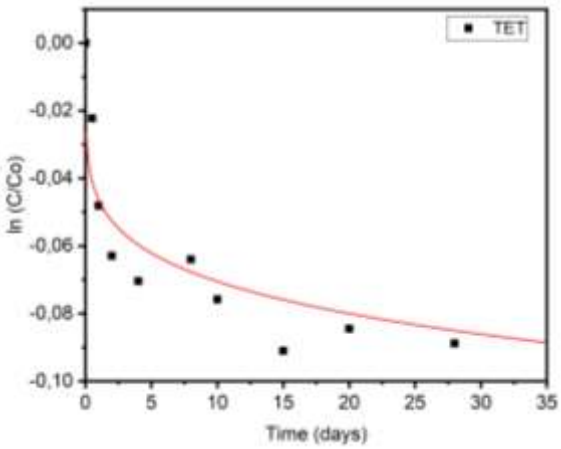



Figure B3: First order biodegradation for TET

# Appendix C: Advanced oxidation and adsorption as a post treatment of wastewaters containing antibiotics

**The three kinetic models used: First and second orders and the intra-particle diffusion models**.

## C1: First order kinetic model

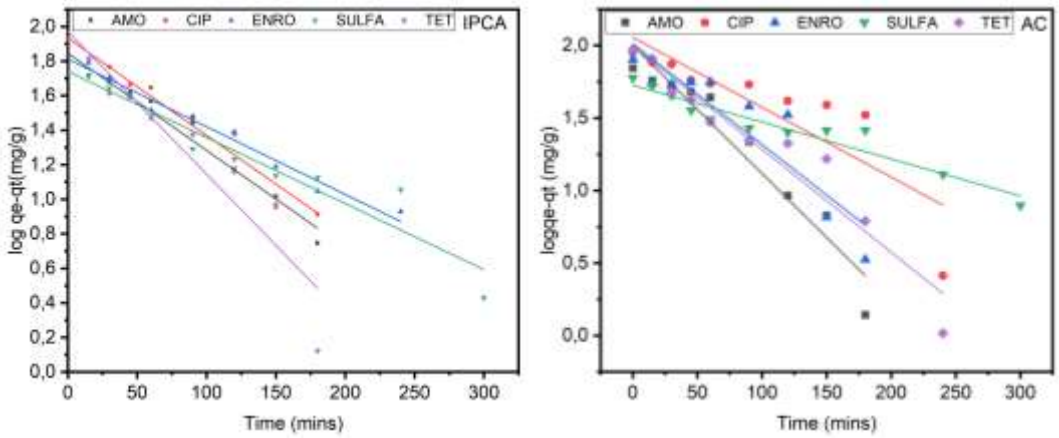



Figure C1: First order for five compounds on using IPCA

## C2: Second order kinetic model

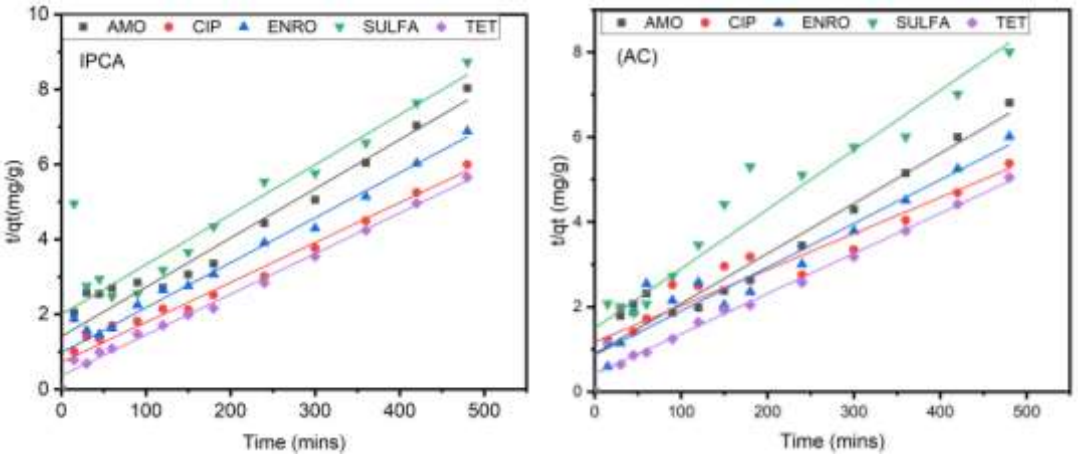
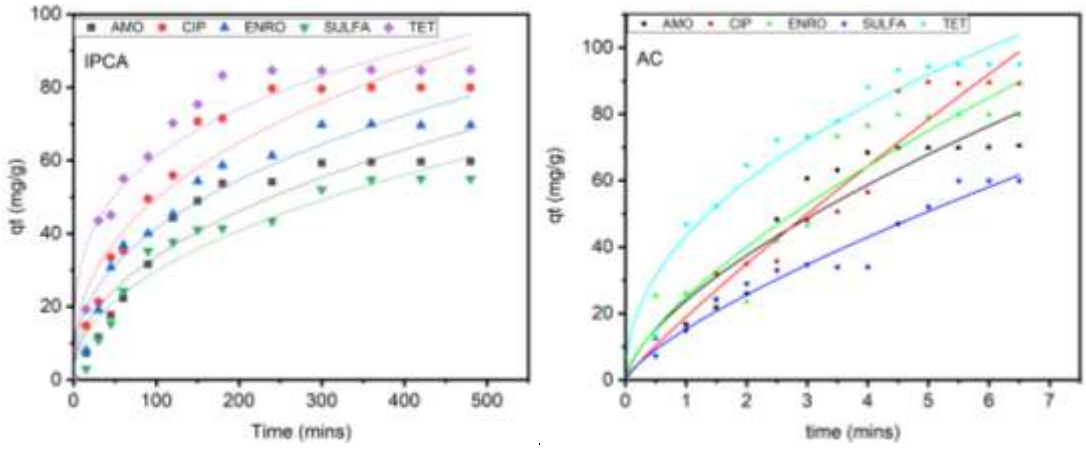



Figure C2: Second order for five compounds on using IPCA and AC

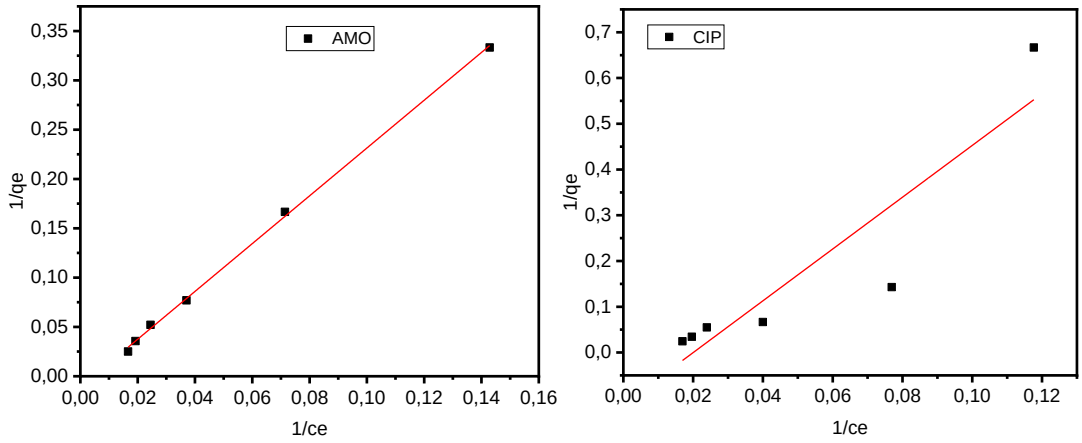
## C3: Intra-particle model




C3: Intra-particle diffusion model on the five compounds on using AC IPCA

**Isotherm Models: Adsorption Langmuir and Freundlich**

**C4: Langmuir Model using AC for the five antibiotics**



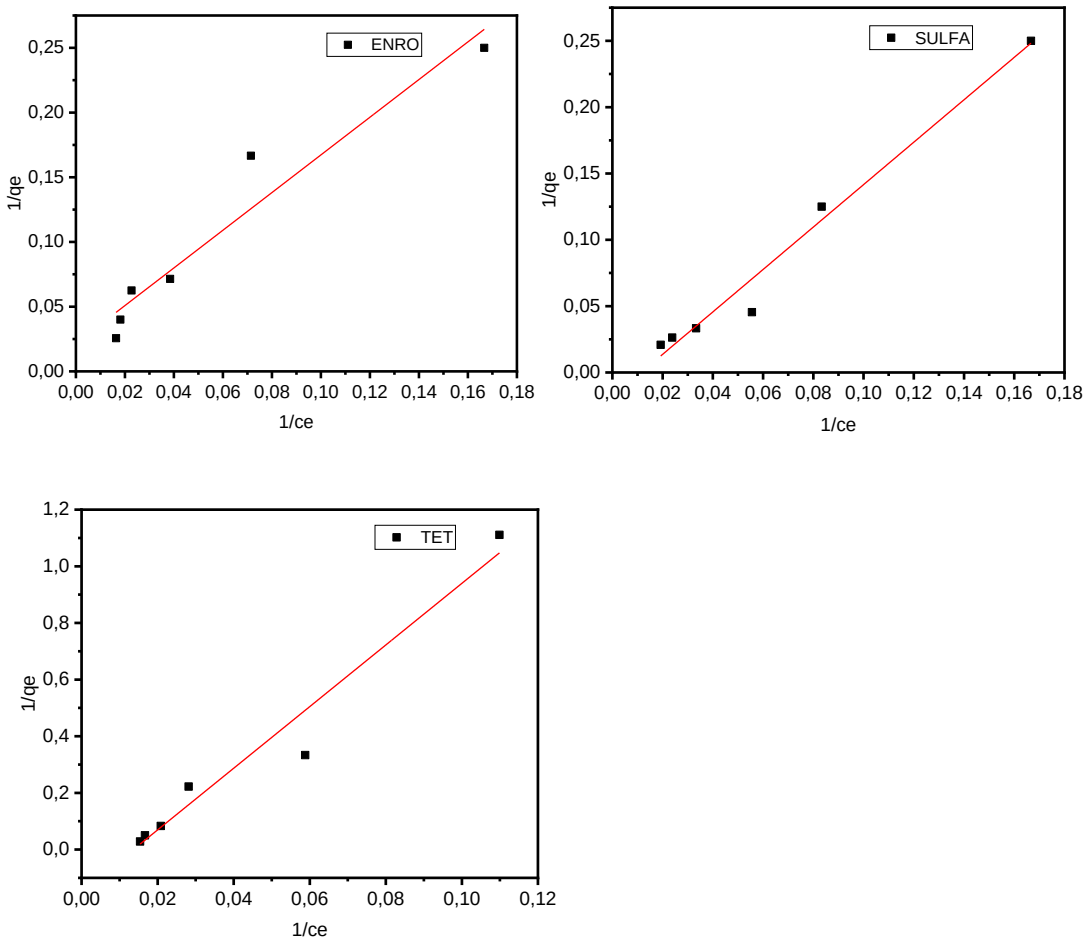
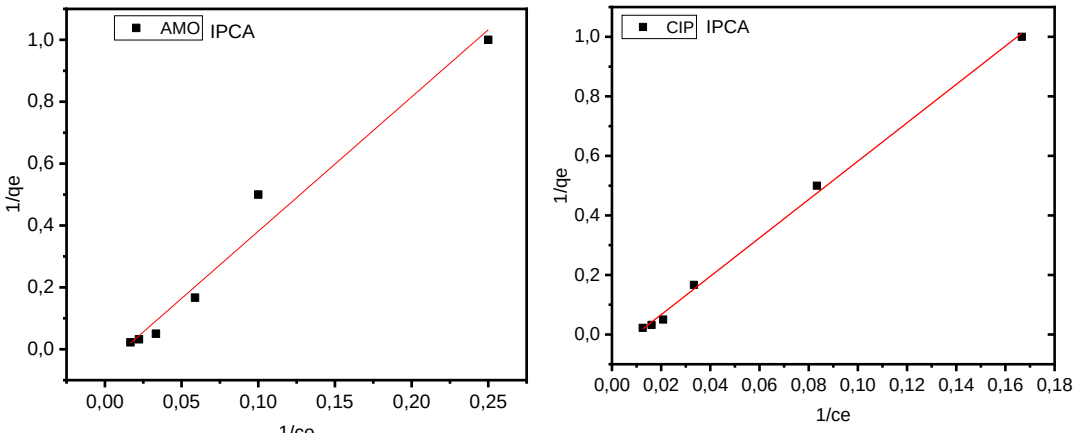



Figure C4: Langmuir Model using AC for the five antibiotics

## C5: Langmuir Model using IPCA for the five antibiotics



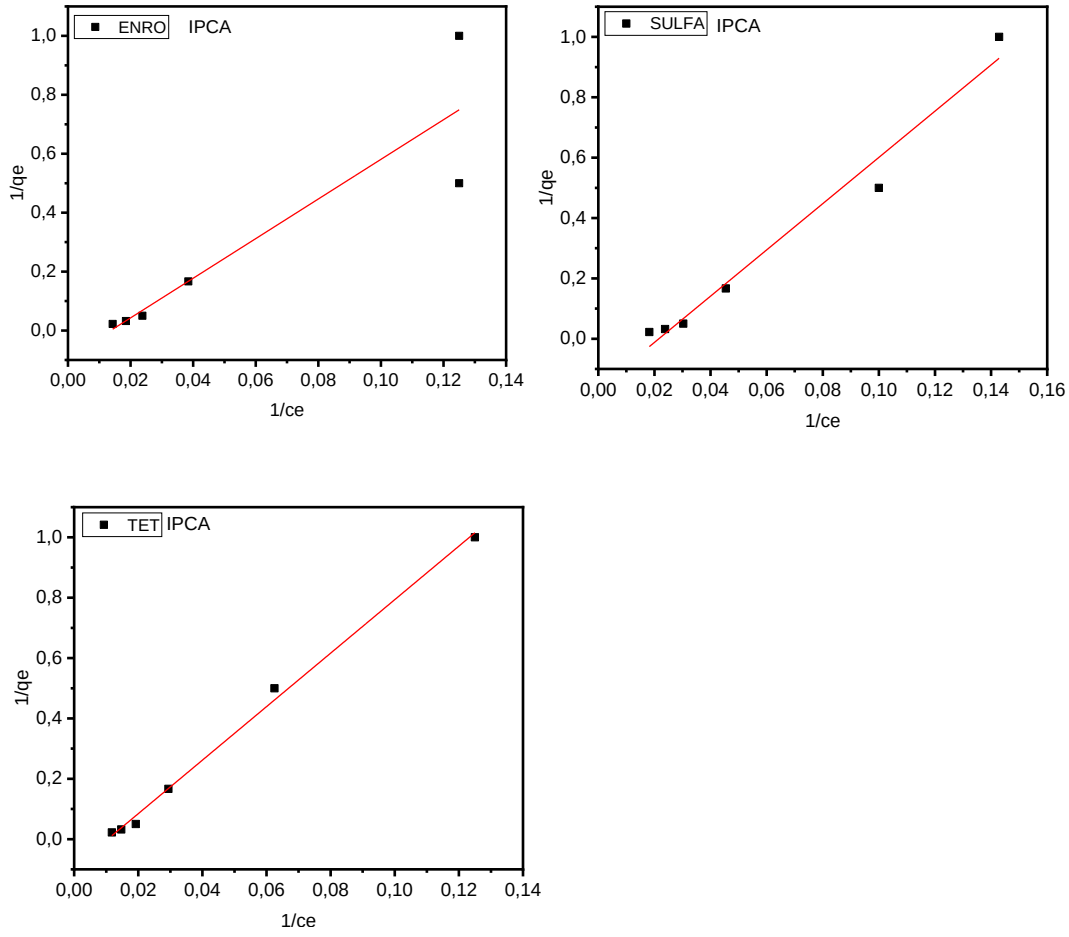
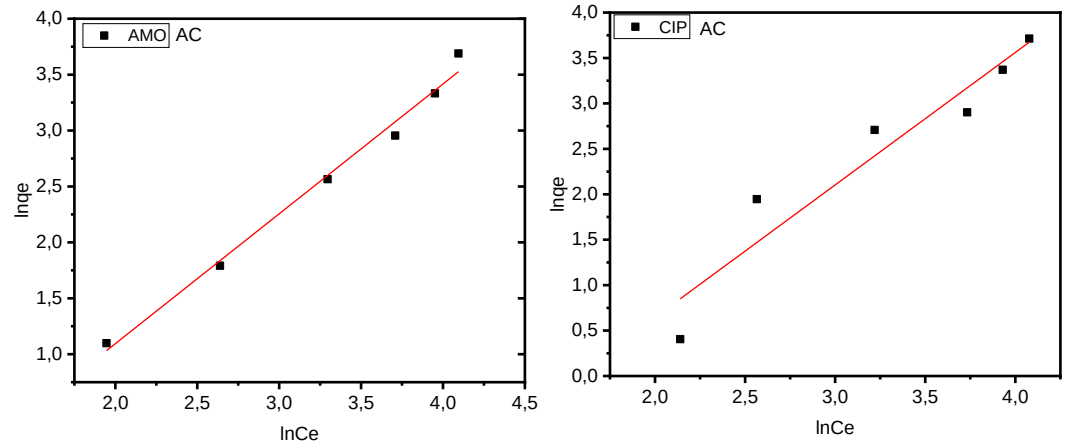
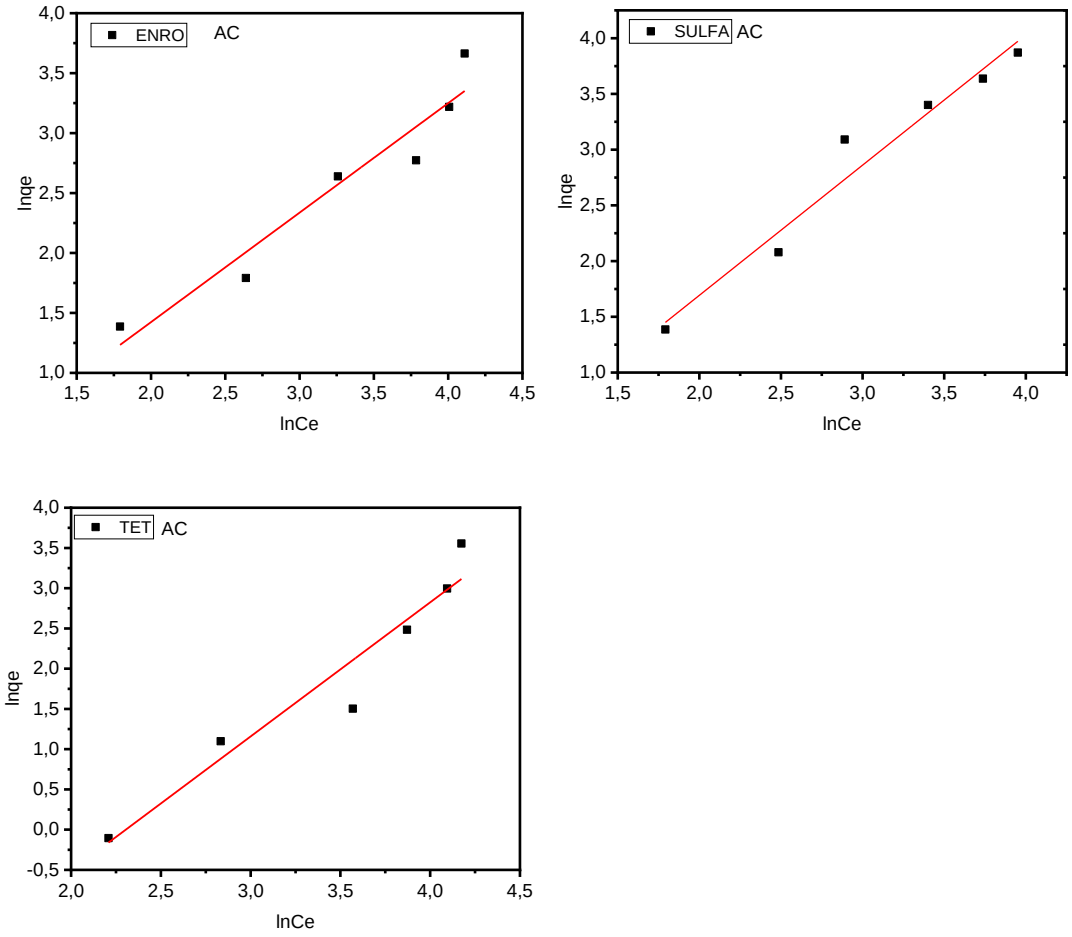



Figure C5: Langmuir Model using AC for the five antibiotics

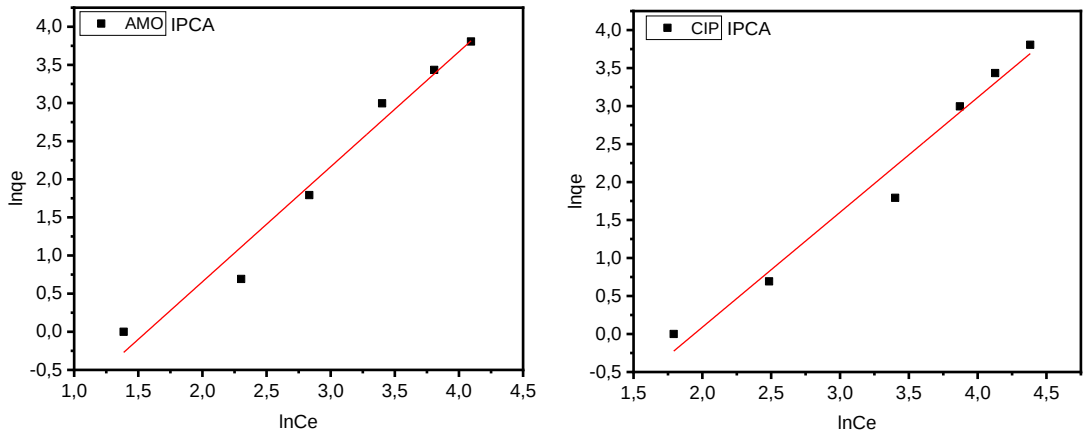
## C6: Freundlich Model using AC for the five antibiotics

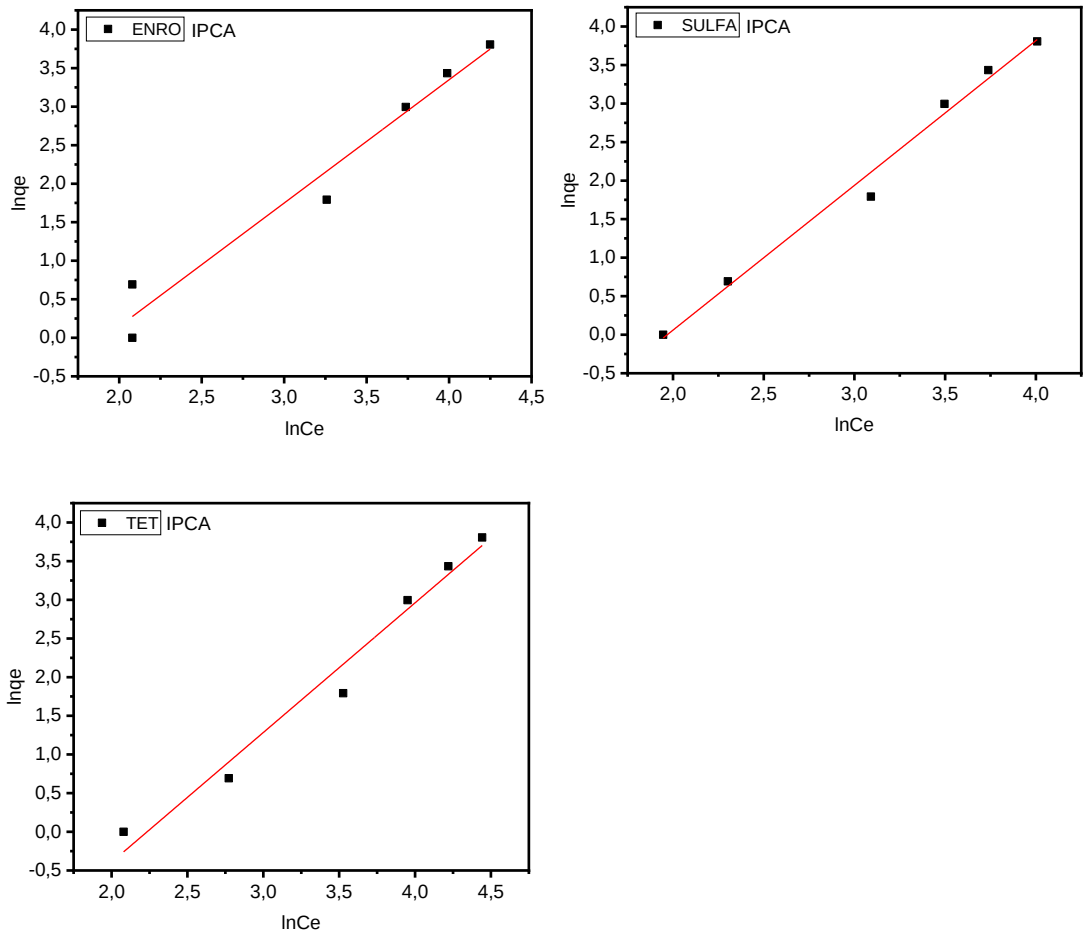





Figures C6: Freundlich Model using AC for the five antibiotics

## C7: Freundlich Model using IPCA for the five antibiotics






Figures C7: Freundlich Model using IPCA for the five antibiotics