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Evaluation of functionalized silver and silica nanoparticles for the removal of deoxyribonucleic acid conveying antibiotics resistance genes from water

By

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201927090

Submitted in fulfillment of the academic requirements for the degree of Doctor of Philosophy in the Department of Pure and Applied Chemistry,

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University of Fort Hare

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Certification

This thesis titled “Evaluation of functionalized silver and silica nanoparticles for the removal of deoxyribonucleic acid conveying antibiotics resistance genes from water” meets the regulation guiding the award of the degree of Doctor of Philosophy at the University of Fort Hare and is approved for its contributions to scientific Knowledge.

Prof O.O Okoh (Supervisor) Date ……... ……

Prof A.I Okoh (Co-supervisor) Date…….

Preface

The experimental work described in this thesis was performed by me at the faculty of Science and Agriculture, Department of Pure and Applied Chemistry as well as Biochemistry and Microbiology, University of Fort Hare, from April 2019 to September 2022, under the supervision of Professor O.O Okoh and Professor A. I Okoh. This study represents the original work of the candidate anhasve not otherwise been submitted in consideration for any degree or diploma in any University. The use of other works in this thesis is acknowledged accordingly.

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I, Adaora Stella Ezeuko, declare that:

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Declaration 3 – publications

Contributions that form part/or include research presented in this thesis (this includes publications, submitted, in press with the detailed contributions of each author to the experimental work and writing of each publication).

Publications

Publication 1

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh (**2021**). Technological advancement for eliminating antibiotics resistance genes from wastewater: A review of their mechanisms and progress. Journal of Environmental Chemical Engineering. Volume 9; Issue 5, *https://doi.org/10.1016/j.jece2021.106183*.

I designed and wrote the draft manuscript under the supervision of Dr. M.O Ojemaye, Professor O.O Okoh and Professor A. I Okoh. Inputs through reading, correction, and editing of the manuscript were also carried out by my supervisors. This publication is presented in this thesis as Chapter 2.

Publication 2

Adaora S. Ezeuko, Mike Ojemaye, Omobola O. Okoh, Anthony I. Okoh (**2021**). Potentials of metallic nanoparticles for the removal of antibiotics resistant bacteria and antibiotics resistance genes from wastewater; A critical review. Journal of Water Process Engineering (JWPE). Volume 4 (2021) 102041, *https://doi.org/10.1016/j.jwpe.2021.102041*.

I planned and wrote the draft manuscript under the supervision of Dr. M.O Ojemaye, Professor O.O Okoh and Professor A. I Okoh. Inputs through reading, correction, and editing of the manuscript were also carried out by my supervisors. This study is presented in this thesis as Chapter 3.

Publication 3

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh. **The effectiveness of silver nanoparticles as a clean-up material for water polluted with bacteria DNA conveying antibiotics resistance genes: Effect of different molar concentrations and competing ions (2022)**. OpenNano Journal. Volume 7 (2022) 100060, ***https://doi.org/10.1016/j.onano.2022.100060.***

I designed, executed, and wrote the initial and final draft manuscript under the supervision of Dr. M.O Ojemaye, Prof O Okoh, and Prof A.I Okoh. This work has been presented in Chapter 4 of this thesis.

Manuscript 1

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh. **Influence of different chaotropic salts on etched mesoporous silica nanoparticles for the removal of bacteria DNA conveying antibiotic resistance genes from hospital wastewater.** Submitted to Chemical Engineering Journal for publication (Under review).

I planned, executed, and wrote the initial and final draft manuscript under the supervision of Dr. M.O Ojemaye, Prof O Okoh, and Prof A.I Okoh. This work has been presented in Chapter 5 of this thesis.

Manuscript 2

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh. **Removal of bacteria DNA conveying antibiotic resistance genes from Ndevana Buffalo River by adsorption onto AgNPs@Fe_3O_4 nanocomposite** (Unpublished article).

I planned, executed, and wrote the initial and final draft manuscript under the supervision of Dr. M.O Ojemaye, Prof O Okoh, and Prof A.I Okoh. This work has been presented in Chapter 6 of this thesis.

Manuscript 3

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh. **Mesoporous silica nanoparticle as a superior adsorbent with high capacity: Synthesis, surface functionalization, and the removal of bacteria DNA conveying antibiotic resistance genes from Uitenhage wastewater treatment plant** (Unpublished work).

I planned, executed, and wrote the initial and final draft manuscript under the supervision of Dr. M.O Ojemaye, Prof O Okoh, and Prof A.I Okoh. This work has been presented in Chapter 7 of this thesis.

Signed: Date:

…… ……..

Conference contribution

Conference presentation:

Adaora S. Ezeuko, Mike O. Ojemaye, Omobola O. Okoh and Anthony I. Okoh. The effectiveness of silver nanoparticles as a clean-up material for water polluted with bacteria DNA conveying antibiotics resistance genes: Effect of different molar concentrations and competing ions (Oral Presentation), December 2021 Kosygin Forum, Moscow Russia.

List of Abbreviation

AgNPs	Silver nanoparticles
AgNPs@Fe_3O_4	Silver nanoparticles functionalized with iron (III) oxide
Fe_3O_4	Iron (III) oxide
MSN	Mesoporous silica nanoparticles
MSN+G	Mesoporous silica nanoparticles with 2 M guanidine HCl
E-MSN+U	Mesoporous silica nanoparticle with Urea
MSN+S	Mesoporous silica nanoparticles with sodium hydroxide
E-MSN	Etched mesoporous silica nanoparticles
MSNPs@TPPY	Mesoporous silica nanoparticles functionalized with 4-(4-hydroxyphenyl)- 2,2;6",2-terpyridine
MSNPS@APTES	Mesoporous silica nanoparticles with 3-(Aminopropyl) triethoxysilane
ARB	Antibiotic-Resistant Bacteria
ARGs	Antibiotic Resistance Genes
WWTPs	Wastewater Treatment Plants
WWTFs	Wastewater Treatment Facilities
WHO	World Health Organization
CDC	Centre for Disease Control
HGT	Horizontal Genes Transfer

DNA	Deoxyribonucleic acid
SEM	Scanning Electron Microscopy
FTIR	Fourier Transformed Infrared Spectroscopy
EDX	Energy-dispersive x-ray spectroscopy
PZC	Point of zero charge
SDS	Sodium dodecyl sulfate
NaCl	Sodium chloride
$CH_5N_3.HCl$	2 M Guanidine HCl
$(NH_3)_2CO$	Urea
bDNA-ARGs	Bacteria DNA conveying ARGs
TEOS	Tetraethyl orthosilicate
HNO_3	Nitric acid
PCR	Polymerase chain reaction
MSNPs-NH_2	Amine-functionalized mesoporous silica nanoparticles
MSNPs-COOH	Carboxylic functionalized mesoporous silica nanoparticles
ETOH	Ethanol

Dedication

This thesis is dedicated to God Almighty, Holy Spirit for inspiration, Love, and guidance throughout the study. Also, I dedicate this thesis to my loving and caring husband, Mr. Izuchukwu Godwin Ezeuko who encouraged and contributed greatly to the fulfilment of my academic pursuit. To my children, Destiny, Goodluck, Anthony and Mercy, and to my loving brother Augustine Chinedu, I love you all.

Acknowledgments

My indisputable gratitude goes to my supervisors and mentor: Professor Omobola. O. Okoh, Professor Anthony. I Okoh and Dr. Mike. O Ojemaye for their contributions such as financial, moral, constructive criticism, and academic guidance throughout this program. So much knowledge has been acquired from you all and this will make me a competent researcher in the future.

I would like to extend my appreciation to the Department of Pure and Applied Chemistry, Applied Environmental and Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, and SAMRC, Microbial Water Quality Monitoring Centre, University of Fort Hare for providing a well-equipped laboratory where these studies were conducted.

My appreciation goes to my colleagues and friends (Ifeoluwa, Sis Funmi, Joan, Mrs. Adeniji, Samuel, Dr Nonso, and Dr Penny) in Nano-chemistry, Microbiology, and Biochemistry unit who assisted in one way or the other in the laboratory. To my awesome brother and friend Dr Chika for the financial support and words of encouragement. Also, to my adopted mother Professor Bamgboye for her constant prayers, advice, and financial support. And to my friend Dr Moronkola for her words of encouragement throughout the programme. May the Lord bless you all.

I wish to acknowledge my wonderful parents Mr. and Mrs. S.O Ezeokwuoha for their prayers, financial support, words of encouragement, and most importantly in taking good care of my children. And to my sweet siblings Dr Johnson, Buchi, Chinedu, Elochukwu, Uche, Chidi, and Chika. May God bless you all.

Finally, unto Almighty God all praise and glory return to him alone, for his immense protection and divine guidance during the period of this study. To this end, “I will sing

praises to thee, and not be silent. O Lord my God, I will give thanks unto thee forever" (Psalm 30:12).

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General abstract

Antibiotic resistance genes (ARGs) are recognized as a serious public health emergency linked to extensive use of antibiotics by humans and animals as a prophylactic agent that treats and prevents infections. The occurrence of high concentrations being identified in wastewater treatment plants, rivers, etc is due to untreated effluents being discharged from households, hospitals, agriculture, and pharmaceutical industries. The application of adequate treatment techniques and material for the removal of bacteria DNA conveying ARGs from the effluents before their release to the environment cannot be overemphasized. Adsorption techniques seem to be effective due to their easy design, operation, and ability to regenerate adsorbents for use without producing toxic by-products. This concept was employed for the removal of bacteria DNA conveying ARGs from simulated aqueous solution, effluents from hospital, river and WWTPs using silver and silica metallic nanoparticles

This thesis investigated the effectiveness of metallic nanoparticles containing silver (AgNPs) and mesoporous silica nanoparticles (MSNPs) as well as magnetite (Fe_3O_4) functionalized with 4(4-hydroxyphenyl)-2,2;6",2-terpyridine onto their surface, for the removal of bacteria DNA conveying antibiotic resistance genes from water samples from hospitals, river, and wastewater treatment plants (WWTPs). Silver nanoparticles (AgNPs) of different molar concentrations (0.1M, 0.5M and 1.0M) and mesoporous silica nanoparticles (MSNPs) adsorbents were successfully synthesized in their original states and surface functionalization achieved by incorporating magnetite (Fe_3O_4) and 4(4-hydroxyphenyl)-2,2;6",2-terpyridine on the silver (AgNPs@ Fe_3O_4) and silica (MSNPs@TPPY) surfaces respectively.

Their effectiveness as adsorbent for the removal of bacteria DNA conveying ARGs from aqueous solutions and real water/wastewater samples were investigated. The DNA uptake by the as-synthesized AgNPs and MSNPs were compared to the functionalized AgNPs@Fe_3O_4 and MSNPs@TPPY by determining the adsorbents with the highest removal efficiencies. All

the as-synthesized and functionalized adsorbents were characterized by SEM, EDX, FTIR, XRD, UV spectroscopy and PZC before the removal process.

The extraction of genomic DNA from antibiotic-resistant *Enterococcus faecium* and *Vibrio parahaemolyticus* was successfully achieved via the boiling method. Antibiotic susceptibility test was conducted using the disk diffusion method before the commencement of genomic DNA extraction. Molecular characterization via gel electrophoresis confirmed the presence of resistance genes at different base pairs.

Adsorption batch experiment were investigated, and the best optimum parameters were evaluated through the influence of pH, contact time, initial DNA concentration, adsorbent dose, and competitive ions for each sorption process. The rate determining step were determined by fitting kinetic models such as Natarajan and Khalaf first order, pseudo first order, pseudo second order, Elovich model to experimental data. Also, the adsorption mechanisms determining adsorption equilibrium were investigated by fitting Freundlich, Langmuir and Sips model into the experimental data.

The application of AgNPs@Fe_3O_4 nanocomposite and MSNPs@TPPY for the removal of bacteria DNA demonstrated much enhancement for DNA uptake than the as-synthesized (AgNPs and MSNPs) materials. The incorporation of magnetite and 4(4-hydroxyphenyl)-2,2;6",2-terpyridine onto AgNPs and MSNPs significantly enhanced the binding affinity towards the removal the bacteria DNA via strong electrostatic attraction between the active sites on the adsorbent and the negative DNA molecules. Finally, high adsorption capacities were recorded with AgNPs@Fe_3O_4 nanocomposite and MSNPs@TPPY compared to AgNPs and MSNPs with chaotropic salts. The kinetic adsorption models were mostly best fitted by the pseudo-second order and Elovich models while the adsorption equilibrium was best described by Langmuir and Sips isotherm models.

MSNPs with different chaotropic salts, AgNPs@Fe_3O_4 nanocomposite and MSNPs@TPPY also proved its effectiveness in DNA removal not only in the simulated aqueous solution but in three different real life water samples obtained from Cofimvaba hospital, Ndevana river and Uitenhage WWTPs. High adsorption efficiencies above 90 % were achieved during the removal of DNA in all the three real water samples. Therefore, application of these adsorbents for the removal of bacteria DNA conveying ARGs may be a promising option that would tackle the consequences of consuming ARGs infected water globally.

Chapter 1

Introduction

1.1 Background

Polluted water has been recognized as a 21st-century threat due to its significant ill health caused by discharging of untreated effluents from households, hospitals, agricultural farms, and pharmaceutical industries into either wastewater treatment facilities (WWTFs) or natural water sources. Bacteria-contaminated water is seen as a global threat that causes water pollution, preventing access to a clean and safe water supply (Reddy, 2022). The report has shown that 3% of the earth's water is fresh, and the rest contains salter water which is unfit for human activities (Barlow and Clarke, 2002; Singaraja *et al.*, 2014). The organic contaminant threatens the limited available freshwater due to reckless industrialization, extensive use of antibiotics, and discharging of the untreated effluents from these sources into water matrices. According to Food and Agricultural Organization (FAO), South Africa is currently estimated to have fresh water availability of 1.154 m^3 per year (Otieno and Ochieng, 2004). The discharged pollution is confronting the little available water without being treated. In 1996, the International Water Management Institute (IWMI) estimated that South Africa may experience physical water scarcity in 2025 (Mancosu *et al.*, 2015; Otieno and Ochieng, 2004).

Therefore, adequate measures should be taken to prevent water pollution in different water matrices. Organic contaminants such as pesticides, phenols, dyes, antibiotics, etc., are critical environmental contaminants that threaten the lives of humans and animals in rural and urban settlements (Zango *et al.*, 2020). The discharge of antibiotic pollution into different water matrices has created a serious health challenge to public health. According to World Health Organization (WHO), antibiotics treat different diseases that threaten human lives (WHO,

2018). Antibiotics are used to enhance their growth in livestock and fish farms, prevent, and even treat infections (Economou and Gousia, 2015; Watts *et al.*, 2017). It is feasible to say that antibiotics are extensively used in anthropogenic activities. About 90% of these antibiotics in human and animal bodies degrade during digestion, and non-metabolized residues are excreted as faces and urine, most of which are unruly discharged into water matrices (Tran *et al.*, 2018). Wastewater treatment facilities are a hotspot for disseminating antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Antibiotic resistance genes transfers ARGs to indigenous bacteria in water through horizontal gene transfer (HGT). Generally, bacteria are a rich source of nucleic acid-targeting toxins.

Nucleic acids are biopolymers vital to all known forms of life, and they are made up of DNA and RNA. They play a vital role in genetic information and consist of nucleotides that regulate metabolism and cellular processes (Bailey, 2017; Bowater and Gates, 2015). Each nucleotide is made of monomers comprising three chemical components and they are 5-carbon sugars, a phosphate group, and a nitrogenous base. The critical components of nitrogenous base found in both DNA and RNA are adenine (A), guanine (G), and cytosine (C). In contrast, thymine is found only in DNA and is described as a centre for genetic activities (Kundu *et al.*, 2017). Horizontal gene transfer (HGT) is the effective medium by which bacteria transfer genetic traits and enables bacteria to adapt to various environments by acquiring an extensive DNA sequence (Brito, 2021). These bacteria acquire ARGs in their DNA, which can be transferred from one bacterium to another through HGT in the water environment. Antibiotic resistance genes widely disseminate and persist in the water environment through horizontal gene transfer, reducing the efficacy of treatment methods and materials that may be employed to eradicate them (Zhou *et al.*, 2021). Studies have shown that water matrices such as WWTFs, and natural waters are reservoirs for the proliferation

and persistence of ARGs (Li *et al.*, 2017a; Meili, 2021). Therefore, adequate treatment materials are needed to tackle the menace of ARGs to the public.

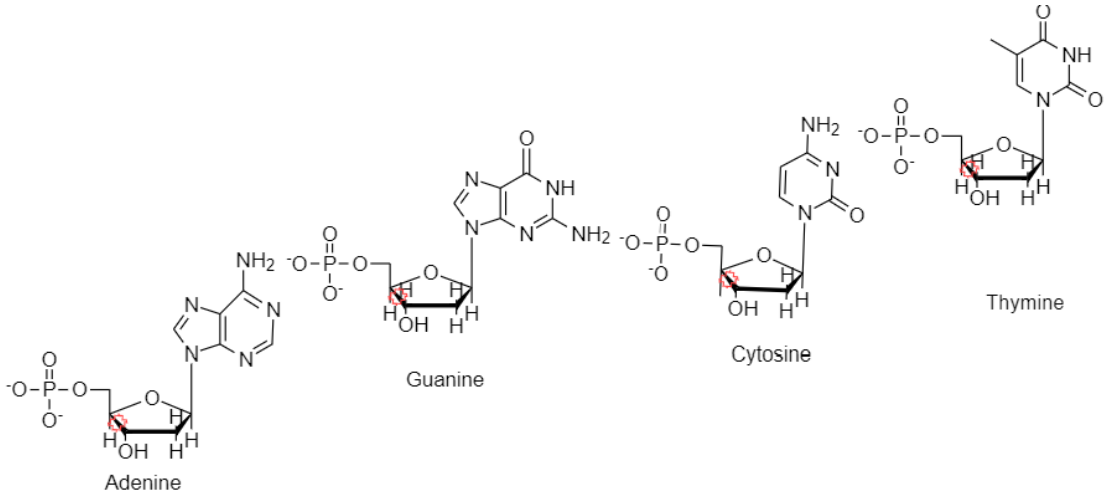

Figure 1.1: Chemical structure of bacteria DNA

Several purification techniques and materials have been reported for ARGs removal from water matrices. Such techniques include advanced oxidation process (AOPs) (Pazda *et al.*, 2019a), disinfection (Barancheshme and Munir, 2018), membrane bioreactors ((Le *et al.*, 2018; Li, Qiu, *et al.*, 2019), conventional activated sludge (Li, Qiu, *et al.*, 2019; Pei *et al.*, 2019) adsorption process (Wang *et al.*, 2020; Yu *et al.*, 2017a), coagulation /flocculation (Barancheshme and Munir, 2018; Li *et al.*, 2017a), and nanofiltration (Cristóvão *et al.*, 2021; Lan, Kong, *et al.*, 2019). Among all, adsorption seems to be more efficient due to its unique properties, including low cost, very efficient not only in laboratory but in a large-scale application, simplicity in design, ease of recovery adsorbent, and ability to reuse adsorbents during treatment processes. Numerous adsorbents, especially nanomaterials-based adsorbents, have been widely employed. They include magnetic, titanium oxides (TiO_4 NPs), cerium oxide (CeO_2), zinc oxide (ZnO), Iron (III) oxide (Fe_3O_4 NPs), mesoporous silica oxide (MSN), graphene oxide (GO NPs) and silver nanoparticles (AgNPs) (Eric *et al.*, 2020; Hwangbo *et al.*, 2019; Kushalkar et al., 2020; X. Li *et al.*, 2012; Ren *et al.*, 2018).

Meanwhile, Nanoparticles are particle sizes between 1 to 100 nm with different synthesis procedures, particle structures, and surface modifications (Jamkhande *et al.*, 2019). Nanoparticles have excellent properties of a specific surface-to-volume ratio, and they influence surface reactivity and size-dependent, which contributes to high adsorption capacities during the adsorption process (Bora and Dutta, 2014).

Recently metallic nanoparticles such as AgNPs and MSN have gained much attention. Silver nanoparticles (AgNPs) have been reported to be an excellent antimicrobial agent due to the presence of silver ion (Ag^+) which has a biocidal action making AgNPs more effective than the bulk silver (Tajkarimi *et al.*, 2014). AgNPs have been reported to cause cell death through various mechanisms, including oxidation of cellular components, enzymes deactivation, oxidation of reactive oxygen species (ROS), and cellular decomposition (Kapoor *et al.*, 2018; Rizzello and Pompa, 2014). Reactive oxygen species (ROS) can damage the DNA present in nucleic acids through hydrogen peroxide (H_2O_2). Besides, its oxidation state of 0, +1, +2, and +3 have an affinity to target negative contaminants (DNA) in an aqueous solution (Singh *et al.*, 2017). Also, surface functionalization of AgNPs with either magnetite may increase the removal efficiency of bacteria DNA from water/wastewater. AgNPs are easy to synthesize, cost-effective, and eco-friendly. AgNPs exhibit strong antibacterial properties that could stop the proliferation of bacteria (Pazda *et al.*, 2019a)

Mesoporous silica nanoparticles (MSNPs) are mesostructured materials with a pore diameter between 2 nm and 50 nm. Several mesostructured properties such as narrow pore size distribution, controllable and uniform particle size, high specific surface area, large pore volume, ease of surface functionalization or surface modification, non-toxic nature, and colloidal stability make it a suitable and efficient adsorbent for wastewater treatment process (Kankala *et al.*, 2020; Panahi *et al.*, 2019; Saman *et al.*, 2020). Since the contaminant in question (bacteria DNA) is highly negative, and MSNPs have a negative surface, adsorption

of DNA onto the surface of original silica may not be possible due to repulsion effects. However, due to the surface hydroxyl group present in MSN, the addition of chaotropic salts and surface functionalization with ligands would assist in removing DNA from water/wastewater. This study investigated the effectiveness of nanomaterials as adsorbents for the removal of bacteria DNA harbouring ARGs from simulated water/wastewater. To achieved this, different nanomaterials were synthesized, and functional group incorporated to improve the efficacy and efficiency of synthesized adsorbents. The following sections described the statement, problems, aim and objectives of this study.

1.2 Problem statement

Extensive use of antibiotics by human and animals deteriorates source of water and environmental issues. The discharge of antibiotics residues at long run promotes ARB and ARGs. The untreated effluents containing ARGs from households, hospital, agricultural and pharmaceutical industries have been recognized as the leading cause of water pollution worldwide (Samreen *et al.*, 2021). Application of antibiotics in diverse area such as clinical and agricultural use contaminate source of waters which exposes humans and animals to various bacterial infections causing lots of health complications.

Meanwhile, unauthorized sales of antibiotics over the counter, overuse by the cattle and poultry at unprecedented rate, improper sanitation process increased constant occurrence of ARGs in different water matrices. And the discharged of non-metabolized residues from these sources as faeces and urine into WWTPs and natural waters have increased the morbidity and mortality rate yearly (NERI *et al.*, 2017).

The prevalence and dissemination of bacteria DNA harboring ARGs have been described by World Health Organization (WHO) as emerging environmental contaminants that may claim 10 million lives in 2050 if adequate treatment procedures are not implemented (Singh *et al.*,

2022). A recent report in our research group reported the prevalence of ARGs in municipal wastewater treatment plants around the Eastern Cape province of South Africa and concluded that wastewater is a point source for ARB and ARGs and can cause outbreak by transferring resistance to multiple unrelated pathogens, causing a severe health risk to the public (Adefisoye and Okoh, 2016).

Natural waters such as rivers, lakes, streams, etc., are increasingly contaminated due to the unruly discharge of antibiotic residues without proper purification. This causes pollution that deprives the public of accessing safe and clean water supply, water shortages, and scarcity (Bora and Dutta, 2014). Consumption of bacteria DNA conveying ARGs contaminated water by humans and animals has been reported as a dangerous factor that leads to an increase in morbidity and mortality rates around the globe (Larsson and Flach, 2022).

Therefore, it is important to develop an effective and simple techniques that would eradicate the existing contaminants before being discharged into WWTPs and natural waters. In recent years, various conventional practices such as ion exchange, reverse osmosis, membrane filtration, photocatalysis, coagulation/flocculation, and oxidation were reported to have limitations, including cost-effective and low absorption capacity (Le *et al.*, 2018; Lu, Wang, *et al.*, 2020; Pazda *et al.*, 2019a). The adsorption process with nanoparticles is considered better due to its ease of operation, low cost, and effectiveness in removing organic pollutants from wastewater (Ojemaye *et al.*, 2017).

The application of different molar concentration of silver nanoparticles (AgNPs) and mesoporous silica nanoparticles (MSNPs) to remove bacteria DNA from wastewater, however, the functionalization of these materials with terpyridine ligand and magnetite (Fe_3O_4) may give a more efficient and higher adsorption capacity. Reports have shown the

effectiveness of novel functionalized nanomaterials for removing different organic and inorganic contaminants from wastewater by electrostatic interaction (Ojemaye *et al.*, 2017).

In this study, we synthesized nano-silver material (AgNPs) of different molar concentrations (0.1, 0.5 and 1.0 M) represented as BD1, BD2 and BD3. AgNPs-magnetite nanocomposites (AgNPs@Fe_3O_4 nanocomposites), MSN with the addition of different chaotropic salts (2 M Guanidine HCl, sodium chloride and Urea) and functionalization of MSN with terpyridine (MSNPs@Tppy) as adsorbents. Their application was extended to removing bacteria DNA conveying ARGs from aqueous solution, hospital effluents, sample water from the river, and wastewater treatment plants.

Therefore, functionalized materials would enhance the removal of more bacteria DNA conveying ARGs water/wastewater via electrostatic interaction.

1.3 Research Hypothesis (Null)

This study hypothesizes that functionalized silver and silica mesoporous nanoparticles will not be effective for the removal of nucleic acids from water/wastewater.

1.4 Aim and objectives of the study

This research aims to study the effectiveness of functionalized inorganic nanomaterial such as silver and mesoporous silica nanoparticles for the removal/adsorption of bacteria DNA conveying ARGs from water/wastewater.

I. To synthesize 4-(4-hydroxyphenyl)-2, 2, 6, 2-terpyridine (Tppy-OH), Fe_3O_4 (magnetite), etched mesoporous silica nanoparticles (E-MSN) and silver nanoparticles (AgNPs) of varying concentrations.

II. To functionalize the surface of AgNPs with Fe_3O_4 (magnetite) and MSNPs with TPPY-OH and characterize the synthesized materials by determining the surface

morphology, absorbance, elemental compositions, functional group, phase crystallinity, and net charge present on the surface of the adsorbents.

III. To carry out antibiotic susceptibility testing (AST) and extraction of genomic DNA from antibiotic-resistant *Vibrio Parahaemolyticus, Enterococcus Faecium,* and *Listeria monocytogenes.*

IV. To carry out molecular characterization on bacteria DNA by determination of antibiotic resistance genes present.

V. To carry out batch adsorption studies, investigate the influence of operating parameters (solution pH, contact time, initial adsorbate concentration, and adsorbent dosages) to remove bacteria DNA conveying ARGs from aqueous solution.

VI. To extend the batch adsorption studies to real-life scenario such as adsorption of bacteria DNA conveying ARGs from hospital effluent, river water and wastewater treatment plant using the optimum conditions.

VII. To investigate adsorption mechanism by fitting in the experimental data into kinetic and isotherm adsorption models.

1.5 Research question

I. Will the synthesized adsorbents such as AgNPs (BD1, BD2, and BD3), E-MSN with combination of different chaotropic salts, AgNPs@Fe3O4 and MSNPs@TPPY adsorbed bacteria DNA conveying ARGs from water/wastewater.

1.6 Thesis summary

This thesis comprises original experimental studies on the synthesis of AgNPs, MSNPs, functionalized nano-silver and magnetic nanocomposites (AgNPs@Fe_3O_4 NPs), and mesoporous silica functionalized with terpyridine ligand (MSNPs@Tppy) and its application

for the removal of bacteria DNA conveying ARGs from aqueous solution, hospital effluents sample water from river and wastewater treatment plant. The thesis is written in manuscript format, and it is made of two literature review chapters (Chapters 2 and 3) and four experimental chapters (Chapters 4, 5, 6, and 7).

Chapter 1: This chapter provided a brief discussion on the contamination of water matrices with the discharge of untreated effluents containing bacteria DNA conveying ARGs. Health challenges associated with ARG consumption of contaminated water and the consequences of not implementing adequate treatment procedures to eliminate these emerging contaminants from water/wastewater.

Chapter 2:

This chapter provides detailed information and review of literature based on the background of the study. This review highlights the importance and potential of silver and silica oxide nanoparticles to remove these substances from wastewater and their unique properties. Incorporating metallic nanoparticles is a promising way of eliminating these pollutants from wastewater. Finally, setting up policies to prevent the indiscriminate use of antibiotics is a sure way to avoid these pollutants' proliferation in wastewater. With this, the challenge of selecting appropriate material will not be a concern. **This chapter has been published in the Journal of Water Processing Engineering 41 (2021) 102041. https://doi.org/10.1016/j.jwpe.2021.102041**

Chapter 3:

In this chapter, a literature review provides concise information on the commonly used antibiotics, their abundance in wastewater, and the natural water environment. Also, the combination of treatment technologies may effectively minimize the consequences of accessing ARGs contaminated water. **This chapter has been published in the Journal of**

Environmental Chemical Engineering 9 (2021) 106183, https://doi.org/10.1016/j.jece2021.106183.

Chapter 4:

This chapter is an experimental study comprising the synthesis, characterization, and adsorption of bacteria DNA conveying ARGs onto different molar concentrations (0.1 M, 0.5 M, and 1.0 M) of as-synthesized AgNPs. Also, kinetic and isotherm adsorption studies were captured. **This chapter has been published in Journal of Open nano**, **Volume 7 (2022) 100060, *https://doi.org/10.1016/j.onano.2022.100060.***

Chapter 5:

In this study, mesoporous silica nanoparticles were synthesized via chemical etching technique using sodium dodecyl sulphate (SDS) as an etchant. This synthesis method was responsible for removing unwanted layers on the surface of the original silica. The original MSNPs were subjected to characterization. The adsorption of bacteria DNA onto the surface of MSNPs was achieved by adding different chaotropic salts (2 M Guanidine HCl, Sodium Chloride, and Urea), followed by investigating the effects of operating parameters. The batch studies were extended to the adsorption of bacteria DNA conveying ARGs from Cofimvaba hospital effluent. Adsorption models such as kinetic and isotherm model were used to explain the best fit for the experimental results. **This chapter has been submitted for peer review in Chemical Engineering Journal.**

Chapter 6:

This experimental chapter discusses the synthesis of nano-silver functionalized magnetic nanocomposites. It investigated its characterization and removal of bacteria DNA conveying ARGs in an aqueous solution. Adsorption kinetics and isotherm model studies were reported.

Furthermore, the efficacy of the nanocomposites adopting the most optimized parameters was confirmed by investigating the removal efficiency of bacteria DNA from Ndevana Buffalo river water. **This chapter has been submitted for peer review in Journal of Environmental Pollution.**

Chapter 7: This experimental chapter provides comprehensive information on the synthesis of 4-(4-hydroxyphenyl)-2, 2, 6, 2-terpyridine (Tppy-OH). The surface functionalization of MSNPs with Tppy-OH by esterification methods. The adsorbent characterization and the adsorption of bacteria DNA conveying ARGs were investigated and reported. Kinetic and isotherm equilibrium were reported. The proof of concept was achieved and confirmed by extending the application to the bacteria DNA contaminated water sample from the Uitenhage wastewater treatment plant. **This chapter has been submitted for peer review in Sustainable Materials and Technology**

Chapter 8:

This chapter gives brief information on the conclusion of the project work and possible recommendations for future research.

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Chapter 2

Technological advancement for eliminating antibiotics resistance genes from wastewater: A review of their mechanisms and progress

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Chapter 2

Literature review

Technological advancement for eliminating antibiotics resistance genes from wastewater: A review of their mechanisms and progress

Abstract

Antibiotics resistance genes (ARGs) concurrence with antibiotic-resistant bacteria (ARB) has been identified globally as contaminants that threaten public health. These contaminants enter wastewater treatment plants through a hospital, domestic, pharmaceutical, and agricultural activities. These channels are responsible for disseminating antibiotic resistance genes (ARGs) among the non-resistant bacteria by horizontal gene transfer, making wastewater a hotspot for antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Conventional and advanced treatment processes have been widely used to mitigate or minimize conjugative gene transfer risk. The application of these processes alone can successfully inactivate ARB during the treatment process. However, there is a possibility that the existing ARGs present in the cell debris could still confer resistance through transformation and transduction without live donor cells. Also, most of the disinfection/treatment processes may enrich ARB and ARGs' concentration in the long run. As a result of these drawbacks, systematic mechanisms that would effectively remove bacteria DNA and inactivate ARB may provide a solution to enable the public to access effluents free from ARGs. This review provides concise information on the commonly used antibiotics, their abundance in wastewater and natural waters, coupled with available technologies for their removal from water and wastewater, which may minimize the risk of accessing ARGs contaminated water. Therefore, the adoption of these well-detailed technologies may be a promising way that would stop ARG proliferation.

2.1. Introduction

Recently, due to the high toxicity of contaminants and their harmful nature on the ecosystem, access to safe water has become impossible and has attracted global attention. Access to

clean and safe water is necessary and crucial for all living beings ranging from household use to more complicated industrial and agriculture/aquaculture operations. According to the World Health Organization (WHO), billions of people lack access to safe water supply (Pink, 2012), responsible for disease outbreaks among the populace. A high mortality rate recorded yearly (about 2 million people died from water-borne diseases). This situation threatens children living in a water-scarce area because it exposes them to some vulnerable diseases. Most of the families in this area seldom receive adequate treatments. Antibiotics residues are dangerous contaminants that should not be allowed or discharged into wastewater treatment facilities (WWTFs). These dangerous contaminants are released into WWTFs due to their excessive and indiscriminate use by humans and animals. Antibiotics are active biological compounds widely used to prevent microbial infections in humans and animals (Mann *et al.*, 2021; Okoh *et al.*, 2007). Excessive use promotes antibiotic-resistant bacteria (ARB) and antibiotics resistance genes (ARGs) in water and wastewater. Adequate treatment technologies and knowledge on the consequences of consuming ARGs-infected water should be implemented. Besides, clean water is essential to all works of life. For example, producing papers, plastics, food packages, cosmetics, medicine, metals, etc., requires safe and clean water. These operations increased the demand for wastewater and greywater usage (EPA, 2012; Mohammadali and Davies, 2017). Due to rapid global growth, proliferation, reckless industrialization, and excessive use of antibiotics, ARB/ARGs have been widely detected in WWTFs across the globe.

Generally, wastewater treatment plants and natural waters are polluted due to waste liquid discharged from households, hospitals, agriculture/aquaculture sectors, and industries associated with feed additives, pharmaceuticals, and personal care products (PPCP) (Crini and Lichtfouse, 2019; Rafraf *et al.*, 2016; Voigt *et al.*, 2020). Among these contaminants, the use of pharmaceuticals such as antibiotics poses a severe threat to public health due to their

extensive usage worldwide. These cause resistance mechanisms among bacteria, humans, and animals, affecting the healthcare system, veterinary, and output from agricultural sectors (Dadgostar, 2019; Manyi-Loh *et al.*, 2018). The antibiotic-resistant bacteria (ARB) results in antibiotics resistance genes (ARGs), causing other indigenous bacteria to develop resistance traits towards antibiotics. This resistant trait enables the bacteria to outpace the efficacy of the antibiotics meant to treat microbial infections (Martínez *et al.*, 2015; Ni *et al.*, 2020). ARGs are emerging environmental contaminants emanating from resistant bacteria and pose a high risk to public health. The resistance mechanisms have been attributed to indiscriminate and excessive use of antibiotics by humans and animals. Many antibiotic residues are being discharged into wastewater treatment plants (WWTPs), making them hotspots for ARB and ARGs (Liu, Zhang, *et al.*, 2019; Pazda *et al.*, 2019a). According to WHO reports on surveillance of antibiotics consumption, China, Europe, Russia, etc., are countries with a high consumption rate among humans and animals (Organization, 2018), resulting in comprehensive detection of ARGs in their WWTFs. Some commonly used human and veterinary antibiotics, antimicrobials, type, chemical formula, and water matrices that harbours antibiotic resistance genes are described in Table 2.1.

Table 2.1 Some commonly used human and veterinary antibiotics, antimicrobials, type, chemical formula, and water matrices that harbour high resistance gene concentrations. References (Agga *et al.*, 2015; Correia *et al.*, 2017; Dadgostar, 2019; Dasenaki and Thomaidis, 2010; Divya *et al.*, 2020; Van Erum *et al.*, 2019; Guo *et al.*, 2017; Kumar, Jaiswal, *et al.*, 2019; Lan, Yin, *et al.*, 2019; Lee *et al.*, 2017; Lien *et al.*, 2016; Rath *et al.*, 2019; Shabana and Al-Enazi, 2020; Yan *et al.*, 2017; Yu *et al.*, 2017a)

Class of antibiotics	Antimicrobial	Type/ Bacteria target	ARGs	Chemical formula	Human use	Veterinary use	Abundance in
Sulphonamides	Sulfamethazine Sulfamethoxazole Sulphapyridine	Bacteriostatic /inhibit gram-positive and some gram-negative bacteria.	*Sul1* *Sul2* *Sul3*	$C_{12}H_{14}N_4O_2S$ $C_{12}H_{14}N_4O_4S$ $C_{10}H_{11}N_3O_3S$	Treatment of urinary tract infections (UTIs), pneumonia, eye infections, bacterial meningitis, diarrhoea, and skin problems.	Treatment of bacterial infection includes scours in cattle, coccidiosis in dogs, foot rot in sheep and goats, acute mastitis, and metritis in sheep, goats, and cattle.	Hospital and Agricultural wastewater
Macrolides	Erythromycin Roxithromycin Azithromycin Clarithromycin	Bacteriostatic /inhibit gram-positive but weakly inhibit gram-negative bacteria	*ermA* *ermB* *ermC*	$C_{37}H_{67}NO_{13}$ $C_{41}H_{76}N_2O_{15}$ $C_{38}H_{72}N_2O_{12}$ $C_{38}H_{69}NO_{13}$	Treatment of asthma, cystic fibrosis, soft tissue infection, bronchiolitis, acute lung injury, sepsis, and sexually transmitted diseases.	Used as an animal growth enhancer, controlling some respiratory diseases and treating diarrhea in dogs and cats.	Hospital, Agricultural wastewater, and natural waters
Fluoroquinolone	Ciprofloxacin lomefloxacin Norfloxacin Levofloxacin	Bactericidal/ active against gram-negative but not active against gram-positive bacteria	*qnrA* *qnrB* *qnrC* *qnrD* *qnrS*	$C_{17}H_{18}FN_3O_3$ $C_{17}H_{19}F_2N_3O_3$ $C_{16}H_{18}FN_3O_3$ $C_{18}H_{20}FN_3O_4$	Treatment of respiratory tract, gynaecological infection, pneumonia, chronic bronchitis, tuberculosis, soft tissue, eye bone, and joint infection	To control intestinal infection in livestock	Hospital, agricultural wastewater, and natural water
Aminoglycosides	Gentamicin Amikacin Neomycin Kanamycin A	Bactericidal/act ive against gram-negative but act synergistically against gram-positive bacteria	*ampC* *ampD* *ampR*	$C_{21}H_{43}N_5O_7$ $C_{22}H_{43}N_5O_{13}$ $C_{23}H_{46}N_6O_{13}$ $C_{18}H_{36}N_4O_{11}$	Treatment of bone, urinary tract infection, sepsis, tuberculosis, and pelvic infection.	To treat ear infections in cats and dogs, it is also used to treat gastrointestinal infections in cattle, goats, pigs, and poultry.	Hospital, agricultural, and aquaculture wastewater
Tetracyclines	Tetracycline Oxytetracycline Doxycycline Chlortetracycline	Bacteriostatic/a ctive against both gram-negative and gram-negative bacteria	*tetA* *tetB* *tetC* *tetG* *tetO* *tetM* *tetW*	$C_{22}H_{24}N_2O_{11}$ $C_{22}H_{24}N_2O_9$ $C_{22}H_{24}N_2O_{11}$ $C_{22}H_{23}ClN_2O_{11}$	They treat gastrointestinal, skin, respiratory tract, lymph nodes, severe acne, syphilis, gonorrhoea, etc.	Used in control and prevention of infectious disease in livestock, also used as in-feed antibiotics for growth promotion	Hospital and agricultural wastewater
β-lactam	Penicillin G Cephalothin Amoxicillin Ampicillin	Bactericidal/act ive against both gram-positive and gram-negative bacteria	bla_{TEM} bla_{CMY} bla_{SHV} bla_{DHA}	$C_{16}H_{18}N_2O_4S$ $C_{16}H_{16}N_2O_6S_2$ $C_{16}H_{19}N_3O_5S$ $C_{16}H_{19}N_3O_4S$	Treatment of Pneumonia, respiratory, meningitis infection, scarlet fever, ear, skin throat infections	Treatment of bovine mastitis and other bacterial infection associated with pneumonia	Hospital, Agricultural wastewater, and natural waters

There is an indication that over 25-75 % of these antibiotics form a residue, excreted in urine and faeces metabolites. Also, the discharge of antibiotic residues from pharmaceutical operations is an excellent reason for the consistent detection and high concentration of ARGs in WWTPs and the natural waters (Lu, Zhang, *et al.*, 2020; Su *et al.*, 2017). For example, a study detected three hundred (300) different ARGs from thirty-two (32) WWTPs belonging to seventeen (17) cities in China with an average ARG concentration of 3.2×10^{11} (influent) and 1.79×10^{12} Copies/L (effluents) (Su *et al.*, 2017). These WWTPs receive influents from pharmaceutical industries located in the cities. Another study that investigated the occurrence of resistance genes in natural waters detected ten (10) resistance genes to tetracyclines and two (2) resistance genes to sulphonamides in the Yangtze River Delta in China (Ni *et al.*, 2020). These studies confirmed the high consumption of antibiotics, resulting in resistance among the populace in that environment. According to Jim O'Neil, the occurrence of antibiotic resistance has claimed over 700,000 lives (Neill, 2014). More lives may be affected before 2050 due to the inability to combat ARGs' proliferation and health consequences (Duan, Gao, Zhang, *et al.*, 2020). Studies have shown that single treatment methods have been ineffective in removing ARGs (Li *et al.*, 2020; Qiu *et al.*, 2020). Some of these treatment technologies enrich or enhance the proliferation of ARGs. At the same time, some are very difficult to recover from the water after treatment processes, thus constituting secondary pollutants in the treated water (Anthony *et al.*, 2021; Sanganyado and Gwenzi, 2019a; Shri Prasad and Madhavan, 2013). Therefore, to avoid these deadly predictions on ARG consequences and provide a complete account of ARG removal from wastewater, we suggest adopting two or more different treatment techniques that would work simultaneously during the treatment procedure. Since the single treatment techniques have disadvantages in removing ARGs from wastewater (Teh *et al.*, 2016), such treatment techniques include disinfection, coagulation, advanced oxidation processes, DNA binding with nanoparticles,

and the functionalization or modification of the nanoparticle's techniques described in Figure 2.1. Applying at least two of these techniques one after the other during the treatment process would provide solutions to the global concern on ARGs contaminated water because the single application of these treatment techniques could not account for the total removal of ARGs from wastewater. Also, the high release of ARGs from WWTPs effluent into natural waters promotes the proliferation, dissemination, and development of resistant bacteria in the aquatic and terrestrial environment through horizontal gene transfer (Berendonk *et al.*, 2013; Rizzo *et al.*, 2013). These consequences can be avoided by applying stringent control on the management of ARGs-infected water. ARGs' adverse effects, which causes chronic toxicity, acute diseases, and induction of microbial ARGs could be avoided by adopting these treatment techniques (Figure 2.1). However, the adoption of these proposed treatment techniques will ensure that humans and animals have access to water free from ARG contaminants.

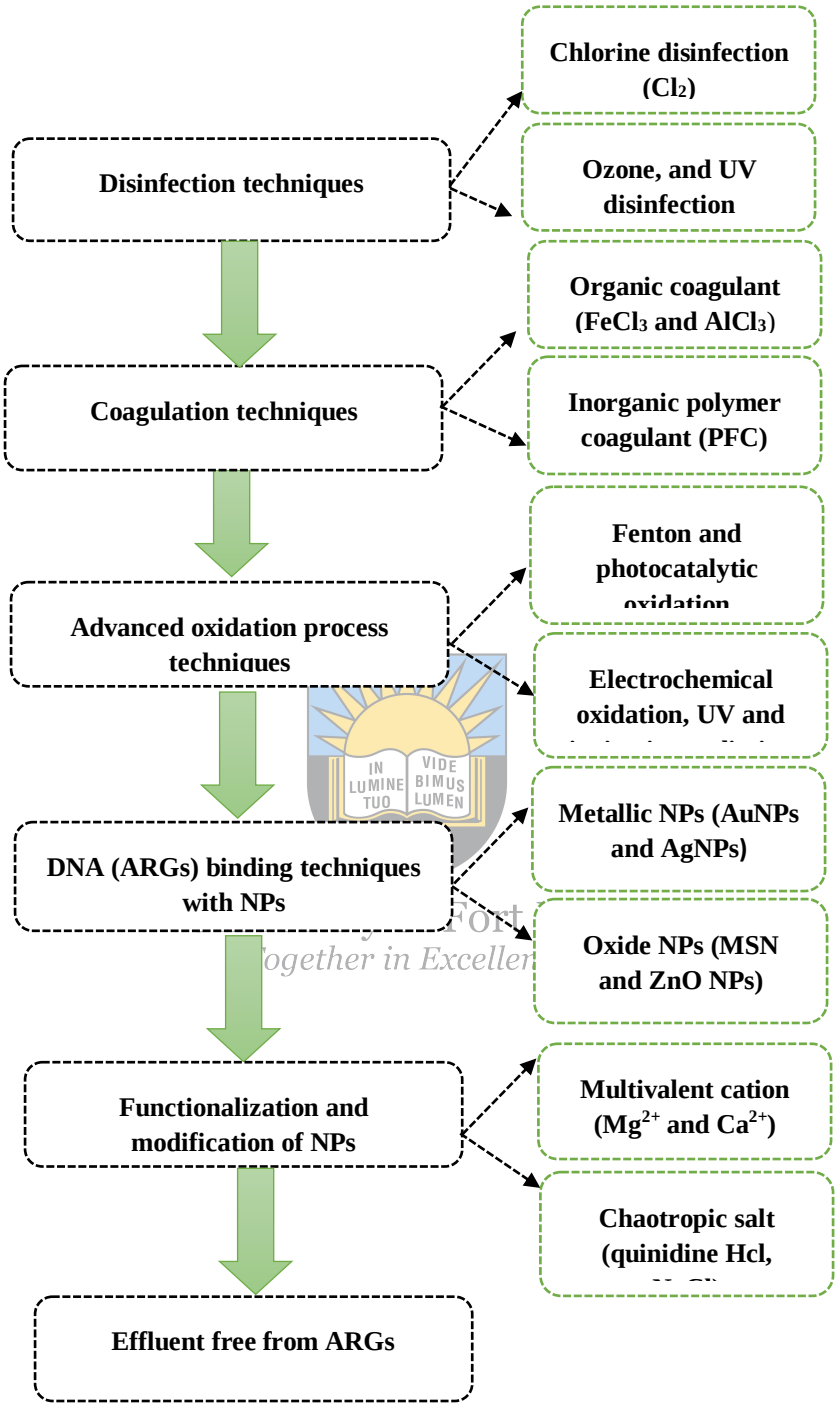

Figure 2.1: A schematic diagram showing the proposed effective techniques for inactivating bacteria DNA followed by damage and elimination of existing ARGs from full-scale WWTPs

From Fig 2.1, the inactivation of ARB harbouring ARGs with disinfection techniques should be considered first during the treatment processes. This is because these techniques will kill

or deactivate ARB from multiplying in their numbers, and previous studies have confirmed that it effectively deactivates ARB (Dodd, 2012). Nevertheless, the existing ARGs may survive even after the deactivation of ARB and may further disseminate in different water matrices (Li *et al.*, 2017b). Because ARGs are carried on an extracellular plasmid or genomics DNA, originating from a competent or non-resistant recipient cell through natural transformation. Natural transformation is typically superficial among the same species, but the interspecies transformation can be achieved within the competent cells (Bello-López *et al.*, 2019; He *et al.*, 2019). It can mediate ARGs transfer to the bacterial genomes temporally or spatial distance from donor cells. Therefore, applying any form of disinfectant will play a vital role in deactivating ARB. Still, the intact ARGs within the cell debris require another treatment technique to eradicate it from water/wastewater. If not, the existing ARGs can replicate and confer resistance to indigenous or downstream bacteria through horizontal gene transfer (HGT) (Khan *et al.*, 2019). In addition, the effective disinfection processes of ARB (chlorination, Ozonation, and ultraviolet irradiation) depends on the irradiation dose rate, the concentration of the material, contact time, and target genes.

The coagulation mechanism is widely recommended for water purification. A tertiary treatment process removes suspended particles, nitrogen, and phosphorous from WWTPs (Kim and Zoh, 2016). Inorganic polyferric chloride (PFC) and inorganic coagulant $FeCl_3$ are the common coagulants used to remove wastewater and drinking water (Tan *et al.*, 2019). PFC comprises a variety of pre-hydrolysed Fe (III) species with high positive charge [49], increasing the interest and are expected to significantly facilitate the removal of ARGs from water/wastewater through electrostatic interaction. Since ARGs are embedded in a harmful bulky DNA molecule, incorporating coagulation techniques during DNA (ARGs) is expected to pull together all the existing ARGs within the water matrices. Then, the advanced oxidation process will assist in disintegrating bulky DNA (ARGs) into smaller molecules,

which could alleviate ARGs' inhibitive effects and enhance biodegradability and the removal rate (Bairán *et al.*, 2020).

Advanced oxidation process (AOPs) is the process in which complex or bulky and harmful organic compounds are converted into smaller and less nontoxic inorganic molecules through hydroxyl radical (HO*) (Deng and Zhao, 2015; Riaz *et al.*, 2020a). AOPs utilizes a high concentration of hydroxyl radical (HO*), sulphate radical (SO_4^-), ozone (O_3), superoxide radical (O_2^-) to disintegrate DNA during the wastewater treatment process. For example, a study that investigated the ARGs removal with functionalized polyvinylidene fluoride (PVDF) ultrafiltration membrane with titanium oxide (TiO_2) nanoparticles shows a significant ARGs degradation in WWTPs (Ren *et al.*, 2018). These forms of techniques function effectively in the disintegration process.

The mechanism of DNA binding to adsorbents through the adsorption process would assist in the removal from wastewater. Most of these adsorbents are readily available and cost-effective. Still, they have some challenges, such as heat resistance, poor mechanical operation, and low adsorption capacity for DNA (ARGs) removal (Liu, Guo, *et al.*, 2019). Due to the low adsorption capacity for DNA removal, functionalization, or modification of adsorbents with cationic linkers would increase ARG removal rate through electrostatic interaction since DNA (the adsorbate) has a high negative charge in its backbone. Therefore, this review critically evaluates the abundance of ARGs in the wastewater treatment plant and natural waters and the five different treatment techniques that would eradicate the existing ARGs and control the proliferation, dissemination of ARGs, and environmental implications of nanomaterials used for ARGs removal.

2.2. Abundance of ARGs in wastewater treatment plants (WWTPs) and natural waters (NWs)

The excessive use of antibiotics to treat human and animal infectious diseases led to the dissemination of antibiotic-resistant bacteria (ARB) and their resistance genes (ARGs) in a water environment. There were reports on the abundance of antibiotic resistance genes (Ben *et al.*, 2017; Devarajan, 2015; Liu, Zhang, *et al.*, 2019) and their fate in wastewater treatment plants and natural waters (Lu, Wang, *et al.*, 2020; Munir *et al.*, 2011; Yuan *et al.*, 2014). WWTPs are vital in disseminating ARGs' concentration in natural waters without restriction (Agunbiade and Moodley, 2014; Guo and Tian, 2019; Hollender *et al.*, 2009). Presently, there are no regulations and guidelines on the concentration of antibiotics molecules released into wastewater treatment plants. The effluents may be discharged into the rivers, seas, oceans, and agricultural farms (Pazda *et al.*, 2019a). Discharging the effluents from wastewater containing ARGs into natural waters and agricultural farms may increase indigenous resistance to bacteria and the genes, including pathogenic bacteria, via horizontal gene transfer, which is a significant threat to humans and animals' lives (Shabana and Al-Enazi, 2020; Wang *et al.*, 2017). The development of multi-drug resistant bacteria (MDRB) such as *Escherichia coli, Klebsiella pneumonia,* or *Staphylococcus aureus* is the greatest threat to human life. They are commonly found among bacteria, causing acquired infectious diseases such as skin, blood, pneumonia, and urinary tract infections (Mao *et al.*, 2015; Pazda *et al.*, 2019a). Many authors have detected ARGs' concentration in many wastewater facilities and natural waters in different countries, class of antibiotics, concentration, and abundance in WWTPs and natural waters as described in Table 2. The WWTPs are designed to eliminate various water pollutants before water reuse or discharge effluents into different watersheds. Still, the continuous discharge of ARGs has changed its design. As a result of this failure, adopting some mechanisms highlighted in this review will eradicate ARGs from water matrices completely.

Table 2.2: Some studies on classes of antibiotics that are widely detected, their concentration, rate of abundance as reported by the authors, different water matrices, and their countries.

Names of ARGs	Class of antibiotics	No of the ARGs detected	Concentration of ARGs Copies/Ml	Rate of abundance	Water matrices	City/ Country	Reference
ampC	Penicillin	4	$6.2\pm3.2\times10^{-9}$	R/H	Municipal WWTPs	Shanghai /China	(Yuan *et al.*, 2014)
ereA	Macrolides		$5.4\pm2.8\times10^{8}$	H			
sul1	Sulphonamide		$1.8\pm1.3\times10^{7}$	L			
vanA	Glycopeptide		$2.2\pm0.8\times10^{-10}$	H			
sul1	Sulphonamide	30	$6.7\pm7.2\times10^{5}$	H	Sewage effluent/ dewretted sludge	Norther/ China	(Mao *et al.*, 2015)
sul2			$2.2\pm2.8\times10^{11}$	L			
ermB	Macrolide		$7.0\pm1.2\times10^{4}$	H			
ermC			$1.2\pm0.9\times10^{10}$	L			
tet genes	Tetracycline		$8.4\pm24\times10^{4}$	R/H			
			$1.3\pm1.6\times10^{10}$	L			
qnr genes	Quinolones		$7.3\pm9.6\times10^{3}$	L			
			$1.5\pm2.3\times10^{9}$	L			
blaCTX-M	B-lactam	3	1.97×10^{-3}	L	Lake of Geneva natural water	Tamil Nadu/ India	(Devarajan, 2015)
blaSHV			1.30×10^{-3}	L			
ampC	Aminoglycoside		3.64×10^{-6}	H			
tetO	Tetracycline	5	$9.7\times10^{4}\pm1.1\times10^{5}$	R/H	Influent of WWTPs	Thuwal/ Saudi Arabia	(Al-Jassim *et al.*, 2015)
tetQ			$8.7\times10\pm1.0\times10^{5}$	R/H			
tetW			$1.8\times10^{5}\pm1.5\times10^{5}$	L			
tetH			$5.6\times10^{4}\pm8.9\times10^{4}$	R/H			
tetZ			$2.2\times10^{5}\pm1.8\times10^{5}$	H			
tetO	Tetracycline	5	$2.5\times10^{2}\pm2.4\times10^{2}$	L	Effluent of WWTPs	Thuwal/ Saudi Arabia	(Al-Jassim *et al.*, 2015)
tetQ			$1.6\times10^{2}\pm1.5\times10^{2}$	L			
tetW			$4.4\times10^{2}\pm4.4\times10^{2}$	H			
tetH			$1.6\times10^{1}\pm1.5\times10^{1}$	R/L			
tetZ			$5.5\times10^{3}\pm4.7\times10^{3}$	R/H			
blaVIM	B-lactam		$5.90\times10^{-5}(H_3)$	H	Hospital wastewater	Cluj	(Szekeres *et al.*, 2017)
			$2.60\times10^{-5}(H_2)$	L			

					1,2,3	County/ Romania
blaSHV			$4.39\times10^{-3}(H_1)$	R/H		
	Aminoglycosides		$1.69\times10^{-3}(H_2)$	L		
			$3.80\times10^{-3}(H_3)$	H		
		16				
aacC₂				H		
			$5.67\times10^{-3}(H_1)$	R/H		
			$9.42\times10^{-3}(H_2)$	R/L		
	Chloramphenicol		$1.00\times10^{-3}(H_3)$			
				R/H		
catA1				L		
			$6.83\times10^{-5}(H_1)$	R/L		
			$5.89\times10^{-4}(H_2)$			
floR			$2.63\times10^{-3}(H_3)$	H		
				L		
ermA			$1.5\times10^{-3}(H_2)$	L		
			$5.0\times10^{-4}(H_3)$			
mefA				R/H		
	Macrolides		$1.89\times10^{-6}(H_2)$	R/H		
				H		
			$1.67\times10^{-3}(H_1)$			
sul1			$1.67\times10^{-3}(H_2)$	R/H		
			$1.47\times10^{-3}(H_3)$	L		
	Sulphonamide			H		
sul2			$1.94\times10^{-1}(H_1)$			
			$5.00\times10^{-2}(H_2)$	R/H		
			$1.50\times10^{-1}(H_3)$	L		
tetA			$5.33\times10^{-3}(H_2)$	R/L		
			$1.94\times10^{-3}(H_3)$	H		
				L		
			$3.46\times10^{-3}(H_1)$			
tetB			$5.05\times10^{-3}(H2)$	L		
			$1.09\times10^{-2}(H3)$	L		
				H		
tetO			$1.40\times10^{-5}(H_1)$			
			$1.40\times10^{-5}(H_2)$	H		
			$2.6\times10^{-5}(H_3)$			
tetC	Tetracycline			H		
			$1.0\times10^{-2}(H_2)$	L		
tetW			$2.5\times10^{-5}(H_3)$	H		
			$1.6\times10^{-3}(H_2)$	H		
			$1.50\times10^{-3}(H_2)$	L		
qacEDI			$1.0\times10^{-3}(H_3)$	R/H		
				H		
	Quaternary ammonium		$5.0\times10^{-2}(H_1)$			
			$1.94\times10^{-2}(H_2)$			
tnpA			$4.89\times10^{-2}(H_3)$	R/H		
				L		
	Transposons related element			H		
			$3.60\times10^{2}(H_1)$			
			$2.11\times10^{-2}(H_2)$			
			$2.60\times10^{-2}(H_3)$			
sul1		9	1.0×10^{6}	R/H		
sul2	Sulphonamide		6.7×10^{5}	R/H	Effluent of WWTPs	(Ben *et al.*, 2017)

tetA *tetC* *tetG* *tetM* *tetO* *tetW* *tetX*	Tetracycline		3.0×10^{4} 4.2×10^{4} 3.5×10^{4} 1.9×10^{4} 1.7×10^{4} 1.0×10^{4} 5.4×10^{6}	H H H L L L R/H		Beijing/ China	
sul1, sul2 *tetQ, tetO, tetW* *Intl 1 gene*		6	5.85×10^{4} to 9.18×10^{4} 1.27×10^{3} to 4.44×10^{4} 8.89×10^{4}	R/H H H	Effluent of WWTPs	Hefei/ China	(Li *et al.*, 2017a)
sul1 *sul2* *ermB* *tetW* *tetA* *tetC* *tetM* *tetB* *qnrS*	Sulphonamide Macrolide Tetracycline Quinolone	8	1.77×10^{-3}(H) 1.18×10^{-1}(G) 6.04×10^{-5}(H) 7.11×10^{-3}(G) 2.53×10^{-5}(H) 4.19×10^{-2}(G) 1.76×10^{-5}(H) 1.23×10^{-2}(G) 6.46×10^{-5}(H) 1.08×10^{-2}(G) 1.31×10^{-4}(G) 5.35×10^{-3}(H) 9.83×10^{-5}(H) 7.97×10^{-3}(G) 4.31×10^{-7}(H) 3.92×10^{-4}(G) 1.67×10^{-6}(H) 9.25×10^{-4}(G)	L L R/H R/H H H L L H L L R/H R/L R/L H H H H	Hospital wastewater and groundwater	Beijing/ China	(Liu, Guo, *et al.*, 2019)
sul1 *tetG* *Intl1* *16S rDNA*	Sulphonamide Tetracycline	4	5.28×10^{2} to 6.3×10^{10} 5.72×10^{5} to 6.5×10^{5} 7.64×10^{1} 9.57×10^{1}	H H R/H R/H	Municipal WWTPs	Nanjing/ China	(Chen, Yin, *et al.*, 2019)
sul1 *sul2* *tetW* *tetX*	Sulphonamide	5	$1.29\pm7.56\times10^{4}$ $8.62\pm8.36\times10^{5}$ 8.44×10^{-3} 4.86×10^{-4}	H L H H	WWTPs	Southern, Northern, and Eastern/	(Zhang *et al.*, 2019)

	Tetracycline			China
intl1		6.08×10^{-1}	H	

L= Low, H= High, R/H= Relatively high, R/L= Relatively low.

Table 2.2 shows the evidence that ARGs are commonly found in wastewater treatment plants and natural waters due to the overconsumption of antibiotics from anthropogenic sources, promoting resistance genes, and polluting natural sources of water. The high concentration of these pollutants reduced access to clean and safe water. There is a need to improve sources of water by eliminating these pollutants from wastewater. Also, information on the consequences of excessive use of antibiotics should be communicated to avoid a high concentration of these pollutants being discharged into the wastewater treatment plant. Some biological, physical, and chemical treatment materials have shown some limitations in removing ARGs from the wastewater treatment plant. Also, combinations of these treatment materials have shown some drawbacks/limitations for ARG removal. Still, they reduce suspended solids, heavy metals, nitrogen, phosphate, and organic matter in wastewater treatment plants (Li *et al.*, 2017a). Such limitations include bacteria and gene enrichment, bacteria growth increase, and even the genetic exchange, which may further lead to ARGs' proliferation among other water bodies (He, Ying, *et al.*, 2016; Jia *et al.*, 2019; Mao *et al.*, 2015; Pazda *et al.*, 2019a). Another limitation is horizontal gene transfer promotion due to high bacteria abundance and diversity from biological treatment materials (Riaz *et al.*, 2020a; Ying *et al.*, 2017).

Furthermore, the discharge of WWTPs effluents containing ARGs can pollute agricultural soil by increasing the indigenous bacteria and developing drug-resistant bacteria in the farm products (Sharma *et al.*, 2016). Therefore, the removal of ARGs from wastewater effluents reduces the dissemination of ARGs in natural rivers. According to the United States of Environmental Protection Agency (USEPA) guidelines on water reuse, traces of antibiotics molecules (organic pollutant) needs to be tackled to reduce the proliferation and

dissemination of ARGs and their health-related problems (EPA, 2012). Healthcare centres and hospitals are facilities with high antibiotic consumption (Rizzo *et al.*, 2019; Windels *et al.*, 2019; Von Wintersdorff *et al.*, 2016). For example, tetracycline genes (*tetM, tetO, tetQ, tetW*) and sulphonamide genes (*sul1, sul2, and sul3*) are common in hospital wastewater due to their consumption rate. Also, studies on the occurrence of antibiotics resistance genes in the hospital wastewater detected a high concentration of *qnrS* (quinolones), *ermB* (macrolides), *sul1* (sulphonamides), and *tetW* (tetracycline) genes (Rodriguez-Mozaz *et al.*, 2015a). The hospital effluents containing ARGs discharged into aquatic environments can make it an ideal site for the occurrence and dissemination of ARGs since antibiotic molecules constantly pollute them (Rizzo *et al.*, 2013; Rodriguez-Mozaz *et al.*, 2015a). Due to the continuous detection in WWTPs and the threat to human and animal lives, it will be of great interest to adopt and apply those five different treatment techniques one after the other during the water treatment process. It will also assist in eliminating the occurrence and proliferation of ARGs in the environment.

2.3. Suggested techniques for ARGs removal from WWTPs

The indiscriminate discharge of antibiotics molecules in WWTPs has raised severe public health concerns because the ecological balance is threatened. Such ecological imbalance promotes the growth of resistant strain, which affects the development of aquatic life, animal, and human lives. It poses persistent pressure on antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Amábile-Cuevas, 2015; Young, 2017). Hence, the occurrence of ARG contamination requires effective techniques to eliminate and stop the proliferation of pollutants. Different treatment technologies with considerable removal efficiencies for ARGs were developed in recent years. Such treatment includes bioremediation, constructed wetland, AOPs, ion exchange, reverse osmosis, disinfection, adsorption, photocatalysis, etc. Still, there are challenges such as high cost, toxic production

by-product, enrichment of ARGs, unable to degrade ARGs, time-consuming, and low removal efficiency (Dantas *et al.*, 2008; Yan *et al.*, 2019). But the ARG removal efficiency with adsorption and nanoparticles is satisfying. Having stated the drawbacks of the application of a single treatment technique earlier, it is essential to consider the application of five different treatment techniques (Disinfection, coagulation, AOPs, DNA binding with nanoparticles, and functionalization of nanoparticles) one at a time to improve the removal efficiencies and eradicate the existing ARGs in WWTPs.

2.3.1. Disinfection techniques

Disinfection techniques and their mechanism are the essential techniques that must be considered first during water treatment processes. Generally, it reduces the number of bacteria and the risk of pathogen transmission in wastewater. Disinfectants are the substances/chemicals that kill, control the spread of pathogens and microbial species during wastewater treatment (Ezeuko *et al.*, 2021a; Zhang, Yan, *et al.*, 2020). Disinfecting water with chemical disinfectant improves the water quality by deactivating the proliferation and activities of resistant bacteria. Selective chemical disinfectants for water/wastewater treatments include a derivative of chlorine such as chlorine gas (Cl_2), chlorine dioxide (ClO_2), hypochlorite (ClO^-), chloramine (NH_2Cl), and ozone (O_3) (Love *et al.*, 2018; Sun *et al.*, 2021). Among all, chlorine, chloramine, and ozone are widely used during wastewater treatment. But for the removal of ARGs, Chlorine, and ozone treatment are reported to increase the propagation of certain ARGs in conventional treatment plants due to resistance due to cell damage repair mechanism and mutagenesis (Amarasiri *et al.*, 2020). Since antibiotics are highly applicable in the treatment of microbial infection in both humans and animals, in the long run, continuous discharge of these antibiotics residue in the water matrices may promote the development of ARGs in bacteria known as ARB. Effective

disinfectants can prevent the inactivation of ARB-bearing ARGs. Some of these disinfectants used for the inactivation of ARB are discussed below:

2.3.2. Chlorine / chloramine disinfection

Chlorine is a chemical disinfectant that inactive bacteria and plays an essential role in preventing ARB proliferation in WWTPs. Chlorine is widely used around the globe. Improving wastewater with chlorine disinfectant for the environment's safety has drastically attracted attention due to its effectiveness in removing traces of organic contaminants (protozoans, fungi, viruses, and bacteria) that have adverse effects on the hormones of mammals (Kunduru *et al.*, 2017). It reduces the bacteria spread, diarrheal incidence and protects the water from recontamination. The action or mechanism of chlorine gas for water treatment is beneficial for pathogen's inactivation. When chlorine gas dissolved in water, it hydrolysed rapidly to hydrochloric acid (HCl) and hypochlorous acid (HOCl), as shown in the equation.

$$Cl_2 + H_2O \leftrightarrow H^+ + Cl^- + HOCl \quad \ldots\ldots\ldots (2.1)$$

Chlorine has solid bactericidal properties that block ARB and pathogens' vital activities with complex mechanisms by destroying the chemical structures of enzymes responsible for bacteria nutrition, inactivating, and inhibiting their life development. The factors that influence chlorine's action during treatment processes include the temperature, pH, and organic content of the polluted water.

Chlorine dioxide is another powerful chemical disinfectant with excessive oxidizing power responsible for its high germicidal potentials (Collivignarelli *et al.*, 2018). It is a water-soluble and volatile chemical with a pungent smell. It is yellowish-green in color, usually produced by sodium hypochlorite and hydrochloric acid (Vandekinderen *et al.*, 2009), as

shown in equation 2.2 Its elimination mechanisms include the inactivation of bacterial enzymatic systems or protein synthesis (He *et al.*, 2019).

$$10NaClO_2 + 8\ HCl \rightarrow 8ClO_2 + 10NaCl + 4H_2O \ldots\ldots\ldots (2.2)$$

Chlorine derivatives have been recorded to be very effective for the inactivation of the ARB activities but less effective in the damage and removal of ARGs (Anastasi *et al.*, 2013; Zhuang *et al.*, 2015). Several studies have shown conflicting reports on chlorine's effects on ARG removal from wastewater (Karaolia *et al.*, 2018; Sanganyado and Gwenzi, 2019b). For example, studies investigating ARGs' removal efficiency with chlorine concluded that chlorine disinfectants could be a promising approach to eradicating ARGs' activation and reactivation in wastewater (Sanganyado and Gwenzi, 2019b; Sharma *et al.*, 2016). Other studies reported the enrichment and increased in ARGs' abundance in wastewater (Shi *et al.*, 2013; Zhang, Zhuang, *et al.*, 2016). According to Wan *et al.*, the potential effects of Chlorine dioxide on ARGs removal from wastewater (Wan *et al.*, 2020). They concluded that it has a positive effect but can enrich ARGs' proliferation after the treatment process. The enrichment of ARGs results from co-resistant bacteria to antibiotics and chlorine during usage (Tan *et al.*, 2019). Also, chlorine can increase the copies of ARGs carrying plasmid in the bacteria cells (Wan *et al.*, 2020). Another limitation is the co-selection of antibiotic resistance, which contributes to ARGs' enhancement and proliferation. For example, Shi *et al.* monitored the effects of chlorine treatment on microbial antibiotic resistance in drinking water using metagenomics sequencing (Shi *et al.*, 2013). They reported that it enriched the abundance of *ampC, tetA, tetG, ermA, and ermB.* The study of Liu *et al*. investigated the effects of chlorine disinfection on the occurrence of extracellular ARGs (eARGs) and intracellular ARGs (iARGs) in an urban wastewater treatment plant for one year (Liu, Zhang, *et al.*, 2019). They reported that the concentration of both eARGs and iARGs increased by disinfection with chlorine dioxide (ClO_2). From their study, chlorine specifically increased the abundance of

eARGs against macrolide (*ermB*), tetracycline (*tetA, tetB* and *tetC*), sulfonamide *(sul1, sul2,* and *sul3*), b-lactam *(ampC),* aminoglycosides (*aph(2')-Id*), rifampicin (*katG*) and vancomycin (*vanA*) up to 3.8 folds. Also, the abundance of iARGs increased up to 7.8 folds after the application of treatment material. This scenario confirmed that the occurrence of iARGs promotes ARB dissemination via conjugation and transduction processes. Simultaneously, eARGs can be taken up by non-competent resistant bacteria found in the biofilm and sedimentation (Liu *et al.*, 2018). Infact, disinfection mechanism can kill resistant bacteria during treatment process, but simultaneously DNA associated with ARGs may be released into the treated water were existing extracellular antibiotic resistance gene (eARGs) present in free living DNA. Since the application of chlorine disinfectant alone for the removal of ARGs is ineffective but effective in inactivating bacteria or pathogens. It is advisable to inactivate the activities of ARB with derivative of chlorine disinfectant before the application of other treatment techniques that would assist in the removal of already existing ARGs or stop the process of ARGs growth in wastewater.

Chloramine is an alternative disinfectant to chlorine because of the reduction in the formation of DBPs during treatment processes. Due to less formation of DBPs, many water treatment utilities are considering using chloramine to ensure regulatory compliance of approved DBPs for treated water (Bougeard *et al.*, 2010). It is a chemical compound produced by reacting ammonia with active ingredients in chlorine bleach (Mattila *et al.*, 2020). It is a weaker oxidant than chlorine, less volatile, reactive with organic matter, and more stable in water than free chlorine. Chloramine is very effective in the deactivation of ARB and removal of ARGs than free chlorine. A study that compared the deactivation of ARB and the removal efficiency with different chlorination chemicals reported that chloramine was more effective in ARB inactivation and performed better in removing ARGs than free chlorine (Gomez-Alvarez *et al.*, 2012). Chloramine may be a more appropriate disinfection technique for

deactivation of ARB and removing ARGs from various water matrices. Notwithstanding, further experimental assessment is required to investigate if the nature of water matrices would enhance the formation of toxic iodinated DBPs during chlorination of ARB.

2.3.3. Ozone / UV Disinfection

Ozone disinfection is another technique that can damage bacterial DNA during the wastewater treatment process. Its oxidizing power is higher than the chlorine disinfectant. It is a substance capable of controlling the spread of ARB with the help of hydroxyl radicals. An efficient process that degrades and reduced facultative pathogenic bacteria in water. Ozone is generated by electrolysis, photolytic, and radiochemical reaction induced by electric shock (Collivignarelli *et al.*, 2018). It is a hazardous gas that discharges fast when produced, as shown in equation 2.3. The principle of Ozone disinfection that facilitates the reduction or inactivation of pathogens is the production of high reactive radicals (Dodd, 2012; Zhuang *et al.*, 2015), as shown in equations 2.4-2.6. For the significant production of hydroxyl radicals in water by ozone, the alkalinity or the concentration of pollutants must be high. Equations 2.4-2.6 described the production of reactive oxygen species by ozone.

$$O_3 \rightarrow O_2 + O^{\cdot} \quad \text{..... (2.3)}$$

$$O_3 + HO^- \rightarrow HO_2^- + O_2 \quad \text{..... (2.4)}$$

$$O_3 + HO^-_2 \rightarrow HO_2^{\cdot} + O_3^- \quad \text{.... (2.5)}$$

$$O_3^- + H^+ \rightarrow HO_3 \quad \text{..... (2.6)}$$

It deactivates antibiotic bacteria activities by breaking their functional sites such as aniline moieties of sulfonamides, thioether group of penicillin, unsaturation bonds of cephalosporin, and phenol ring trimethoprim (Riaz *et al.*, 2020b, 2020a). The ozone mechanism includes destroying the bacteria cell wall, followed by cellular constituents' leakages and DNA

damage (Alexander *et al.*, 2016). The ozone inactivation efficiency depends on the target bacteria's susceptibility, the concentration of ROS generated, and contact time (Michael-Kordatou *et al.*, 2018). There is an indication of its effectiveness in inhibiting bacterial DNA, but the existing ARGs could remain in the treated water. Several studies reported the removal of ARGs with ozone in full-scale WWTPs. Czekalski *et al.* (2015) found that ozone doses do not remove the intracellular ARG from wastewater after the treatment process. A study on the use of ozone for inactivation of gene copies of *sul(1), tet(G), intI(1),* and 16S rDNA stated that ozone reduces 16S rDNA, but ARGs were not inactivated (Zhuang *et al.*, 2015). Also, Macauley et al. (Macauley *et al.*, 2006) investigated the effect of chlorine dose, ultraviolet light, and ozone against swine lagoon bacteria. The result indicated that the chlorine dose and ultraviolet light were effective than ozone. The significant drawbacks of ozone disinfectants are dangerous by-products, high concentration during the treatment process, operational and maintenance costs. Therefore, removing ARGs with ozone alone is inefficient and helpful in reducing antibiotics and bacteria in wastewater (Liu *et al.*, 2014; Marcelino *et al.*, 2017). As a result of these reports, it is advised that ozone treatment should be considered as another disinfectant mechanism for ARB's inactivation before applying other mechanisms that will draw all the existing ARGs together for easy removal.

Ultraviolet (UV) disinfection is another alternative to chlorine or ozone due to its capacity to inactivate a broad spectrum of bacteria (Guo *et al.*, 2012). Many water utilities widely adopt it due to the inability to form hazardous or toxic by-products, easy operation, and environmentally friendly. UV lamp emits radiation in the range 240-260 nm. Nucleic acids (DNA and RNA) can absorb energy at this range, damaging bacteria DNA [108], ensuing pyrimidine dimers responsible for changes in DNA structure. This scenario contributes to the elimination of ARB and ARGs (Zhou *et al.*, 2020). According to Umar et al.(Umar *et al.*, 2019a) UV irradiation inactivates bacteria with short-wavelength, known as UV-C. Previous

studies reported that UV irradiation could considerably inactivate ARB and remove ARGs at a very short wavelength (Guo *et al.*, 2015; Zhuang *et al.*, 2015). A study investigated the UV influence and reactivation of ARB on the conjugative transfer of survival bacteria after-treatment process. The experimental data generated proved that one mJ/cm^2 UV disinfection could decrease the conjugative transfer of the survived bacteria. The viable cells induced after treatment can be recovered through photoreactivation and dark repairs (Guo and Tian, 2019). In the meantime, reactivation such as photoreactivation and dark repair can mend the damaged DNA, thereby weakening efficiency and promoting the microbial risk of UV disinfection procedures (Chen *et al.*, 2020). The major challenge of UV disinfection is a short wavelength, energy consumption, cost-effectiveness, and the ability to recover and repair damaged DNA. Due to these challenges, other treatment techniques such as coagulation should be applied immediately after the treatment procedure to prevent the reactivation of ARB and DNA repairs.

2.3.4. Coagulation techniques

Coagulants are widely used as tertiary treatment materials that remove colloidal suspended particles, natural organic matters, and phosphorous. It is simple, efficient, and extensively used for the removal of various contaminants from wastewater. The principle aim of coagulation involves aluminium sulphate (alum) to cause the particles to settle in water before being removed from water (Teh *et al.*, 2016). Due to their effective removal of emerging contaminants, which improves water quality, an interest in ARGs' fate during wastewater treatment with coagulants is growing. The inorganic coagulant and inorganic polymer coagulants are prepared based on the contaminants (Barancheshme and Munir, 2018). For effective removal of any contaminants with a coagulant, the contaminants must possess either negative (Barancheshme and Munir, 2018; Sebok *et al.*, 2020) that will be neutralized by a positive coagulant, destabilizing the forces keeping the colloid apart. The

coagulation techniques are induced by inorganic metal salts such as $Al_2(SO_4)_3$ (aluminium sulphate), $AlCl_3$ (aluminium chloride), $NaAlO_2$ (sodium aluminate), $Fe_2(SO_4)_3$ (Iron (III)sulphate), $FeCl_3$ (ferric chloride), and PFC (polyferric chloride). Among these coagulants, the inorganic coagulant ($FeCl_3$) and inorganic polymer coagulant (PCF) are widely used for the removal of ARGs from wastewater because PFC consists of pre-hydrolysed Fe (III) species with high positive charges that can facilitate the removal of ARGs (Sebok *et al.*, 2020; Tan *et al.*, 2019). The coagulation involves the electrostatic interaction between a positive charge coagulant and a negatively charge contaminant (Lee *et al.*, 2014; Pivokonsky *et al.*, 2015). Figure 2 shows the mechanisms of coagulation during the removal of DNA conveying ARGs from wastewater. The action mechanisms involve rapid mixing of dispersed coagulants in water/wastewater via vigorous agitation followed by flocculation of accumulated small particles into well-defined flocs via gentle agitation. The flocs are then allowed to be settled and removed as sludge from treated water (AlMubaddal *et al.*, 2009; Teh *et al.*, 2016)

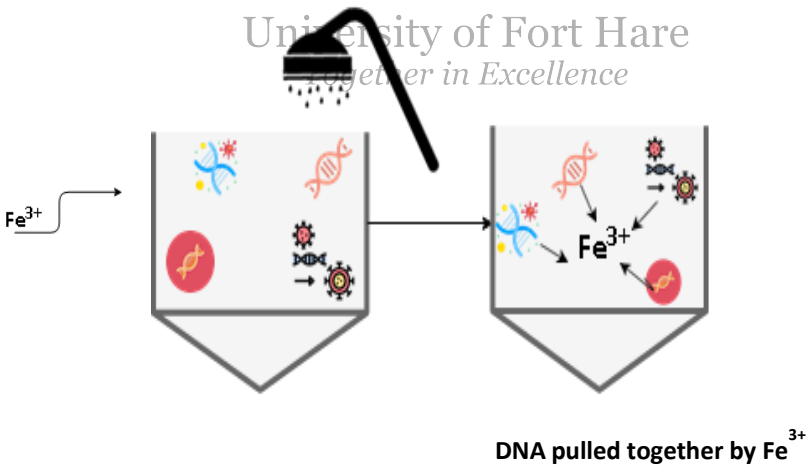

Figure 2.2: The coagulation mechanism

Although the coagulant is time-consuming in large-scale applications, they are cost-ineffective and may help draw all the existing ARGs in water. Therefore, incorporating it during the removal process may assist in pulling together all the existing DNA harboring ARGs and getting it ready for the disintegration process. Afterward, the other techniques will

be applied to facilitate removing these contaminants from water or wastewater. The application of coagulant alone has proven ineffective in the removal of DNA harboring ARGs from wastewater. However, few researchers have monitored the removal efficiency of ARGs with an inorganic coagulant ($FeCl_3$) and inorganic polymer coagulant (PCF) and are restricted (Li *et al.*, 2017a; Tan *et al.*, 2019; Zhuang *et al.*, 2015). For example, Li and co-workers investigated the removal efficiency of $FeCl_3$ and PFC coagulants on two *sul* genes, three *tet* genes, and integrase genes in the influents and effluents of wastewater (Li *et al.*, 2017a). From their study, ARG removal efficiency ranging from 0.5 to 2log removal was observed. No further removal was observed by increasing the dose of $FeCl_3$; an increase in PFC dose noticed slight removal. Another study reported that, for the effective removal of ARGs with coagulants, the ARG concentration must not be less than 1.15-log to 2.46-log (Tan *et al.*, 2019), which is seen as a constrain of these treatment materials. Besides, the solution, coagulant dosage, mass, pH, the turbidity of the water, time of flocs, and property of contaminants are the significant factors affecting ARGs' removal efficiency coagulants. Therefore, it is a suitable material that would bring the DNA molecules together for easy disintegration into smaller molecules, which would enhance the removal efficiency from wastewater.

2.3.5 Advanced oxidation processes (AOPs)

The advanced oxidation process (AOPs) has received considerable attention as an effective wastewater treatment method in the environmental research domain. It is another technique that should be adopted after coagulation techniques and can assist in the removal of ARGs from wastewater. It functioned effectively in the disintegration of contaminants into smaller and nontoxic molecules through the help of hydroxyl radicals (HO*) (Riaz *et al.*, 2020a). Since the DNA is a bulky molecule with a highly negative charge in its backbone (Sahoo *et al.*, 2010). Applying any type of AOPs will effectively aid in the disintegration and remove

contaminants from water/wastewater. AOPs are highly recognized for removing organic and recalcitrant pollutants. It aims at improving wastewater effluents for human and animal consumption as well as irrigation purposes. AOPs, a chemical treatment process designed to remove organic and emerging pollutants from wastewater by oxidation of hydroxyl radicals (Wang and Zhuan, 2020). The effectiveness of AOPs in wastewater treatment depends on free radicals' production as a potent oxidizing agent (Deng and Zhao, 2015). The free radical species are atoms or molecules containing one or more unpaired electrons. Such free radicals are AOPs superoxide radicals (O_2^-), hydroperoxyl radical ($HO_2^{\cdot}$), hydroxyl radical (HO*), and alkoxyl radicals ($RO^{\cdot}$) (Wang and Zhuan, 2020). Among these radicals, hydroxyl radicals (HO*) play a vital role in wastewater treatment involving AOPs. These potent oxidation agents can be produced through different classes of AOPs. They include Fenton oxidation, photocatalytic oxidation, electrochemical oxidation, ionization radiation, and so on.

Several studies have utilized AOPs successfully for the degradation of different pollutants in water (Michael-Kordatou *et al.*, 2018; Wang and Xu, 2012; Zhang, Zhuang, *et al.*, 2016). Studies also stated that AOPs disintegrate and damage DNA associated with ARGs during wastewater treatment (Iakovides *et al.*, 2019). But unable to eradicate the debris from water or wastewater. Therefore, combining it with other treatments mentioned in Figure 1 will successfully remove ARGs. Before the selection of AOPs treatment processes for wastewater treatment, specific factors should be considered. Such factors are characteristic of wastewater, technical applicability, regulatory requirements, economic aspects, and long-term environmental impacts (Egle *et al.*, 2016). Therefore, applying AOPs with their mechanisms would damage and disintegrate the large DNA molecule into smaller molecules, which would enhance DNA binding through the adsorption process.

2.3.6. Fenton and photo-Fenton oxidation

Fenton and photo-Fenton oxidation is a method from AOPs that effectively disintegrates hazardous organic pollutants in wastewater with potent oxidation agents such as hydroxyl radical (HO*). Fenton's reaction was first described by Fenton, who observed the oxidation of tartaric acid by hydrogen peroxide in the presence of ferrous ions (Fenton, 1894). The action mechanism involves the combination of ferrous salt (Fe^{2+}) and hydrogen peroxide (H_2O_2) to form hydroxyl radicals (HO*), and Fe^{2+} catalyst can be regenerated as shown in Equations 2.7-2.12.

$$OH + {}^{\cdot}OH \rightarrow H_2O_2 \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.7)$$

$$Fe^{3+} + H_2O_2 \leftrightarrow Fe\,(OOH)^{2+} + H^+ \ldots\ldots \quad (2.8)$$

$$Fe\,(OOH)^{2+} \rightarrow Fe^{2+} + O_2 + H^+ \ldots\ldots\ldots \quad (2.9)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH^- + HO^{\cdot} \ldots\ldots \quad (2.10)$$

$$^{\cdot}OH + H_2O_2 \rightarrow HO_2 + H_2O \ldots\ldots\ldots\ldots \quad (2.11)$$

$$2\,{}^{\cdot}OH \rightarrow H_2O_2 \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.12)$$

Fenton reaction is propagated by reducing Fe^{3+} with H_2O_2 to regenerate $Fe^{2+,}$ as shown in equation (2.10), followed by the reaction of Fe^{2+} with H_2O_2 to form hydroxyl radicals ($HO^{\cdot}$**)**, which could oxidize or disintegrate bacterial DNA harbouring ARGs (Chen *et al.*, 2020). Both Fenton and photo-Fenton processes are adequate for the inactivation of ARB and disintegration of ARGs (Duan, Gao, Zhang, *et al.*, 2020; Sharma *et al.*, 2016; Zhuang *et al.*, 2015). Rubio et al. (Rubio *et al.*, 2013) reported photo-Fenton efficiency using 1 mgL^{-1} and 10 mgL^{-1} for the inactivation of *E.coli* and disintegration of ARGs from seawater. The fastest bacteria inactivation was observed. Disinfection with photo-Fenton under UV_{254} recorded a high rate of bacteria inactivation. This study confirms that photo-Fenton's reaction with UV-Vis radiation would increase the bacteria's inactivation rate by providing additional hydroxyl

(HO*) radicals (Chen *et al.*, 2009). Their degradation efficiency depends on temperature, the concentration of H_2O_2 and $Fe^{2+,}$ and pH. The pH is necessary because iron chemistry requires that the Fenton reaction be carried in acidic conditions (pH ≃ 3-4) to avoid hydroxide precipitation (Bokare and Choi, 2014). An increased rate requires a higher temperature to increase the decomposition of H_2O_2 into O_2 and H_2O in temperature (Egle *et al.*, 2016). This treatment method has shown some significant advantages, such as iron availability and its non-toxicity nature. Also, hydrogen peroxide is easy to handle. Fenton process can inactivate ARB, damage bacteria DNA by mutation, and strand breaks, but cannot eliminate ARGs from wastewater (Wang and Xu, 2012). Thus, Michael et al. (Michael *et al.*, 2019) suggested that a combination of Fenton with UV radiation should be considered for recalcitrant ARGs.

2.3.7. Photochemical oxidation

Photochemical oxidation based AOPs have appeared as an attractive option for ARG disintegration. Recent studies on eliminating ARB/ARGs in WWTPs and NW following the photochemical oxidants are reviewed extensively (Barancheshme and Munir, 2018; Riaz *et al.*, 2020a). Among the various treatment materials, photochemical oxidants have attracted considerable attention because of their capacity to degrade organic pollutants in water, and they are eco-friendly. The action mechanism of this treatment technique involves the use of semiconductor catalysts shown in Table 4 to generates reactive oxygen species (ROS) such as hydroxyl radicals (HO*), oxygen radicals ($O_2^{\cdot}$) in solution through the process of photocatalysis (Riaz *et al.*, 2020a). In aqueous solution, $HO_2^{\cdot}$ and $O_2^{\cdot}$ are generated rapidly from the primary active species as described in Equations 2.13 - 2.14. The oxidation (HO*****, HO_2, $O_2^{\cdot}$) and reduction species ($H^{\cdot}$,e^-_{aq}, $HO_2^{\cdot}$,) produced makes it practical for the degradation/disintegration of pollutants, treatment of oxidizable compounds such as chlorinated and fluorinated hydrocarbon in water (Hermosilla *et al.*, 2014; Litter, 2005). This technique is highly efficient because the VUV lamps possess high radiant power of

illumination. It does not require adding a chemical agent but an oxygen supply with quartz with high provision power (Litter, 2005).

$$O_2 + H^{\bullet} \rightarrow HO_2^{\bullet} \qquad K_{HO2}^{\bullet} = 1\times1010M^{-1}\ s^{-1} \ldots\ldots\ldots (2.13)$$

$$O_2 + e^-_{aq} \rightarrow O_2^{\bullet} \qquad K_{O2}^{\bullet} = 2\times1010M^{-1}\ s^{-1}\ldots\ldots\ldots\ldots (2.14)$$

Thus, allowing the radicals to efficiently degrade pollutants such as antibiotics molecules, organic and micropollutants from wastewater (Karaolia *et al.*, 2018; Riaz *et al.*, 2020a). In the form of a catalyst, these semiconductors must have two energy bands: the valence band and a conduction band to generate HO^* radical. The first band represents the low energy, and the latter represents the high energy. HO*, radical's primary role is the ability to kill bacteria cells in solution due to its half-life and is approximately between 10^{-6} to 10^{-9} seconds, and it's highly reactive (Li, Xu, *et al.*, 2019; Vatansever *et al.*, 2013). They are also considered the best attribute to wastewater treatment due to their usual non-selective attack on contaminants in solutions (Malato *et al.*, 2009). Molecules of antibiotics and ARB found in wastewater can experience degradation with HO* radicals generated in solution under sunlight and artificial visible light (UV light) (Li, Xu, *et al.*, 2019). These semiconductor catalysts include titanium dioxide (TiO_2), zinc oxide (ZnO), strontium titanium trioxide ($SrTiO_3$), iron (III) oxide (Fe_2O_3), cadmium sulphide (CdS), and zinc sulphide (ZnS) with a noticeable bandgap. These catalysts become photocatalysts when they absorb a specific wavelength from the light spectrum. Among the photocatalysts, TiO_2 is extensively applicable in the degradation of various contaminants, including the inactivation and removal of ARB/ARGs (Guo and Tian, 2019; Pereira *et al.*, 2014). The broad bandgap enables it to inhibit microorganisms' growth and disintegrate the DNA strand found in wastewater (Karaolia *et al.*, 2018; Mccullagh *et al.*, 2007). Besides, the UV irradiation of TiO_2 generates reactive oxygen species (ROS), which

would attack microorganism's organic components (DNA, RNA, cell membrane, lipids, and protein), leading to cell death (Ren *et al.*, 2018; Vatansever *et al.*, 2013).

Furthermore, HO* radicals can be produced by combining photolysis and ozone products such as H_2O_2/UV, O_3/UV, and H_2O_2/O_3/UV (Malato *et al.*, 2009; Michael *et al.*, 2019). The group researchers investigated recent studies on photocatalysis potentials by TiO_2 combined with H_2O_2 as an alternative treatment technology for ARB/ARGs in water (Guo *et al.*, 2017). The study reported that TiO_2 achieved less than 0.2 log reduction of ARB but recorded a 4.5 to 5.0 log removal on TiO_2/H_2O_2 of 12 mJ/cm^2 UV_{254} fluence dose. For the ARGs, 0.12 log removal was observed in the presence of TiO_2 and TiO_2/H_2O_2, and log removal of 4.7 to 5.8 on 120mJ/cm^2 UV_{254} fluence dose at 480mins. The study shows that TiO_2 and TiO_2/H_2O_2 are not active and required much time during ARG removal from wastewater. Another study by Ferro *et al.* on total inactivation of ARGs (bla_{TEM}) using UV/H_2O_2 treatment discovered that bla_{TEM} genes are still present after 300 mins (Ferro *et al.*, 2017). These conflicting results proved that photochemical oxidants are limited in their potential to remove ARGs but useful in disintegrating ARGs in wastewater. Alternatively, ZnO can be used to remove ARB/ARGs due to its low cost and light absorbance capability in the broad UV spectrum range from 254-380 nm (Kunduru *et al.*, 2017; Wan *et al.*, 2020). Therefore, the photochemical oxidation process is an alternative method of disintegrating DNA associated with ARGs in wastewater. Applying this AOPs based method will enhance the removal of existing ARGs and inactivate the bacteria DNA present in the wastewater treatment plant.

2.3.8. Electrochemical oxidation

The electrochemical oxidation (EO) process is another option that can disintegrate bacteria DNA by producing reactive species via electricity. EO's uniqueness is producing reactive species via electricity without chemicals and secondary waste (Garcia-Segura *et al.*, 2018). According to Sires and Barillas (Sirés and Brillas, 2012), electrochemical oxidation is

classified into electrochemical separation (electrodialysis and electrocoagulation) and degradation technology (anodic oxidation). The production of hydroxyl radicals involves two mechanisms; direct oxidation at the anode (direct charge transfer occurs between the pollutant and the anode surface) and indirect oxidation, generating reactive oxygen species (ROS) as oxidant at the surface of the electrode via in-situ (Feng *et al.*, 2013; Kanakaraju *et al.*, 2018). An active oxidant such as oxygen in the oxide lattice and hydroxyl radical can be generated at the anode region when immersed in water. Furthermore, the oxidant can be chlorine, hypochlorous acid, hypochlorite (Rajkumar *et al.*, 2007; Rajkumar and Kim, 2006), ozone, and hydrogen peroxide (Chu *et al.*, 2012; Yang *et al.*, 2018) forming at the electrode, as shown in equations 2.15 - 2.21.

$$Cl_2 + 2e^- \rightarrow 2Cl^- \quad \text{(2.15)}$$

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad \text{(2.16)}$$

$$HOCl \rightarrow H^+ + OCl^- \quad \text{(2.17)}$$

$$H_2O \rightarrow HO^* + {}^*H + e^- \quad \text{(2.18)}$$

$$HO^* \rightarrow H_2O_2 \quad \text{(2.19)}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad \text{(2.20)}$$

$$O_2 + {}^*O \rightarrow O_3 \quad \text{(2.21)}$$

From the equations, indirect oxidation occurs when active chlorine species are generated from chloride ions in the anode region, destroying pollutants (Särkkä *et al.*, 2015). In the electrochemical oxidation process, metal ions oxidized on the anode region from a stable state to a more reactive valence state, which can attack pollutants directly by producing free

radicals that can promote degradation (Chen *et al.*, 2020). However, EC's fundamental idea is the occurrence of redox reaction at the anode and cathode, facilitating the disintegration of pollutants from wastewater (Garcia-Segura *et al.*, 2018), as shown in figure 4. There are popular conventional anode materials that play a vital role in the degradation of pollutants. Such anodic materials platinum (Pt), iridium (IV) oxide (IrO_2), or Lead (IV) oxide (PbO_2), and boron-doped diamond (BDD). They have good corrosion stability, high over-potential oxygen that generates more hydroxyl radical (HO***),** and inert surfaces (García-Espinoza *et al.*, 2018; Kanakaraju *et al.*, 2018)**.** BDD is a non-active anode material that exhibits high oxygen over-potentials, achieving oxidation of pollutants by electrochemical step mediated by physiosorbed hydroxyl radicals. This potential makes it useful in the degradation of pollutants in wastewater. Then, active anode materials such as IrO_2 or RuO_2 exhibit lower oxygen potential but have sufficient oxidation; thus, the surface of these materials can undergo oxidation by limiting the accumulation of HO* radicals. Recently, EC has been applied extensively to treat wastewater, drinking water, and soil remediation (García-Espinoza *et al.*, 2018; Li *et al.*, 2020; Särkkä *et al.*, 2015). The techniques are relatively simple and useful in the degradation of harmful organic pollutants and ARB's inactivation. The benefit of anodic oxidation over indirect oxidation is the addition of no chemical in the treatment of wastewater and no secondary contaminants after the treatment processes. A limited number of studies investigated the effects of electrochemical oxidation in removing ARGs on the laboratory-scale study (Bharath *et al.*, 2017; Kanakaraju *et al.*, 2018). However, HO* generated through electrical supply can disintegrate bulky DNA (ARGs) and making it easier for it to bind to the surface of nanoparticles which would assist in withdrawing the smaller molecules from water or wastewater.

However, the pH values and contact time have proven to play an essential role during electrochemical oxidation processes.

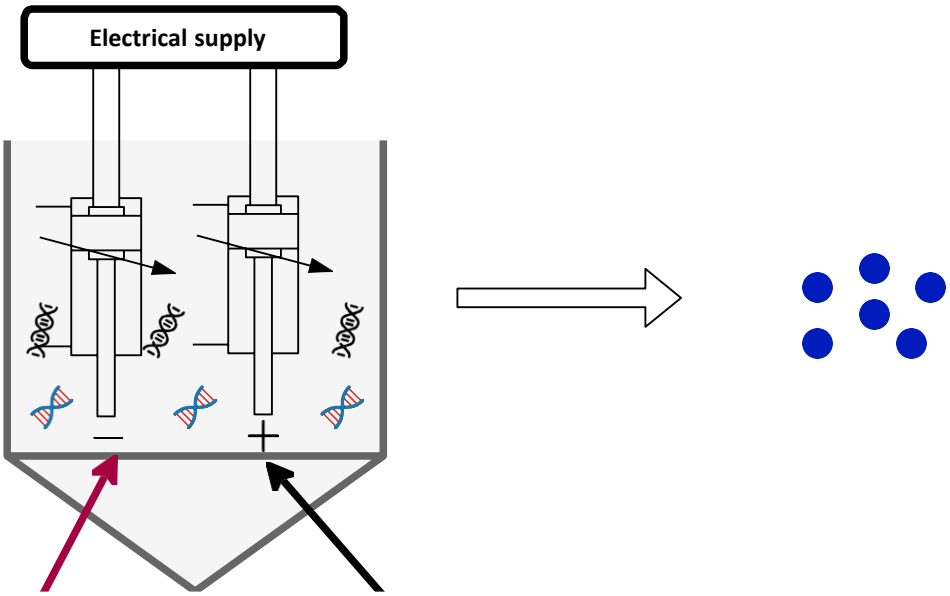

Figure 2.3: Mechanism of electrochemical oxidation processes

For example, Chu et al. degraded 2,4 dichlorophenol by anodic oxidation in pH values of 3.0, 6.0, and 9.0 (Chu *et al.*, 2010). Degradation efficiency reached 100% in 240 mins for pH value 6.0 and 9.0 but 60% for pH 3.0. Also, Chen et al. (Chen *et al.*, 2020) monitored EC and electron-Fenton processes' potential as an alternative option for ARB inactivation and removal of iARGs and eARGs. The result showed that EC was effective for ARB inactivation but less effective in removing iARGs and eARGs. Therefore, this treatment method would assist in inactivating and control the spread of ARB. The high ROS at the anodic oxidation with the effects of pH would assist in the degradation or breakup of the DNA (associated with ARGs) molecules drawn together by the coagulation mechanism during wastewater treatment.

2.3.9. Ionization irradiation

Ionization irradiation-based AOPs is another technology that can degrade ARGs directly or indirectly through hydroxyl radical generated by the gamma-ray and electron beam. Studies have proven its effectiveness in degrading various toxic organic pollutants in wastewater (Ságiet *al.*, 2016; Szabó *et al.*, 2016; Wojnárovits and Takács, 2017). In wastewater or aqueous solution, gamma-ray radiates and excites water molecules to produce reactive species, thus demonstrating the radiolysis of water with chemical radiation yield, as shown in the equation (2.22).

$$H_2O \rightarrow HO^* (2.7) + e_{aq}^- (2.6) + {}^*H (0.55) + H_2 (0.45) + H_2O_2 (0.71) + H_3O^+ (2.6) \ldots\ldots (2.22)$$

The chemical radiation yield represented in the brackets indicates the number of molecules formed per energy (100ev) of the absorbed pH range of 6.0-8.0 (Wang *et al.*, 2019; Wang and Chu, 2016). From the equation, two main reactive species responsible for the decomposition /degradation of organic pollutants are the hydroxyl radical (HO*) (2.7V) with oxidation potential and solvated electron (e_{aq}^-) with the yield value of 2.9V reduction potential. The HO* radical plays a vital role in oxidative conditions, while the solvated electron plays a reductive condition. Thus, the interaction of oxidizing and reducing species leads to the degradation of a wide range of pollutants. These reactive species' contribution to the degradation of toxic pollutants by gamma radiation depends on irradiation conditions (Shah *et al.*, 2020). The parameters that can positively influence ionization radiation efficiency include absorbed dose, irradiation dose, initial concentration of the pollutant, pH, inorganic anions, and organic matter (Sayed *et al.*, 2016; Wang *et al.*, 2019). The pollutant concentration decreases exponentially with the absorbed dose during the degradation of pollutants, and the degradation efficiency is determined by Pseudo first-order kinetic model represented in equation 17 (Rivas-Ortiz *et al.*, 2017; Wang *et al.*, 2019).

$$C = C_0 \, ^{e\text{-}KD} \ldots\ldots\ldots\ldots (2.23)$$

Where C = the concentration of pollutant after radiation

C_0 = the initial concentration before radiation

K = dose constant and

D = absorbed dose.

Ionization radiation has been applied extensively in the wastewater treatment process. Recent studies have shown effectiveness in the degradation of antibiotics with the effects of different parameters mentioned above (Mousavi *et al.*, 2019; Rivas-Ortiz *et al.*, 2017; Wang and Chu, 2016) compared to other AOPs based treatment technology. For example, Murtaza et al. investigated the degradation of ciprofloxacin in an aqueous solution, and the result showed that it degrade entirely at an absorbed dose of 870 Gy (Sayed *et al.*, 2016). Also, Wang et al. monitored amoxicillin, ofloxacin, and cefradine and concluded that these antibiotic's degradation efficiency increases with increased pH value (Wang *et al.*, 2017). A Study that monitored ARG removal with ionization radiation showed effective inactivation of ARGs but less effective in the total removal of existing ARGs in water (Shen *et al.*, 2019; Spotheim-Maurizot and Davídková, 2011). During the inactivation of ARGs, hydroxyl radical (HO*) and solvated electron (e_{aq}^-) are formed when ionization radiation interacts with the cellular water, reacting with biological molecules and facilitating the damage or degrade DNA bases and sugar (Spotheim-Maurizot and Davídková, 2011). The molecular modelling calculations show that the DNA region with a strong negative charge can bind to the positive charge molecules through electrostatic interaction (Spotheim-Maurizot and Davídková, 2011). Their binding masks and destroys DNA bases whose abstraction by HO* radicals would lead to strand breakage. This process would enhance the removal of ARGs from wastewater through DNA binding mechanisms with nanoparticles.

2.3.10. Application of DNA binding techniques and functionalization of nanoparticles improves the removal of ARGs from water/wastewater

The DNA binding techniques and their ARG removal mechanism effectively control disease or alter cellular activity in wastewater. Elimination of ARGs from wastewater has become a realistic goal within the domain of environmental chemistry. Exploring materials capable of binding DNA has resulted in nanoparticles that effectively remove ARGs through the adsorption process. The binding mechanism of DNA can be divided into covalent and non-covalent interactions.

Covalent interaction involves sharing pair electrons and can be most robust in modifying DNA molecules through alkylation. DNA alkylation involves adding an alkyl group to the DNA bases, resulting in alkylation products such as O^2-alklythymine, O^4-alklythymine O^6-methylguanine, and O^6-ethylguanine (Tosal *et al.*, 2000). Alkylation can damage DNA by pairing abnormal bases causing excision and leading to strand breakage.

Non- covalent interaction involves intercalation between DNA bases or the compound's binding to minor or major grooves of DNA helix. Interaction of compounds or adsorbents capable of binding DNA can leads to strand breaking. A suitable adsorbent for DNA binding would enhance ARG removal and must be recovered after the treatment process. The most efficient method of binding DNA from wastewater is through electrostatic interaction with nanoparticles. The mechanism of DNA binding with nanoparticles through the adsorption process has received less attention. Recently, metal base nanoparticles such as Ag, Au, Cu, Pt, SiO_2, ZnO, and TiO_2 quantum dots nanoparticle have shown their effectiveness in destroying bacterial DNA and improving DNA binding wastewater (Kerman *et al.*, 2004; Yaqoob *et al.*, 2020; Zhang *et al.*, 2011). The mechanisms of their interactions with metallic nanoparticles are described in Figure 2.4. The positive charge present at these nanoparticles' surfaces aids their binding ability on DNA molecules' surfaces with a high negative charge in

its backbone (Blackman *et al.*, 2019). A study has shown quantum dots nanoparticles' effectiveness with a small radius (10 nm) permeating bacteria cells and leading to DNA binding (Zhang *et al.*, 2011). DNA interaction with metal-based nanoparticles occurs at the thiol group and the DNA bases, as shown in Figure 2.4. This process can facilitate the removal of ARGs through the adsorption process since they can bind to phosphate bases of DNA.

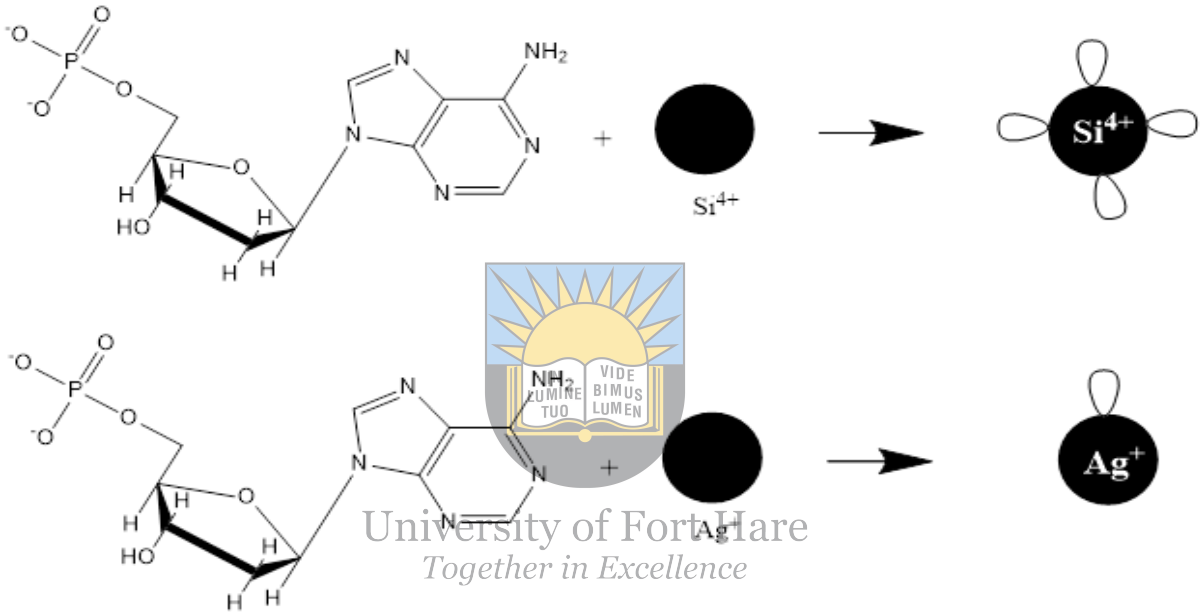

Figure 2.4: Reaction mechanisms for DNA binding to metallic nanoparticles

Some of the negatively charged surface nanoparticles can binding DNA through the process of functionalization or modification with multivalent cations ($Ca^{2+,}$ $Mg^{2+,}$ etc.) and chaotropic salt (guanidine HCl, NaCl salt, etc.). This process will assist in mediating DNA onto the surface of nanoparticles, thereby increasing the removal efficiency of ARGs from wastewater.

The functionalization of NPs has been reported to increase the removal efficiency of contaminants during water purification (Ebadi and Rafati, 2015; Ojemaye *et al.*, 2017;

Ojemaye and Okoh, 2019). Functionalization of NPs means a coating, modifying, or fabrication of surface nanoparticles with a functional group (amine or thiol), multivalent cations, or use of chaotropic salt, etc., without altering or damaging the material's significant constituents (Ojemaye *et al.*, 2017). It adds a new electronic structure different from the original materials, making it controlled size, shape, and surface area. All these improved the adsorption capacity of the nanoparticles. The high degree of surface area relative to their mass makes it more efficient for surface functionalization. Also, the greater the surface area of the NPs (adsorbent), the stronger the binding site, thereby making it efficient for the adsorption process. To achieve high removal of ARGs with metallic NPs, functionalizing these materials is an essential procedure that should be considered.

Meanwhile, the primary purpose of functionalizing materials during wastewater treatment is to control, maintain, or improve the efficiency at which the material removes contaminants. There are different types of materials or compounds used for the surface functionalization of nanoparticles during wastewater treatment. But the choice of the material depends on the intended contaminants meant for the removal (Ojemaye *et al.*, 2017; Walcarius and Mercier, 2010). For ARGs contaminated water, removal with functionalized metallic NPs can be achieved in two ways: through electrostatic interaction between metal-based NPs and a harmful contaminant (DNA) since the ARGs are embedded in the bacterial DNA. Removal of ARGs through oxides of NPs is mediated through chaotropic salt. These oxides have a negative surface charge and may not adsorb a harmful contaminant (DNA) in an aqueous medium. It can be due to electrostatic repulsion between the oxides NPs and DNA molecules (Cashin *et al.*, 2018). Most oxide NPs are considered excellent adsorbents due to their large surface area relative to their size and high catalytic activities. Therefore, modifying their surface provides a better surface ion for electrostatic interaction and binding sites for DNA molecules. Several studies have investigated the efficiency of surface-functionalized

nanoparticles to remove cationic contaminants from wastewater or aqueous solution (Lytton-Jean and Mirkin, 2005; Ojemaye and Okoh, 2019; Pandya *et al.*, 2018). Ojemaye et al. investigated the adsorption capacity of functionalized superparamagnetic NPs to remove cationic pollutants from an aqueous solution (Ojemaye and Okoh, 2019). The result showed that functionalized NPs were useful and spontaneous in removing cationic pollutants from an aqueous solution. Studies on the efficiency of metal-based nanoparticles binding DNA molecules through electrostatic interaction have received less attention. This calls for more research that would prove their efficiency in binding DNA molecules. Several studies have investigated DNA binding with metal oxide nanoparticles such as silica mesopore due to their retaining features in controlling the loading and release of guest molecules in the drug delivery process. The entry of DNA into MSN with a negative charge becomes difficult due to the electrostatic repulsion effect. Therefore, compounds that mediate DNA molecules onto the silica mesopore surface have been investigated (Cashin *et al.*, 2018; Solberg and Landry, 2006). These compounds have been investigated with an excellent report (Cashin *et al.*, 2018; Fujiwara *et al.*, 2005; Karim *et al.*, 2012). Gao et al. mediated plasmid DNA successfully onto the surface of mesoporous silica NPs with cationic linkers (Gao *et al.*, 2009). Another study also achieved the adsorption of plasmid DNA onto silica mesopores' surface with a multivalent cation such as the Mg^{2+} and Ca^{2+} (Solberg and Landry, 2006). Other researchers avoided the use of cationic linkers for plasmid DNA mediation onto the mesopore. They made use of an aqueous solution with high NaCl concentration at low pH and chaotropic salt (guanidine hydrochloride at pH~ 5) (Fujiwara *et al.*, 2005; Li *et al.*, 2012). For example, Li and co-workers utilized the guanidine hydrochloride salt solution at pH 5 to mediate DNA into MSN. DNA adsorption was successfully achieved by varying salt solutions under different adsorption conditions (Li *et al.*, 2012). The study reported that the amount of adsorbed DNA onto MSN changes with salt solution concentration. From these studies, MSN

demonstrated excellent stability and reusability for DNA binding, affecting time, pH, temperature, and concentration. For the DNA to successfully bind on oxides of NPs such as MSN mesopore, there are methods involved, and they are:

- The ion present in the DNA supports the binding when arranged in its backbone since it consists of sugar and phosphate groups.
- Another helpful method is the variation of MSN concentration with pH value, which controls the surface charge because electrostatic interactions play a vital role in DNA binding.

Therefore, oxides of NPs such as MSN-ARGs can control ARG pollutants because plasmid DNA would be disconnected during horizontal gene transfer (Blokesch, 2017). Also, MSN can control the ARG transfer, making it possible to have effluents free from ARGs during the treatment process. Therefore, the surface-modified/functionalized oxides of NPs may be a promising material for eliminating ARGs contaminated wastewater.

Apart from the binding DNA harbouring ARGs, the metallic nanoparticles also effectively kill or fight against ARB due to their antibacterial or bactericidal properties (Singh *et al.*, 2020). These excellent and unique properties can fight or prevent the growth of ARB due to their large surface area to mass ratio and small sizes of nanoparticles which provide strong reactive interaction with cellular and intercellular partition (Oukarroum *et al.*, 2012). Also, metallic nanoparticles generate free radicals, which damage cellular function in microorganisms (Kim and Aga, 2007). Several studies have reported the effectiveness of metallic nanoparticles fighting against ARB. Quintero et al. (Quinteros *et al.*, 2016) stated that the picomolar concentration of AgNPs exhibited bactericidal effects against three clinical strains *methicillin-resistant S. aureus, A. baumannii, and E. coli*. Another study by Krishnaraj et al. (Krishnaraj *et al.*, 2010) reported that the antibacterial activity of synthesized AgNPs

has inhibitory effects on *E.coli* and *Vibrio cholera* water-borne bacteria. The bactericidal activity of AgNPs is ascribed to silver cations, which bind strongly to the thiol group of bacteria proteins, disrupting their physiological activity. A study on another metallic nanoparticle (gold nanoparticles) confirmed that AuNPs effectively against Gram-positive and Gram-negative bacteria (Su *et al.*, 2020). Generally, metallic nanoparticles exhibit good antibacterial activity against standard strain and multi-drug resistant bacteria. They are nontoxic to human cells at low concentrations (Martínez-Castañón *et al.*, 2008). As a result, it can be used to inactivate or kill ARB in water or wastewater without adverse effects on humans.

2.4. Conclusion and future research need

To access water free from ARGs and meet the ever-growing stringent water regulatory standard placed by the government and other regulatory bodies, various advanced and conventional treatment processes are used as an emerging treatment option for ARG removal. But they are restricted due to some drawbacks, including the enrichment of ARGs, inability to separate the treatment materials from the effluent, which become secondary pollutants, etc. Substantial efforts have been made to use only disinfection, coagulation, AOPs, DNA binding, and functionalization mechanisms to remove ARGs from wastewater. But they did not give a total amount of removal from wastewater. Considering these drawbacks, which facilitate horizontal gene exchange mechanisms, and difficulty of removal from wastewater, experimental studies on using these proposed techniques described in Figure 1 would help eliminate ARGs. It may be an explicitly important technique for future study. A better understanding of these techniques and their mechanisms of action (as described in Figure 1) by which ARGs are entirely removed from wastewater should be considered. The process and factors facilitating DNA binding onto the adsorbent (NPs) surface should be clearly stated. The cytotoxicity of the treated water with these nanoparticles should be studied to ascertain human and animal health implications. The

overarching questions or research problems of this context is: Are the application of these techniques during the treatment process capable of eliminating existing ARGs in the cell debris and at the same time inactivate ARB after the treatment process and if there is a health implication of using nanoparticles for treatment of ARGs contaminated water. These research questions will be answered if;

(1) Future research adopts these mechanisms during experimental analysis.

(2) The residual ARG copy number and ARB cell viability, including the quantification of ARGs at each level, will be measured to ascertain these mechanisms' efficiency.

(3) These experiments should be investigated under the conditions selected by countries' environmental protection agencies targeted for regulated bacterial inactivation and condition optimized for ARGs removal from wastewater.

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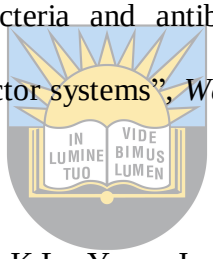

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Chapter 3 (Literature Review continued)

Potentials of metallic nanoparticles for the removal of antibiotics resistant bacteria and antibiotics resistance genes from wastewater; A critical review

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Chapter 3

Literature Review (Continued)

Potentials of metallic nanoparticles for the removal of antibiotics resistant bacteria and antibiotics resistance genes from wastewater; A critical review

Abstract

Globally, the challenges with extensive consumption and improper disposal of antibiotics have become a menace to public health. This anomaly has contributed to the emergence and spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment. Many of these resistance genes are carried on plasmids, transposons, or integrons, and their origin is linked to anthropogenic sources. Wastewater treatment plants (WWTPs) harbors a vast diversity of microorganisms and are responsible for disseminating ARB/ARGs. The failure of different treatment methods/materials to address the challenges observed with antibiotics resistance in wastewater treatment facilities alters access to quality water in the ecosystem. This paper emphasized the health implication of antibiotic resistance in wastewater and its effect and the pathways for their dissemination from wastewater into receiving waters. Furthermore, the lack of quality data and efforts in removing antibiotics-resistant bacteria and their genes were mentioned in this paper compared to the abundance of data on the prevalence or incidences of these substances in wastewater. Metallic nanoparticles' potential and their unique properties as a treatment option for removing DNA conveying ARGs from wastewater treatment plants were critically discussed in this paper. Also, other treatment options and their limitations have been extensively discussed. Lastly, this review also highlights the importance of regulating antibiotics and their indiscriminate use.

3.1 Introduction

Water is a vital component of life that plays an essential role in human and animal lives. It is described as a significant component of the blood that carries nutrients oxygen to all cells, thus crucial for body temperature regulations (Bure, 2019). Because of these critical roles in life, access to quality water and quantity should be a priority. However, pollution compromises water quality and the quantity discharged into wastewater treatment plants from different sources (Agunbiade and Moodley, 2014; Böckelmann *et al.*, 2009; Jia *et al.*, 2019; Välitalo *et al.*, 2017). Water reclamation has been considered a solution to water shortages and adopting efficient treatment strategies will help achieve high-quality water to survive all living organisms. Improving water quality requires fundamental strategies such as the prevention of pollutants [inorganic (heavy metals), organic (dyes, pesticides, pharmaceuticals) and biological (antibiotic resistant bacteria and antibiotic resistance genes)], treatment of polluted water, restoration and protection of the ecosystem (Pink, 2012). Water is a significant pathway for disseminating microorganisms in nature among human and animal populations (Sanganyado and Gwenzi, 2019b). As mentioned earlier, wastewater treatment plants (WWTPs) and their effluents harbours and disseminate antibiotics resistant bacteria and antibiotics resistance genes (ARB/ARGs) due to inadequate treatment technology/materials. What are ARB and ARGs? According to the World health organization (WHO), ARB is the bacteria that acquire resistance mechanisms to the antibiotics meant to eradicate them. At the same time, ARGs are the resistance genes acquired through spontaneous mutation or genetic exchange with other bacteria that can resist one or more antibiotics (WHO, 2014). These contaminants are released into the environment due to extensive and indiscriminate antibiotic use by humans and animals. The consequences include rendering the efficacy of antibiotics that are meant to treat an infection ineffective,

thus, increasing the morbidity and mortality rate among the populace in the environment (Mossialos *et al.*, 2010). Reports from the World Health Organization (WHO) and the Centre for Disease Control have shown that the existence and spread of antibiotic resistance genes without proper sanitation facilities pose a severe risk to global health (CDC, 2019; Thines *et al.*, 2017; WHO, 2014). In particular, wastewater treatment plants are the hotspot for activation and the spread of ARB/ARGs into different water bodies (Aruguete *et al.*, 2013; Singh, Singh, *et al.*, 2019). Thus, the release of ARB/ARG contaminated water is dangerous to humans, animals, and aquatic life. Therefore, wastewater disposal from industries, hospitals, and agricultural/aquaculture sectors should be treated efficiently to prevent the rapid proliferation of ARB, ARGs, and even widespread multidrug-resistant bacteria (MDRB) in diverse environments (Zhang *et al.*, 2013; Zhuang *et al.*, 2015). It is necessary to state that antibiotic resistance results from extensive or overconsumption of antibiotics, which build up to form resistance mechanisms in organisms. The ability of bacteria to outpace antibiotics' efficacy is termed antibiotics resistance (Manaia *et al.*, 2018; Vaz-Moreira *et al.*, 2014). Over-prescription of antibiotics, patients, not completing the entire antibiotics course, overuse of these antibiotics in livestock and fish farming, poor infection control, hygiene, sanitation in healthcare settings, and lack of newly developed medicines (antibiotic) are the significant causes of antibiotic resistance in the natural environment (Andrew Duong, 2015).

Due to their limited understanding, fate, and the risk posed to human and animal health in the ecosystem, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are referred to as emerging or unregulated pollutants in water matrices. Numerous reports have outlined the concentration of these emerging pollutants (ARB/ARGs) in wastewater (Manaia *et al.*, 2018; Pallares-Vega *et al.*, 2019), rivers (Singh, Singh, *et al.*, 2019), and soil through swine feedlot and waste (He, Ying, *et al.*, 2016). The widespread ARB/ARGs have led to spontaneous gene mutation and gene transfer among bacteria found in various water matrices

(Gyles and Boerlin, 2014; Lin *et al.*, 2016; Von Wintersdorff *et al.*, 2016). Hence the control of ARGs from the influents and effluents of WWTPs may reduce contamination of watersheds, which will avert health-related illnesses among the populace in the environment. Also, controlling the dissemination of ARB/ARGs and averting their harmful health effects required several treatment strategies to remove these pollutants from the influents and effluent of wastewater. Such treatment strategies are filtrations, bioremediation (Sharma and Malaviya, 2016), UV irradiation (Tobechukwu *et al.*, 2020), photocatalysis, (Ojemaye *et al.*, 2017), chlorination (Owoseni *et al.*, 2017), Fenton, and ozone disinfection processes (Bharath *et al.*, 2017; Liu, Guo, *et al.*, 2019). These strategies have proven their efficiency in removing organic and inorganic pollutants. Still, they are ineffective for removing ARB/ARGs as they may not account for the total removal of these pollutants from wastewater. Most of them are reported to enrich or enhance the proliferation of ARB/ARGs in the wastewaters.

Recently, advances in nanoscience and nanotechnology have been considered the best option for eliminating these harmful pollutants from wastewaters (Dutt *et al.*, 2020; Lee *et al.*, 2016; Wang, 2012). Their unique properties aid in the adsorption of pollutants from wastewater. They are also quickly recovered from treated water. Metallic nanoparticles such as silver nanoparticles (AgNPs), silica oxide nanoparticles (SiO_2 NPs), sulfidated nanoscale zero-valent, and persulfate treatment of NPs have demonstrated their potentials in binding DNA through electrostatic interactions and mediating DNA into the oxide nanoparticles' surface with the use of chaotropic salt (Gao *et al.*, 2009; Kamali *et al.*, 2019). Silver nanoparticles have played a vital role in combating emerging pollutants responsible for infectious diseases. Their wide application in several fields is due to their physical and chemical properties, including thermal, optical, biological, and high electrical conductivity. These properties enable their use in industrial, healthcare-related products, cosmetics, optical sensors, pharmaceuticals products, biomedical devices, households, and water purification processes.

Silver is a metal with a history of antimicrobial properties that provides health impact in early life, such as treating venereal disease with silver nitrate in 1700CE, the treatment of fresh burn in the 18th century to date, and as a water treatment material in 1000BCE (Tajkarimi *et al.*, 2014). AgNPs have been applied in various biological and biomedical fields such as antibacterial, anti-tumour, antifungal, antiviral, antioxidative, drug carrier, anti-inflammatory, and biosensor. It is used in the water treatment process, especially in eliminating bacterial DNA conveying ARGs due to its ability to curb bacteria resistance against antibiotics. It has low toxicity, high thermal stability, and volatility. AgNPs are more effective than the bulk due to silver ion (Ag^+), which can cause cell death (Michalcová *et al.*, 2018).

Similarly, silica oxide nanoparticles (SiO_2 NPs) have been reported to be a promising adsorbent with high potentials for ARB/ARGs removals from wastewater due to their large surface area, affinity to bind DNA, morphology, particle size, and porosity (Li *et al.*, 2012; Peralta *et al.*, 2019). A suitable surface for SiO_2 functionalization could improve the adsorption capacity and removal efficiency of ARB/ARGs from wastewater. Sulfidated nanoscale zero-valent iron (n-ZVI) has been proven to be effective, and a new promising alternative that may provide a solution to ARB/ARGs contaminated water. It has a large surface area and particle size to inject n-ZVI to the source zone, which attacks pollutants first before they spread out into the water (He *et al.*, 2018). Treatment of nanoparticles with persulfate salt has shown its effectiveness in removing ARB/ARGs through generating sulphate radicals. This review evaluates the occurrence and removal of ARB/ARGs in wastewater, their health implication, the mechanism of their transfer in the water environment, limitations of selected treatment materials for ARGs removal, and some metallic potentials nanoparticles for the removal of ARB/ARGs from wastewater.

3.2. ARB and ARGs in wastewater

3.2.1. Sources and occurrence

The discovery and proliferation of ARB/ARGs in different water milieu, including wastewater treatment plants, are of great concern to the general public (Voigt *et al.*, 2020). The knowledge of these dangerous pollutants calls for lifestyle changes and drastic measures. WWTPs are regarded as the human source for promoting ARGs in the environment, while the application of manure on land is the animal source for spreading ARGs (Barancheshme and Munir, 2018). Adefisoye and Okoh investigated ARGs' prevalence in municipal wastewater treatment plants in Eastern Cape, South Africa, and concluded that WWTPs are point sources for ARB and ARGs (Adefisoye and Okoh, 2016). It could be due to sewage and hospital disposal containing ARGs associated with clinical pathogens. The incidence of antibiotic resistance in wastewater treatment facilities and the threat posed by this menace cannot be over-emphasized. This is complemented by the number of studies reported on ARB/ARGs in wastewater from different world countries. A search that we conducted between 4th and 6th June 2020 using two free online databases; Scopus and Google Scholar, with the keywords shown in Table 3.1 on literature published in English indicated that from 2015 to 2020 and across six continents, namely, Africa, Asia, Australia, Europe, North America, and South America. Seven hundred thirty-six (736) studies were reported on the incidence of ARB/ARGs in wastewater, while 331 studies were reported to remove these substances from wastewater. This does not include duplicate studies as the thorough screening was done to eliminate duplication of articles that may arise from using two search engines. Statistical analysis of this data was carried out using Microsoft Excel 2016, and the details are presented in Figure 3.1

Table 3.1: Keywords used for search on works of literature published in English on the occurrence and removal of ARGs across the six (6) continents.

Search Engine	Keywords
Scopus	"Occurrence" AND "antibiotics resistance genes" AND 'wastewater", "Prevalence AND Proliferation," "Dissemination," "Elucidation AND Variation," "Preliminary investigation," "Detection and removal" AND "antibiotics resistances genes," "Elimination," "Inactivation and Removal" AND "antibiotics resistant bacteria" AND "antibiotics resistance genes" AND "wastewater, "Evolutionary treatment" AND antibiotics resistance genes" AND "wastewater, "Abundance" AND "Removal of antibiotics resistance" AND "wastewater."
Free online google scholar	Occurrence and detection of ARB and ARGs from wastewater. Prevalence and Proliferation of ARB/ARGs from hospital wastewater. Detection and inactivation of ARB and ARGs using chlorine disinfectant, Fenton reagent, and UV irradiation. Elucidating a selection process for ARGs. Seasonal occurrence and Removal of ARGs with coagulation method, Deactivation of ARGs from wastewater. Surveillance and quantification of ARB/ARGs from wastewater. Persistence and dissemination of ARB and ARGs from wastewater. Sources of ARB and ARGs from wastewater treatment of industrial wastewater with ozone and photocatalyst. TiO_2/H_2O_2 for the inactivation of tetracycline resistance genes from hospital wastewater.

Fig. 3.1 shows that all six continents have significantly reported ARB/ARGs in wastewater, with vast amounts of the study conducted in Asia and Europe. Furthermore, comparing published articles on the incidence/occurrence and removal of ARB/ARGs, we found that most efforts have been concentrated on the fate or presence of these substances in wastewater. Little has been done on their removal from wastewater. Consistent report/incidence of these contaminants necessitated continuous research to provide solutions for the removal these contaminants from water/wastewater.

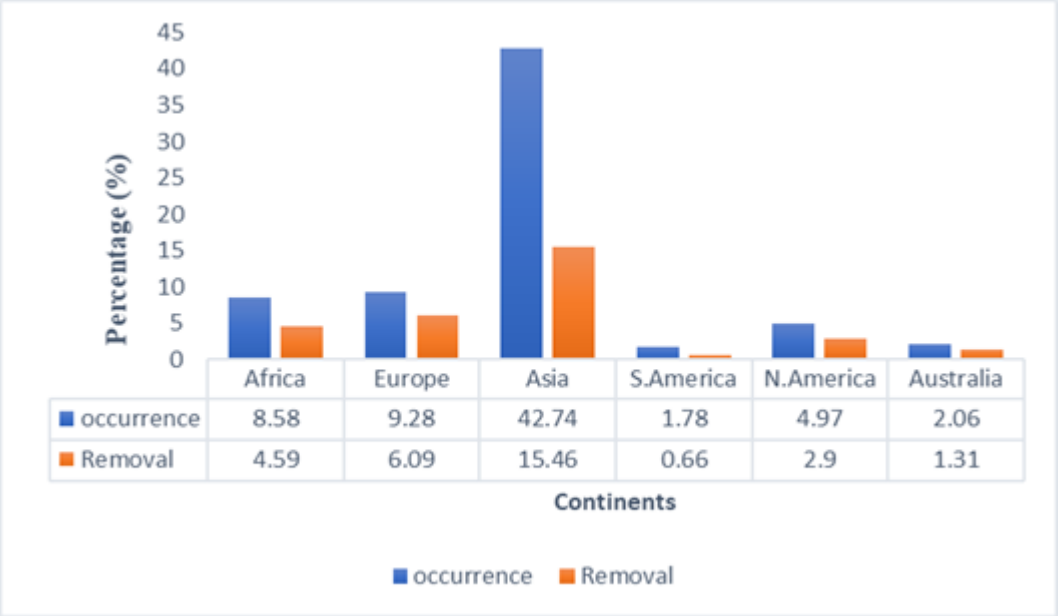

Figure 3.1: Percentage (%) of studies reported on the occurrence and removal of ARGs from wastewater between 2015 and 2020

Furthermore, the WWTPs received a considerable amount of antibiotics residue, ARB/ARGs through pharmaceutical industries due to inadequate treatment materials. A detailed process showing the route of these pollutants and their health implications resulting from inadequate treatment methods and indiscriminate discharge of untreated effluents is presented in Fig. 3.2

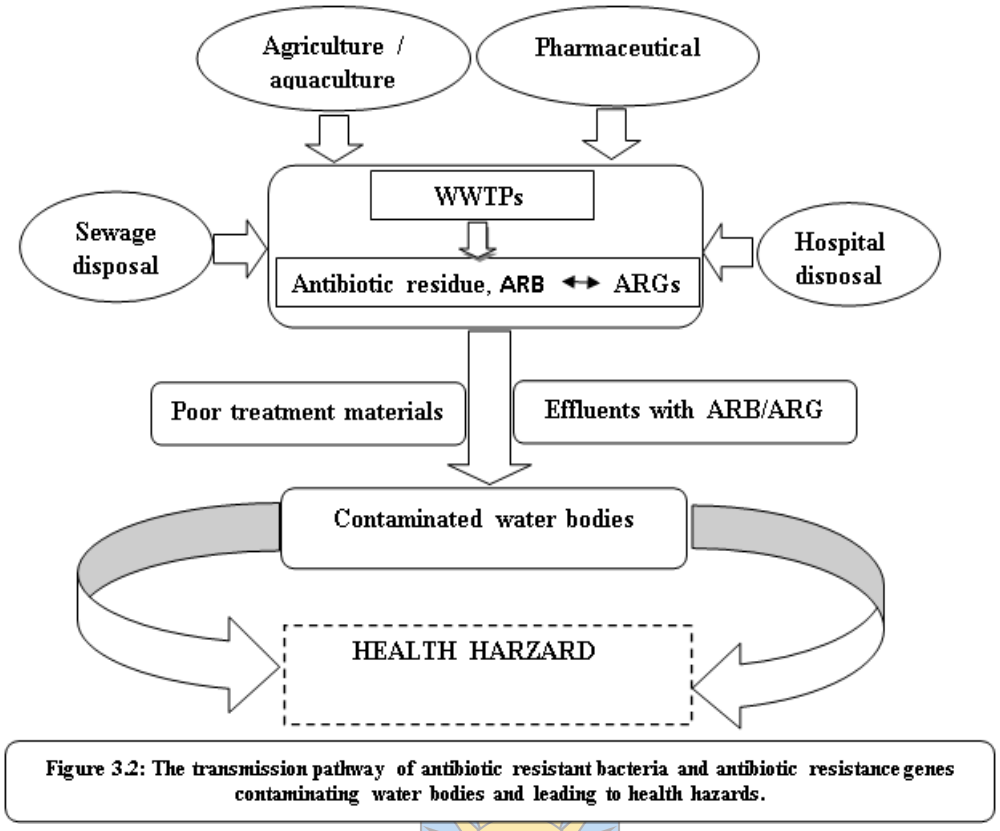

Figure 3.2: The transmission pathway of antibiotic resistant bacteria and antibiotic resistance genes contaminating water bodies and leading to health hazards.

Fig. 3.2 shows that the inability to remove ARB/ARGs can cause health hazards in an ecosystem (Agunbiade and Moodley, 2014; Anthony A *et al.*, 2018). The stable antibiotics discharged into the environment could be washed as runoffs by rainwater into water bodies, including groundwater located at dumpsites. Furthermore, antibiotic resistance can be induced by water disinfectant by-products (DBPs), including surface water and other water compartments. For example, Lie and co detected two DBPs of water (chlorite and iodoacetic acid). They concluded that they possess antibiotic properties, leading to the evolution of resistant *E. coli* strains at low and high exposures to inhibitory concentrations (Lin *et al.*, 2016). The report showed that the concentration of some antibiotic resistance genes was detected in the biosolids and effluents after treatment (Al-Jassim *et al.*, 2015; Auerbach *et al.*, 2007; Barancheshme and Munir, 2018).

3.2.2. Health implications of antibiotic resistance

Since antibiotic resistance in various water milieu poses a risk to public health globally (CDC, 2019; WHO, 2014), their detection and the subsequent removal of antibiotic resistance determinants require urgent attention (Manaia *et al.*, 2018; Thapa *et al.*, 2020). In 2017, the World Health Organization (WHO) reported that antibiotic resistance had implicated global health and food security (Safdari *et al.*, 2017). Also, it is predicted as a future infection that can ravage lives. According to WHO, methicillin-resistant *staphylococcus aureus* (MRSA) responsible for skin infections, *pneumonia*, toxic shock syndrome, vancomycin-resistant *Enterococcus*, multidrug-resistant, *Mycobacterium tuberculosis*, and carbapenem-resistant *Enterobacteriaceae* gut bacteria are examples of resistant bacteria that causes severe health damage (Safdari *et al.*, 2017). WHO scientist in July 2017 reported that antibiotic resistant gonorrhoea is another life-threatening infection that causes severe ill-health and sometimes becomes very difficult to treat (CDC, 2019).

Furthermore, the aquatic environment experiences an ecotoxicological challenge as a result of ARB/ARGs. It also stimulates the algal community structure resulting in a shift in the food web (Kim and Aga, 2007; Singh, Singh, *et al.*, 2019). The continuous release of these pollutants in water into the soil can contaminate the farm crops; thus, endangering human and livestock survival (Karkman *et al.*, 2018). All these drives the resistance evolution, which requires urgent action across different countries to halt its effects on environmental health. Blocking the primary route for receiving large-scale antibiotics into the wastewater treatment plants (Chamosa *et al.*, 2017; Manaia *et al.*, 2018) may avert the health challenges synonymous with antibiotics resistance.

Meanwhile, the World Health Organization (WHO), in 2015, also reported that antibiotic resistance occurs naturally via natural selection through random mutations (Safdari *et al.*, 2017). A study showed that removing resistant bacteria without targeting the genetic

mutation that deals with the DNA sequence had been a threat to treatment strategies (Windels *et al.,* 2019). These genetic mutations serve as defence mechanisms to the organisms enabling the bacteria to outpace the effects of novel antibiotics designed to destroy them (Adefisoye and Okoh, 2016). They are the main factor that promotes diversity among organisms. The genetic mutation occurs in a cell that produces the next generations and affects the hereditary material of the microorganisms by altering the pre-existing genetic makeup of a cell's DNA (Radzig *et al.*, 2013; Sharma *et al.,* 2016). Thus, this genetic makeup in DNA can cause changes in all aspects of living organisms. Many settlements in South Africa rely on surface water for their daily use. Thus, exposing the communities to water contaminated with DNA conveying ARGs can result in waterborne diseases. ARGs cause infection when a bacterium develops the ability to outpace the efficacy of one or more antibiotics designed to eradicate them or halt their growth (Mossialos *et al.*, 2010). Antibiotic resistance in water is an emerging health challenge that has claimed over 700,000 lives. Adequate measures should be considered in other to eliminate the proliferation of ARB/ARGs from water matrices. These measures may avert ARB/ARGs' prediction of claiming 10 million lives in the year 2050 (Tadesse *et al.*, 2017).

3.2.3. Transfer of ARB/ARGs in wastewater

Several studies with characterized laboratory strains have explained the nature of ARGs' mechanisms, and the knowledge obtained was used to investigate the environmental factor's effects on gene transfer (Baquero *et al.*, 2008). ARB/ARGs possess environmental threats due to their association with mobile genetic elements, which can be transferred easily between microorganisms through horizontal gene transfer. For the genes to be effectively disseminated from the environment to pathogenic bacteria, the bacteria must be closely related by temporarily sharing the same habitat (Bengtsson-Palme *et al.*, 2018). These bacteria acquire resistance genes in mobile genetic platforms such as plasmids (Jeong *et al.*,

2014). Figure 3 shows the ARGs dissemination among bacteria into the receiving environment via horizontal and vertical gene transfers distributed from human and animal sources.

Furthermore, the transfer of bacterial genetic materials is influenced by stressors, like antibiotics, metals, and biocides (Jutkina *et al.*, 2016; Zhang *et al.*, 2017). Subsequently, antibiotic resistance occurs either by mutations or acquiring resistance-conferring genes via horizontal gene transfer (HGT). Horizontal or lateral gene transfer is a primary mechanism for spreading antibiotic resistance and an evolution, maintenance, and transmission of virulence genes (Gyles and Boerlin, 2014; Varga *et al.*, 2012). It also permits new bacteria variants to arise without the mutation. Besides, antibiotic resistance developed from the natural selection can receive genetic material through horizontal gene transfer. Its consequences in wastewater are the dissemination of ARGs and an increase in drug resistance. For example, bacteria that acquire resistance transfer their resistance genes to other species. It occurs from resistant donor bacteria, free DNA, phage, and dead cells to living cells (Dodd, 2012; Sharma *et al.*, 2016). HGT includes those mechanisms responsible for infections and propagative activities of mobile genetic elements (conjugation, transduction) and those controlled by the bacterial cell (transformation) (Carvalho *et al.*, 2019; Hall *et al.*, 2017). The mechanisms also consist of uptake of naked DNA and mobile genetic elements such as integrons, transposons, plasmids, gene cassettes, and bacteriophages (Aminov, 2011; Lekunberri *et al.*, 2018). Some of these are known to transfer multiple ARGs at the same time. For example, methicillin-resistant *Staphylococcus aureus* represents a clinical acquisition of gene cassette while the integrons capture and reshuffled gene cassette, which encodes antibiotic resistance determinants (Berglund, 2015). The four significant mechanisms of transfers are briefly discussed below:

- **Transformation:**

This transfer mechanism deals with naked double-stranded DNA taken up by a proficient bacterial cell to attain a transformation state. It has the potential to transfer DNA to distantly related bacteria. Its evolutionary benefits are its diversification and mixing within the bacteria population (Carvalho *et al.*, 2019). The genetic mixing during transformation can provide selective advantages by allowing bacteria to combine favorably by mutations (Blokesch, 2017; Carvalho *et al.*, 2019). A cell can then absorb and integrate into extracellular DNA (eDNA) and its chromosome; the absorbed eDNA serves as a source of information and nutrients (Lin *et al.*, 2016; Takeuchi *et al.*, 2014).

- **Conjugation:**

The conjugation transfer occurs when DNA is transferred from the donor cell to the recipient cell through sexual pilus and adhesins, and it requires cell-to-cell contact (Bellanger *et al.*, 2014). The donor cells become resistant by freshly acquired resistance genes (Somensi *et al.*, 2015). A conjugative pilus provides the physical link for DNA to move from donors to the recipient. It transfers plasmids and plays a vital role in the dissemination of plasmids borne ARGs.

- **Transduction:**

Transduction involves DNA transfer from one bacterium to another through bacteriophages. It is the most critical mechanism for the exchange of genes among bacteria (Fogg, 2019). The bacteriophages have a wide-ranging host and can infect other bacteria hosts because they are carriers of other bacteria (Berglund, 2015). They can as well transfer their genes into the DNA of the new bacterial host. Transduction contains phage particles that mediate DNA transfer in the environment, they are resistant to environmental degradation, and their compact size aids the dissemination quickly (Bengtsson-Palme *et al.*, 2018). Transduction's

unique properties make it possible to transfer genes from bacterial communities' environment to human microbiomes (Sheth *et al.*, 2016).

- **Gene transfer agent (GTA):**

GTA virus-like particles that carried DNA present in the host and transduced to a recipient cell (Québatte and Dehio, 2019). It facilitates random gene transfer between species, and the particles can be freed through cell lysis and spread to recipient cells (Fogg, 2019).

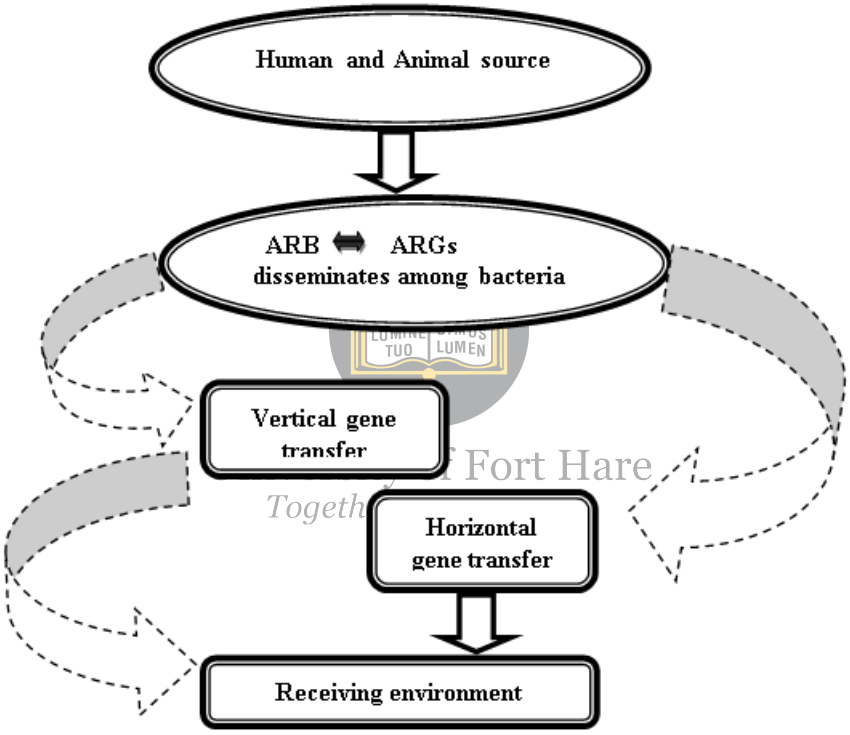

Figure 3.3: Pathway for dissemination of ARGs via vertical and horizontal gene transfer

3.3. Metallic nanoparticles and ARGs in wastewater

The application of nanotechnology has gained much interest in solving environmental-related problems, especially for removing biological, organic, and inorganic substances that pollute the water environment through industrialization, population growth, global climate change,

etc. (Das *et al.*, 2013; Lan, Kong, *et al.*, 2019; Motahari *et al.*, 2015; Ojemaye and Okoh, 2019). These pollutants, including ARB and ARGs, are toxic and even carcinogenic and cause a severe health risk to humans, livestock, and aquatic life. Nanotechnology, however, has excellent potential for water purification and treatment. Nanomaterials have a large surface area, high reactivity and degree of modification, dependable size properties, and affinity to target specific contaminants (Dhakras, 2011; Kunduru *et al.*, 2017; Younes *et al.*, 2019). These unique features contribute to their excellent adsorbent, sensors, and catalyst. These materials are considered as alternative therapies in medicine and wastewater reclamation. The ability to be utilized solely by itself or modification with other materials to increase the removal efficiency qualifies it as excellent treatment material for wastewater purification. Another useful aspect of this material is being able to recover it after-treatment process. Nanotechnology's actual environmental application includes TiO_2 nanoscale for photocatalytic degradation of air contaminants (e.g., $NO_{(s)}$, VOCs). Also, for removing hardness in water (water purification) using nanofiltration and nanoscale zero-valent iron for reclaiming soil and groundwater (Mueller and Nowack, 2010). Zero-valent nanoparticles are active in removing genetic components of heterotrophic bacteria from wastewater (Yeganeh *et al.*, 2018). Several synthesis routes of nanoparticles, including ionic gelation, solvent dispersion, fluid extraction, solid-state reaction, polymerization, chemical reaction, co-precipitation techniques, sol-gel, combustion methods, and even the green synthesis, which is eco-friendly and cost-effective depending on their application (Larayetan *et al.*, 2019; Sharma *et al.*, 2009; Vazquez *et al.*, 2017). The combination of diverse nanoparticles has proven to be effective against ARB and ARGs in water. ARB and ARGs' treatment with nanoparticles was formally limited to medical research due to the rapid development of antibiotic multidrug-resistant bacteria in the human gut (Sharma *et al.*, 2016). Their effectiveness in combating drug resistance motivated interest in the removal of ARB/ARGs

from wastewater. The removal efficiency of ARB/ARGs using nanoparticles during wastewater treatment involves some mechanisms. They include nanoparticles' functionalization with antibiotics or organic compounds with a positive charge that can attract a reasonable number of toxic ions (DNA conveying ARGs) (Li *et al.*, 2012). The addition of chaotropic salt can mediate DNA conveying ARGs to the surface of metal oxides NPs and the combination of different nanoparticles that combat the ARB/ARGs by synergistic means (Aruguete *et al.*, 2013; Barancheshme and Munir, 2018; Kamali *et al.*, 2019). Some of these NPs exhibit strong antimicrobial properties and are useful for a broad spectrum of microorganisms (Pazda *et al.*, 2019a). They are environmentally friendly, cost effectiveness and easy to synthesize. Several studies have shown the effectiveness of some nanoparticles against the inactivation of ARB and removal of ARGs in water (Li *et al.*, 2005; Sanganyado and Gwenzi, 2019c; Yao *et al.*, 2019). The utilization of AgNPs, SiO_2 NPs, sulfidated nanoscale zero-valent iron, and persulfate treatment in nanoparticles (due to their unique properties) may help eliminate the problems associated with antibiotic resistance in wastewater (Stark *et al.*, 2015; Tajkarimi *et al.*, 2014; Zhang *et al.*, 2013).

3.3.1. Silver nanoparticles (AgNPs)

The removal of antibiotic resistant bacteria and their genes from wastewater requires highly advanced treatment technology with materials of vast antimicrobial properties, which can halt the spread of these resistant bacteria and eliminate ARGs from wastewater before their disposal into the receiving water bodies. Nanoparticles (1-100 nm) may provide solutions to this kind of water-related problem. AgNPs has shown great prospect among the metallic nanoparticles due to its essential properties and application to different areas, including antimicrobial agents, catalytic agent, antiseptic spray, ink production, coating agents, and food storage packaging (Aisida *et al.*, 2019; Singh, Singh, *et al.*, 2019; Ugwoke *et al.*, 2020). They effectively reduce or inactivate the growth of microorganisms in different applications,

such as in the production of emollient creams and wound dressing. Studies confirmed that the antibacterial property of AgNPs is effective against Gram-positive and Gram-negative bacteria, including the multidrug-resistant strains (methicillin-resistant *S. aureus*) (Katsunuma *et al.*, 2007; Sharma *et al.*, 2016). Aruguete *et al.* (2013) investigated the potentials of AgNPs in restricting the proliferation of multidrug-resistant pathogens by functionalizing AgNPs with a water-soluble polymer in other to combat the resistant organism. They concluded that functionalized AgNPs performed like antibiotics and are toxic to *Pseudomonas aeruginosa* bacteria resistant to multiple drugs previously administered.

Furthermore, several authors reported that the antibacterial properties of AgNPs synthesized from plant extract are high and active against bacterial strains (Adegboyega *et al.*, 2014; Aisida *et al.*, 2019; Barros *et al.*, 2018). Adegboyega and co investigated the antibacterial activity of AgNPs synthesized from Fe_2^+-Ag^+ coated with natural organic matter (NOM) against various Gram-negative (g-) and Gram-positive (g+) bacterial species. The authors concluded that the coated AgNPs were toxic to different bacterial species (Adegboyega *et al.*, 2014). A study also reported the use of silver nanoparticles as a reliable antiseptic and antibacterial agent against Gram-positive and Gram-negative bacteria in the past due to its low toxicity to mammalian cells (Franci *et al.*, 2015). According to Maillard and Hartemann (2013), colloid silver, metallic silver, and salt silver inhibit the growth of antibiotic resistance genes in water by inducing a high degree of the structural and morphological trait that kills bacteria cells. Their inhibitory effects are due to the presence of Ag^+ and their sorption with a negatively charged bacterial cell wall, which deactivates cellular enzymes, disrupting membrane permeability leading to cell lysis and death (Choi *et al.*, 2008). Various silver forms are active with the increase in catalytic properties, making them more toxic to the bacteria cell. Their toxicity depends on size and shapes because the smaller sizes can pass and accumulate in the membrane (Akter *et al.*, 2018). The intracellular accumulations can lead to

cell death (Choi *et al.*, 2008). This confirmed the effectiveness of silver ion (Ag^{+}) than the bulk silver for the inactivation of bacteria species.

Catalytic oxidation of metallic silver and dissolved monovalent silver ions contributes to its bactericidal effects. A recent study indicated that silver ion undergoes catalytic oxidation with nascent oxygen, and the resulting compound can attack bacteria on the cell membrane, thereby leading to cell death (Rizzello and Pompa, 2014). Therefore, incorporating AgNPs in wastewater treatment can cause an attack on bacterial DNA, causing the organism to undergo structural changes by forming pores and allowing the cellular components into fluids osmotic pressure. And through electrostatic attraction, a considerable amount of ARGs may be removed during treatment processes. Modifying AgNPs with antibiotics may increase the removal efficiency of ARGs through DNA binding from wastewater. The combination of AgNPs with antibiotics has also demonstrated their effectiveness in combating multidrug-resistant bacteria and their genes. For example, the combination of AgNPs with molecular antibiotics proved that they are active in killing multidrug-resistant pathogens and combating the activities of ARB and ARGs in the water matrix (Aruguete *et al.*, 2013; Sharma and Malaviya, 2016).

3.3.1.1. Unique properties of nano silver for ARB/ARGs removal from wastewater

3.3.1.1.1 Varying size and shape

The application of silver nanoparticles for removing ARB/ARGs from wastewater largely depends on their physical properties, including the variation of size and shape, which influenced their preparation methods. The size and shape of AgNPs can be controlled by optimizing different parameters such as temperature, pH, precursors, reducing agents, and other experimental conditions (Wypij *et al.*, 2019). Varying the size and shape of nanoparticles plays a vital role in the inactivation of bacteria. Such roles are the generation of ROS that can influence the cytotoxicity of AgNPs, leading to cell death. Variation of size and

shape has a rejuvenating effect on cell viability, lactate dehydrogenase (LDH), and ROS production in a dependable size mode. Surface area, volume ratio, and surface reactivity can improve the particle size and shape, facilitating ROS production. Several studies reported the effect of AgNPs size and shape on the production of ROS. Carlson et al. investigated the effect of different sizes (15 nm and 55 nm) and shape (cubic and nanorod) of hydrocarbon coated AgNPs. They found that 15 nm can generate a high ROS amount compared with 55 nm in a macrophage cell line (Carlson *et al.*, 2008). Liu et al. found that 5 nm AgNPs were more toxic than 20 and 50 nm AgNPs (Liu and Wong, 2013). Production of high ROS can be toxic than their bulk counterpart (Akter *et al.*, 2018; Piao *et al.*, 2011) and can cause cell damage, as shown in Fig 3.4.

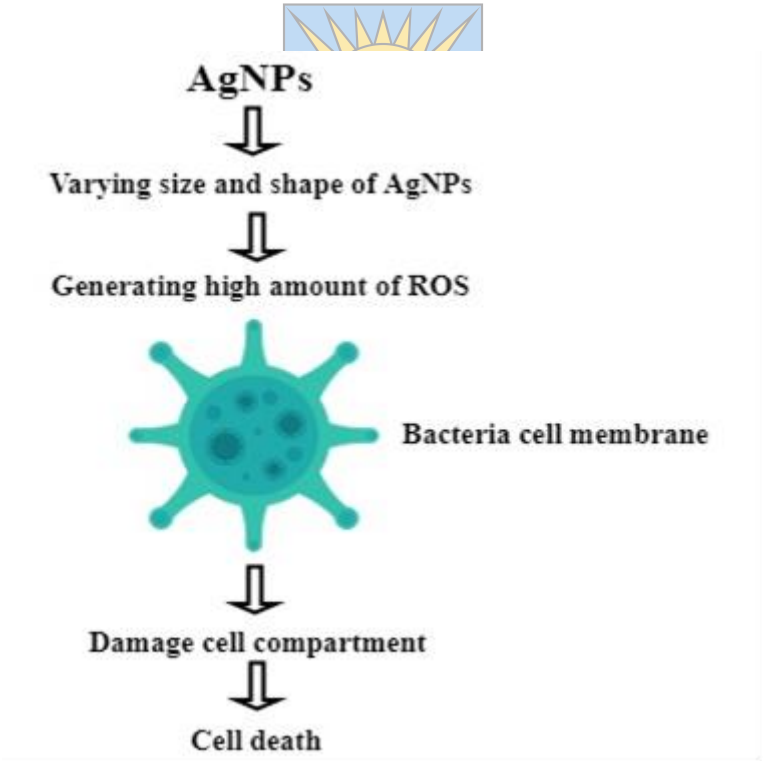

Figure 3.4: A pathway for AgNPs induced ROS generation because of size and shape variation, leading to cell damage and cell death

Silver nanoparticles with smaller sizes are very potent for the inactivation of ARB. They tend to rapidly release silver ions, resulting in their penetration in the cell, which can stop the further spread of ARB and remove ARGs through the binding process (Helmlinger *et al.*, 2016). Similarly, a study reported that silver nanoparticles' size is an essential factor for their effectiveness against ARB (Martínez-Castañón *et al.*, 2008). They reported that the antibacterial activity of silver nanoparticles increases with a decrease in particle size. Although different shapes of silver nanoparticles are known, e.g., cubic, rings, rod-like, platelets, spherical, nanowire, nano-triangle, etc. However, the shape of the nano silver is related to the atom density of surface facets. The shape with the highest atom density results in the particle with the highest removal of ARB/ARGs from wastewater (Oukarroum *et al.*, 2012; Pal *et al.*, 2007). The SEM and TEM images of typical silver nanoparticles with comparably small size and spherically shaped morphology are shown in Figure 3.5.

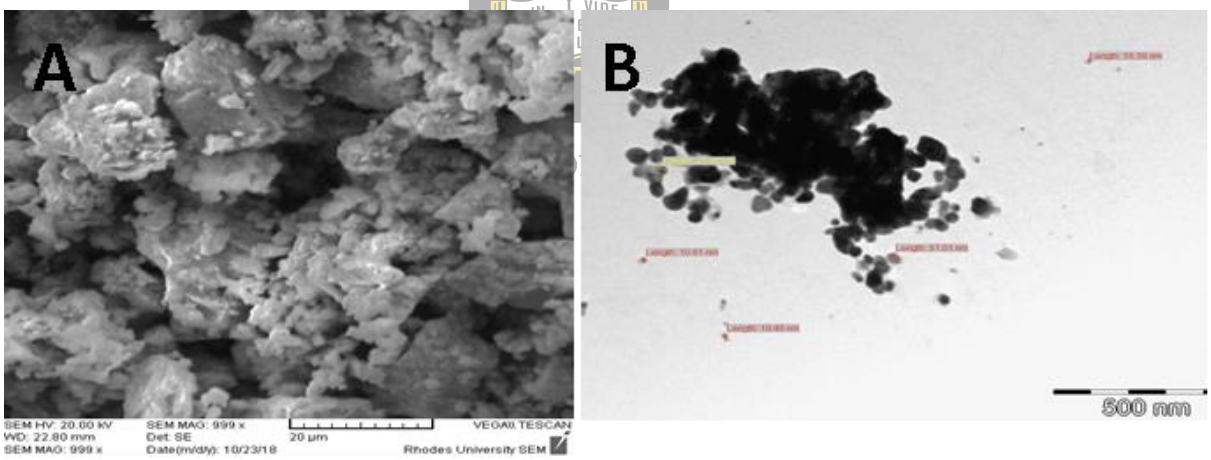

Figure 3.5: Images of (A) SEM and (B) TEM micrographs of silver nanoparticles

3.3.1.1.2 Antibacterial property

Silver nanoparticles are known for their excellent bactericidal properties, which are applicable in the pharmaceutical and biological sciences, such as in the production of medicine, disinfectant, and biocidal spray, wound dressing, and heart valve (Guan *et al.*, 2019; Le *et al.*, 2016). It is widely applicable in producing antiseptic creams, clothing, underwear, and textile products, even in washing machines. Antibacterial property can also

reduce infection and prevent prostheses colonization, dental material, and human skin infection (Rajawat and Qureshi, 2013). A report has shown the effectiveness of AgNPs against antibiotic resistant bacteria. Quintero et al. (Quinteros *et al.*, 2016) monitored the usefulness of biosynthesized AgNPs as bactericidal agents for three clinical strains, namely the *methicillin-resistant S. aureus*, *A. baumannii*, and *E. coli*. The authors discovered biosynthesized AgNPs at picomolar concentration exhibit bactericidal activity against human gram-positive and gram-negative pathogens, including the clinical isolates mentioned. A study investigated the antibacterial effects against gram-negative aerobic, rod shape bacterium called *Pseudomonas Aeruginosa* with the combination of AgNPs, gentamicin, and ampicillin. The authors concluded that the concentration of AgNPs with gentamicin shows bactericidal effects against *Pseudomonas Aeruginosa*, while that of ampicillin shows resistance to the bacteria (Rajawat and Qureshi, 2013). It implies that AgNPs with some antibiotics can effectively reduce antibiotic resistant bacteria and eradicate ARGs enrichment. Miller and co-workers (2013) reported that the antibacterial property of AgNPs prevented an increase in the abundance of *sul1*, *sul2*, (sulphonamide antibiotics resistance genes), and *intl1* (integrons) (Miller *et al.*, 2013).

Furthermore, a recent study points towards the generation of reactive oxidative species (ROS) in the presence of AgNPs, contributing to cell death by targeting cell wall and intracellular targets via ion release, physical interaction, and production of ROS at the highest concentration might lead to DNA damage. (Mi *et al.*, 2018). For example, Ji Lu *et al*. (Lu, Wang, *et al.*, 2020) investigated AgNPs and Ag ion's effect on ROS generation of *E. coli* K-12LE392 and P. putida KT2440 using flow cytometry RNA sequencing proteomics. The authors reported that a higher concentration of AgNPs and Ag ions significantly increases ROS production. The ROS generated because of the rise in the intensity of AgNPs induces oxidative stress amongst bacterial, which eventually leads to damage of DNA embedded in

ARGs. Embedding of AgNPs with other antimicrobial nanoparticles (TiO_2) can enhance the antibacterial effects of AgNPs and may give high removal efficiency of ARB/ARGs. A study investigated the antibacterial effects of embedding nanoparticles of TiO_2/AgNPs under UV irradiation to reduce *E. coli* bacteria, generation of ROS, and the removal of tetracycline resistance genes (Chen *et al.*, 2020). The authors concluded that the embedment of TiO_2/AgNPs inhibited the growth of *E. coli* bacteria. It generated ROS due to TiO2 (large band gap), which could damage the cell wall. Due to the electrostatic interaction between TiO_2 and AgNPs, a considerable amount of resistance genes was removed. Therefore, incorporating AgNPs as a treatment material in the wastewater treatment process may inhibit bacterial DNA and facilitate their removal from wastewater. The ability to generate excessive ROS with other antibacterial nanoparticles can damage plasmid DNA leading to a decrease in ARGs abundance in wastewater. The antibacterial property of AgNPs can inhibit the activation of antibiotic resistant bacteria and the dissemination of resistance genes detected from wastewater and receiving water.

3.3.1.1.3. Catalytic oxidation property

Silver nanoparticles can undergo catalytic oxidation by visible light, releasing silver ions, which can gradually accumulate and damage bacteria's DNA. Catalytic oxidative stress is known as one of the mechanisms of DNA damage. The incorporation of catalytic oxidation of silver ions into the bacteria cells induces a high degree of structural and morphological features that can kill bacteria cells (Michalcová *et al.*, 2018). It can attack the DNA bases and inactivate their ability to replicate. The silver ion's attachment on the cell membrane can modify the cell potentials by disorganizing the permeability and hindering cell respiration (Radzig *et al.*, 2013), leading to reactive oxygen species. It is known that oxygen is adsorbed on the silver surface in its atomic state, causing the oxygen to diffuse freely within the silver than any other metal indicating the source of nascent oxygen from metallic silver. A study

has proven the effectiveness of catalytic oxidation of silver ion with nascent oxygen reacting with thiol groups (-SH), forming R-S-S-R (R is an organic moiety) bond, causing respiratory blockage and cell death (Das *et al.*, 2013; Li *et al.*, 2005). Also, the silver ion binding with bacteria DNA prevents it from unwinding, which can inactivate cellular replication (Batarseh, 2004). The bacteria cell found in wastewater undergoes structural changes with silver ion, forming pores and allowing cellular components into the fluid due to osmotic difference. The application of AgNPs for ARB/ARGs removal would be achieved through catalytic oxidation/reduction of silver ion reacting with the negative charge of the toxic DNA by oxidizing bacteria DNA, which would result in complete disintegration.

3.3.1.1.4 Mechanism of nano silver for the removal of ARB/ARGs from wastewater

The resistance of microbes against antimicrobials has been known to take place through several mechanisms. These include efflux of antimicrobials from the cell of microbes, transportation of the nano antimicrobial agent to the site of action, antimicrobial agent degradation, modification of the enzymes of antimicrobials and antimicrobial targets, changes in the permeability of the microbial cell walls, which prevents antimicrobial access to target points in the cell and over-secretion and production of the target enzyme (Wright, 2005). The mechanism of the silver nanoparticle's action against ARB is such that: firstly, the nanoparticles attach themselves to the bacteria DNA bases and, after that, alters the function of the membrane. Secondly, the nanoparticles in the bacterial cell result in cell injury. The breakdown of silver nanoparticles can release silver ions that interact with thiol-proteins in the cell wall to affect cell functions (Durán *et al.*, 2016). As a result, targeting the infection of pathogens harbouring antimicrobial resistance genes using silver nanoparticles is an exciting aspect of eliminating antibiotic resistance. It is the major challenge encountered with delivering a therapeutic agent to the site of action. Controlling the delivery of nanosilver antimicrobial will put an end to these challenges. It allows the release of a load of drugs to

eliminate microbes. The combination of silver nanoparticles and antimicrobials can, through endocytosis, penetrate its host (Dakal *et al.*, 2016; Pinto-Alphandary *et al.*, 2000). Some reports have it that antimicrobials loaded with silver nanoparticles administered intratracheally penetrated directly through the alveolar-capillary blockage into the systemic circulation in blood vessel organs, including kidney, liver, bone, and spleen (Bourquin *et al.*, 2018; Yildirimer *et al.*, 2011). Altering the surface of nanoparticles to bind to mannose receptors expressed in alveolar macrophages adequately was considered an effective way to enhance the delivery of antimicrobial agents to the targeted spots (Gupta *et al.*, 2009; Gustafson *et al.*, 2015). Studies reported that mannose conjugated liposomes loaded with ciprofloxacin have been active as drugs targeting alveolar macrophages through pulmonary administration (Chono *et al.*, 2008; Huh and Kwon, 2011). Several different useful carriers for nano silver antimicrobials delivery include dendrimers, polymers, liposomes, solid lipids, etc. (Steiniger *et al.*, 2004). Nanoparticles designed to target the active site can readily penetrate the cell membrane and reside in the cell's cytoplasm. By this action, phagocytosis and pinocytosis are known as the two mechanisms for internalizing other materials in the cell (Darvishi1 and Mohammad Reza Nazer, 2017; Mayor and Pagano, 2007).

3.3.2. Silica oxide nanoparticles

The high concentration of ARGs in WWTPs threatens lives and the efficacy of the treatment methods/materials globally, which links to the unregulated use of antibiotics. Various treatment methods/materials' success depends on their effectiveness in removing pollutants, especially the DNA conveying ARGs. ARGs are seen as a significant cause of morbidity and mortality in the 21st century, and the use of nanoparticles as treatment materials significantly increased life expectancy. Since discovering the effectiveness of silica materials in duplex DNA packaging, researchers have attempted to use this material for the adsorption of DNA from an aqueous solution (Fujiwara *et al.*, 2005; Gao *et al.*, 2009; Li *et al.*, 2012). Bacterial

DNA is a threat to water quality, and it contains genes that can be resistant to antibiotics. It is a bulky molecule that has a high negative charge in its backbone. Therefore, mediating DNA molecules into the surface of silica materials becomes a difficult task since the SiO_2 NPs have a negative charge. Because of this challenge, most researchers utilize a means of successfully mediating DNA into silica materials' surface. It possesses a pore volume, a high surface area up to 1200 m^2 g^{-1}, identical pore size, controllable particle size, and excellent biocompatibility (Panahi *et al.*, 2019). Adsorption of DNA molecules on the silica surface was achieved by mediating DNA into the silica surface with a multivalent cation such as Mg^{2+} and Ca^{2+} (Solberg and Landry, 2006). Gao et al. (Gao *et al.*, 2009) also mediated DNA into silica surface with cationic linkers to adsorbed plasmid DNA through electrostatic interaction. All these studies successfully induced DNA into silica oxide nanoparticles. Consequently, other researchers avoided the use of cationic linkers for DNA adsorption. They made use of an aqueous solution with high sodium chloride (NaCl) concentration at low pH and chaotropic salt (guanidine hydrochloride at pH~ 5) to mediate DNA into the surface of the silica oxides nanoparticles (Fujiwara *et al.*, 2005; Li *et al.*, 2012). For example, Li *et al.* (Li *et al.*, 2012) utilized the guanidine hydrochloride salt solution at pH 5. DNA adsorption was successfully achieved by varying the salt solutions under different adsorption conditions. The study reported that the amount of adsorbed DNA into the silica surface changes with salt solution concentration. SiO_2 NPs demonstrated excellent stability and reusability for DNA binding with effects on time, pH, temperature, and concentration from these studies. For the DNA to successfully bind on SiO_2 NPs, there are essential factors that should be considered, and they are

- The ion present in the DNA must support binding when arranged in its backbone since the backbone consists of sugar and phosphate.

- The variation of SiO_2 NPs concentration with pH value controls the surface charge because electrostatic interactions play a vital role in DNA binding.

The success of DNA binding in modified SiO_2 NPs could be attributed to hydrogen bonding, electrostatic attraction, and the interaction between DNA and SiO_2 NPs. Impressive success on drug/gene delivery and DNA binding during the adsorption process are recorded due to their retaining features and their controllable loading in releasing guest molecules during the drug delivery process. The SiO_2 NPs with pore sizes ranging from 2 nm to 50 nm are excellent materials for the water/wastewater treatment process. Its beneficial features such as uniform and tuneable size, secure independence, ability to functionalize the surface, internal and external pores, and opening pores' mechanisms make it a distinctive and proficient material for pollutant removal and drug carrier (Narayan *et al.*, 2018). SiO_2 NPs enable the covalent linkage of different compounds, involves considerable changes in both physical and chemical properties. It was firstly prepared by Strober synthesis, followed by sol-gel with little modifications (Meier *et al.*, 2018; Rossi *et al.*, 2005). The synthesis involves combining selected silane compound and nitric acid (HNO_3) as a pore generating template depending on the application (Vazquez *et al.*, 2017). The size and morphological shape of the synthesized silica oxide nanoparticles are determined by SEM and TEM, as shown in figure 5. Silica oxide nanoparticles are also considered as an ideal disinfectant for DNA in wastewater because of their extensive antimicrobial activity at ambient temperature, binding ability, inability to produce harmful substance during and after use, less expensive, ease to store, highly soluble in water, and easy to recover from water/wastewater after the treatment process (Kunduru *et al.*, 2017).

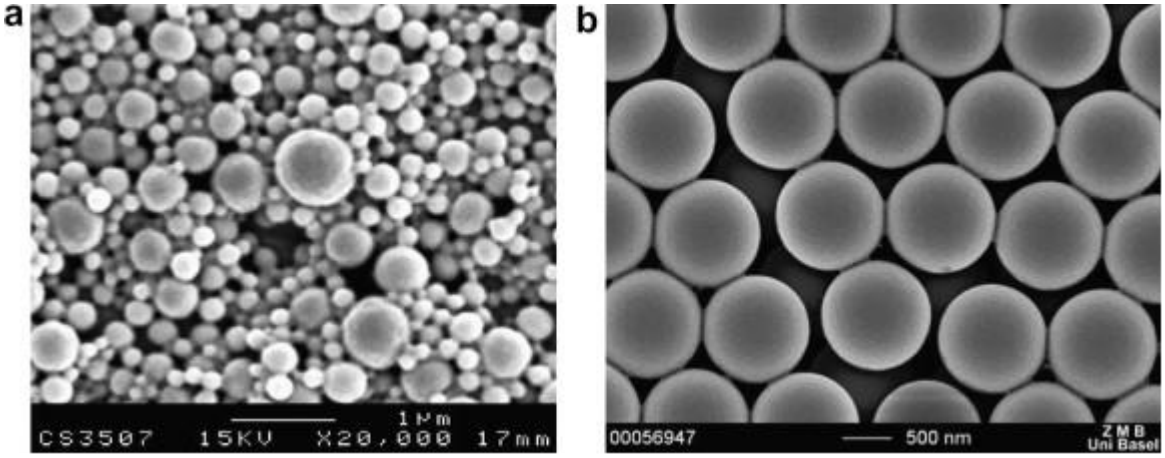

Figure 3.6: Images of SEM (A) And TEM (B) Micrographs of Silica Oxides Nanoparticles (SiO_2 NPs) (Adopted from Https://Researchgate.Net, Accessed 19th December 2020)

Besides, silica oxide nanoparticles are useful in removing contaminants such as organic dyes, methylene blue, and antibiotics from wastewater (Karim *et al.*, 2012; Panahi *et al.*, 2019; Ray *et al.*, 2013). Modification of SiO_2 NPs can lead to high removal efficiency of these pollutants from different water matrices. Further applications of SiO_2 NPs include catalysis, biological, and biomedicine due to their unique feature (Vazquez *et al.*, 2017).

3.3.2.1. Surface modification of silica oxide nanoparticles (SiO2 NPs)

For the increase in the removal efficiency of SiO_2 NPs, there is a need for adequate modification with cationic linkers. The surface modification of every treatment material enhances the adsorption capacity during the wastewater treatment process. A wide variety of modifying silica surfaces has been developed to improve the adsorption capacity and selectivity of the material for organic and inorganic pollutants. Also, modification of SiO_2 NPs surface provides a better surface ion for electrostatic interaction and binding sites for DNA molecules. Various functional groups such as amine or thiol, ligands, multivalent cations, and organosilane have been employed for SiO_2 NPs surface modification (Cashin *et al.*, 2018; Lehman and Larsen, 2014; Li *et al.*, 2012). The standard material for modification can be through organosilane, which involves two methods. The first method is the co-

condensation, which involves organosilane, aminopropyltriethoxysilane (APTES) with tetraethyl orthosilicate (TEOS). The second method is through photosynthesis grafting involving the calcined porous materials modified by heating the organosilane reactant with an aprotic solvent such as toluene (Lehman and Larsen, 2014). With the vast literature available online on the use of SiO_2 NPs for the drug/gene delivery process, which prompted the little work on DNA binding, there is a need for further research or more actual field trial on the binding of DNA conveying ARGs from wastewater. Also, the synthesis of fewer complex particles with a controllable size less than 100 nm and materials free from harmful chemicals such as ammonia (N_2H_4) as catalysts should be considered. This condition will ensure that no secondary contaminants would be detected after the treatment process.

3.3.3 Sulfidated nanoscale zero-valent iron

In the last two decades, nanoscale zero-valent iron (nZVI) has gained more attention as a promising new technology for eliminating contaminant. It is relatively inexpensive, eco-friendly, and has strong reducing capacity ($Fe^{2+} + 2e^- \rightarrow Fe(s)$, $E0 = -0.44$ V) (He *et al.*, 2018). It also can cause physical damage and oxidative stress to bacteria (Cheng *et al.*, 2016; Wang *et al.*, 2020). The simple mechanism is the process of Fe^o core oxidizing to iron oxides and hydroxides simultaneously (Zhang, Gao, *et al.*, 2020). As a nanoscale material, S-nZVI may provide a cost-effective way for ARGs removal compared with AOPs due to its antibacterial properties (Diao and Yao, 2009). The particle size allowed the injection of nZVI into the source zone. It attacked the pollutant before they spread out in the water, which is crucial for in situ water purification process (Wang *et al.*, 2020). A recent development on the sulfidated nanoscale zero-valent iron to eliminate ARB/ARGs has been demonstrated. For example, Lee *et al*. reported its strong bactericidal effects on *E. coli* under anaerobic conditions than the silver nanoparticles (Lee, Jee, *et al.*, 2008). Oprckal *et al*. reported the effective inactivation of *E. coli*, Enterococcus, and Clostridium from biological wastewater

effluents when 0.5 g/L of nZVI was introduced during the treatment process (Oprčkal *et al.*, 2017). However, two factors may affect its increase in sterilization efficiency. First, the easy passivation that retard the outward FeO core transfer to the oxide's surface, resulting in the decrease in the yield of reactive oxygen species (ROSs) (Qin *et al.*, 2017). Secondly, the easy aggregation and poor uniform distribution of nZVI prevent adhesion between nZVI particles and the bacteria surface (Phenrat *et al.*, 2007). Since the removal capacity of nZVI depends on the electron selectivity for the target contaminant and its utilization ratio of Fe^{o} particles (Qin *et al.*, 2017); therefore, it is necessary to improve the removal and inactivation efficiencies through surface modification of nZVI.

Several reports have proven that surface modification of nZVI with Sulphur compounds enhances the decontamination process. He et al. reported that sulfide-modification of nZVI could efficiently fabricate or build the electron transfer stream because, in his study, approximately 72% of electron transfer stream were due to the FeS_x instead of iron oxide (He *et al.*, 2018). Other studies reported that the electron transfer pathway between sulfur-modified nanoscale zero-valent iron (S-nZVI) and O_2 and its continuous electron transfer dominated and improved ROSs yield. Wang et al. monitored the inactivation of gram-negative sulfonamide ARB, the removal of its intracellular ARGs and integrase gene (intl1) with nZVI with different sulfur/ iron (S/Fe) molar ratio, the removal rate of ARG, Sul1, and intl1 were high on S-nZVI than removal with ordinary nZVI (Wang *et al.*, 2020). The further experiment proved that ARB cell structure and intracellular DNA were severely damaged after applying S-nZVI treatment material. A study also investigated the mechanism of ARGs, mobile genetic element (MGEs) removal, and the cause of the weakening nature of their bacteria carrier regrowth capacity after the application of S-nZVI on secondary effluent (Zhang, Gao, *et al.*, 2020). The result showed that the DNA-accepted electron provided by the Fe^{o} core of S-nZVI is responsible for reducing 16srRNA, ARGs, and the loss of regrowth

capacity, especially for the MGE (Intl1). Based on these successful results mentioned above, the S-nZVI could be a potential approach that will stop ARB and ARGs' proliferation from wastewater.

3.3.3.1. Modification of metal nanoparticles with persulfate treatment

Modification of nanoparticles with persulfate salt has recently played a vital role in minimizing the risk of ARB/ARGs spreading into other water recipients. Persulfate can be activated by heat, microwave, ultrasound, pH adjustment, the addition of transition metals, Fenton and Fenton like process (Malakotian *et al.*, 2019; Olmez-Hanci *et al.*, 2013) to generate sulfate radicals with redox potential (SO_4^{-}, E^0 = 2.5 - 3.1V), which has more aggressive oxidizing agents than the persulfate anions ($S_2O_n^{2-}/2SO_4^{2-}$) (Qiu *et al.*, 2020). It has proven its effectiveness in the water treatment process due to its long lifetime, wide pH range, and strong oxidizing agent. Its mechanisms involved the use of persulfate ion as a precursor in producing free sulfate radicals that could degrade various pollutants, especially the antibiotics residue (Zhou *et al.*, 2020). According to Olmez et al., SO_4 can react with water of different pH to produce $HO^{\bullet}$ during the wastewater treatment process (Olmez-Hanci *et al.*, 2013). The reaction is described in the equation below:

All pHs: $SO_4^{\bullet-} + H_2O \rightarrow SO_4^{2-} + HO^{\bullet} + H^+$ $k\,[H_2O] < 2 \times 10^{-3} s^{-1}$ ……. (3.1).

This process eliminates toxic pollutants by converting them into fewer toxic products such as mineral products (Malakotian *et al.*, 2019). Persulfate treatment has been considered a promising method for ARB/ARGs removal due to its high solubility, low cost, and production of high radical stability at different conditions, and being independent of organic products during the removal of pollutants (Toolabi *et al.*, 2019). Recent studies have demonstrated the removal of antibiotic residues, leading to the proliferation of ARB/ARGs with persulfate treatment in conjunction with nanoparticles. Malakotian et al. investigated the

removal of antibiotics residue with persulfate Fe_3O_4 nanoparticle, and the result shows that a high concentration of antibiotics residues was removed at pH 10 (Malakotian *et al.*, 2019). Duan et al. investigated the removal of selected ARGs (sul1, intl1, and 16s rRNA genes with persulfate modified nanoscale zero-valent iron (PS + nZVI), persulfate modified Ginkgo biloba L (PS + G-nZVI), and ordinary nZVI from sewage. The result proved that ARGs' removal efficiency was high with PS + nZVI than the ordinary nZVI and PS+ nZVI (Duan, Gao, Li, *et al.*, 2020). These successful results proved that persulfate modified nanoparticles could reduce the abundance of ARB/ARGs in the receiving water and minimize the risk of consuming the ARGs contaminated water.

3.4. Other treatment materials previously employed for the removal of ARB/ARGs from water/wastewater and their drawbacks

Many countries are experiencing shortages of quality water because of emerging pollutants from several sources. An emerging contaminant like ARGs is reported as one of the essential factors affecting water quality in developed countries (Anyaduba, 2016; Lu, Zhang, *et al.*, 2020; Yuan *et al.*, 2015). However, the most significant source remains the discharge of a large scale of antibiotics from the hospital, pharmaceutical, and agricultural sectors, which increases the resistance mechanisms. These sources generate waste in the form of water discharged into wastewater treatment plants. The wastewater treatment aims are to ensure that wastewater is safe without traces of pollutants at toxic levels. Another aim is to recover nutrients, energy, reclaim water, and other valuable resources, which are the most vital resource to humans (Abou-Elela *et al.*, 2019). To avoid being infected by ARGs contaminated water, many treatment technologies have been employed to eliminate these dangerous pollutants from water sources. Among them all, the advanced oxidation process has been widely used by the environmentalist to eliminate ARB/ARGs from the WWTPs. Advanced oxidation process (AOPs) is the new technology that oxidized and degraded

various forms of pollutants using highly generated hydroxyl radicals ($HO^{\bullet}$) as the main oxidative species and other reactive oxidation species (Sayed *et al.*, 2016). It is an alternative material that can transform decontaminate antibiotic residues from wastewater. Oxidative decontamination includes ultraviolet (UV), Fenton, ozone, photocatalyst, ultrasound based sonolysis, and electrochemical process have been employed for ARB/ARGs removal. These treatment processes have some challenges and restrictions. Some of the oxidation processes have useful properties that can kill bacteria DNA and reduce ARB proliferation in the wastewater treatment plant's final effluent, as shown in Table 3.2. Still, they may not remove a considerable amount of ARGs after the treatment process (Ren *et al.*, 2018).

Table 3.2: Examples of advanced oxidation technologies, their characteristics, mode of generating reactive radicals, and status after the treatment process

Advanced oxidation process	Materials generating $HO^{\cdot}$ and other ROS	Characteristics	Status	Reference
Photocatalyst	TiO_2, ZnO, Fe_2O_3, ZrO_2, V_2O_5	The direct and wide bandgap in the near UV spectral region, strong oxidation ability, and large free excitation banding energy	Effective in ARB inactivation but not in ARG removal	(Feng *et al.*, 2013; Lee *et al.*, 2016)
Fenton oxidation	H_2O_2, Fe^{2+}, Fe^{3+}	High performance and simple operation for the oxidation of the pollutant. It can be operated at room temperature and atmospheric pressure	Effective in ARB inactivation at high hydroxyl radical, not adequate for ARGs removal.	(Oturan and Aaron, 2014; Singh, Sharma, *et al.*, 2019)
Ozonation	O_2 and O_3	The ability to destroy cell walls and infiltration into cells through the cell membrane can oxidize enzymes needed to decompose glucose inside bacteria.	Effective in the ARB inactivation but requires high dosage for ARGs removal.	(Pei *et al.*, 2019; Sharma *et al.*, 2016)
U.V radiation	UV/H_2O_2, UV/O_3, UV/Fe^{2+}	Ability to inactivate ARB without a chemical that can form disinfectant by-product (DBPs)	Effective in ARB inactivation, not effective in removing the existing ARGs	(Macauley *et al.*, 2006; Umar *et al.*, 2019b)
Ultrasound based Sonolysis	Ultrasound energy and H_2O	Ability to degrade pollutants in water either by direct thermolysis or $HO^{\cdot}$	Effective in inactivating ARB, ineffective in the removal of ARGs.	(Kanakaraju *et al.*, 2018; Rayaroth *et al.*, 2017)

radical attack.

The extent of removal depends on the efficacy of the treatment method/materials adopted, the adsorbent's surface charge, the type of wastewater involved, time, and the amount of chemical used (Sanganyado and Gwenzi, 2019b). Studies have shown different removal strategies (Gomes *et al.*, 2019; Liu *et al.*, 2018; Pliego *et al.*, 2015; Sharma and Malaviya, 2016) with their shortcomings, such as high maintenance cost, enrichment, reactivation of ARGs, and low removal capacity. Some of these treatment materials are as follows:

3.4.1. Photocatalyst

Photocatalysts are the artificial green-sustainable materials used to solve energy and environmental issues, including wastewater treatment (Ojemaye *et al.*, 2017; Ren *et al.*, 2018). It is an advanced oxidation process (AOPs) and an improved technology suitable for removing contaminants from wastewater. These treatment materials aim at the natural self-purification of water using solar light initiated by the transition complex metal (Prihod'ko and Soboleva, 2013). It can also conveniently be applied in water/wastewater for the degradation of pollutants (Behnajady *et al.*, 2011). Numerous studies have shown the effects of photocatalysts on removing ARB/ARGs from the effluents of WWTPs and drinking water (Gomes *et al.*, 2019; Ren *et al.*, 2018). The photocatalytic materials with UV light inactivate a wide range of pathogens, viruses, and protozoa from water/wastewater. It has a potential valence band hole and conduction band electron for dissimilar semiconductors ranging from +4.0 to -1.5 volts. (Dunlop *et al.*, 2015; Mccullagh *et al.*, 2007). The photocatalytic disinfection materials can be classified into two, depending on the nature of the occurrence of targeted pollutants, and they are (1) homogenous and (2) heterogeneous catalysts (Guo and Tian, 2019). The homogenous photocatalyst depends on the chemical interaction between the reagents and the targeted contaminants.

In contrast, the heterogonous photocatalyst depends on the chemical oxidation process by metal oxidation semiconductors such as TiO_2, ZnO, and CdS immersed in water and irradiated near the UV light ($\lambda < 385$ nm), which forms free hydroxyl radicals (Dunlop *et al.*, 2015; Rizzo *et al.*, 2019). The presence of TiO_2 photocatalytic irradiation generates a reactive oxygen species (ROS) (Ren *et al.*, 2018). While CdS acts as a visible sensitizer, and ZnO is responsible for charge separation, conquering recombination progression. Furthermore, the illumination of TiO_2 near UV radiation generates an electron-hole pair (e^-/h^+) charge carrier that can migrate to the surface of semiconductor particles (Dunlop *et al.*, 2015; Mccullagh *et al.*, 2007). It reacts with the hydroxyl group and dissolves oxygen to form a reactive oxygen species (ROS). The ROS damages cell membranes, DNA, and RNA via oxidative stress (Sanganyado and Gwenzi, 2019c). The treatment of wastewater with the photocatalyst materials offers some attractive advantages such as TiO_2 being stable under UV irradiation, deficiency of chemical additives in the water, low ion inhibition present in water, and low cost of TiO_2 (Herrmann *et al.*, 1993). TiO_2 photocatalyst material is biologically and chemically inert, insoluble under most conditions, photostable and non-toxic (Mukherjee and Ray, 1999). Also, it requires low-energy ultraviolet light to function well.

Furthermore, the simplicity, efficiency, possibility of using solar light radiation for removal of ARB/ARGs and using fossil fuel for energy production due to ongoing ozone depletion is a distinct advantage that contributes to the development of water treatment technology. The inability to work with a solution containing a low concentration of a pollutant to achieve the removal of ARB/ARGs from wastewater is one of these treatment materials' challenges. Also, cell death depends on the illumination time and concentration of TiO_2 (Mccullagh *et al.*, 2007). Studies have tried to explain the interaction between generated ROS and the cell, which inactivate ARB/ARGs from wastewater. The early work of Dunlop and co on the use of photocatalyst materials on three strains of antibiotic resistant *E. coli* under different

reference strains cause a reduction of the viable number of ARB from 3log10 to 0.5log10 after 180 mins. After post-treatment incubation for 180 mins for both ARB (37°C and 24 h), a bacteria recovery of 3log10 was observed. And at 150 mins, no *E. coli* survived. This suggests that photocatalyst disinfection materials should take a long time to avoid post-treatment recovery, enhancing the unwanted transfer of ARGs among bacteria (Dunlop *et al.*, 2015). The major constraint/disadvantage of these materials includes the inability to guarantee their application on a large scale, inability to generate a uniform distribution of light to a large surface area, difficulty in recovering the adsorbent from treated water. It is time-consuming (it takes a longer time than usual, and there will still be an insufficient overall conversion of energy required for treatment) even after a prolonged time.

3.4.2. Fenton and ozone disinfectants

Fenton oxidation is the effective process of disinfecting wastewater containing ARB/ARGs and the degradation of antibiotics. This treatment material requires accessible pH and hydroxide ions to function effectively in the treatment processes. It involves the manufacturing of hydroxyl radical ($HO^{\bullet}$) from hydrogen peroxide (H_2O_2) reacting with ferrous ions in an acidic medium (Michael *et al.*, 2019). Fenton oxidation material can be a solar-driven Fenton (photo-Fenton) and a conventional Fenton process (Pliego *et al.*, 2015). In Fenton reaction, the oxidative species ($HO^{\bullet}$) is produced through the reacting Fe^{2+} with H_2O_2 while in solar-driven Fenton reaction, the Fe^{3+} produced from Fenton reaction is photo-reduced and regenerates Fe^{2+} as indicated in equations 3.2, 3.3, and 3.4:

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- +. OH \ldots\ldots\ldots\ldots\ldots (3.2)$$

$$Fe^{3+} + OH^- +\cdot HO \longrightarrow [Fe\ (H_2O)]^{3+} \ldots\ldots\ldots\ldots\ldots (3.3)$$

$$[Fe\ (H_2O)]^{3+} + hv \longrightarrow Fe^{2+} + H^+ +. OH \ldots\ldots\ldots\ldots (3.4)$$

The free hydroxyl radical ($HO^{\bullet}$) produced during the reaction has a strong oxidation potential for the full range of micropollutants, bacteria cells, and DNA damage (Karaolia *et al.*, 2018). The $HO^{\bullet}$ radical produced by the decomposition of H_2O_2 is catalysed by Fe^{2+} (K= 76 M^{-1} S^{-1}), and Fe^{2+} catalyst regenerates in solar-driven Fenton at a low rate (K= 0.01-0.02 M^{-1} S^{-1}) (Zhuang *et al.*, 2015). It records high efficiency in the degradation of various types of organic pollutants. And the use of solar irradiation to stimulate the hydroxyl formation, which results in low cost of application, are some of the advantages of the Fenton oxidation treatment material (Dodd, 2012; Zhu and Zhang, 2016). Also, Fenton disinfection aims to improve the quality of secondary effluents before reuse or disposal (Lin *et al.*, 2016). It can regenerate highly reactive radicals and other species, which can cause the oxidation of a broad range of organic pollutants (Pereira *et al.*, 2014). The Fenton process's efficiency is affected by the concentration of H_2O_2, Fe^{2+} dose catalyst, and pH solution (Pereira *et al.*, 2014; Zhu and Zhang, 2016). A recent study investigated ARB/ARGs' fate, the conditions affecting the removal efficiency, and the oxidative damage pathway involved during the Fenton oxidation process (Fe_2^{+}/H_2O_2). (Karaolia *et al.*, 2018). The authors reported that, besides these conditions, there is viable behaviour observed by the genetic constituent of the microbial community, which may result from the treatment materials and oxidative damage mechanisms during the application. The Fenton solar-driven, and Fenton conventional processes are grouped among the advanced oxidation processes (AOPs), which remove recalcitrant organic and bio-pollutants. However, the reduction of ARB/ARGs with this application is still limited. The main limitation of this treatment material is the formation of the oxidative products derived from the dissolved effluent of organic matter, which can induce toxic effects during the biological treatment method (Michael *et al.*, 2019; Pereira *et al.*, 2014). Furthermore, this treatment method's cost is high due to the massive consumption

of reagents, which can also introduce the reagents as secondary pollutants into WWTPs (Lu, Zhang, *et al.*, 2020).

Ozone materials are also among the advanced oxidation process (AOPs) of the water treatment process. An interest in the application of an ozone-based system is a great deal. This is because it has demonstrated the effectiveness of numerous micropollutants' significant abatement and provides suitable disinfectants to wastewater at different ozone doses and contact times (Gomes *et al.*, 2019). Moreover, they are useful for the inactivation of gram-positive and gram-negative bacteria, such as *E.coli* and *E. faecalis* (Girgin Ersoy *et al.*, 2019). Ozone materials are very active in water treatment because of their stable oxidizing agents. The oxidizing agents can react with commonly used antibiotics such as b-lactams, sulfonamide, macrolides, quinolones, trimethoprim, and tetracyclines. It can be distorted through direct oxidation during the ozonation of wastewater and can help remove these antibiotic residues from wastewater (Riaz *et al.*, 2020a).

Deactivation of antibiotic bacterial characteristics by breaking their functional group is the primary function of ozone material during the wastewater treatment process (Dodd, 2012). The knowledge of the efficiency of ozone materials for removing ARB/ARGs is limited because most studies focused on the effects of ozone on bacteria population reduction (Karaolia *et al.*, 2018). Thus, the results of the removal efficiency towards ARGs are still unclear. However, the removal of antibiotics with ozone is effective in wastewater and depends on ozone concentration and contact time during the water treatment process (Hollender *et al.*, 2009; Riaz *et al.*, 2020a). Ozone is a powerful oxidant used during the wastewater treatment process over the last decades. It can react with organic compounds under alkaline pH conditions. Ozone is not widely used for wastewater disinfection due to the cost of operation, maintenance issues of the first step, and a high ozone concentration during the treatment process (Gehr *et al.*, 2003).

Ozone materials have a high potential for the oxidation of various pharmaceutical compounds, including antibiotics and inactivation of total pathogens in drinking water and wastewater (Von Gunten, 2018). A study on the treatment methods, including ozone for the inactivation of gene copies of *sul(1), tet(G), intI(1),* and 16S rDNA, stated that ozonation reduced 16S rDNA. Still, ARGs were not notably inactivated (Zhuang *et al.*, 2015). The significant drawbacks of ozone materials are the possibility of generating dangerous by-products during partial oxidation of dissolved organic compounds. Also, for adequate disinfection, a high concentration of ozone materials is needed. Ozone gas preparation is not always direct and smooth, and there is a need to prepare it on site. These reports show that ozone materials are not the best option for targeting bacteria harbouring ARGs.

3.4.3. UV disinfectants

The UV disinfection process has been applied to wastewater treatment technology over the past few decades. The reason that UV irradiation can effectively inactivate bacteria within a short period and does not release toxic disinfection by-products (Guo *et al.*, 2013; Lee *et al.*, 2015). Similarly, UV disinfection is popular and promising disinfection material compared to chlorine in the wastewater treatment process. It recently demonstrated its effectiveness for removing ARB/ARGs in WWTPs and drinking water (Sanganyado and Gwenzi, 2019a). UV irradiation has some potentials that distinguish it from other materials used for wastewater treatment. Such possibilities are being non-toxic, environmentally friendly. Also, it uses short-wavelength (UV-C) light to kill microorganisms by destroying and disrupting nucleic acid and their DNA, which performs vital cellular functions among organisms. UV irradiation promotes oxidative species formation at a wavelength between 315-400 nm (UV-A), leading to oxidative damage on ARB/ARGs (Umar *et al.*, 2019b). The significant advantage of this treatment material is the ability to accomplish disinfection without using chemicals that may produce hazardous by-products. In North America, UV is commonly used as an alternative to

chlorine in wastewater treatment (Gehr *et al.*, 2003). It does not require a contact tank and steps for the neutralization process (like in chlorination). It produces few by-products compared to the use of chlorine as a water disinfection material. It involves photoreactivation or dark repair (Herrmann *et al.*, 1993). In UV disinfection, the reactor is an open channel involving water in contact with the UV light. Mercury arc-discharge lamps with quartz sleeves produce UV light. Iron and humic matter are used as UV-absorbing constituents in wastewater. The removal efficiency of ARB/ARGs in the effluent of sewage has some limitations because of the performance of both chemical and UV disinfection process and shielding target pathogens from the disinfecting agent.

Several studies have investigated the influence of disinfection on ARB/ARGs and concluded that UV disinfection could remove many ARB/ARGs from the effluent (Umar *et al.*, 2019b; Zhuang *et al.*, 2015). However, a report from numerous studies has shown that there are still some traces of ARB/ARGs in wastewater effluents after the UV disinfection procedure (Yuan *et al.*, 2015). The photoreactivation or dark repair can fix the damaged DNA by UV irradiation, thus reducing the efficiency and increasing microbial risk, i.e., reactivation promotes the survival of ARB after UV disinfection (Guo and Tian, 2019). The ARB also can gain their conjugative ability after photoreactivation (Guo *et al.*, 2012). The major drawback of UV irradiation points to the sustainability of UV technology on energy consumption, the flimsiness of UV lamp, short life span, and the use of mercury, which is difficult to separate from the effluent before disposal (Umar *et al.*, 2019b). The UV irradiation's inability to generate a specific wavelength could affect the removal efficiency since the inactivation mechanisms of ARB/ARGs depend on the wavelength.

3.4.4. Ultrasound radiation based Sonolysis

Ultrasound irradiation-based sonolysis is a new advanced oxidation process for the degradation of recalcitrant pollutants. It is the process of using ultrasonic waves to

disintegrate or decompose pollutants through the acoustic cavitation process. Acoustic cavitation is the interaction of ultrasound waves at high intensity with dissolved gases in an aqueous solution. This interaction involves forming and collapsing the bubbles in an aqueous solution irradiated by intense ultrasound (Pinon and Pinon, 2020). The bubble collapse makes the ultrasound generate hydroxyl radicals that have been proven to be effective in removing pollutants from water (Ince, 2018; Rayaroth *et al.*, 2017). The liquid's temperature and pressure reach thousands of Kelvins and hundreds of atmospheres during the bubble collapse (Pinon and Pinon, 2020; Rooze *et al.*, 2013). The energy from ultrasound-induced acoustic cavitation split the oxygen and hydrogen molecules' chemical bonds in water, forming hydroxyl ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2). Increasing the ultrasonic amplitude results in sonochemical activities during water treatment (Pinon and Pinon, 2020). Sonolysis undergoes two irradiation methods during treatment process; they are the pulse and continuous irradiation. Each method is effective based on the nature of pollutants. The major drawbacks of this method are the removal of some selected antibiotic which are hydrophobic in nature. Incomplete elimination of antibiotics residues increases the risk of ARB/ARGs proliferation in water. Also, the cost of maintaining a constant temperature with chiller and ethylene glycol is another challenge because ethylene glycol may form dangerous by-products and can be difficult to separate from treated water. Production of high amount of hydroxyl radicals required for effective degradation of ARB/ARGs may be costly.

3.5. Restriction to indiscriminate use of antibiotics

Antibiotics gained popularity because of their effective action against microbial pathogens and attracted massive attention in the 1950s. Bbosa *et al.*, (2014) gave a detailed review of antibiotics as such; we will not dwell too much on it in this paper. However, the use of antibiotics at a large scale in human and animal medicine accelerates the proliferation of resistant bacteria and their genes. It even develops multidrug-resistant bacteria being the most

dangerous (Aarestrup, 2015). The estimated report states that animals' and humans' global consumption of antibiotics in foods lies between 100 000 to 200 000 tons per annum. In comparison, China's use alone is more than 25 000 tons (Van Boeckel *et al.*, 2017). The wrong usage of these antibiotics leads to ARB/ARGs' emergence and weakens therapeutic properties against human and animal pathogens. It causes resistance to the most commonly used antibiotics (Singh, Singh, *et al.*, 2019), resulting in a prolonged stay in the hospital, increased mortality and morbidity rate, as well as the drug-resistant bacteria developing in the gut of humans and animals (Joo *et al.*, 2019; Vaz-Moreira *et al.*, 2014). The consumption of undercooked meats that contain antibiotic residue can facilitate the development of drug-resistant bacteria in the human gut. At the same time, animals developed it from the consumption of feeds that contain antibiotics or antibiotics residue (Mekuria *et al.*, 2019).

The continuous discharge of both high and low concentrations of antibiotics into wastewater treatment plants from these sources promotes the development and dissemination of antibiotic resistance genes in the environment, causing severe public health risk (Neil, 2016; Singh, Singh, *et al.*, 2019). Because of the harmful effect of antibiotics in the environment, specifically wastewater, access to these drugs in many advanced countries of the world is heavily restricted, and restrictions are strongly enforced (Mossialos *et al.*, 2010; Simon *et al.*, 2017). For instance, Belgium's government established and implemented policies geared towards changing physicians' prescription practices (family Doctors get feedback on the prescription methods). The Belgium Government resorted to public awareness of antibiotic consumption and generated patient education materials, using leaflets, booklets, advertisements on television and radio, and websites (Power and Elliott, 2006). This effort was reported to have significantly reduced the indiscriminate use of antibiotics in Belgium (Bauraind *et al.*, 2004). In addition to reducing consumption and indiscriminate use of antibiotics, data from Hungary, Finland, Japan, the USA, and Iceland suggest that national

policies on the restriction of access to antibiotics resulted in the drastic reduction of drug-resistant bacteria infections (Barrett *et al.*, 2009; Mariana *et al.*, 2008). This scenario cannot be said to be obtainable in Africa as in countries where these drugs are restricted; enforcement is not active, while in others, access is unrestricted. Therefore, calls for the adoption of regulations, policies, and public campaigns among Africa nations on the appropriate use of antibiotics to limit the level of drug-resistance bacterial infections in the environment.

3.6. Conclusion

Antibiotic resistant bacteria (ARB) in the wastewater treatment plant are an additional strain on the effluent's quality for reuse because of ARGs' increasing presence in the WWTPs. Some of the ARGs commonly detected include sulfonamides, tetracycline, beta-lactam, and fluoroquinolones resistance genes. They are the leading cause of disease outbreaks in the environment. Several treatment methods/materials used for the removal of these ARGs from wastewater were ineffective. This calls for more research in other to improve the removal of ARGs in different water matrices. This review highlights the importance and potentials of silver and silica oxide nanoparticles for removing these substances from wastewater, going by the unique properties they boast of. Incorporating metallic nanoparticles is a promising way of eliminating these pollutants from wastewater. Finally, setting up policies to prevent the indiscriminate use of antibiotics is a sure way to avoid these pollutants' proliferation in wastewater. With this, the challenge with the selection of appropriate material will not be a concern.

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Chapter 4

The effectiveness of silver nanoparticles as a clean-up material for water polluted with bacteria DNA conveying antibiotics resistance genes: Effect of different molar concentrations and competing ions

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Chapter 4

The effectiveness of silver nanoparticles as a clean-up material for water polluted with bacteria DNA conveying antibiotics resistance genes: Effect of different molar concentrations and competing ions

Abstract

This study employed silver nanoparticles to remove DNA conveying antibiotic resistance genes from water. Three different molar concentrations of silver nanoparticles represented as BD1 (0.1 M), BD2 (0.5 M), and BD3 (1.0 M) were synthesized as adsorbents and evaluated in a batch adsorption system for the removal of bacteria DNA conveying antibiotic resistance genes from simulated aqueous solution. The authenticity of the adsorbents was confirmed by characterization techniques using Fourier transformed infrared spectroscopy (FTIR), scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectroscopy (EDX), and x-ray diffraction spectroscopy (XRD) indicated the successful synthesis of these AgNPs. Adsorption studies involving the different operating conditions on the synthesized materials showed that pH affects the removal of DNA with increased removal efficiency observed at acidic pH (removal percentage ranging from 50.26-87.61%, 65.80-87.79%, and 69.23-87.92% for BD1, BD2, and BD3 respectively). Maximum adsorption equilibrium was achieved after 180, 195, and 225 mins for BD1, BD2, and BD3. The isotherm study revealed that Langmuir model is the best fit compared to Freundlich model with highest correlation coefficient and reduced Chi-square (X^2) of R^2 = 0.97625 and X^2 = 0.12142, R^2 = 0.96049 and X^2 = 0.24403, R^2 = 0.85108 and reduced X^2 = 1.00914 for BD1, BD2, and BD3 respectively. The kinetic study for the adsorption process indicates that the adsorption of bacteria DNA onto AgNPs obeyed pseudo-second order with the highest R^2 values (ranging from 0.90 to 0.98). Similarly, competing ions (cations and anions) influenced the adsorption capacity in this study. Therefore, this study concludes that AgNPs demonstrated effectiveness in

removing bacteria DNA-conveying ARGs from water and will serve as an excellent option to tackle the menace of ARGs in water.

4.1 Introduction

The interference of antibiotic resistome in wastewater treatment facilities (WWTFs) has become a global challenge of increasing environmental concern. It poses a considerable health risk to the public due to the release of untreated effluent from the hospital, domestic, agricultural, and pharmaceutical industries into WWTFs (Sharma *et al.*, 2016). Research cleaning up bacteria DNA contaminants from WWTFs has recently interested environmental researchers and wastewater treatment management. Bacteria is responsible for about 91% of food-borne diseases, clinically treated with antibiotics (Salaheen *et al.*, 2014). The residues may be directly or indirectly discharged into WWTFs and other water matrices such as faeces and urine from households and hospitals. Another way of transferring bacteria into WWTFs is the discharge of untreated effluents from agricultural farms and pharmaceutical industries into the water source. Consumption of bacteria-contaminated water causes diarrhoea, vomiting, headaches, fever, fatigue, cramps, nausea, etc. These illnesses increase morbidity and mortality rate among humans and animals. The bacteria acquire antibiotic resistance when exposed to different antibiotics during treatment. Antibiotic resistance (A.R.) is carried by antibiotic-resistant bacteria (ARB) and conveyed as antibiotics resistance genes (ARGs) (Checcucci *et al.*, 2020; Triggiano *et al.*, 2020). The bacteria acquire ARGs in their deoxyribonucleic acid (DNA) through promiscuous gene transfers such as a conjugative plasmid, transposable elements, and integron, including genes encoding antibiotic resistance from one bacterium to another (Che *et al.*, 2021). ARB transfers ARGs vertically from one bacterium to another in the water environment, making ARGs exist permanently. In addition, ARGs disseminate in the water environment via horizontal gene transfer (HGT), with mobile genetic elements which transfer ARGs through conjugation, transduction, and transformation

medium (Chen, Yin, *et al.*, 2019). HGT is the most effective medium for spreading ARGs from ARB to water environmental bacteria (Abe *et al.*, 2021; Barancheshme and Munir, 2018). It transfers genetic material from one bacterium to another bacteria of the same and different species (Dimitriu *et al.*, 2014; Rout *et al.*, 2021).

Bacteria DNA conveying ARGs have been reported to exist in the influent and effluent of WWTFs and natural waters. Excessive application of antibiotics promotes the accumulation of antibiotic-resistant bacteria (ARB) and antibiotics resistance genes (ARGs). Over 90% of antibiotics residues are discharged directly into WWTFs or other water matrices (Iwu *et al.*, 2020; Nippes *et al.*, 2021). The exposure of these bacteria to different antibiotics synergistically promotes resistance mechanisms (Rafraf *et al.*, 2016; Shi *et al.*, 2022). Therefore, adequate clean-up strategies are vital in preventing ARG diffusion in WWTFs and other water environments.

DNA is a molecule made up of nucleotides. Each nucleotide comprises components: a phosphate group (phosphorous atom bonded to an oxygen atom), a sugar group, and a nitrogenous base (Abe *et al.*, 2021; Gomes *et al.*, 2015). It is a nucleic acid containing genetic instructions for all cellular life development and proper functioning. Untreated effluents from hospital, domestic and pharmaceutical industries are the carrier of bacteria DNA conveying ARGs because these sources extensively use large quantities of antibiotics (Ezeuko *et al.*, 2021a; Yao *et al.*, 2021); it is feasible that ARGs originate from these sources. Their casualties are on the rise due to the design of WWTFs and the lack of treatment material that would halt the widespread of this toxic contaminant (ARGs).

WWTFs are the facilities that allow the combination of treatment methods such as physical, chemical, and biological processes to purify or remove contaminants from wastewater (Edokpayi *et al.*, 2020; Gray, 2021). WWTFs have been known as a "hotspot" for bacteria

DNA conveying ARGs, especially those receiving effluents from hospital and pharmaceutical industries (He *et al.*, 2019; Pärnänen *et al.*, 2019; Pazda *et al.*, 2019b). The effluents from these sources affect the water treatment process because the design of WWTFs cannot effectively eradicate pharmaceutical products. WWTFs across the globe have been reported to have high nutrient content, large microbial density, and subinhibitory concentration of antibiotics (Aubertheau *et al.*, 2017; Kumar and Pal, 2018). These features make it a comfortable environment for disseminating bacteria DNA conveying ARGs. A survey carried out between 2015 to 2020 on ARG incidence across six continents (Africa, Asia, Australia, Europe, North America, and South America) confirmed the occurrence of high ARG concentrations (Ezeuko *et al.*, 2021a). Still, few studies have proposed methods of eradicating these toxic contaminants from WWTFs. However, this concern has led to finding efficient and adequate treatment materials and methods to eradicate bacteria DNA conveying ARGs from WWTFs effectively. Several treatment methods and materials could remove a large amount of antibiotic residue and ARB from wastewater, but they are ineffective in removing ARGs (Dong *et al.*, 2018; Foroughi *et al.*, 2022a). Conventional, advanced oxidation processes and biodegradation treatment materials and methods effectively eradicate a substantial amount of antibiotics and ARB but are ineffective in removing ARGs (Garrido-Cardenas *et al.*, 2020; Zhang, Li, *et al.*, 2020).

Nanomaterials' adsorption treatment strategies could be one of the best physical treatment strategies for removing ARGs due to the high specific surface area possessed by the adsorbents (Ahmed *et al.*, 2021; Rashid *et al.*, 2021). The adsorption process design allows other intense wastewater treatment strategies to ensure more effective removal of contaminants from wastewater. Moreover is cost-effective, efficient, and the best physical method for removing antibiotics-related contaminants and other pharmaceuticals from wastewater (Riaz *et al.*, 2020a). Their unique features, such as size-dependent properties,

large surface area, high degree of functionalization, and affinity to bind positive or negative contaminants, are responsible for their excellent removal of toxic contaminants from wastewater (Dhakras, 2011; Ugwoke *et al.*, 2020). Also, they are known to contain a high capacity for contaminants removal and are very easy to recover after treatment. Several nanomaterials such as titanium (Jang *et al.*, 2018), graphene oxides (Bytesnikova *et al.*, 2019), silica (Li *et al.*, 2012), and cerium oxide (Anthony et al., 2020) have been employed to remove ARGs from wastewater. Among all, silver nanoparticles (AgNPs) seem to have unique potential for the inactivation of bacteria DNA and removal from wastewater through electrostatic interaction (Ezeuko *et al.*, 2021a; Reidy *et al.*, 2013).

AgNPs are a material that is widely used for surface coating, reducing bacterial infection in food, dermal formulations, and water (Prasad *et al.*, 2021). AgNPs are synthesized through ultrasound irradiation, chemical reduction, microwave dielectric heating, and electrochemical and green methods (Karadirek and Okkay, 2019). AgNPs possess unique properties such as optical, electrical, catalytic, and binding properties (Ali *et al.*, 2021). It can inhibit DNA replication through oxidative stress and prevent bacteria from producing resistance (Shaikh *et al.*, 2019). The particle size, morphology, structure, and size distribution can be controlled by the synthesis concentration of the precursor (Hassanien and Khatoon, 2019). The large surface area of AgNPs increases high surface energy, which promotes surface reactivity and sorption in the adsorptive site. AgNPs formed particle size in the range of 1-100nm with an oxidation state of 0, +1, +2, and +3, which have an affinity to target negative contaminants in an aqueous solution (Singh *et al.*, 2017). AgNPs are easy to synthesize, cost-effective, eco-friendly, and exhibit strong antibacterial properties that could stop the proliferation of bacteria (Pazda *et al.*, 2019b). Due to these excellent properties and the fact that bacteria DNA are highly negative in their backbone (Fiorentino *et al.*, 2019), The authors hypothesized that removing bacteria DNA conveying ARGs could be significantly achieved

using AgNPs (positively charged nanoparticles) as adsorbent. Therefore, we investigated the efficiency of different molar concentrations (0.1M, 0.5 M, and 1.0 M) of silver nanoparticles (AgNPs) as adsorbents to remove DNA conveying ARGs from water. The kinetics and isotherm parameters for the adsorption process were also investigated. This study will be the first to report on removing DNA conveying ARGs from water using AgNPs

4.2 Materials and methods

4.2.1 Chemicals

Silver nitrate ($AgNO_3$), tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), glucose ($C_6H_{12}O_6$), and sodium hydroxides (NaOH) were purchased from Sigma Aldrich United State of America. Nuclease-free water and DNA kit were purchased from Thermo Fischer South Africa. All the chemicals used in this study were of analytical grade and used as purchased.

4.2.2 Synthesis and characterization of different molar concentrations of (AgNPs)

The synthesis of silver nanoparticles (AgNPs) was performed through the chemical reduction method described by (Wang *et al*., 2005) with slight modifications. The molar concentration of silver nitrate ($AgNO_3$) was varied to obtain different concentrations of AgNPs in ascending order of 0.1 M, 0.5 M, and 1.0 M. In contrast, the other precursors such as tri-sodium nitrate ($Na_3C_6H_5O_7.2H_2O$), glucose ($C_6H_{12}O_6$), and sodium hydroxide (NaOH) remain constant. The synthesis was performed in the following consequence. Solution A was prepared by dissolving 100 mM of $AgNO_3$ into 20 mL of distilled water. Solution B contains an equal concentration (20 Mm) of tri-sodium nitrate ($Na_3C_6H_5O_7.2H_2O$), glucose ($C_6H_{12}O_6$), and sodium hydroxide (NaOH) dissolved in 60 mL of distilled water. NaOH was added to speed up the rate of reaction. Then, solution B was allowed to heat up to 60 ^oC under vigorous stirring for 2 h. Solution A ($AgNO_3$) was added drop by drop and heated for 6 h at 65 ^oC, and stirring continued for 24 hrs. The reaction was performed in the dark. The nanoparticles were then separated by centrifugation at 4,400 rpm for 15 mins and washed

several times to obtain pure AgNPs labelled BD1 (0.1 M), BD2 (0.5 M), and BD3 (1.0 M). The morphologies, functional group identification, and elemental compositions of AgNPs were determined using a scanning electron microscope (SEM) coupled with an energy dispersive x-ray spectroscope (EDX) (JOEL JSM-6390LVSEM) and Fourier transform infrared spectroscope (FTIR) (Perkin-Elmer Universal ATR 100) respectively. Ultraviolet-Visible Spectroscopy (UV-VIS) was used to analyse the concentration of synthesized samples. X-ray diffraction (XRD) (Bruker D8) determined the samples' crystallinity and phase compositions.

4.2.3 Extraction and molecular characterization of bacterial DNA

The antibiotic-resistant *Vibrio Parahaemolyticus* bacteria used in this study was obtained from our laboratory archives isolated from a wastewater treatment plant University of Fort Hare, Alice. The bacteria isolate was subjected to an antibiotic susceptible test before the commencement of genomic DNA extraction. The test showed that *Vibrio Parahaemolyticus* bacteria were resistant to five (5) different antibiotic drugs. These are tetracycline, PB 300, meropenem, amikacin, and ciprofloxacin. Genomic DNA extraction was conducted using the boiling method (Foroughi *et al.*, 2022b) with slight modification. 12.5 g of Luria Bertani (L.B.) broth was dissolved in 500 mL of sterile distilled water and autoclaved at 121 ^{o}C for 15 mins. The solution was allowed to cool at room temperature. The bacteria isolates (*Vibrio Parahaemolyticus*) were suspended in the solution and incubated for 22 hours at 37 ^{o}C. After incubation, the solution was centrifuged at a speed of 5000 rpm for 10 mins, and the supernatant was decanted. The pellet obtained was suspended in 200 µL nuclease-free water, vortexed briefly, and boiled for 10 mins at 100 ^{o}C. The resulting solution was centrifuged at 13,400 rpm for 15 mins to obtain the genomic DNA. The DNA stock was stored in a -20 ^{o}C refrigerator for future analysis. The concentration and purity of DNA were measured by finding the absorbance ratio at 260 nm, 280 nm, and 320 nm using Multiparameter HACH

DR 6000 Ultraviolet Spectroscopy. The polymerase chain reaction (PCRs) assay was used to confirm Vibrio Parahaemolyticus isolates, and the presence of the resistance gene was also confirmed using the primer presented in table 4.1. The resulting PCR products were examined using a mixture of 2% agarose gel, stained with ethidium bromide (Han *et al.*, 2015), pictured using the Alliance BioDoc-It System.

Table 4.1: PCR primers, sequences, and protocols used in this study

Class of Antibiotic	PCR primers	Primer sequences	Product size (bp)	PCR protocols	References
Tetracyclines	*tetA*	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	94 °C – 5 min; 35[94 °C – 1 min; 55 °C – 1 min; 72 °C 1½ min]; 72 °C – 5 min.	(Titilawo *et al.*, 2015)

4.2.4 Batch adsorption experiment

The bacterial DNA (2 mL) was used to contaminate nuclease-free water (NFW). 20 mg of adsorbents (AgNPs) of different molar concentrations represented as BD1, BD2, and BD3 were separately weighed and added to the prepared contaminated NFW for the batch adsorption study. The effects of pH (pH range from 2 to 9 by dropwise adding 0.1 mol/L NaOH or 0.1 mol/L HCl solutions), adsorbent dosage (10-30 mg), contact time (180, 195, and 225 mins), and competing ions (0.075, 0.3, 0.093 g for cations and 0.59, 0.73 and 0.61 g for the anions) on the DNA removal from aqueous solution on each adsorbent were investigated. The experiments were conducted on a KS260 control orbital shaker at a speed of 300 rpm using 25 mL bottles with different DNA concentrations (obtained from the stock solution). After equilibration, the mixtures were subjected to centrifugal force to separate the adsorbents, and supernatants were decanted. The concentrations of DNA left in the supernatant solution were determined using a dsDNA assay kit (Q32850) Qubit 1.0

Fluorometer (Thermofischer). The amount of DNA adsorbed per unit mass (qe) of AgNPs in all the samples was calculated according to Eq (4.1) and (4.2), respectively (Fu *et al.*, 2015).

$$\mathbf{qe} = \frac{(Ci - Cf)}{m} \times V \quad \text{..... (4.1)}$$

$$\mathbf{\%R} = \frac{(Ci - Cf)}{Ci} \times \mathbf{100} \quad \text{..... (4.2)}$$

Where qe = the adsorption capacity at equilibrium (µg/mL), V = volume of adsorbate solution (mL), m = equal to adsorbent mass (mg), C_i and C_f are the initial and final concentrations of DNA measured in µg/mL, and % R is the removal efficiency.

4.2.4.1 Isotherm and kinetic studies

Kinetic experiments were performed using a similar procedure to the equilibrium experiment. The working concentrations ranging from 4.98, 9.92, and 14.98 µg/mL (obtained from DNA stock solution) were prepared, and a known adsorbents dose of 20 mg was added to each working solution. These working concentrations were decided because of the concentration of residual DNA obtained previously from municipal wastewater plants (Mcconnell *et al.*, 2018). The pH 6.9 was maintained at different working concentrations. The solutions were agitated in a KS260 control orbital shaker at a speed of 300 rpm for a contact time varied from 0 to 360 minutes. The procedure was applied to all the adsorbents (BD1, BD2, and BD3) investigated at room temperature. The adsorbents from different working solutions (BD1, BD2, and BD2) were separated by centrifuging at various time intervals, and the supernatant decanted was analysed by the same procedures as the batch adsorption experiment. The amount of residual DNA adsorbed at each time interval per unit mass of adsorbents was calculated using Equation (4.1)

The adsorption isotherm model was evaluated using Freundlich and Langmuir models. In contrast, kinetic models were evaluated using Natarajan and Khalaf's First order (NKFO) and Pseudo-second order (PSO) equations presented in Table 4.2.

Table 4.2: Representation of isotherm and kinetic models, their equations, and parameters adopted in this study

Adsorption Modelling		Equation	Parameters	Reference
Isotherm Model	**Freundlich**	$Logqe = logKf + \frac{1}{n} logCe$	***qe*** (µg/mL): the amount of DNA adsorbed. ***Kf*** (µg/g): Freundlich adsorption constant. ***n*** = empirical constant showing that adsorption occurred on heterogeneous surfaces through a multilayer adsorption mechanism. ***Ce***: equilibrium concentration of adsorbate.	(Aarab *et al.*, 2020)
	Langmuir	$qe = \frac{KL\ qmCe}{1 + KLCe}$	***qe*** (**µg/g**): the amount of DNA adsorbed per unit mass of adsorbent at equilibrium. ***qm*** (**µg/g**): theoretical maximum adsorption capacity. K_L.: is a Langmuir isotherm constant related to the adsorption energy (**mL/µg**). **Ce**: equilibrium concentration	(Yusuff *et al.*, 2019)
Kinetic Model	**Natarajan and Khalaf First order equation**	$log(\frac{Ci}{Ct}) = \frac{K1}{2.303\ t}$	*Ci* and *Ct* (**µg/mL**) are the initial concentration of DNA and the final amount of DNA adsorbed at contact time t (mins), respectively. K_1: Adsorption rate constant for first-order equation (min^{-1})	(Idris *et al.*, 2012)

	Pseudo-second order (PSO)	$\frac{t}{qt} = \frac{1}{K_2 qe^2} + \frac{1}{qe} t$	K_2 is the rate constant of Pseudo-second order adsorption (g/(µg mins)). ***qe*** and ***qt*** represent the same as in the First order equation	(Karim *et al.*, 2012)

4.3. Data analysis

The data obtained from this study were plotted and analysed using OriginPro Graphing and Analysis 2021 (v.9.8.0 200), Microsoft Excel 2019, and Image J software.

4.4. Results and discussion

4.4.1 Isolate identification and molecular characterization of bacteria DNA

The PCR product amplifications were visualized using gel electrophoresis showing the identification of *Vibrio parahaemolyticus* isolates of molecular weight of 503bp (Figure 4.1a) and *tetA* resistance gene of molecular weight of 210bp (Figure 4.1b). Table 4.2 shows the primers and PCR conditions of the targeted resistance gene.

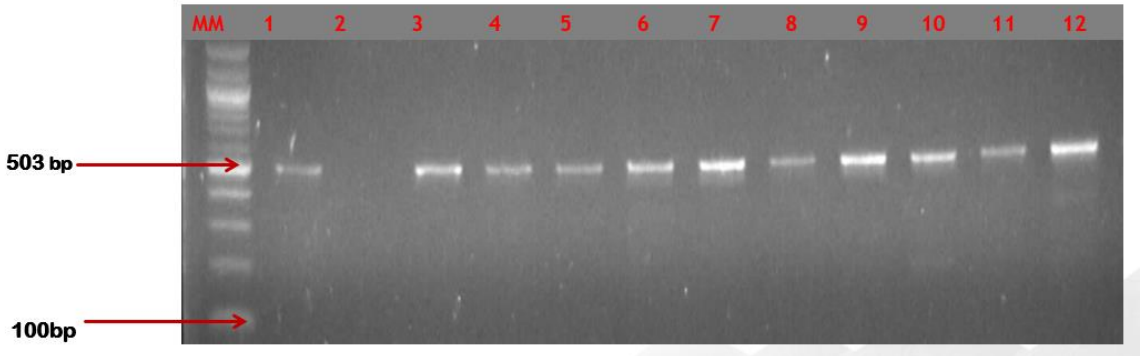


Figure 4.1 A: Gel electrophoresis confirming vibrio parahaemolyticus isolates using toxR gene; Lmm: base pair gene marker; lane 1: positive control; lane 2: negative control; lane 3-12: positive isolate

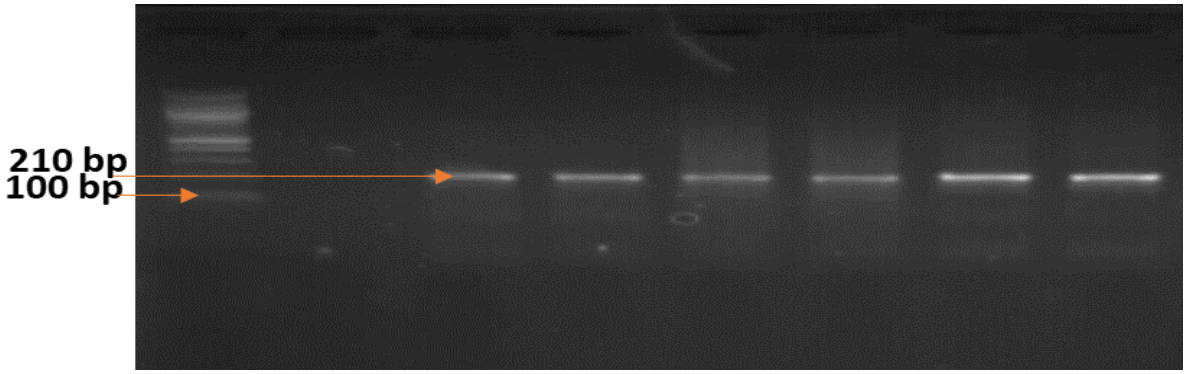


Figure 4.1 B: Gel representing tetracycline resistance gene (tetA) amplified at 210 bp

4.4.2 Characterization of adsorbents

4.4.2.1 Point of zero charges of as-synthesized AgNPs samples (BD1, BD2, and BD3)

The Point of zero charges (PZC) of all the AgNPs samples (BD1, BD2, and BD3) was investigated. Meanwhile, the PZC is generally described as the pH at which the net charge of the total particle surface is equal to zero or neutral (Yang *et al.*, 2020). Figure 2 shows that the PZC result on all the as-synthesized samples of AgNPs is in the range of 8.6, and it is similar to an already published report on the synthesis of silver nanoparticles (Dawodu *et al.*, 2019). It implies that, below this value (pH < 8.6), the surface of adsorbents acquires a positive surface charge due to protonation from the nitrate group and dissociation of $H^{+\cdot}$ (Guo *et al.*, 2020). Beyond 8.6 (pH > 8.6), it tends toward negative due to deprotonation of the nitrate group (Jimmy et al., 2021; Lv et al., 2021).

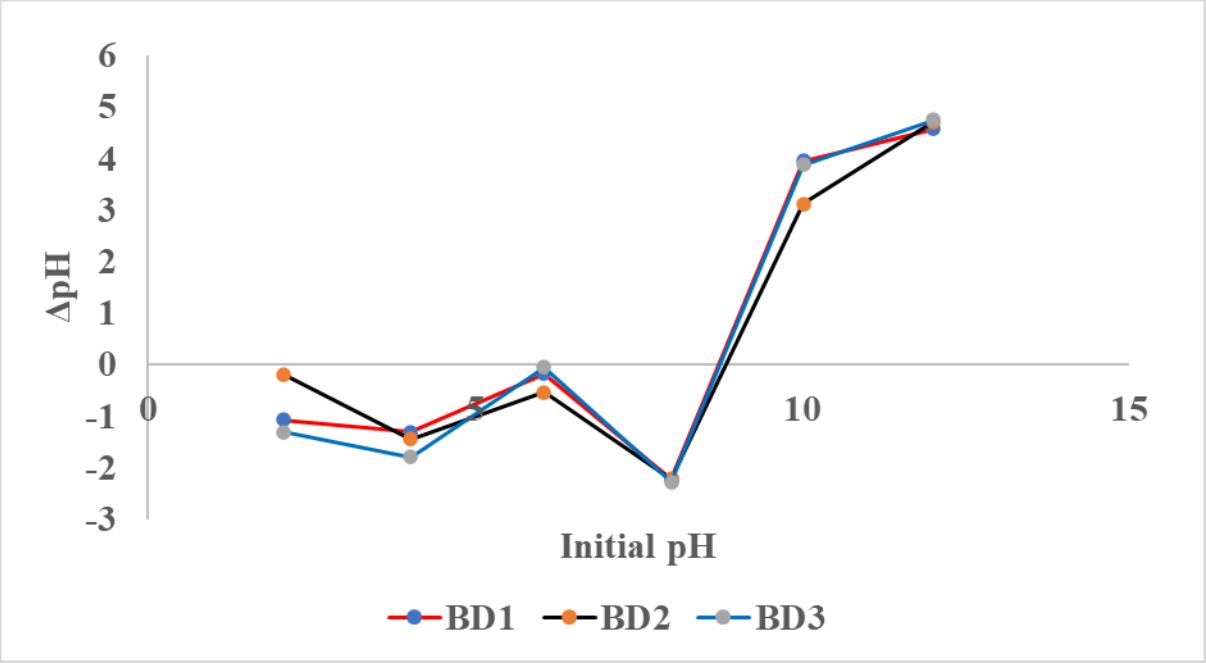


Figure 4.2: PZC for the molar concentrations (0.1, 0.5 and 1.0 M) of as-synthesized AgNPs represented as BD1, BD2 and BD3

4.4.2.2 SEM analysis

The Scanning Electron Microscopy (SEM) analysis of as-synthesized AgNPs (BD1, BD2, and BD3) was captured at different magnifications as presented in Figures 4.3 A-B, C-D, E-F, respectively. The spherical and irregular multi-branched particles' morphology signified the particles' authenticity. The SEM micrograph revealed that adsorbents formed aggregation into clusters and bunches (Alzahrani, 2020; Vilchis-Nestor *et al.*, 2014). The presence of large and small spherical particles was present, resulting in the aggregation of the particles through weak force (Metreveli *et al.*, 2015).

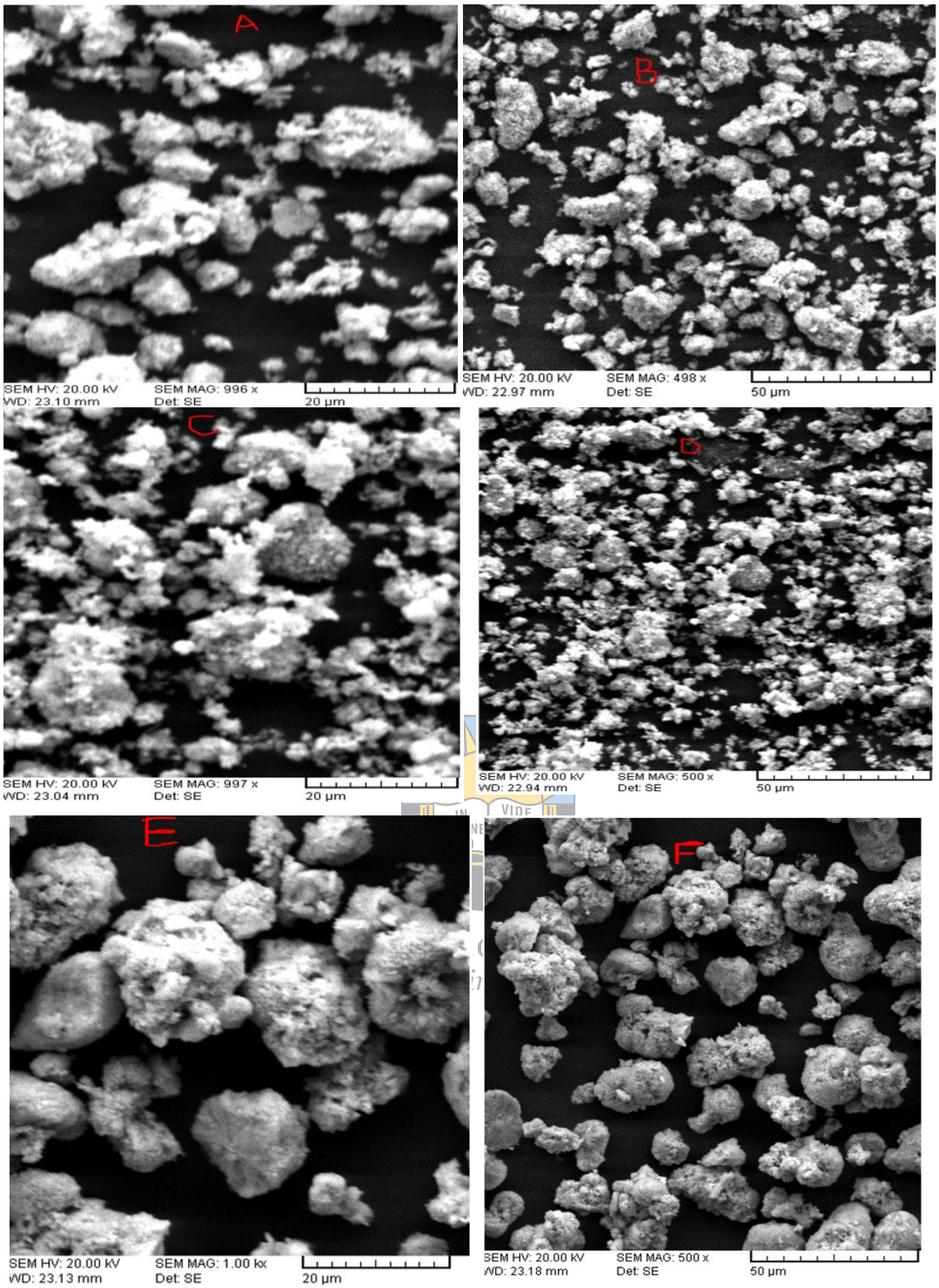


Figure 4. 3 A, B, C, D, E, and F: SEM images of BD1, BD2, and BD3 at low (A, C, E) and higher (B, D, F) magnifications showing spherical and irregular multi-branched morphology of the particles

4.4.2.3 EDX analysis

Energy-dispersive X-ray spectroscopy (EDX) determined the elemental composition of synthesized silver nanoparticles represented as BD1, BD2, and BD3. Figures 4.4 A, B, and C showed the strongest signal from the silver (Ag) region and the weak signals from carbon (C)

and oxygen (O). Observing optical peaks for Ag in BD1, BD2, and BD3 was seen approximately at 3 KeV, showing weight percentages (%) of 87.98, 89.65, and 90.50 and atomic percentages (%) 47.89, 50.54, and 54.86, respectively. The result is similar to previously reported studies (Hamouda *et al.*, 2019; Raza *et al.*, 2016). The absence of other elements confirmed the pure crystalline nature of silver nanoparticles.

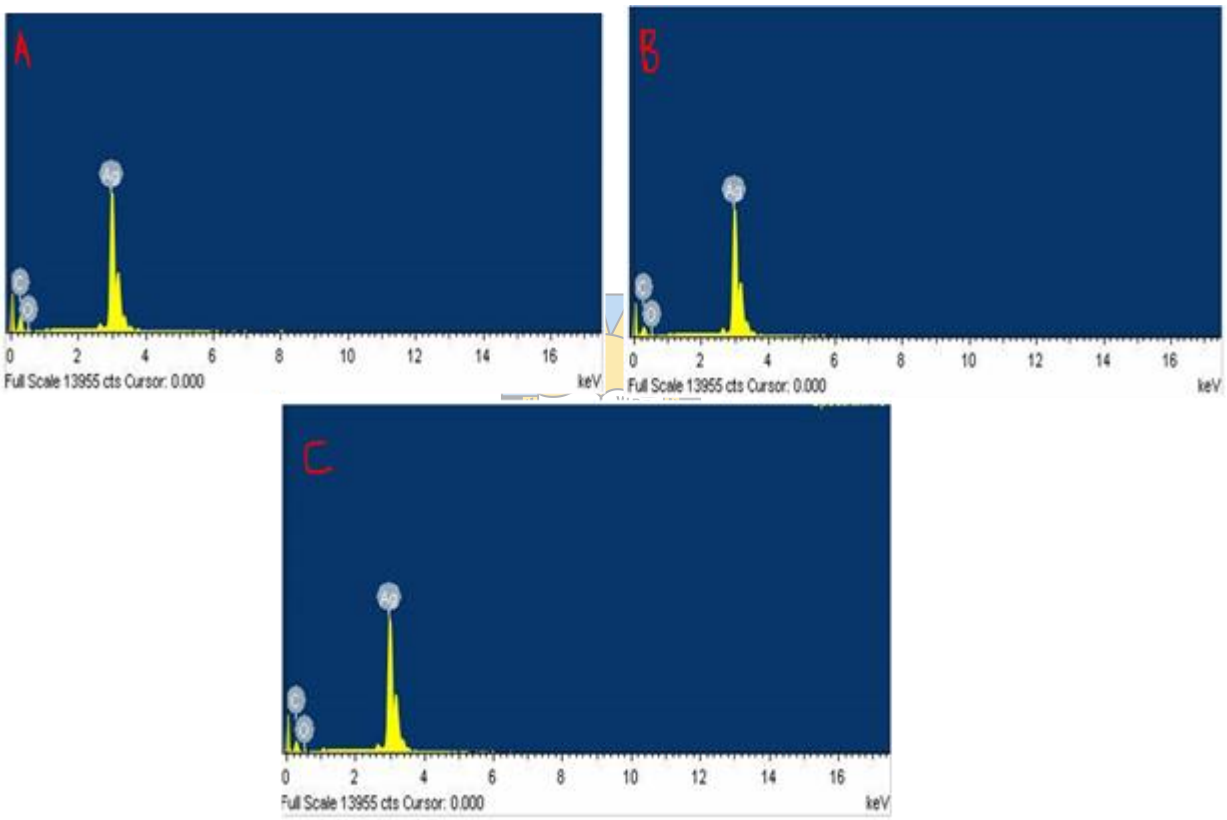


Figure 4.4 A, B, C: Energy-dispersive x-ray spectroscopy (EDX) of AgNPs represented as BD1, BD2, BD3 showing the elemental composition of the nanoparticles

4.4.2.4 UV Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) is an essential and reliable technique for the characterization of synthesized metallic nanoparticles. UV-Vis was used to determine the maximum absorption of all the as-synthesized AgNPs. UV-Vis spectral (Figure 4.5) showed

an intense absorption peak around 420 nm and 550 nm at 0.6, 0.55, and 0.49 h of incubation for BD1, BD2, and BD3, respectively. The peaks confirmed the formation of the homogenous spherical and irregular shapes of AgNPs. The result of the present study corroborates with the report obtained from the synthesis of AgNPs from Aspergillus (Kumar, 2012). The report has shown that homogenous AgNPs can be produced at the surface of the plasmon resonance band at 420 nm. The position and shape of the plasmon absorption band at 420 nm that led to the formation of homogenous spherical silver nanoparticles depends on the particle size, shape, and dielectric constant (Guzmán *et al.*, 2009).

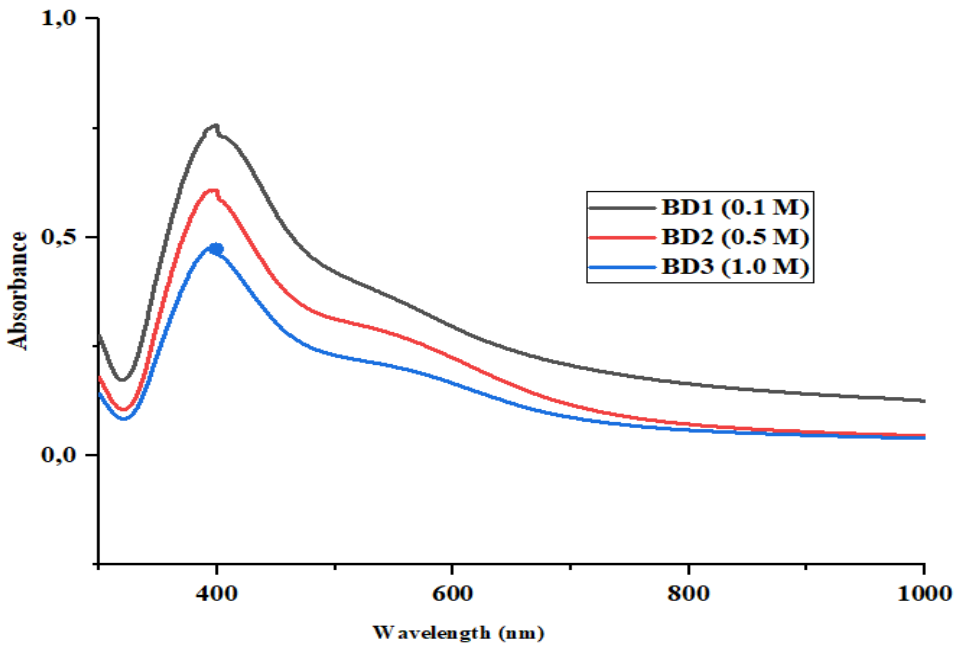


Figure 4.5: UV-visible absorption spectra of different molar concentrations of the as-synthesized AgNPs

4.4.2.5 XRD analysis

X-ray diffraction (XRD) is an analytical instrument used to determine or measure a synthesized material's degree of crystalline structures, size, and phase. Figure 6 showed the XRD pattern of the molar concentrations of as-synthesized AgNPs (BD1, BD2, and BD3) with intense diffraction peaks of 38.07º {111}, 44.87º {200}, 67.75º {220} and 77.49º {311} which corresponds to JCPDS files number 00-004-0783 (Agasti and Kaushik, 2014). The

average crystallinity size of all the adsorbents was determined using the Scherrer equation (Equation 3):

$$D = \frac{K\lambda}{\beta cos\theta} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.3)$$

Where D = Average crystallite size (nm)

K = Scherrer constant of 0.94 for spherical crystallites with cubic symmetry

ʎ = X-ray wavelength, CuKα = 1.5406 Å

β = line broadening at FWHM in radians

θ = Bragg's angle in degree, half of 2θ

The average crystalline size of all the adsorbents is BD1 = BD2 = BD3 = 30.27 nm. The appearance of significant intense peaks confirmed the pure crystalline nature of AgNPs.

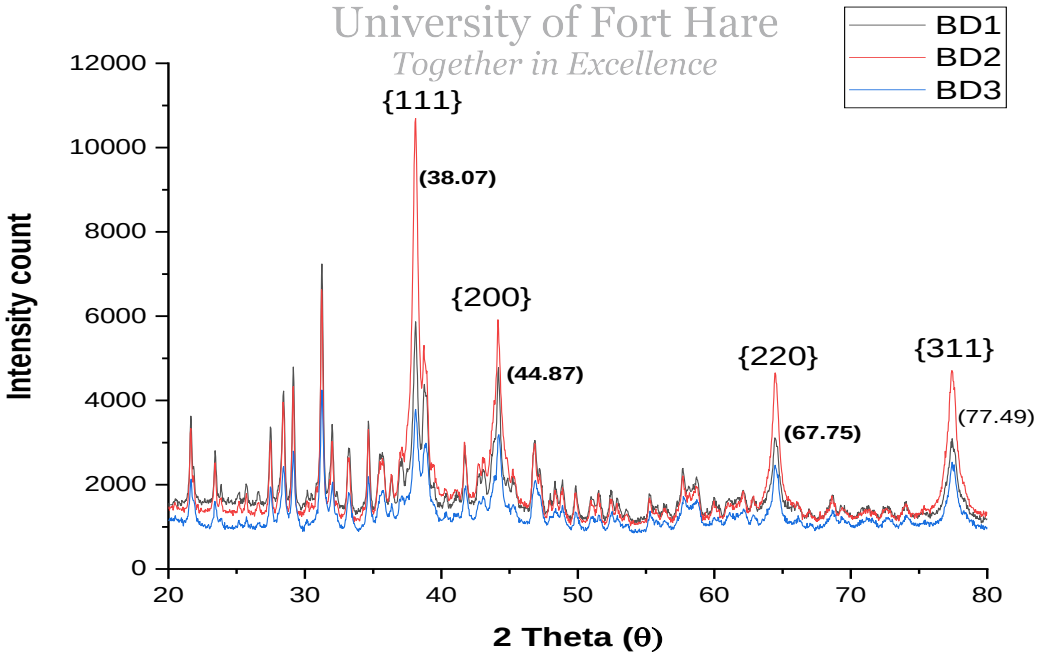


Figure 4.6: XRD pattern for the as-synthesized AgNPs of different molar concentrations represented as BD1, BD2, and BD3

4.4.2.6 FTIR spectroscopy

FTIR spectroscopy is a non-invasive technique used to confirm vibrational bands of the active functional group present in the material. (Zhang, Liu, *et al.*, 2016). FTIR measurement (Figure 4.7) revealed the potential functional group in this study's as-synthesized three adsorbents (BD1, BD2, and BD3). The FTIR profile exhibited nine (9) peaks having the following wavenumber 3320, 1622, 1550, 1450, 1388, 1296, 1071, 841, and 606 cm ^{1}. The absorbance band located at 3320 cm $^{-1}$ is assigned to the stretching vibration of N-H of primary amine. The primary amine, i.e., stretching vibration of N-H, was reported at 3413 cm^{-1} (Dawodu *et al.*, 2019). The absorbance band at 1622 cm^{-1} corresponds to $^{-}$O.H. (hydroxyl) stretching assigned to the hydroxyl group from glucose. Other peaks at 1550, 1450, 1388, 1296, 1071, 841, and 606 correspond to the stretching vibration of N-O from a nitro compound, C-H bending from an alkane, C-N bending vibration of amine, C-N stretching from aromatic amine, C-O stretching vibration of alcohol, C=C bending of alkene and C-Cl from halo compounds. The result is similar to an already published report on the FTIR of pure crystalline AgNPs (El-Kheshen and El-Rab, 2012; Shahjahan, 2017). The functional group of NO_3^- is from the starting material, which is $AgNO_3$ (Kumar, 2012; SODHA* *et al.*, 2015). The Ag-O vibrational peak was observed around 410 $cm^{-1.}$ All are the functional group covering AgNPs.

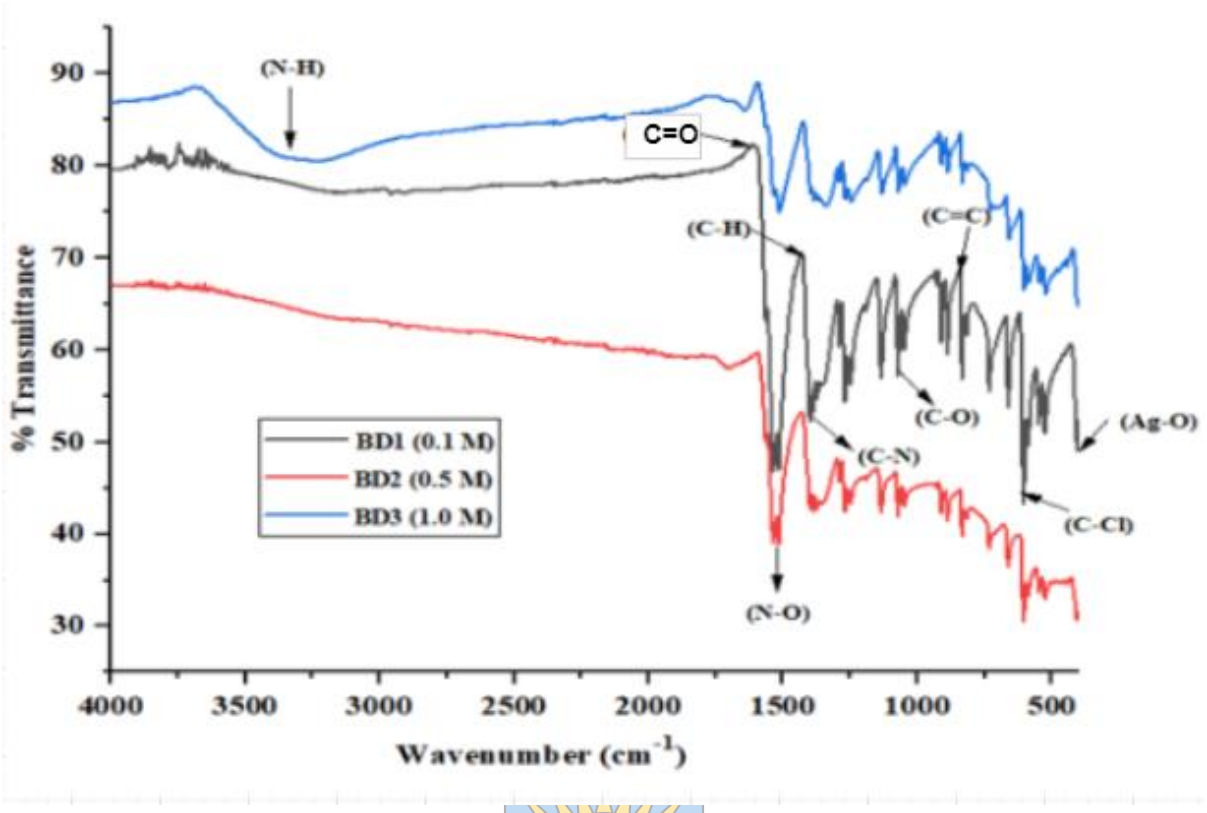


Figure 4.7: FTIR spectra of the as-synthesized AgNPs represented as BD1, BD2, and BD3

4.5 Adsorption of bacterial DNA

It is well-known that the adsorption of DNA onto synthesized nanoparticles in solution depends on factors such as the nature, size, charge, binding characteristic of the nanoparticles, and the buffering condition (Radi *et al.*, 2016). These factors are essential in solution chemistry and solid-phase extraction of certain metals like nanomaterials and polymeric species. Besides, adding acid and base solution to adjust the pH of a working solution plays a vital role in sorption processes, especially in adsorption capacity (Fu *et al.*, 2015).

4.5.1 Effects of varying pH of DNA solution

The effect of varying pH (pH 2 to 9) on the DNA adsorption onto as-synthesized samples represented as BD1, BD2, and BD3 was carried out at an initial DNA concentration of 9.92 µg/mL, adsorbent dose of 20 mg, equilibrium time of 360 mins and at room temperature. We

observed that bacterial DNA could adsorb on the as-synthesized samples of AgNPs (BD1, BD2, and BD3) at a wide pH range. DNA percentage removal (%R) increases significantly from basic to acidic pH (50.26 - 87.61 %, 65.80 - 87.79 %, and 69.23 - 87.92%) for BD1 BD2 BD3, respectively, and the result is shown in Figure 4.8. This result is similar to data obtained on the adsorption of ARGs embedded in DNA onto graphene oxide nanosheets (Yu *et al.*, 2017b). The removal efficiency can increase to 99% when the initial pH is strongly acidic (initial pH 1.0) and if there is a competing ion in the solution and surface modification of adsorbents. The adsorption of bacterial DNA onto the as-synthesized samples depends on the adsorbent's surface charge, and the influence of pH solution since the adsorbate (DNA) has a high negative charge. From this study, removal efficiency from pH 2 to 6 was in the range (87.61-70.7%, 87.79-77.20%, 87.92-78.36% for BD1, BD2, and BD3, respectively) and exhibited high % removal efficiencies. It is because adsorbent surface charge acquires more positive charge particles from the acidic pH solution increasing the electrostatic attraction between adsorbate charge species (negative) and adsorbent charged particles (positive), thereby leading to high removal efficiencies in all the adsorbents by electrostatic interaction (Akter *et al.*, 2018; Zhang *et al.*, 2012). Also, it can be attributed to changes in the surface charge of the adsorbents in an aqueous solution and dissociation of the functional group on the adsorptive site (Uddin *et al.*, 2017). At pH 7 (neutral), the removal efficiency is 70 %, similar to the result obtained at neutral pH during the adsorption of DNA onto graphene oxide under Fluorescently Labelled Oligonucleotide (Yu *et al.*, 2017b). The removal efficiencies of pH 8-9 decreased to 55.8-50.26 %, 67.42-65.80 %, and 69.84-69.23 % for BD1, BD2 and BD2 respectively. It is due to charge repulsion in the adsorptive site (Jorge de Souza *et al.*, 2019). The DNA adsorption onto as-synthesized AgNPs samples is more favorable under acidic conditions than the neutral or alkaline conditions due to excess H^+ ions, increasing the positive charge in the adsorbents.

Interestingly, the final pH increases slightly from pH 2-6 (acidic condition) but decreases from pH 8-9 (alkaline condition), except for the initial pH of 6.9, whose final pH is 5.41, 5.61, and 5.60 for BD1, BD2, and BD3 (Table 4.3 a, b, c). These slight increases and decreases in the final pH may be attributed to the addition of hydrogen (H^+) and hydroxide ($O.H.^-$) ions released into the solution, leading to depurination and hydrolysis of the phosphate group and a nitrogenous base (An *et al.*, 2014). For the stability of the working DNA, pH 7 (neutral) was chosen for further experiments. Because, at pH 2-6 and 8-9, the depurination of DNA may gradually occur, leading to DNA damage. But at neutral pH, all the four DNA bases (pH 7.0) are charged-neutral, and each phosphate group carries a negative charge ($pK_a < 2$).

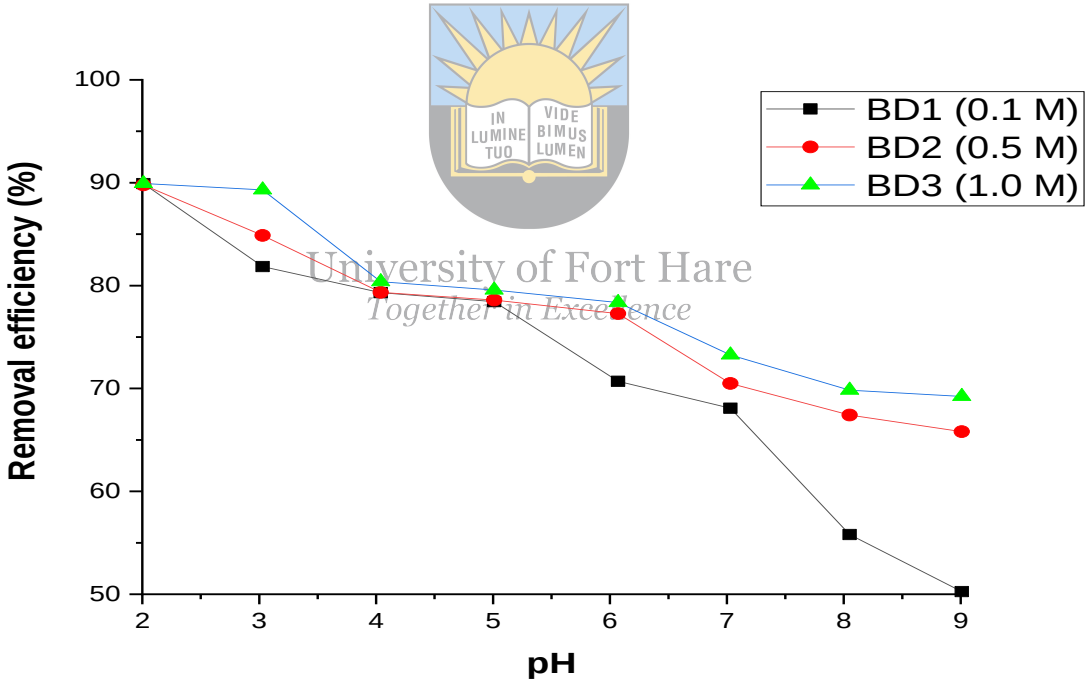


Figure 4.8: Effects of pH for removal of bacteria DNA harbouring ARGs with as-synthesized samples of AgNPs (concentration = 9.92 µg/mL; adsorbents dose = 20 mg, reaction time = 360 mins and at room temperature)

Table 4.3 a: Represents the initial and final pH of BD1 before and after adsorption process

pH	2	3	4	5	6	7	8	9
Initial pH	2.01	3.01	4.03	5.04	5.9	6.90	8.23	9.31
Final pH	2.89	3.50	4.91	5.21	5.41	5.83	5.91	6.30

Table 4.3 b: Represents the initial and final pH of BD2 before and after adsorption process

pH	2	3	4	5	6	7	8	9
Initial pH	2.01	3.03	4.24	5.32	5.91	6.85	8.11	9.21
Final pH	2.91	3.69	5.11	5.75	5.61	6.01	6.11	6.71

Table 4.3 c: Represents the initial and final pH of BD3 before and after adsorption process

pH	2	3	4	5	6	7	8	9
Initial pH	2.03	3.16	4.13	5.34	6.39	6.83	8.30	9.2
Final pH	2.88	3.70	5.41	5.41	5.63	5.72	6.18	6.87

4.5.2 Effects of contact time and initial DNA concentrations

The effects of contact time on the adsorption capacity of DNA onto the samples of as-synthesized AgNPs at different initial concentrations (4.98, 9.92, and 14.98 µg/mL) are shown in Figure 4.9. The experiment was conducted at room temperature, with a fixed adsorbent dose of 20 mg and neutral pH. The data obtained for BD1, BD2, and BD3 at an initial concentration of 4.98, 9.92, and 14.98 µg/mL were instantaneous during the initial period and gradually slowed and stagnated with the increase in contact time. We observed that equilibration time for removing DNA initial concentrations (4.98, 9.92, and 14.98 µg/mL) starts and ends from 0-180 mins, 0-195 mins, and 0-225 mins for BD1, BD2, and BD3, respectively. It may be because of the acidic functional group on the surface of the adsorbents. For the effects of initial concentration, the adsorption capacities for BD2 and BD3 increased as the initial concentrations rose from 4.98 to 14.98 µg/mL but decreased in BD1.

Furthermore, DNA concentration increased the adsorption capacity for all three adsorbents. This report confirmed that most adsorptive sites are available at the initial stage and the vacant site facilitates the binding of DNA onto the adsorbent through electrostatic interaction forces (Patel *et al.*, 2012). It was also observed that equilibrium adsorption capacities on

BD1, BD2, and BD3 at different concentrations show an increasing trend because of the driving force provided by initial concentration (Michael *et al.*, 2019), electrostatic screening effects, and generation of intermolecular hydrogen bond (Li *et al.*, 2012). These reports confirmed that the success of any adsorption process depends highly on the initial concentration of DNA. It was noticed that adsorption of DNA onto samples of BD1, BD2, and BD3 reached equilibrium at different time intervals. It may be attributed to the different molar concentrations of the variables adopted during the synthesis of AgNPs. Also, the equilibrium time is dependent on the DNA solution's initial concentration. Fixed adsorbate initial concentration of 9.92 µg/mL and contact time of 180, 195, and 225 mins for BD1, BD2, and BD3 respectively were maintained for the subsequent adsorption experiments.

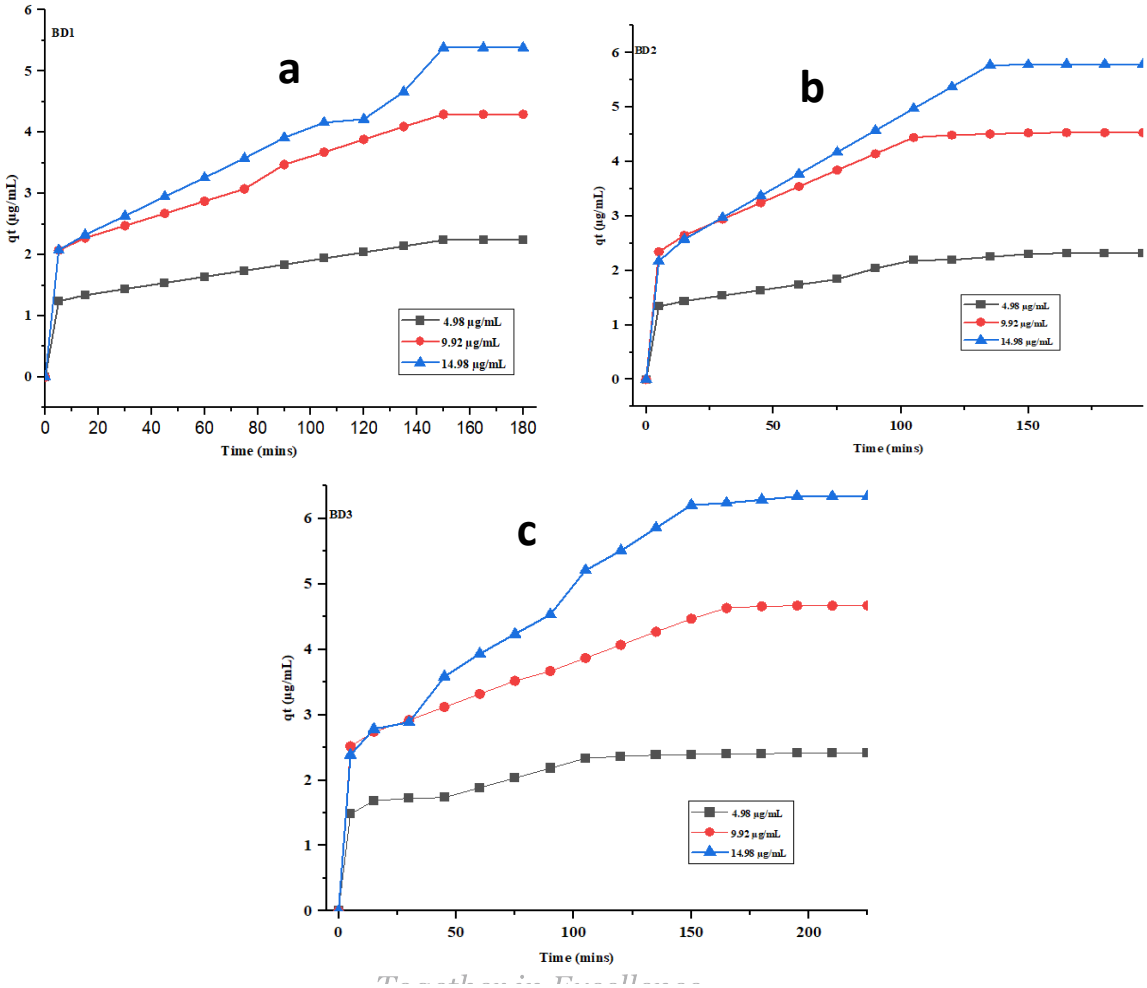


Figure 4.9 A, B, C: Represent the effect of contact time for BD1, BD2 and BD3 respectively at different adsorbate (DNA) concentrations (4.98, 9.92 and 14.98 µg/mL); reaction time of 180, 195, and 225 mins, speed = 300 rpm, and pH = 6.9

4.5.3 Effects of adsorbents dosage

The adsorbent dosage in working solutions plays a vital role in DNA uptake and the percentage removal efficiency of DNA. It is one of the best parameters for determining the optimum condition for the adsorption process. It influenced adsorption through adsorbent material capacity (Deng *et al.*, 2011; Sigrist *et al.*, 2014). In this study, the adsorption of DNA onto BD1, BD2, and BD3 were investigated by varying the adsorbent quantity from 10 mg to 30 mg in solutions while maintaining a fixed DNA working concentration of 9.92 µg/mL, pH of 6.9, contact time of 180, 195, and 225 mins for BD1, BD2, and BD3 respectively, speed of 300 rpm at room temperature. Figure 4.10 shows the percentage (%)

adsorption efficiency plot against adsorbent concentration (mg) of BD1, BD2, and BD3. Adsorption efficiency increases with adsorbent concentration (Radi *et al.*, 2016). Thus, the initial concentration of DNA decreased with an increase in the adsorbent dose from 10 to 30 mg. The % removal efficiencies increases from 70.76 to 80.14%, 71.77 to 90.02%, and 76.81 to 92.43% for BD1, BD2, BD3 respectively. This result may be attributed to two reasons: (1) the amount of adsorption efficiency per unit mass depends on the concentration gradient between adsorbate and the increase in adsorbent concentration (Al Bsoul *et al.*, 2021; Yusuff *et al.*, 2019). (2) the abundance of the active sites on the adsorbent for adsorbate sorption increases as the adsorbent dose increases (Aarab *et al.*, 2020). This result agrees with published reports on the effects of adsorbent dose on the adsorption process (Peralta *et al.*, 2019; Tan *et al.*, 2014). It suggests that a high adsorbent dose increases the adsorptive sites, increasing attraction between DNA molecules and AgNPs (Kushalkar *et al.*, 2020). Therefore, the optimum dosage for DNA adsorption onto BD1, BD2, and BD3 is 30 mg, and economical dosage for removing DNA from water.

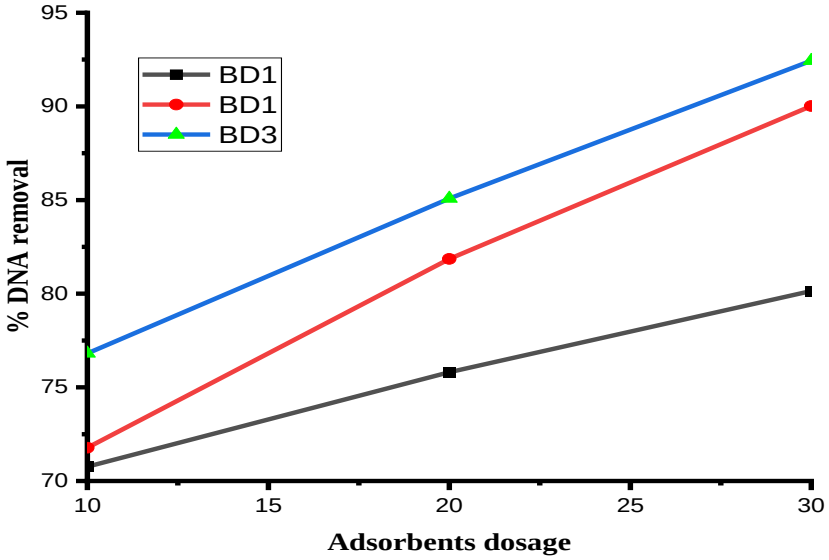
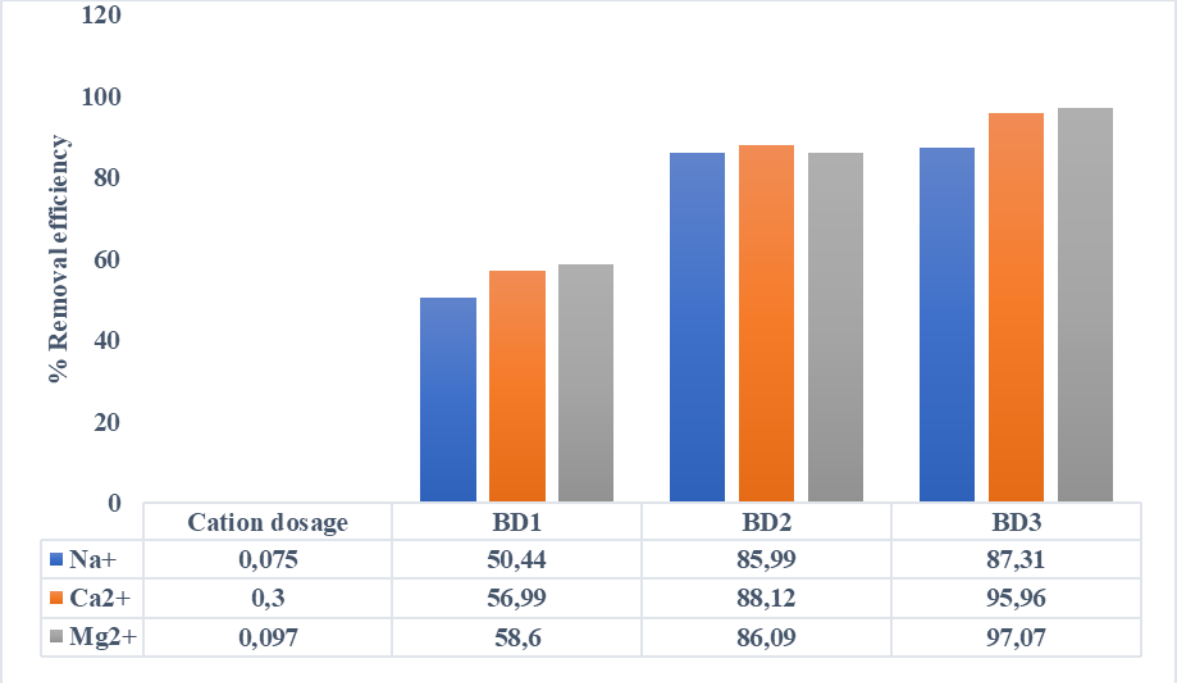


Figure 4.10: Effect of adsorbent dose on the removal of DNA, adsorbate concentration = 9.92 µg/mL, reaction time = 225 mins; speed = 300 rpm, pH = 6.9

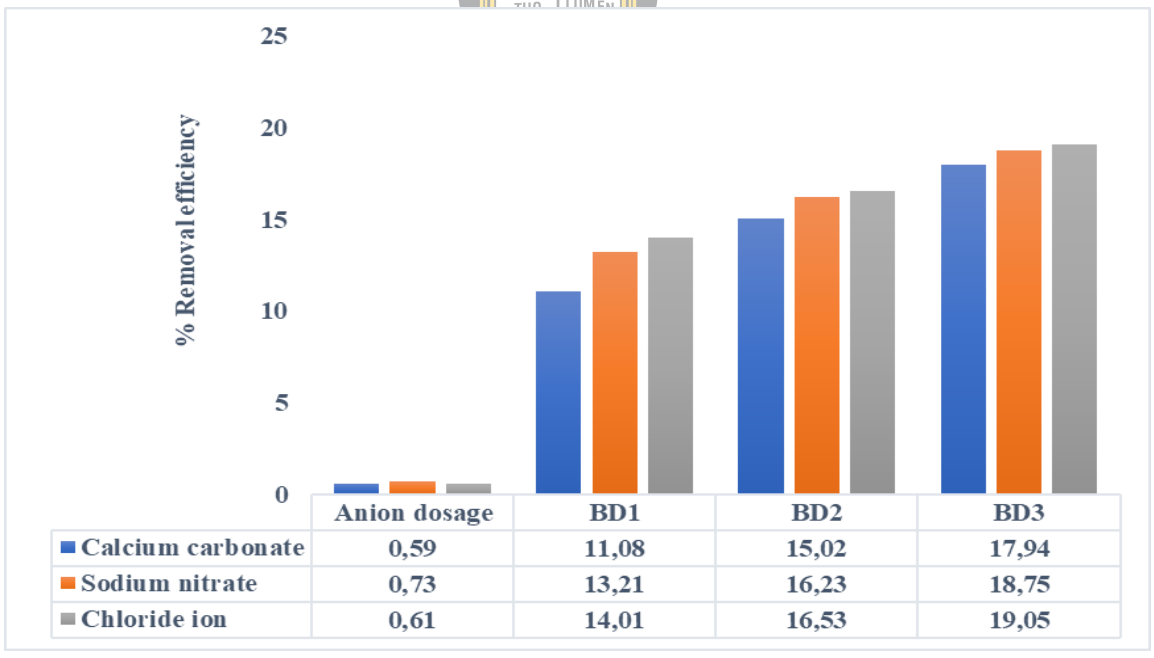
4.5.4 Effects of competitive cations

Several background ions are detected in the wastewater treatment plants, which may interfere with the adsorbent's DNA uptake through competitive binding or complexation (Håvarstein, 1998). Batch equilibrium studies were investigated to ascertain the effect of cations (Na^+, $Ca^{2+,}$ and Mg^{2+}) and anions (Cl^-, CO_3^{2-}, and NO_3^-) onto DNA uptake by adsorbents (BD1, BD2, and BD3). The solutions containing 0.075, 0.3, and 0.097 g (Na^+, Ca^{2+}, Mg^{2+}) for cations and 0.59, 0.73, and 0.61 g for anions ($CO_3^{2-,}$ NO_3^-, and Cl^-) with fixed adsorbents dose of 30 mg, pH of 6.9, DNA working concentration of 9.92 µg/mL, contact time of 180, 195, and 225 mins for BD1, BD2, and BD3 at room temperature were agitated separately. The results are shown in Figure 4.11a and b. Figure 4.11a demonstrated that DNA adsorption onto all the adsorbents was optimum for Mg^{2+} and Ca^{2+} with removal efficiencies of 89.23 to 91.39%, 93.89 and 95.0, 97.10, and 99.94 % for BD1, BD2, and BD3 compared to Na^+ (50.44, 56.99, and 58.66) in all the adsorbents. However, DNA adsorption efficiencies increase compared to the values obtained in the absences of cations, indicating competitive complexation with some other ions (Radi *et al.*, 2016). For the presence of anions shown in Figure 11b, all the adsorbents' DNA removal efficiencies drastically decreased from 11.08 to 17.94, 13.21 to 18.75, and 14.01 to 19.05 % for BD1 BD2, and BD3, respectively. It was observed that the presence of $CO_3^{2-,}$ NO_3^-, and Cl^- shows no significant effect on DNA adsorption.



Cation dosage	BD1	BD2	BD3
Na+	0,075	50,44	85,99	87,31
Ca2+	0,3	56,99	88,12	95,96
Mg2+	0,097	58,6	86,09	97,07

Figure 4.11a: Effects of competing ions (cations) on DNA adsorption onto BD1, BD2 and BD3 (pH = 6.9, adsorbent dose = 30 mg, DNA working concentration = 9.92 µg/ml, contact time = BD1(180 mins), BD2 (195 mins) and, BD3 (225 mins) at room temperature.



Anion dosage	BD1	BD2	BD3
Calcium carbonate	0,59	11,08	15,02	17,94
Sodium nitrate	0,73	13,21	16,23	18,75
Chloride ion	0,61	14,01	16,53	19,05

Figure 4.11b: Effects of competing ions (anions) on DNA adsorption onto BD1, BD2 and BD3 (pH = 6.9, adsorbent dose = 30 mg, DNA working concentration = 9.92 µg/mL, contact time =BD1 (180 mins), BD2 (195 mins) and, BD3 (225 mins) at room temperature

This information is considerably helpful for the adsorption of DNA in natural water samples that contain a large amount of alkaline earth metal ions (Vandeventer *et al.*, 2012). Thus, AgNPs have good adsorption efficiency and a high affinity for DNA in solution. Therefore, this study shows that different molar concentrations of as-synthesized AgNPs are potential adsorbents for DNA removal from an aqueous solution containing competing ions.

4.6. Adsorption mechanism

Modelling adsorption is vital in removing targeted pollutants during the water treatment process. They predict or describe adsorbate interaction's comprehensive nature with adsorbents, adsorbents' surface properties, and adsorption system design. In this study, mathematical equations of each model presented in table 1 were employed to improve the adsorption system design and ensure that different molar concentrations of synthesized AgNPs (BD1, BD2, and BD3) have good removal efficiency for bacteria DNA. Besides, they were used to establish the best reasonable correlation for equilibrium curves and characteristics. Parameters obtained from these models provided an insight into the adsorbent's surface properties, such as maximum adsorbent capacity over adsorbate and its affinity (Chen *et al.*, 2010).

4.6.1 Isotherm model

This study used isotherm equations from Freundlich and Langmuir models to describe the equilibrium characteristics of adsorbate-adsorbents interaction. They helped in determining the maximum adsorption capacity at equilibrium. Their basic assumption differs in the adsorption process. The Freundlich isotherm assumed that cation and anions are adsorbed on a heterogeneous surface (multilayer adsorption), forming an attractive surface force (Huang *et al.*, 2018). Whereas the Langmuir model assumes monolayer adsorption at a homogenous site within the adsorbent surface i.e., no further adsorption occurs once the DNA molecules occupy the adsorptive site (Chen *et al.*, 2010). The result of the isotherm study on DNA

adsorption onto different molar concentrations of as-synthesized AgNPs and the isotherm parameters are summarized in Table 4.4. The Chi-square (X^2) evaluated the experimental and calculated data difference. The model with a smaller X^2 indicated the best fit for the study.

$$X^2 = \frac{(qe^{exp} - qe^{cal})^2}{qe^{cal}} \ldots\ldots\ldots\ldots (4.4)$$

Among the two-isotherm models employed, experimental data of BD1 and BD2 were best described by the Langmuir model, with the highest coefficient parameter and a reduced Chi-square (X^2) (R^2 = 0.97625 and X^2 = 0.12142 for BD1) (R^2 = 0.96049 and X^2 = 0.24403 for BD2). For BD3, Langmuir is fairly fitted with R^2 = 0.850108 and reduced X^2 = 1.00914 compared to Freundlich model (R^2 = 0.72646 and reduced X^2 = 1.85363). The best fitting of the Langmuir model on BD1 and BD2 denoted that adsorption of bacteria DNA molecules took place at specific homogenous sites within the adsorbent surfaces (Fu *et al.*, 2015). The maximum adsorption capacity (qmax), widely used to compare the efficiency of adsorbents, was obtained in Langmuir isotherm, and they are 0.06595 µg/mL, 0.0665 µg/mL, and 0.66094 µg/mL for BD1, BD2, and BD3.

The separator factor ($R_{L.}$) relating to the Langmuir isotherm was employed in the study. It was used to evaluate the feasibility of adsorption onto the adsorbent (Fu *et al.*, 2015) using the Equation below:

$$R_L = {}^{1}/_{1 + Ci \times KL} \ldots\ldots\ldots\ldots\ldots (4.5)$$

Ci (µg/mL) is the initial DNA concentration, and K.L. is the Langmuir constant. Note $R_{L.}$ indicates the adsorption status, and when R_L= 0, it is irreversible; when $0 < R_{L.} < 1$, it is favourable; when $R_{L.}$ = 1, it is linear, and when $R_{L.} > 1$, it is unfavourable. From Langmuir's study, the range of $R_{L.}$ for BD1, BD2, and BD3 are 0.0706, 0.1958, and 0.0000365. This

result confirmed that the adsorption of DNA onto different as-synthesized AgNPs is favourable.

Table 4.4: Isotherm parameters for the bacteria DNA adsorption onto BD1, BD2 and BD3

Isotherm	Parameter	BD1	BD2	BD3
Langmuir	qmax (µg/mL)	0.06595	0.06865	0.660939
	$K_{L.}$ (µg/g)	0.01179	0.82443	0.53278
	$R_{L.}$	0.07061	0.19586	0.0000365
	X^2	0.12142	0.24403	1.00914
	R^2	0.97625	0.96049	0.90108
Freundlich	K_f (µg/g)	3.38305	4.05012	4.83834
	n	2.89176	3.15705	4.49655
	X^2	0.51699	0.82316	1.85363
	R^2	0.89886	0.86674	0.72646

4.6.2 kinetic model

The adsorption kinetics is the utmost parameter considered during the adsorption process, and it determines the adsorption efficiency and rate of adsorbate uptake per contact time. To measure the adsorption equilibrium per contact time of different initial concentrations, the experimental data obtained in this study were fitted into two kinetic models, such as Natarajan and Khalaf first order and Pseudo-second-order kinetics (Table 2). The result obtained shows that Pseudo second order (PSO) kinetic is the best fit with the highest correlation value ranging from R^2 = 0.90727 to 0.98797 compared to First-order (R^2 = 0.88234 to 0.9567). The plot of t/qt versus t is a straight line, as shown in Figure 4.12.

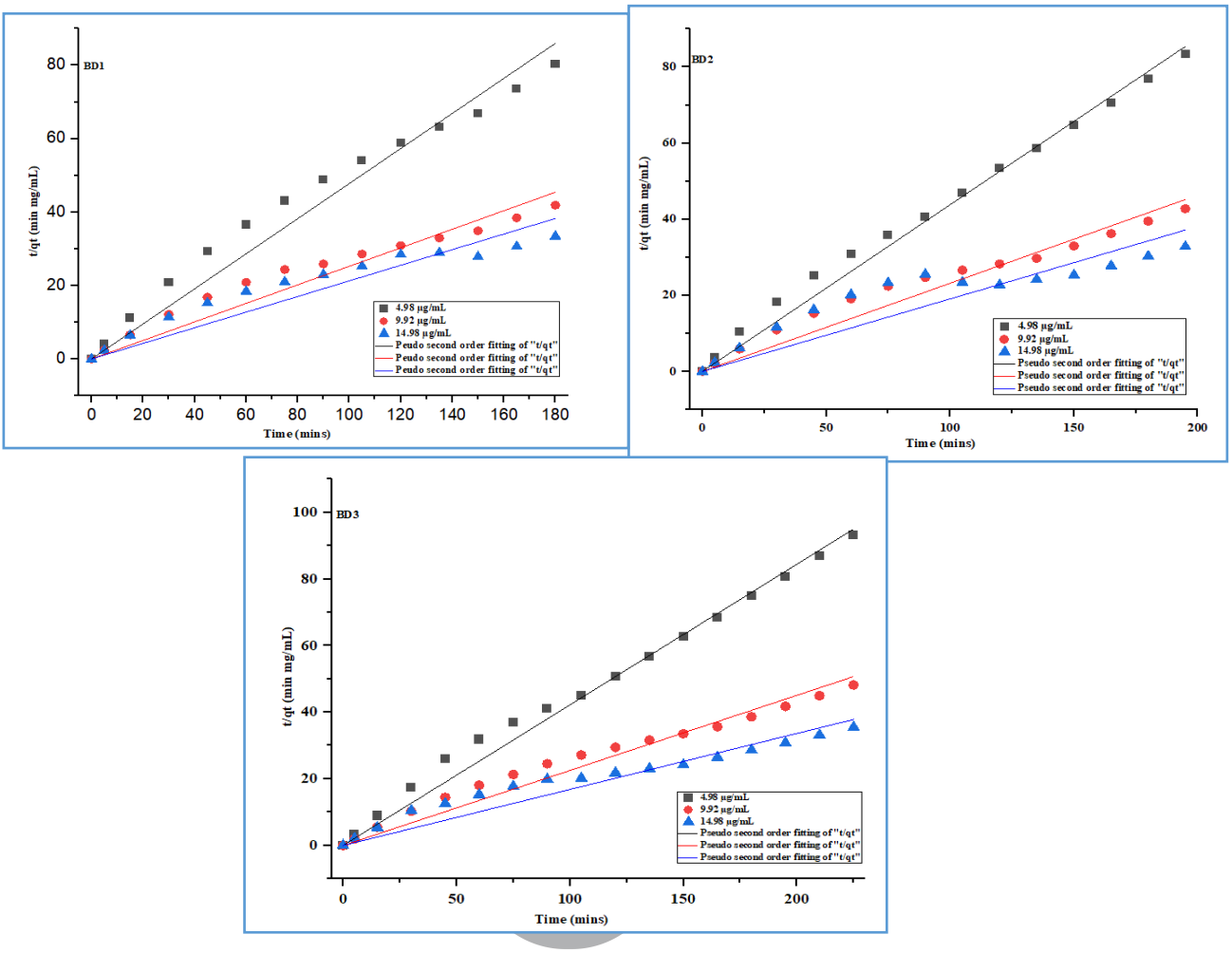


Figure 4.12a, b, c: Represent the effect of contact time for BD1, BD2 and BD3 at different adsorbate concentrations (4.98, 9.92 and 14.98 µg/mL); reaction time of 180, 195, and 225 mins, speed = 300 rpm, and pH = 6.9 at room temperature.

The slope and intercepts of the plots were obtained from the values of K_2 and qe, respectively. From the plots, the adsorption of bacteria DNA onto these adsorbents rose rapidly at the beginning and gradually slowed down over time. The adequate contact time was determined, and it is the time taken by these adsorbents (BD1, BD2, and BD3) to achieve between 70-90% DNA removal at equilibrium. From Figure 4.12, the adequate contact time for DNA adsorption onto BD1, BD2, and BD3 were 140 mins, 150 mins, and 200 mins, and the equilibrium was achieved at 180 mins, 195 mins, 225 mins, respectively. According to Pseudo-second-order kinetic, which was confirmed as the best fit for this study, the initial sorption rate or rate of adsorption (*h*) can be determined by using Equation (4.6):

$$h = k_2 \times qe^2 \ \ldots\ldots\ldots\ldots (4.6)$$

The value of *h* obtained from different initial concentrations using the three adsorbents increases in the same other as K_2. This indicates that a high adsorption rate occurs when the initial DNA concentration increases. The experimental value (qe^{exp}) and the calculated (qe^{cal}) of all the three adsorbents in PSO show a good correlation with little consistency compared to First-order kinetic. We noticed that the pseudo-second-order constant (K_2) reduces with the increased initial concentration of adsorbate (DNA). It could be attributed to increased bacteria DNA concentration, reducing the diffusion of DNA molecules from the adsorptive site. The high correlation coefficient ranging from R^2 = 0.90727 to 0.98797 indicated that DNA adsorption by BD1, BD2, and BD3 was controlled by chemisorption (Chen *et al.*, 2010). It implies that during the adsorption process, the valency force exerted could be because of sharing or exchange of electrons between the synthesized AgNPs (BD1, BD2, and BD3) and the DNA molecule. The PSO kinetic parameters obtained determined the equilibrium adsorption capacity, rate constant, initial sorption rate, and the percentage removal of DNA from these adsorbents. To compare the applicability and authenticity of each model fitted into the experimental data, the standard deviation (Δq) was calculated using Equation (4.7):

$$\Delta q\ (\%) = 100 \times \sqrt{\frac{\sum[(qe^{\exp} - qe^{cal})]/qe^{exp}}{n-1}} \ \ldots\ldots\ldots\ldots\ldots\ldots (4.7)$$

Other parameters such as correlation value (R^2), *h*, K_2, Δq, including the qe^{exp} and $qe^{cal,}$ are summarized in Table 4.5.

Table 4.5: Kinetic parameters for bacteria DNA adsorption onto different molar concentrations of as-synthesized AgNPs represented as BD1, BD2, and BD3

Kinetic models and Parameters	Adsorbent	C_i (µg/mL)	qe^{exp} (µg/g)	qe^{cal} (µg/g)	Δq (%)	K_1 ($mins^{-1}$)	h (µg/g)	R^2
Natarajan and Khalaf First order equation	BD1	4.98	2.24	3.80	49.24	4.23×10^{-5}	-	0.88234
		9.92	4.29	3.85	37.15	3.39×10^{-5}	-	0.91493
		14.98	4.21	2.29	32.25	3.31×10^{-5}	-	0.89862
	BD2	4.98	0.30	3.79	82.24	6.76×10^{-5}	-	0.95413
		9.92	0.81	6.38	21.87	2.56×10^{-5}	-	0.91465
		14.98	3.11	6.15	69.06	1.03×10^{-5}	-	0.92492
	BD3	4.98	0.15	2.32	102.2	3.98×10^{-5}	-	0.93133
		9.92	0.50	2.78	32.23	1.93×10^{-5}	-	0.91909
		14.98	2.30	3.29	30.49	2.56×10^{-5}	-	0.95670
Kinetic model parameters	**Adsorbent**	**C_i**	**qe^{exp} (µg/g)**	**qe^{cal} (µg/g)**	**Δq**	**K_2 ($mins^{1}$)**	**h (µg/g)**	**R^2**
Pseudo-Second Order	BD1	4.98	2.24	2.16	2.52	1.10×10^{4}	5.25	0.95781
		9.92	4.29	4.25	2.08	0.46×10^{4}	8.77	0.92682
		14.98	4.21	4.19	1.06	0.41×10^{4}	7.78	0.90727
	BD2	4.98	0.30	0.29	2.34	0.65×10^{4}	0.06	0.98794
		9.92	0.81	0.76	4.37	0.46×10^{4}	0.28	0.94421
		14.98	3.11	2.98	2.94	0.41×10^{4}	3.71	0.90146
	BD3	4.98	0.15	0.18	14.14	0.64×10^{4}	0.02	0.98755
		9.92	0.50	0.49	1.41	0.46×10^{4}	0.11	0.96194
		14.98	2.30	2.27	2.91	0.39×10^{4}	2.01	0.90579

4.7. Conclusion

We concluded that the three different molar concentrations of AgNPs (BD1, BD2, and BD3) were subjected to the same operating parameters (pH, time, DNA concentrations, adsorbent dose, cation, and anions), and BD3 recorded the highest DNA removal efficiency.

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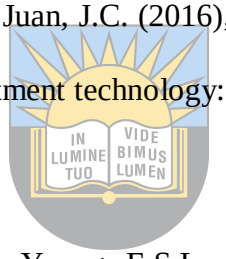

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Chapter 5

Influence of different chaotropic salts on etched mesoporous silica nanoparticles for the removal of bacteria DNA conveying antibiotic resistance genes from hospital wastewater

Submitted to Chemical Engineering Journal

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Influence of different chaotropic salts on etched mesoporous silica nanoparticles for the removal of bacteria DNA conveying antibiotic resistance genes from hospital wastewater

Abstract

The adsorption of bacteria DNA onto mesoporous silica nanoparticles in their original state has been a great challenge due to the high negative charge exhibited by both the DNA and silica surface. In this study, mesoporous silica nanoparticles were synthesized via chemical etching techniques using sodium dodecyl sulphate (SDS) as an etchant. Sodium dodecyl sulphate was used to remove unwanted layers and provides a convenient platform for underlining mesopore with different chaotropic salts. The Scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectroscopy (EDX), Fourier transformed infrared spectroscopy (FTIR), x-ray diffraction spectroscopy (XRD), and point of zero charges (PZC) results showed that synthesized etched mesoporous silica nanoparticles have a crystalline, non-spherical shape and elemental composition of silica at approximately 2 Kev, functional groups that depict silica particles. Molecular characterization of extracted genes showed that *Enterococcus faecium* harbours *tetA*, *tetM*, and *ermB* in 201bp, 158bp, and 320bp, with the DNA purity ranging from 1.7 to 1.9. The DNA adsorption was studied as a function of operating parameters in different solutions of chaotropic salts (sodium chloride (NaCl), 2 M guanidine HCl (CH_5N_3.HCl), and urea ($(NH_3)_2CO$). Among the different chaotropic salts used to compliment the silica nanoparticles, 2 M guanidine HCl exhibited the highest percentage (%) removal efficiency (80%) compared to urea (75%) and sodium chloride (70%) in simulated water and hospital wastewater. Experimental results revealed that the pseudo-second-order kinetic and Sips isotherm was the best fit for the adsorption process. The reaction is governed by electrostatic interactions that occur on heterogeneous surfaces. Therefore, the performance of mesoporous silica nanoparticles enhanced by

different chaotropic salts in this study showed that this material may be a promising and economical one for the uptake of bacteria DNA conveying antibiotics resistance genes from hospital wastewater.

Keywords: Mesoporous silica; inorganic nanomaterials; chaotropic salt; hospital effluent; heterogenous; chemisorption

5.1 Introduction

Recently, inorganic-based nanomaterials have gained much interest in wastewater treatment applications due to their inherent functionalities such as size/shape-dependent morphology, scalability, easy synthesis, and stability (Kankala *et al.*, 2020; Leonel *et al.*, 2021; Maduraiveeran *et al.*, 2019). These attractive features are responsible for their wide application in various fields such as engineering, agriculture, energy production, medicine, and wastewater treatment process. Among numerous inorganic-based nanomaterials, mesoporous silica nanoparticles (MSN) have attracted the attention of researchers as a promising adsorbent for the removal of different contaminants from water/wastewater. According to IUPAC, mesoporous is mesostructured material with a pore diameter between 2 nm and 50 nm and can be divided into silica-based material and non-silica-based material. The several mesostructured properties such as narrow pore size distribution, controllable and uniform particle size, high specific surface area, large pore volume, ease of surface functionalization or surface modification, non-toxic nature, and colloidal stability make it a suitable and efficient adsorbent for wastewater treatment process (Kankala *et al.*, 2020; Panahi *et al.*, 2019; Saman *et al.*, 2020). Mesoporous silica nanoparticle is thermally and chemically stable with controllable morphology and porosity in its original state (Kankala *et al.*, 2020; Kumar *et al.*, 2017). Both interior and exterior surfaces of MSN can undergo

functionalization or modification approaches either by attachment of multiple organic or inorganic functional groups via its Si-OH bond (Krajewska *et al.,* 2022; Zaharudin *et al.,* 2020). Another intrinsic feature is that the silica interior surface can function as a reservoir for loading guest molecules (Jafari *et al.*, 2018). This feature could enhance the selective target of contaminants at the adsorptive site. Their pore size distribution (2 nm - 30 nm) is usually narrow and can be controlled by changing the synthesis composition's mixture. The large surface area and pore size have been reported to have good sorption capability (Kumar *et al.*, 2017), allowing well controllable loading and release of DNA molecules (Li *et al.*, 2012). Its adsorption mechanism is achieved mainly by electrostatic attraction and hydrogen bonding between positive contaminants and MSN. MSN wide applications include bioengineering, catalysis, carrier for protein, enzyme, drug delivery system, and adsorption of pollutants.

Numerous synthesis route has been employed to achieve controllable morphological and porous structure/ materials: they are (a) template-assisted techniques (Kumar *et al.*, 2017), (b) sol-gel technique (Vazquez *et al.*, 2017), (c) microwave-assisted techniques (de Greñu *et al.*, 2020), and (d) chemical etching techniques (Salehtash *et al.*, 2018). All these procedures successfully achieved a controllable morphology and particle size adequate for their diverse applications in science.

Studies have reported the effectiveness of MSN in the adsorption of cationic contaminants from water/wastewater (Abbaraju *et al.*, 2009; Spoial *et al.*, 2020). But few studies have reported the successful adsorption of anionic contaminants onto the negative surface of MSN. Apart from being an efficient carrier for protein, enzyme, drug loading, and delivery system through therapeutic agents (Shi *et al.*, 2020; Zhou, Quan, *et al.*, 2018). Numerous researchers have attempted to mediate duplex DNA onto MSN. These attempts were not successful due to the repulsive effect between the bare MSN and DNA molecule (Jiang *et al.*, 2021; Li *et al.*,

2012). Besides, bacteria DNA is a bulky molecule with a high negative charge in its backbone. Therefore, in an aqueous solution, it is difficult to mediate it on the to MSN mesopore with a negatively charged silica surface as an adsorbent. But reports have shown that adsorption of DNA onto MSN mesopore can be achieved when the silica surface undergoes modification or adding carriers (chaotropic salts) that would assist DNA adsorption onto MSN. For example, a study reported the successful adsorption of DNA onto MSN in the presence of multivalent cations (Solberg and Landry, 2006). Other studies functionalized the surface of MSN with a cationic linker (Gao *et al.*, 2009) and the addition of 2 M Guanidine salt (chaotropic salt) at low pH in mediating DNA onto magnetic mesoporous silica nanoparticle (M-MSN).

Consequently, this study utilized for the very first time, different chaotropic salts (sodium chloride, 2 M guanidine HCl and Urea) onto an etched mesoporous silica nanoparticle (E-MSN) to adsorb bacteria DNA harbouring antibiotic resistance genes from wastewater at neutral pH, compared their behaviours during an investigation at different operating parameters. Meanwhile, the term chaotropic means chaos-forming in which the entropic nature of the salt is capable of disrupting the structure of macromolecules such as protein in water, allowing the DNA in water to bind to the silica-based substance (Vandeventer *et al.*, 2012). Chaotropic salts include phenol, urea, sodium chlorides, guanidine hydrochloride, and lithium perchlorate. During DNA uptake in an aqueous solution, these salts disrupt the hydrogen bonding strand of DNA and facilitate their binding onto the silica surface. This study reports on the synthesis of a type of etched MSN (E-MSN) through chemical etching techniques known as silica etched chemistry to remove the unwanted layer from the silica surface. The main reason for this procedure is to remove the hydroxyl group on the silica surface, which tends to agglomerate causing the nanoparticles to have a weak affinity (Qiao *et al.*, 2016). Sodium dodecyl sulphate (SDS) used as an etchant was due to its high oxidizing

power. This procedure was responsible for the etching of the amorphous silica framework by breaking Si-O-Si bonds, removing the OH group and the condensed silica species, and creating a new mesoporosity in the shell. It gradually etched out the negative charge electron possessed by MSN in other to gain electrons from other sources. This study adopted this method of synthesis in other to eliminate the negative silica surface and allows the lining of the MSN surface with chaotropic salts which would influence the adsorption of bacteria DNA conveying ARGs onto the E-MSN mesopore.

Bacteria DNA conveying antibiotic resistance genes (bDNA-ARGs) is a public health problem of growing concern. Extensive consumption of antibiotics by humans and animals has been considered the leading cause of dissemination and proliferation of antibiotic-resistant bacteria (ARB) and ARGs detected in different water environmental bodies (Shao *et al.*, 2018; Zainab *et al.*, 2020). In recent decades, studies on the consequences of consuming bDNA-ARGs contaminated water and the lack of adequate treatment materials threaten public health, especially among the populace living in water-scarce areas. A study reported that ARGs enter and persist in the water environment through multiple pathways such as sewage, domestic, agricultural, and hospital disposal (Ezeuko *et al.*, 2021b). They spread across the gut of microbial communities of livestock animals, humans, and crop soil, threatening human health and ecological sustainability (Amarasiri *et al.*, 2020; Kimbell *et al.*, 2020). Antibiotic resistance genes are often located at mobile genetic elements (MGEs) and spread through horizontal gene transfer (HGT) phage, plasmid, integron gene cassettes, or transposons. The release of untreated effluents containing bDNA-ARGs may prompt the indigenous bacteria to acquire ARGs through spontaneous mutation and HGT. Mutations are responsible for the continuous evolution of ARGs, producing so many variants that replicate in the water environment. The menace of bacteria DNA conveying ARGs are on the rise, such that it increases mortality and morbidity rate due to the lack of clean and safe water

supply. Therefore, developing an efficient, less toxic material may serve as an excellent option to tackle the consequences of bacteria DNA conveying ARGs (bDNA-ARGs) in wastewater.

Even though there are reports on the adsorption of DNA onto MSN using multivalent cations, cationic linkers, and surface-modified MSN with functional moieties (Gao *et al.*, 2009; Solberg and Landry, 2006; Zhang *et al.*, 2012), to the best of our knowledge, no information on the influence of different chaotropic salts on E-MSN for the removal of bacteria DNA conveying ARGs have been published.

5.2 Materials and methods

5.2.1. Chemicals

Tetraethyl orthosilicate (TEOS), Nitric acid (HNO_3), Urea, Guanidine HCl, sodium chloride, and sodium dodecyl sulphate (SDS) were purchased from Sigma Aldrich, United State of America. Nuclease-free water and DNA kit were purchased from Thermo Fischer, South Africa. All the chemicals used in this study were of analytical grade and used as purchased.

5.2.2. Extraction and molecular characterization of bacterial DNA

Antibiotic-resistant *Enterococcus Faecium* used in this study was obtained from our laboratory archives isolated from the beach water in East London, Eastern Cape Province, South Africa. Before the commencement of extraction of genomic DNA, antibiotic susceptibility testing (AST) was conducted on the bacteria isolate using the disk diffusion method, following the procedure of Kirby-Bauer recommended by CLSI (Humphries *et al.*, 2021). The result of AST confirmed that *Enterococcus Faecium* was resistant to four (5) different antibiotic drugs. They are linezolid, erythromycin, ampicillin, tetracycline, and vancomycin. Genomic DNA extraction was carried out using the boiling method (Lee *et al.*, 2020) and stored at -20 ^{o}C as a stock solution. This DNA extraction method was adopted to

denature proteins, inactivate enzyme reaction inhibitors, and extract quality DNA from spots (Barbosa *et al.*, 2016). The concentration and purity of DNA were measured by finding the absorbance ratio at 260 nm, 280 nm, and 320 nm using Multiparameter HACH DR 6000 Ultraviolet Spectroscopy ranging between 1.7 and 1.9, indicating that the interfering compounds were efficiently removed. Polymerase chain reaction (PCRs) assay confirming the presence of resistance genes in the bacteria isolates was prepared using the primers presented in Table 5.1. The amplicons were examined using 1.5 % (w/v) agarose gel, stained with 10 µL of ethidium bromide at a processing time of 45 mins (Han *et al.*, 2015). Then, it was pictured using the Alliance BioDoc-It system.

Table 5.1: PCR primers, sequences, and protocols used in this study

Antibiotics Class	PCR primers	Primer sequences	Product size (bp)	PCR protocols	References
Tetracyclines	*tetA*	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	201	94 °C – 5 min; 35[94 °C – 1 min; 55 °C – 1 min; 72 °C 1:30 min]; 72 °C – 5 min.	(Titilawo *et al.*, 2015)
	tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	94 °C – 5 min; 35[94 °C – 1 min; 55 °C – 1 min; 72 °C 1:30 min]; 72 °C – 5 min.	
Macrolides	*ermB*	BN1: CGAGTGAAAAAGTACTCAACA BN2: CGGTGAATATCCAAGGTACG	320	94 °C – 3 min; 35 [94 °C – 1 min; 55 °C – 1 min; 72 °C 1 min]; 72 °C – 10min.	(Adeniji *et al.*, 2020; Osode and Okoh, 2009)

5.2.3 Synthesis and characterization of etched mesoporous silica nanoparticles (E-MSN)

A solution of tetraethyl orthosilicate (TEOS) in deionized water was heated to 250 °C. Nitric acid (HNO_3) was added dropwise, and the mixture was stirred at 500 rpm for 2 h to obtain a homogenous solution. Finally, urea was added slowly to the mixture. After 1 h of heating, the mixture was allowed to stir for 24 h further. Then, the mixture was calcined for 5 h at 550 °C. The calcined product was subjected to a chemical etching technique using SDS as an etchant

to remove the unwanted layers and yield mesoporous materials on the synthesized materials. 3 g of SDS was dissolved in deionized water. A calcined products of 10 g were added to the solution containing SDS and allowed to stir for 24 h at 80 °C. After interacting with SDS, the resulting suspension was washed several times with ethanol and water to remove the unreacted SDS. It was subjected to oven-drying for 24 h to obtain a mesoporous silica nanoparticle labelled as E-MSN.

The point of zero charges (PZC) was used to determine the pH at which the net charge of the total particle surface is equal to zero or neutral. Functional groups identification, morphologies, elemental composition, and crystallinity and phase composition were determined using the Fourier transform infrared spectroscope (FTIR) (Perkin-Elmer Universal ATR 100), Scanning electron microscope (SEM) coupled with an energy dispersive x-ray spectroscope (EDX) (JOEL JSM-6390LVSEM), and X-ray diffraction (XRD) (Bruker D8) respectively.

5.3 Batch adsorption study

A batch adsorption experiment was prepared in the following procedure: 25 mL Erlenmeyer flask containing 8 mL of nuclease-free water (NSFW) was polluted with 2 mL of genomics DNA extracted from antibiotic-resistant *Enterococcus Faecium.* Adsorbents were prepared by adding 5 mg of each chaotropic salt, namely, sodium chloride (NaCl), 2 M guanidine HCl (CH_5N_3.HCl), and urea ($(NH_3)_2CO$) into 15 mg of synthesized etched mesoporous silica nanoparticles (E-MSN) labeled as E-MSN+S, E-MSN+U E-MSN+G. Then, 20 mg of each adsorbent marked as E-MSN+S, E-MSN+U, and E-MSN+G were separately weighed and added to the DNA contaminated NFW for batch adsorption study. The effects of pH (pH range from 2 to 9 by dropwise adding 0.1 mol/L NaOH or 0.1 mol/L HCl solutions) and adsorbent dosage (ranging from 10-40 mg) on three adsorbents (E-MSN+S, E-MSN+U, and

E-MSN+G) for bacteria DNA removal were monitored at a fixed concentration of 3.66 µg/mL, pH 7.2, contact time of 360 mins and at room temperature. The experiments were conducted on a KS260 control orbital shaker at a speed of 300 rpm using a 25 mL Erlenmeyer flask with different DNA concentrations (obtained from the stock solution). After equilibration, the mixtures were subjected to centrifugal force to separate the adsorbents from the supernatant solutions. The concentrations of DNA left in the supernatant solutions were determined using a dsDNA assay kit (Q32850) Qubit 1.0 Fluorometer (Thermofischer). The percentage removal efficiency (%R) and the amount of DNA adsorbed per unit mass (qe) in all the solutions were calculated using the Equations (5.1) and (5.2) described by (Ghaemi and Absalan, 2014):

$$\%\,R = \frac{(Ci - Cf)}{Ci} \times 100 \quad \ldots\ldots\ldots (5.1)$$

$$qe = \frac{(Ci - Cf)}{m} \times V \quad \ldots\ldots\ldots\ldots\ldots (5.2)$$

Where qe = the adsorption capacity at equilibrium (µg/mL), V = volume of adsorbate solution (mL), m = equal to adsorbent mass (mg), C_i and C_f are the initial and final concentrations of DNA measured in µg/mL, and % R is the removal efficiency.

5.3.1 Effect of contact time and adsorption kinetic

The effect of adsorption time was studied as follows: 25 mL of Erlenmeyer flask containing initial working concentrations (1.83, 3.66, 5.49, and 7.32 µg/mL obtained from stock DNA solution) at fixed pH 7.2 were in contact with 20 mg of each adsorbent labelled as E-MSN+S, E-MSN+U, and E-MSN+G at room temperature. The mixture was vortexed for 2 mins and agitated using a KS260 control orbital shaker at 300 rpm for 360 minutes. The DNA solutions containing each of the adsorbents were collected at different time intervals ranging from 0 to

360 mins. After the equilibration time, which were recorded at a different time interval (180, 195, and 210 mins for E-MSN+S, E-MSN+U, and E-MSN+G), the mixtures were centrifuged (PRISMER Centrifuge Labnet International) at 5000 rpm for 10 mins. The final concentration of DNA residues on the supernatants was quantified.

At equilibrium time, the rate of DNA uptakes on E-MSN+S, E-MSN+U, and E-MSN+G was fitted into three (3) kinetic equations listed in Table 5.2.

5.3.2 Effect of initial DNA concentrations and isotherm study

Here, 20 mg of each of the three adsorbents labelled as E-MSN+S, E-MSN+U, and E-MSN+G were added to 10 mL of different initial DNA concentrations (1.83, 3.66, 5.49, and 7.32 µg/mL) at fixed pH of 7.2, and the mixtures were allowed to agitate at 300 rpm. Samples were collected and processed after equilibrium of 180, 195, and 210 mins for E-MSN+S, E-MSN+U, and E-MSN+G. The initial working concentrations ranging from 1.83, 3.66, 5.49, and 7.32 µg/mL were decided using previously reported concentrations of residual DNA conveying ARGs obtained from hospital effluent (Rodriguez-Mozaz *et al.*, 2015a).

The experimental data obtained from the adsorption process involving the effect of initial DNA concentrations were fitted into three (3) isotherm models presented in Table 5.2.

Table 5.2: Kinetic and isotherm equations adopted in this study

Adsorption model	Equations	Plots	References
Kinetic model	Natarajan and Khalaf first order $$log(\frac{Ci}{Ct}) = \frac{K1}{2.303\ t}$$	$Log\left(\frac{Ci}{Ct}\right) vs\ Time\ (mins)$	(Idris *et al.*, 2012)
	Pseudo-First order $$Log\ (qe - qt) = logqe - \frac{K_1}{2.303t}$$	$Log\ (qe - qt) vs\ Time\ (mins)$	(Karim *et al.*, 2012; Simonin, 2016)
	Pseudo-second order	$\frac{t}{qt}\ vs\ Time\ (mins)$	

	$\frac{t}{qt} = \frac{1}{K_2 qe^2} + \frac{1}{qe}t$	$qt\ vs \ln(t)$	(Idris *et al.*, 2012; Largitte and Pasquier, 2016)
	Elovich model $qt = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t)$		
Isotherm model	Freundlich $qe = KFCe^{1/n}$	$qe\ vs\ Ce$	(Uddin *et al.*, 2017; Yusuff *et al.*, 2019)
	Langmuir $qe = \frac{KL\ qmCe}{1 + KLCe}$	$\frac{1}{qe}\ vs\ \frac{1}{Ce}$	(Ebadi and Rafati, 2015; Saman *et al.*, 2020)
	Sips $qe = \frac{qm(KLCe)\,1/n}{1 + (KLCe)1/n}$	$qe\ vs\ Ce$	

qe = µg/mg; qm = µg/mL; K_f = µg/g; K_L= mL/µg; α = $mg^{-1}\ min^{-1}$; β = µg/mg; Ce = µg/mL; Ci and Ct = µg/mL, K_1 (1/min), K_2 (g/µg min).

5.4 DNA adsorption from hospital effluent using the synthesized material

The adsorption capacities of the three (3) adsorbents (E-MSN+S, E-MSN+U, and E-MSN+G) were measured in real wastewater using effluent collected from the hospital at Cofimvaba Han district municipality Queenstown, Eastern Cape Province of South Africa. The sampling was done in November 2021. The physicochemical parameters were conducted using a Multiparameter device (HANNA H19829). The hospital effluent was initially quantified to have bacteria DNA conveying ARGs. The DNA solution was used to spike hospital effluent in the ratio of 1:1, 1:2, and 1:3 to obtain 2.30, 4.13, and 5.96 µg/mL. Adsorbents dosage of 45 mg was employed at a pH of 7.29, contact time of 180, 195, and 210 mins for E-MSN+S, E-MSN+U, and E-MSN+G, respectively. All the experimental data were collected in triplicate, and the average result was used for subsequent data analysis.

5.5 Data analysis

The data obtained from this study were plotted and analysed using OriginPro Graphing and Analysis 2021 (v.9.8.0 200), Microsoft Excel 2019, and Image J software.

5.6 Results and discussion

5.6.1 Molecular characterization of bacteria DNA from *Enterococcus faecium*

The amplicon or PCR product amplified at 320bp, 201bp, and 158bp for *ermB, tetA,* and *tetM* genes are presented in Fig. 5.1. The gel image visualized shows that ***Enterococcus faecium*** harbours antibiotic resistance genes at different base pairs.

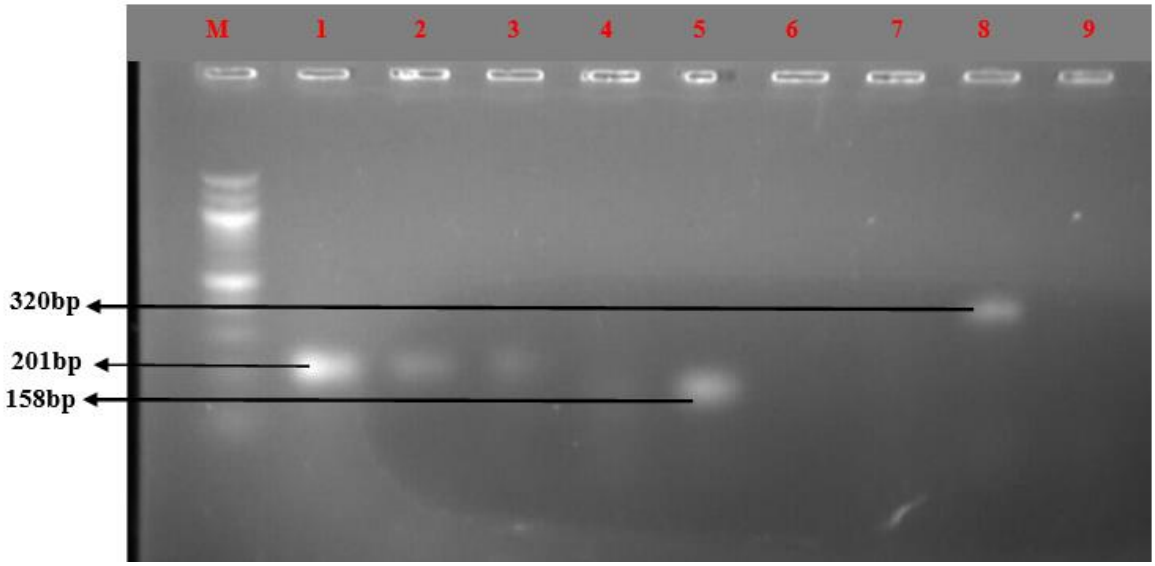


Figure 5.1. Gel electrophoresis representing resistance genes for erythromycin (ermB) amplified at 320 bp and tetracycline (tetA, tetM) amplified at 201 and 158bp

5.6.2 Characterization of adsorbent (E-MSN)

5.6.2.1 Point of zero charges of synthesized etched mesoporous silica nanoparticles (E-MSN)

The understanding of how DNA is adsorbed onto the material surface (E-MSN) and what may be the probable arrangement was confirmed by the point of zero charges (PZC) of E-MSN. Meanwhile, PZC determines the total concentration of positive and negative control results as the surface charge equals zero. At pH > PZC, the surface charge becomes negative, while at pH < PZC, the surface charge tends to be positive (Dorigon *et al.*, 2017). The PZC of

a mesoporous silica surface is usually between 2-3, indicating that the hydroxyl group is protonated at a low pH value, resulting in a negative surface charge (Vilà *et al.*, 2019). Interestingly, this result indicated the material's abnormal surface charge after silica underwent chemical etching technique with SDS, and the PZC found was 6.3 (Fig. 5.2). The PZC results from this study indicated that the material's surface possesses a weak negative charge which may not be difficult for the material (E-MSN) to adsorb a large quantity of DNA by electrostatic interaction with the addition of certain salts or compounds acting as a mediator.

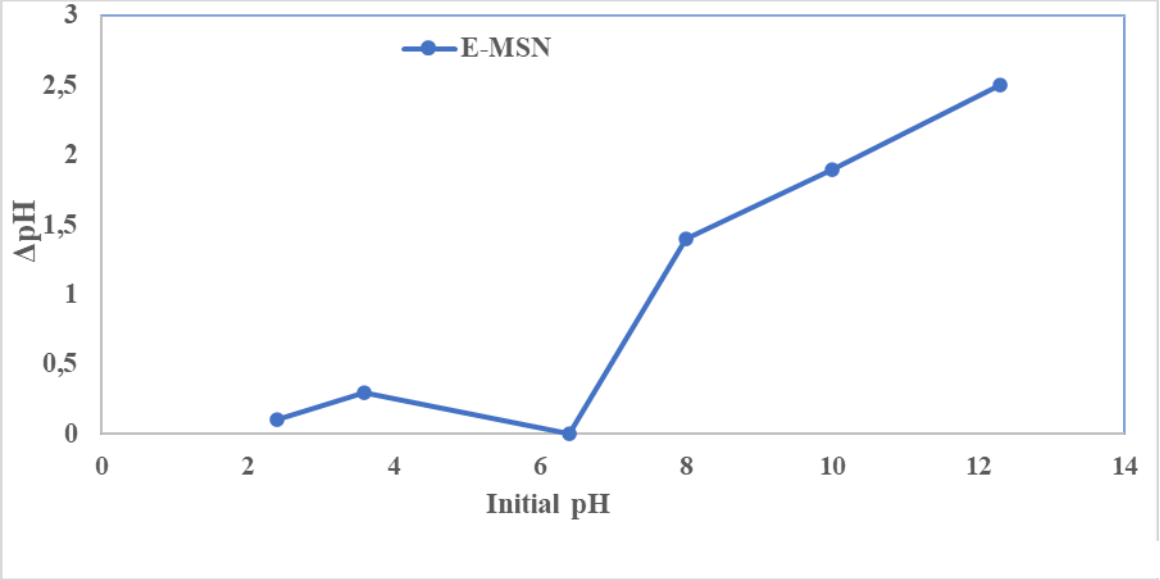


Figure 5.2: Point of zero charges (PZC) of the synthesized etched mesoporous silica nanoparticles (E-MSN)

5.6.2.2. Fourier transform infrared spectroscope (FTIR) analysis

FTIR spectral presented in Fig. 5.3 confirmed that silica particles underwent etching chemical techniques. Successful synthesis is the evidence of IR absorption band at 462 cm^{-1}, 800 cm^{-1}, 958 cm^{-1}, and 1069 cm^{-1}. The band at 462 cm^{-1} indicated the Si-O bending mode at 805 $cm^{-1,}$

and 1067 cm^{-1} confirmed the asymmetric and symmetric stretching of the Si-O-Si bridge and the obscure band at 997 cm^{-1} corresponding to the Si-OH group. The spectra obtained are similar to MSN synthesized through the chemical etching technique (Jabir *et al.*, 2018; Salehtash *et al.*, 2018). The FTIR result shows no SDS on the E-MSN surface, showing that subsequent washing by ethanol and water for SDS removal was effective.

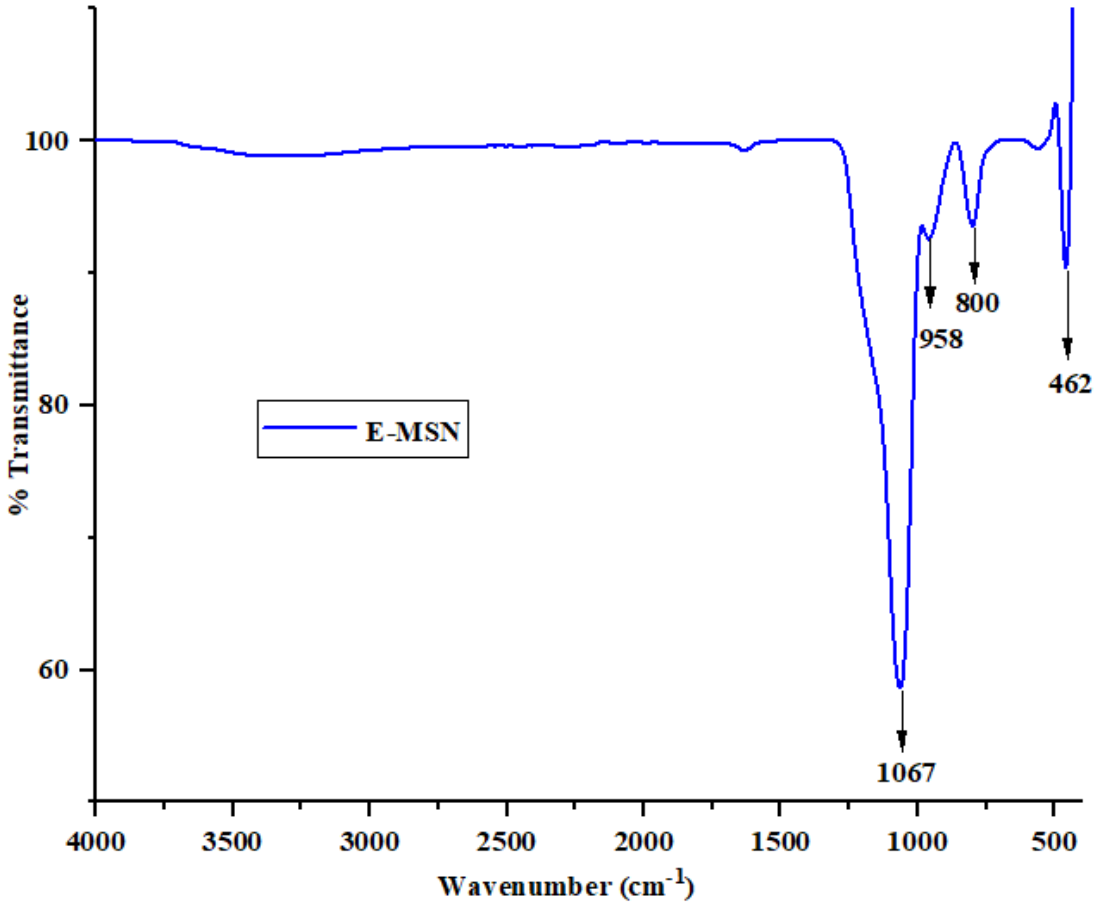


Figure 5.3: FTIR spectra of Mesoporous silica nanoparticles synthesized by a chemical etching technique

5.6.2.3 Scanning electron microscope (SEM) analysis

SEM investigated the morphology of E-MSN. As indicated in Fig. 5.4a and b, the E-MSN captured in different magnifications (20 µm and 50 µm) showed a non-spherical shape with nearly uniform size and formation of agglomeration. The particles' surface was rough, which

may be attributed to surface pore formation (Jia *et al.*, 2013). Agglomeration of E-MSN may be attributed to the chemical etching technique used during the synthesis and may occur due to the removal of unwanted layers from the material. The SEM image is similar to previously published reports on the synthesis of MSN through chemical etching chemistry (Salehtash *et al.*, 2018).

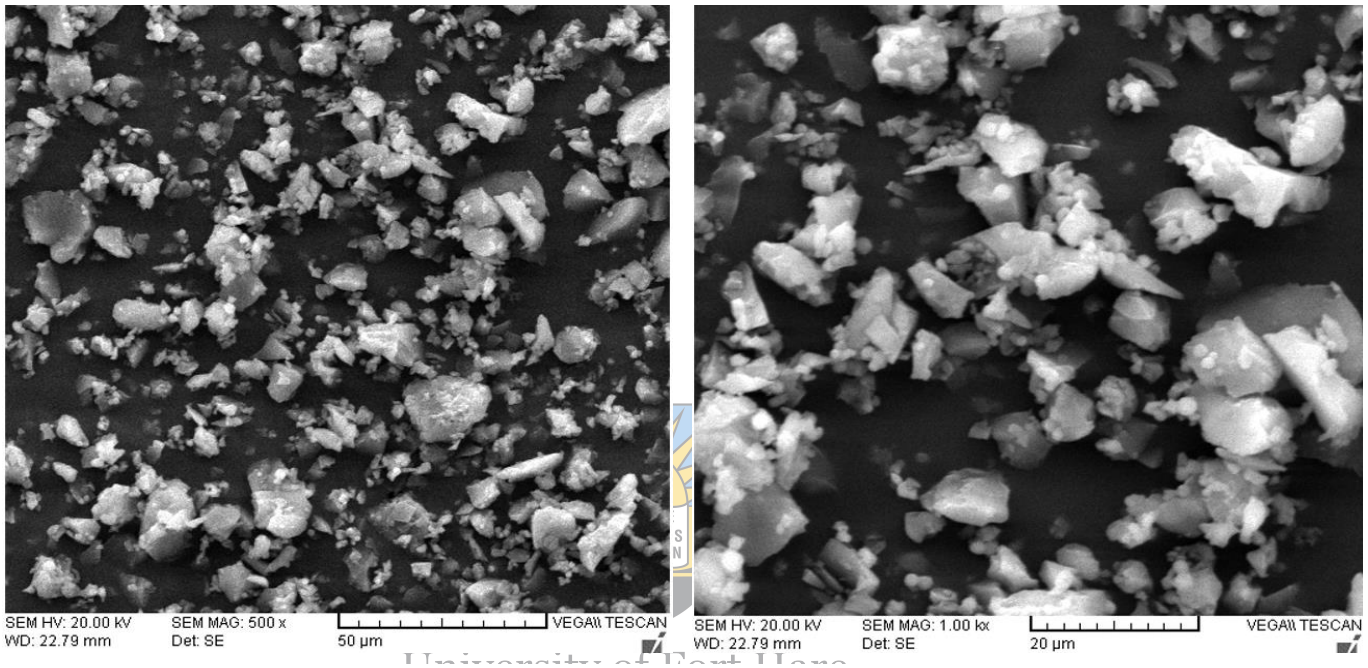


Figure 5.4 A and B: SEM images of E-MSN at high (A = 50 µm) and low (B = 20 µm) magnifications showing non-spherical morphology and less agglomerated particles

5.6.2.4. Energy dispersive x-ray spectroscope (EDX) analysis

EDX determined the elemental composition of E-MSN. Figure 5.5 showed a strong signal from the silica (Si) region at approximately 2 Kev with 31.12 % weight. Other elements detected at weak signals are carbon (C), oxygen (O), and nitrogen (N) at 17.44%, 46.75%, and 4.46%, respectively. With their % compositions, these elements confirmed the material (E-MSN) purity. The result is similar to previously reported studies (Salehtash *et al.*, 2018; Saman *et al.*, 2020).

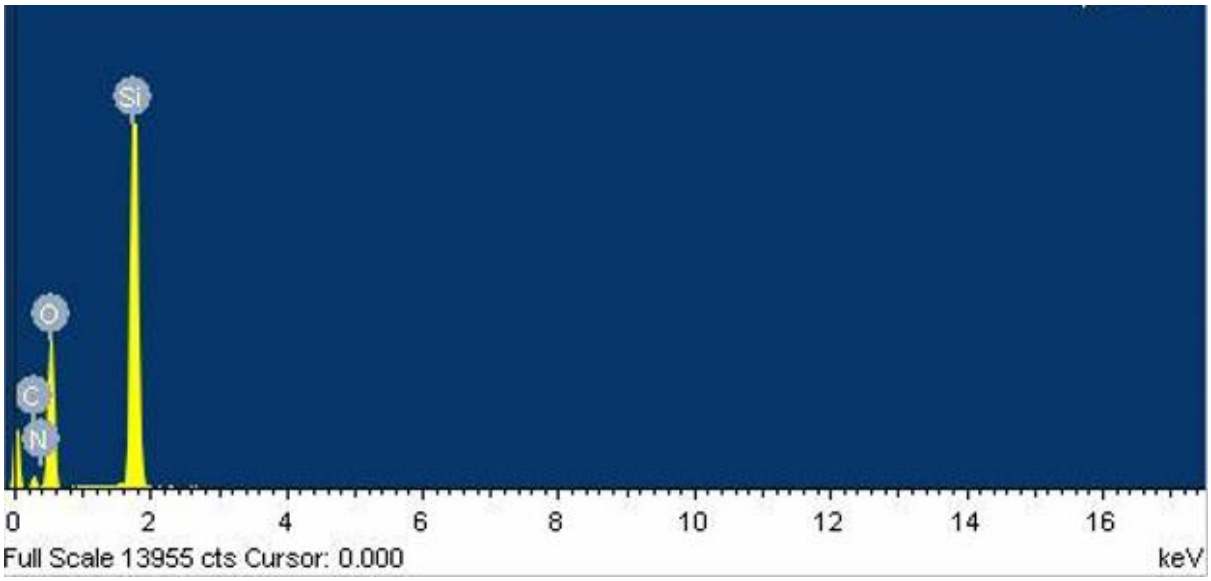


Figure 5.5: Energy-dispersive X-ray spectroscopy (EDX) of synthesized E-MSN showing a strong signal of silica (Si) at approximately 2 kev

5.6.2.5 X-ray diffraction (XRD) analysis

The X-ray diffraction pattern determined the average crystalline structure of the synthesized E-MSN. Fig. 5.6. shows the evidence that particles are crystalline. The intense peak at θ = 23.18^{o} confirmed the evidence of silica nanoparticles in the amorphous state. The intense peak showed the high purity of the material (E-MSN). Due to more negligible particle size effects, incomplete inner structure, and regular periodic variation of electron density occur during the ordering of pores in mesoporous silica nanoparticles (Sharmiladevi *et al.*, 2016). Also, the intense peak can be indexed to hexagonal lattice structure related to the mesoporous silica net (Soares *et al.*, 2015).

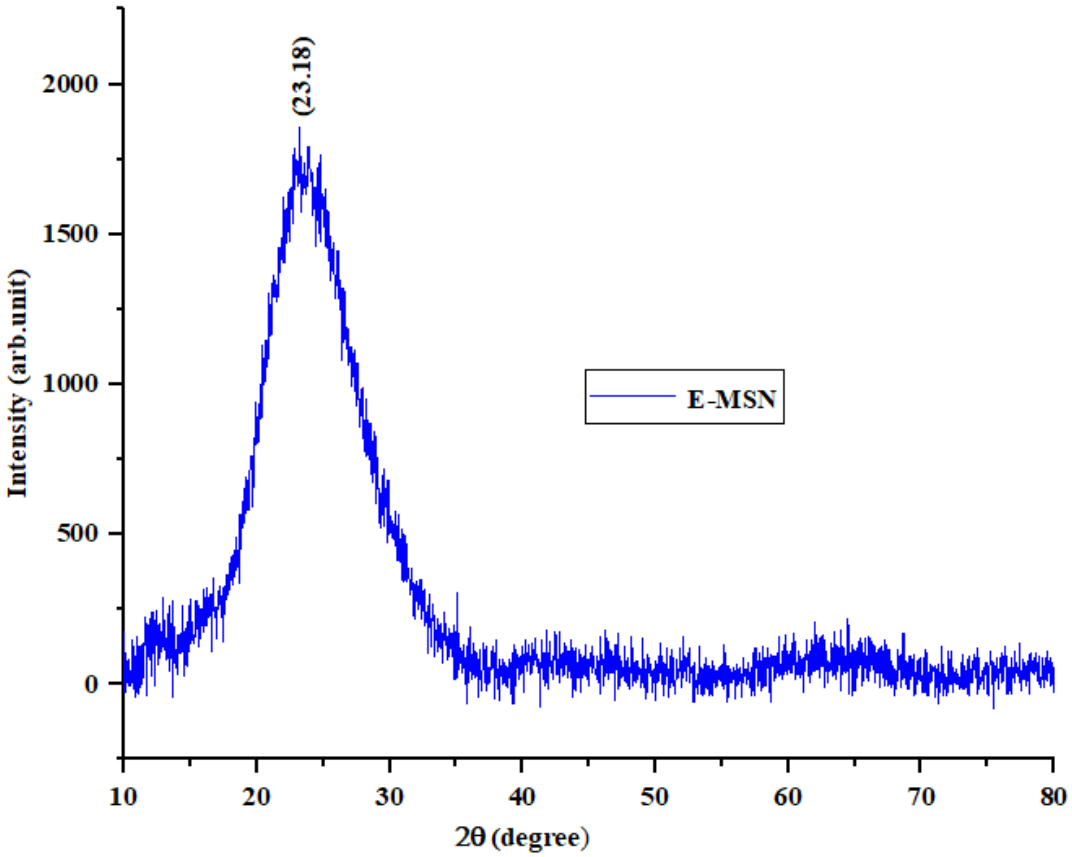


Figure 5.6: Typical XRD pattern of synthesized silica etched mesoporous nanoparticle (E-MSN)

5.7 Studies on the operating parameters for the adsorption process

5.7.1 pH effect on bacteria DNA adsorption

Varying the pH of the DNA solution plays a vital role in determining adsorption capacity, the efficiency of the adsorbent's surface charge, and the adsorbate molecules' ionization state (Aarab *et al.*, 2020). The effects of pH on bacteria DNA removal by the three (3) adsorbents labelled as E-MSN+S, E-MSN+U, and E-MSN+G were studied over a wide range, pH values 2 to 9, with the initial concentration of 3.66 µg/mL, contact time of 0-360 mins and at a fixed adsorbents dosage of 20 mg of each adsorbent. The experiment was conducted at room temperature, with a speed of 300 rpm, and the results were captured in Fig 5.7. It was observed that, at acidic pH (2-6), the removal efficiencies increased above 80% in all the adsorbents. This was due to the deprotonation of DNA molecules and protonation of functional groups (guanidinium, amine, carbonyl, Na^+, and Cl^-) found in the adsorbents,

which promotes electrostatic attraction between the negative charge of DNA and positively charged surface adsorbents (He *et al.*, 2020). The adsorption of bacteria DNA onto the adsorbents decreased drastically to 55 to 60% at basic pH (8-9). This may be attributed to the competition between OH^- ions and deprotonated DNA molecules in the adsorption site (El-Bindary *et al.*, 2018). Meanwhile, at neutral pH (7.2), the removal efficiencies for E-MSN+S, E-MSN+U, and E-MSN+G were 70%, 73%, and 76%. The optimum pH for DNA adsorption onto E-MSN+S, E-MSN+U, and E-MSN+G was pH 2.01. In this study, pH 7.2 was considered for further experimental analysis because DNA bases are still intact at neutral pH without any interference from either acid-based solution or external factors.

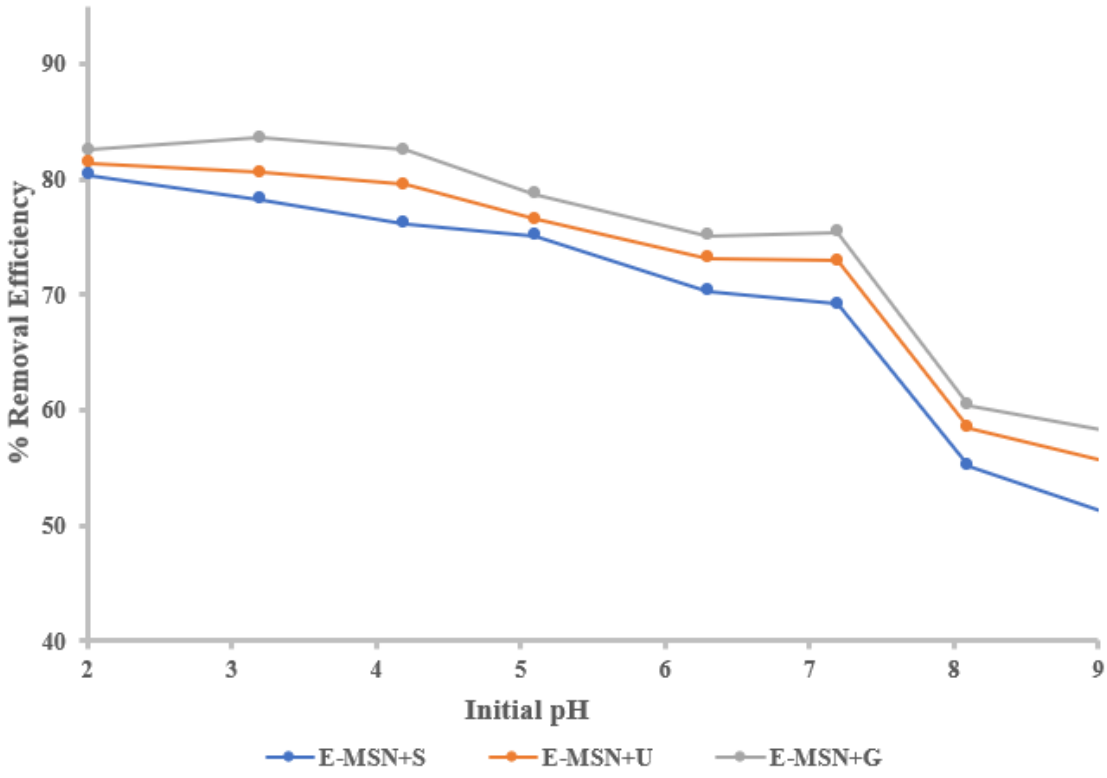


Figure 5.7: Effect of pH on the removal efficiency of DNA; adsorbent dose = 20 mg, adsorbate concentration = 3.66 µg/mL, reaction time = 360 mins and speed = 300 rpm and at room temperature

5.7.2 Sorbent effects on bacteria DNA adsorption

The sorbent dose is considered an essential parameter in determining an adsorbent's sorption capacity for a given initial concentration during the adsorption process (Batool *et al.*, 2018). In this study, the adsorbent mass on the DNA adsorption was studied and illustrated in Fig. 5.8. The result obtained complied with an already published article on DNA adsorption onto ultrathin nanosheets (Wang *et al.*, 2014). As expected, the percentage removal of bacteria DNA rapidly increased with an increase in sorbent mass from 10 to 40 mg. At 40 mg, the optimum removal efficiencies reached 89%, 92%, and 94% for E-MSN+S, E-MSN+U, and E-MSN+G, respectively. We observed that removal efficiencies increased in the following order: E-MSN+G > E-MSN+U > E-MSN+S by increasing the adsorbent dose to 40 mg. The high removal efficiency of E-MSN+G is attributed to the protonation of guanidine HCl, producing a more positive charge on the adsorbent's surface, thus increasing electrostatic interaction between the DNA and the sorbent. More surface-active sites were available for the bacteria DNA to penetrate easily into the sorption site (Bundjaja *et al.*, 2021). To obtain a constant value, the sorbent dose was increased to 50 mg at fixed initial concentrations of 3.66 µg/mL of the DNA solution; the value obtained showed no significant removal of DNA adsorbed onto the adsorbents. Therefore, an adsorbent dose of 40 mg was chosen for further studies.

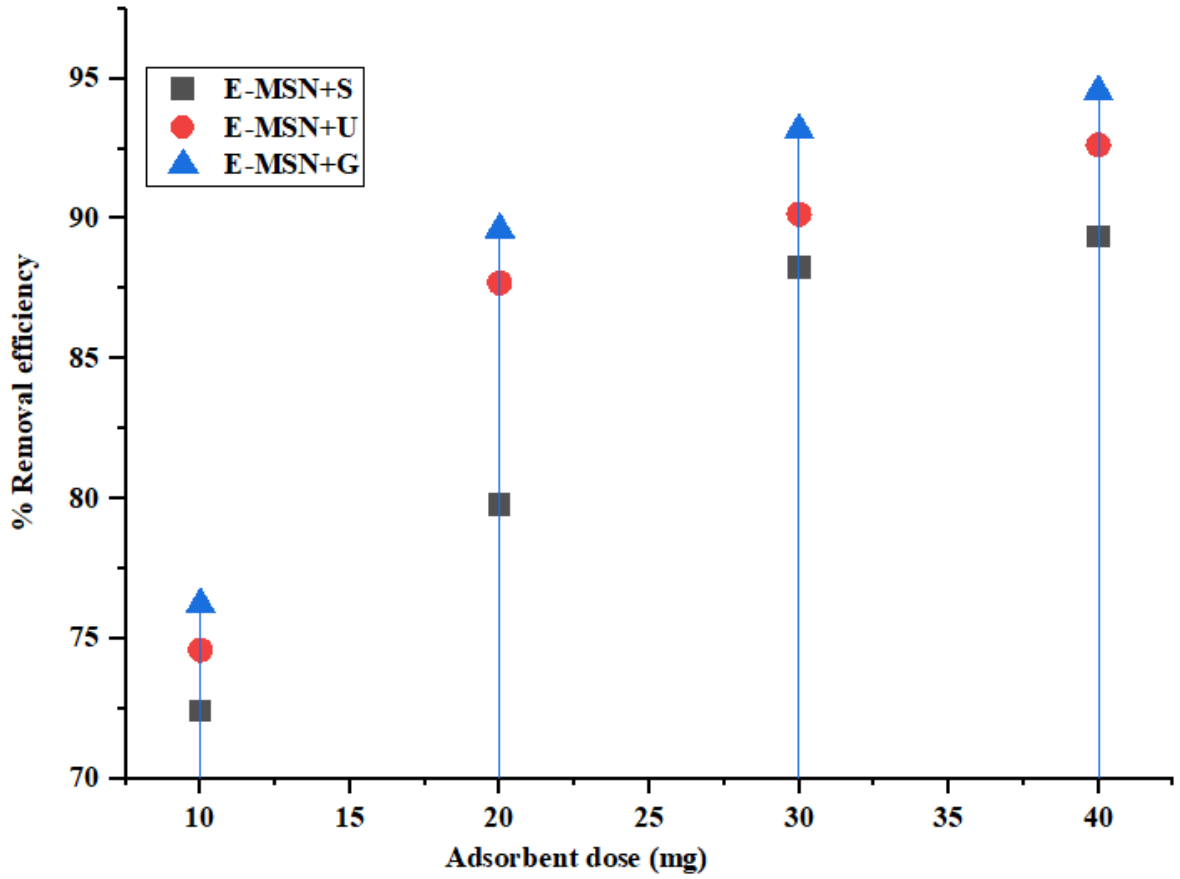


Figure 5.8: Effect of adsorbent dose on the removal of bacteria DNA onto E-MSN+S, E-MSN+U, and E-MSN+G, adsorbate concentration = 3.66 µg/mL, reaction time = 180, 195, and 210 mins; speed = 300 rpm, pH = 7.2, and at room temperature

5.7.3. Effect of contact time and kinetics study

In this study, the effect of contact time was studied because it reflected the adsorption kinetics of an adsorbent for a given concentration of DNA solution. It determines the efficiency of the adsorbent in terms of adsorption of pollutants from wastewater through rapid uptake of the adsorbate (Chen, Zhao, *et al.*, 2019). The effect of contact time on the adsorption of bacteria DNA onto the adsorbents (E-MSN+S, E-MSN+U, and E-MSN+G) at different initial concentrations (1.83, 3.66, 5.49, and 7.32 µg/mL) are shown in Fig. 5.9A. The result showed that the adsorption capacity of bacteria DNA on the E-MSN+S, E-MSN+U, and E-MSN+G was rapid between 5-15 mins at different initial concentrations and gradually stagnated with the increase in contact time. We noticed that equilibration time for

DNA adsorption occurred at 180, 195, and 210 mins for E-MSN+S, E-MSN+U, and E-MSN+G, respectively. Adsorption capacity obtained at each adsorbent increased simultaneously in the order of E-MSN+G >E-MSN+U > E-MSN+S. The increase in adsorption capacity within 5 to 15 mins is attributed to the availability of positively surface charged E-MSN enhanced by the chaotropic salt. At the same time, the gradual process of reaching the equilibrium time could be slow pore diffusion of DNA molecules into the bulk of the adsorbents. However, we observed that the adsorption capacity measured by the adsorbents reduces at an increase in concentrations of DNA solution. It is because high adsorption capacity is highly dependent on the initial concentrations of DNA.

To study adsorption kinetics of the experimental data obtained from E-MSN+S, E-MSN+U, and E-MSN+G, four (4) kinetic models, namely, Natarajan and Khalaf first order (NKFO), Pseudo-first order (PFO), Pseudo-second order (PSO), and Elovich model equations were applied to the adsorption data. The results of kinetic parameters obtained are shown in Table 5.3; where K_1 (min^{-1}), K_2 (µg/mL mins), β (µg/mg), and α (mg^{-1} min^{-1}) are the equilibrium NKFO, PFO, PSO, and Elovich model rate constants respectively. According to Table 5.3 and Fig. 5.9B, pseudo-second-order exhibited the highest correlation coefficient (R^2 ranging from 0.97 to 0.99 for E-MSN+S, E-MSN+U, and E-MSN+G) than other kinetic models investigated. The highest R^2 values exhibited by PSO confirmed the kinetic mechanism of adsorption onto E-MSN+S, E-MSN+U, and E-MSN+G was based on chemisorption, which involved electron sharing between DNA molecules and the adsorbent's surface charge. Also, the calculated qe (qe^{cal}) equilibrium values were closely related to the qe experimental (qe^{exp}). This confirmed that PSO correlated with DNA adsorption onto surface-enhanced mesoporous silica nanoparticles (E-MSN+S, E-MSN+U, and E-MSN+G). To further confirm the authenticity of pseudo-second-order as the best fit for this study, the initial sorption rate was calculated using equation (5.3):

$$h = K_2 \times qe^2 \ \ldots\ldots\ldots\ldots (5.3)$$

The value of *h* obtained from initial DNA concentrations decreases in the same order as K_2, indicating a low adsorption rate at the increase in initial DNA concentration.

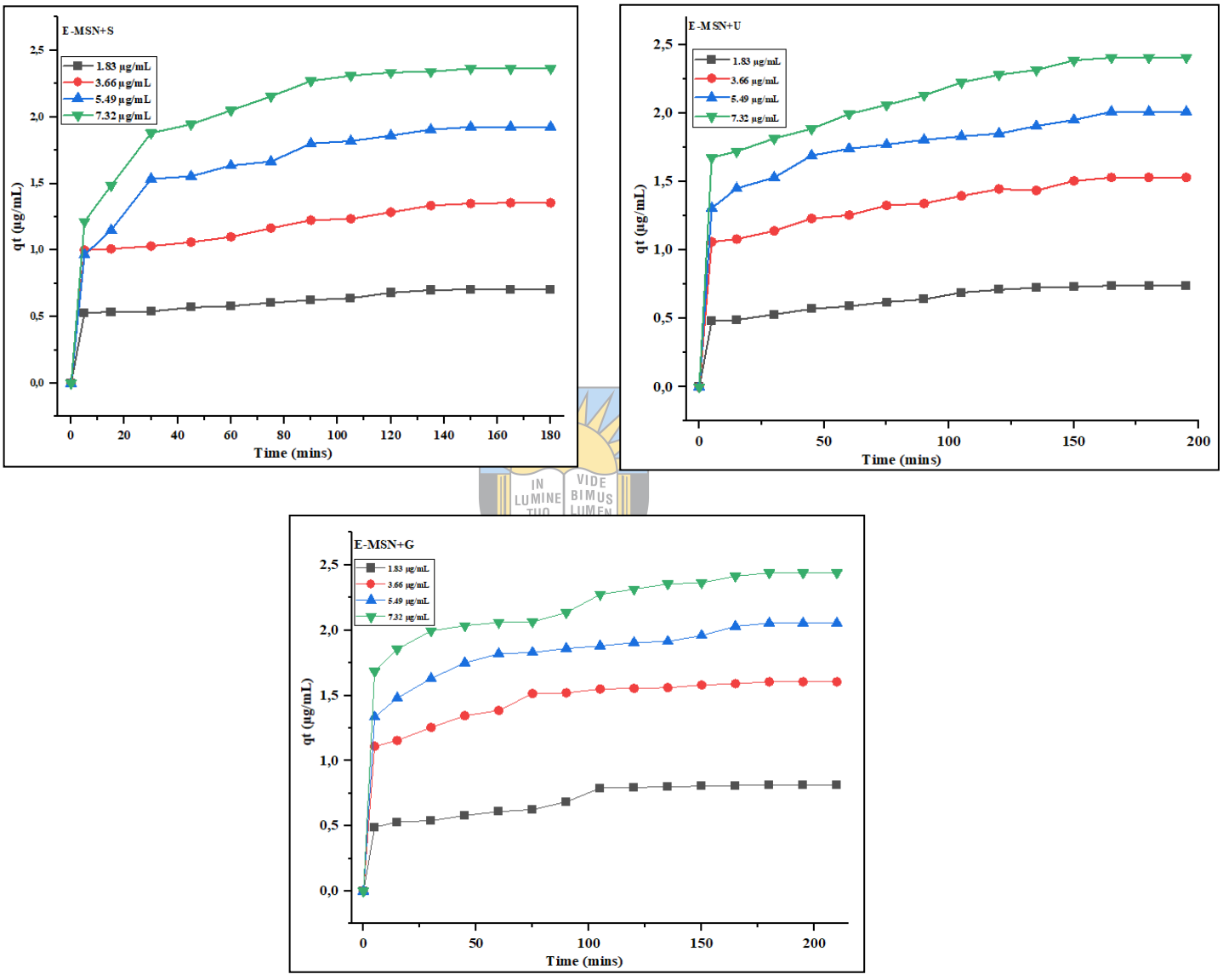


Figure 5.9A: Represent the effect of contact time for E-MSN+S, E-MSN+U and E-MSN+G at different adsorbate concentrations (1.83, 3.66, 5.49 and 7.32 µg/mL); reaction time of 180, 195, and 210 mins, sorbent dose = 20 mg, speed = 300 rpm, and pH = 7.2

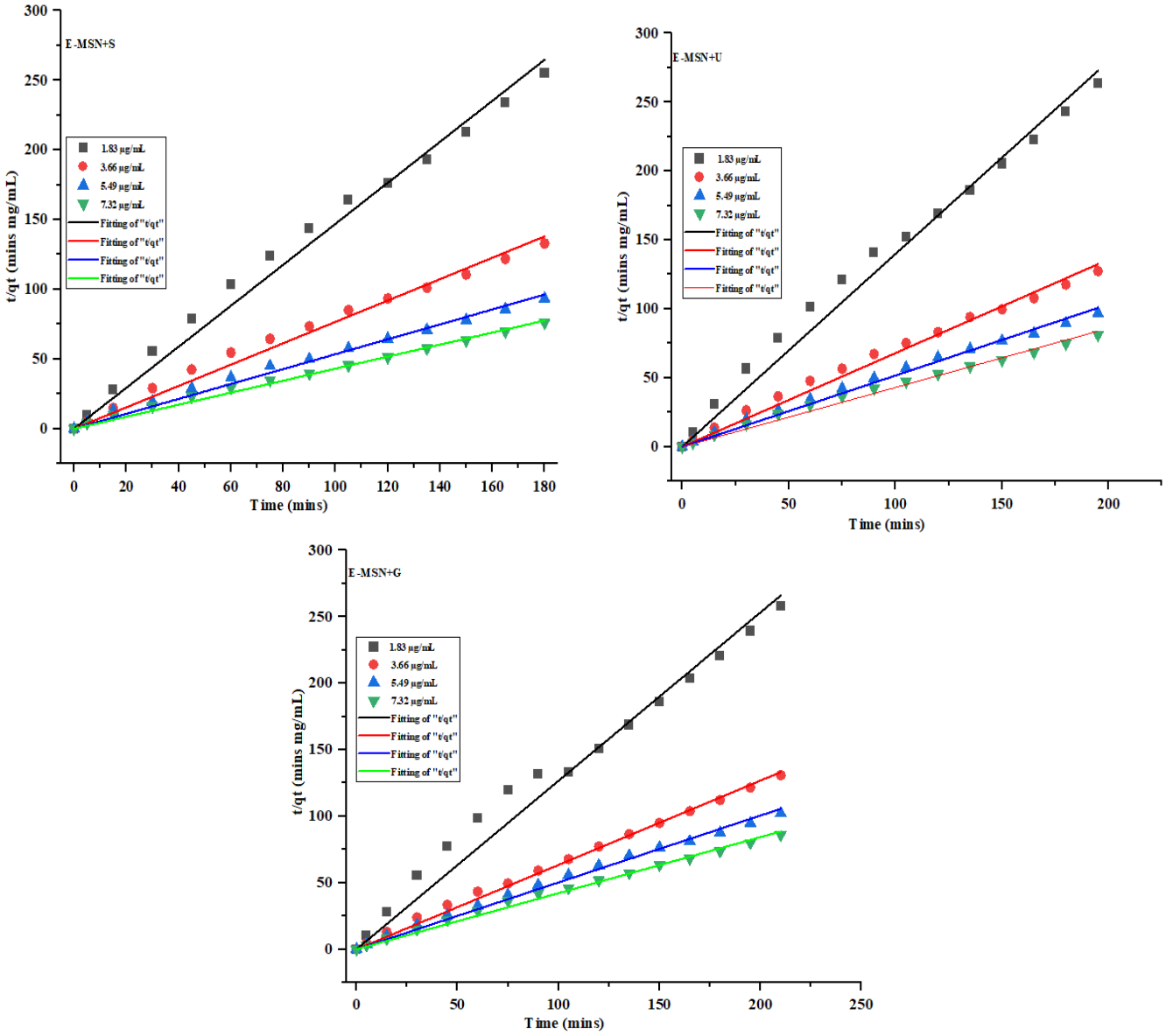


Figure 5.9B: The kinetic of the nonlinear plot of the pseudo-second-order model for DNA adsorption onto E-MSN+S, E-MSN+U, and E-MSN+G

Table 5.3: Calculated parameter values obtained from kinetic adsorption models; K_1 and K_2 are the Natarajan and Khalaf first order, Pseudo first order, and Pseudo second-order rate constant, qe (^{cal}) and qe $^{(exp)}$ are then calculated, and experimental sorption capacity, *h*, and R^2 are the initial sorption rate related to Pseudo second order and correlation coefficient, α and β represent the initial adsorption rate and desorption constant.

Kinetic model	Adsorbents	Parameters	Initial DNA concentration (µg/mL)			
			1.83	**3.66**	**5.49**	**7.32**
Natarajan and Khalaf First order	E-MSN+S	K_1(^{1}/min)	0.0000134	0.0000122	0.0000124	0.0000103
		R^2	0.73559	0.73455	0.77126	0.71712
	E-MSN+U	K_1(^{1}/min)	0.0000311	0.0000301	0.0000248	0.0000199
		R^2	0.90299	0.86761	0.81683	0.80438
	E-MSN+G	K_1(^{1}/min)	0.0000416	0.0000442	0.0000247	0.0000188
		R^2	0.92344	0.90119	0.80505	0.75812
Pseudo First order	E-MSN+S	qe (^{cal})(µg/mg)	0.70	1.33	1.93	2.37
		K_1 (^{1}/min)	0.0000446	0.0000637	0.0000644	0.0000898
		qe(^{exp})(µg/mg)	0.60	0.81	1.04	1.17
		R^2	0.88591	0.88591	0.95622	0.95493
	E-MSN+U	qe (^{cal})(µg/mg)	0.74	1.53	2.01	2.41
		K_1 (^{1}/min)	0.0000647	0.0000403	0.0000483	0.0000295
		qe(^{exp})(µg/mg)	0.76	0.85	0.93	0.96
		qe (^{cal})R^2	0.9271	0.9388	0.96523	0.94332
	E-MSN+G	qe (^{cal})(µg/mg)	0.82	1.61	2.06	2.44
		K_1 (^{1}/min)	0.0000743	0.0000362	0.0000391	0.0000702
		qe(^{exp})(µg/mg)	0.91	0.88	0.95	0.85
		R^2	0.97504	0.93488	0.93883	0.92852
Pseudo Second order	E-MSN+S	qe (^{cal})(µg/mg)	0.70	1.33	1.93	2.37
		K_2 (g/µg min)	1.20606	0.87374	0.72433	0.64653
		qe(^{exp})(µg/mg)	0.89	1.48	2.15	2.45
		h	1.47036	0.76664	0.53474	0.43124
		R^2	0.98648	0.98362	0.98824	0.99181
	E-MSN+U	qe (^{cal})(µg/mg)	0.74	1.53	2.01	2.41
		K_2 (g/µg min)	1.17798	0.82072	0.71122	0.64547
		qe(^{exp})(µg/mg)	0.77	1.63	2.22	2.48
		h	1.39865	0.67916	0.51649	0.42991
		R^2	0.98362	0.98765	0.99093	0.98808
	E-MSN+G	qe (^{cal})(µg/mg)	0.82	1.61	2.06	2.44
		K_2 (g/µg min)	1.12339	0.79202	0.70124	0.63911
		qe(^{exp})(µg/mg)	0.95	1.77	2.31	2.56
		h	1.26660	0.63421	0.502838	0.42196
		R^2	0.97461	0.99566	0.99677	0.99170
Elovich model	E-MSN+S	α($mg^{-1}min^1$)	1.36136	2.13191	0.69745	0.94067
		β (µg/mg)	5.49388	2.65062	0.59199	0.53406
		R^2	0.95251	0.95009	0.98972	0.99272
	E-MSN+U	α($mg^{-1}min^1$)	0.43821	1.51491	1.79431	2.39783

		β (µg/mg)	2.80432	1.89631	1.34461	1.20166
		R^2	0.95203	0.9621	0.98365	0.96081
	E-MSN+G	α($mg^{-1}min^1$)	0.31115	1.51491	1.9668	3.19876
		β (µg/mg)	1.64509	1.89631	1.35864	1.33924
		R^2	0.92575	0.96218	0.98939	0.97847

5.7.4 Effect of initial concentrations and isotherm study

The plots of E-MSN+S, E-MSN+U, and E-MSN+G percentage removal efficiency against initial concentrations are presented in Fig 5.10A. In all the three adsorbents, it was observed that the amount of DNA adsorbed onto sorbent mass decreased with the increase in the initial concentrations (1.83, 3.66, 5.49, and 7.32 µg/mL). At equilibrium, the percentage (%) removal efficiencies obtained from each adsorbent are 70.04, 74.04, 70.12 and 64.61 (E-MSN+S); 80.87, 83.60, 73.22, and 65.71 (E-MSN+U); and 89.07, 87.70, 74.86, and 66.66 (E-MSN+G). From the results, E-MSN+G exhibited the highest performance in removing DNA conveying ARGs followed by E-MSN+U and E-MSN+S. Generally, the decrease in removal efficiencies of E-MSN+S and E-MSN+U compared to the literature may be attributed to the adsorptive site being saturated due to an increase in initial concentrations of DNA. Also, the materials' inability to produce a more positive charge would facilitate the adsorption of DNA onto the adsorbents at the increase in adsorbate concentration. Therefore, to describe the transmission of DNA from the solution phase to the adsorbent phase at equilibrium conditions, adsorption isotherms such as Freundlich, Langmuir, and Sips models were used to evaluate the experimental data. Generally, the Freundlich model is best fitted when adsorption occurs on a heterogeneous surface, having unequal active sites and different energies of adsorption (Al-Ghouti and Da'ana, 2020; Yang *et al.*, 2021). The Langmuir model applies the adsorption at the specific homogenous sites within the adsorbent, thereby

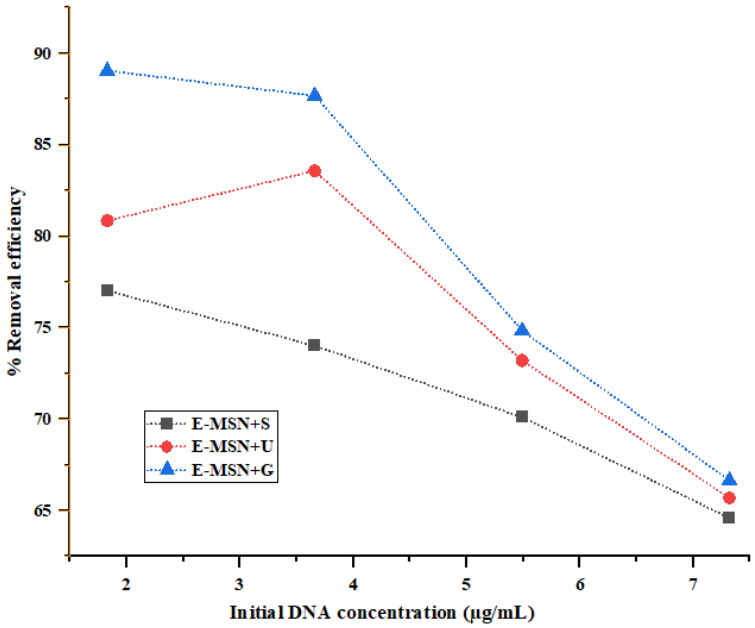
forming a single layer over the surface of the adsorbent (Miyah *et al.*, 2018; Panão *et al.*, 2019). The Sips model, a three-parameter isotherm, describes a combination of Freundlich and Langmuir models. The model is suitable for adsorption on a heterogeneous surface, avoiding the limitations of increased adsorbate concentration normally associated with the Freundlich model (Kumar, Gadipelli, *et al.*, 2019; Siqueira *et al.*, 2020). Therefore, this model concluded that at low adsorbate concentration, Freundlich is predicted, but it depicts Langmuir at high adsorbate concentration (Ayawei *et al.*, 2017). The given equation for the three isotherm models (Table 5.2) was plotted (Fig. 5.10B), and the values obtained were evaluated from the slope and intercept of nonlinear plots (Table 5.4). The three-parameter adsorption model (Sips) obtained from the experimental values reasonably agrees with DNA adsorption onto E-MSN+S, E-MSN+U, and E-MSN+G. The adsorption data obtained from all the three adsorbents were best well fitted into Sips model (R^2 = 0.99982, 0.98798, and 0.98971 for E-MSN+S, E-MSN+U and E-MSN+G) with reduced Chi-square (0.002, 0.01, and 0.02) than Freundlich (R^2 = 0.95571, 0.92632, and 0.92971) and Langmuir (R^2 = 0.979418, 0.98645, and 0.96727). The highest R^2 values obtained from the sips model indicated that DNA adsorption onto E-MSN+S, E-MSN+U, and E-MSN+G were achieved via heterogeneous surfaces.

Although the maximum % removal obtained from E-MSN+S (77.04) and E-MSN+U (80.8) is lower than the value (89.5%) reported in the literature for DNA adsorption onto magnetic silica surface (Li *et al.*, 2012); they are still advantageous because DNA can adsorbed onto the mesopore of silica nanoparticles by addition of chaotropic salts alongside the adsorbent without any costly modification. It can be quickly recovered from the treated water. These characteristics make E-MSN+S, E-MSN+U, and E-MSN+G an economical option that can tackle bacteria DNA menace across the globe.

Table 5.4. Isotherm parameters for the adsorption of bacteria DNA (ARGs) onto E-MSN+S, E-MSN+U, and E-MSN+G at room temperature

Isotherm	Parameter	E-MSN+S	E-MSN+U	E-MSN+G
Langmuir	qmax (µg/mL)	4.3792	3.4614	2.896
	$K_{L.}$ (µg/g)	0.4532	0.9045	1.887
	$R_{L.}$	0.5466	0.3766	0.225
	X^2	0.0012	0.0024	0.0050
	R^2	0.9794	0.9865	0.9673
Freundlich	K_f (µg/g)	1.3545	1.6068	1.8194
	n	1.6319	2.1071	2.8351
	X^2	0.0111	0.0567	0.0519
	R^2	0.9557	0.9263	0.9297
Sips	qmax	3.8281	2.8373	2.5775
	Ks	0.7886	2.4059	4.4167
	n	1.2265	1.0818	1.1281
	X^2	0.002	0.018	0.028
	R^2	0.9998	0.9879	0.98971

(A)



(B)

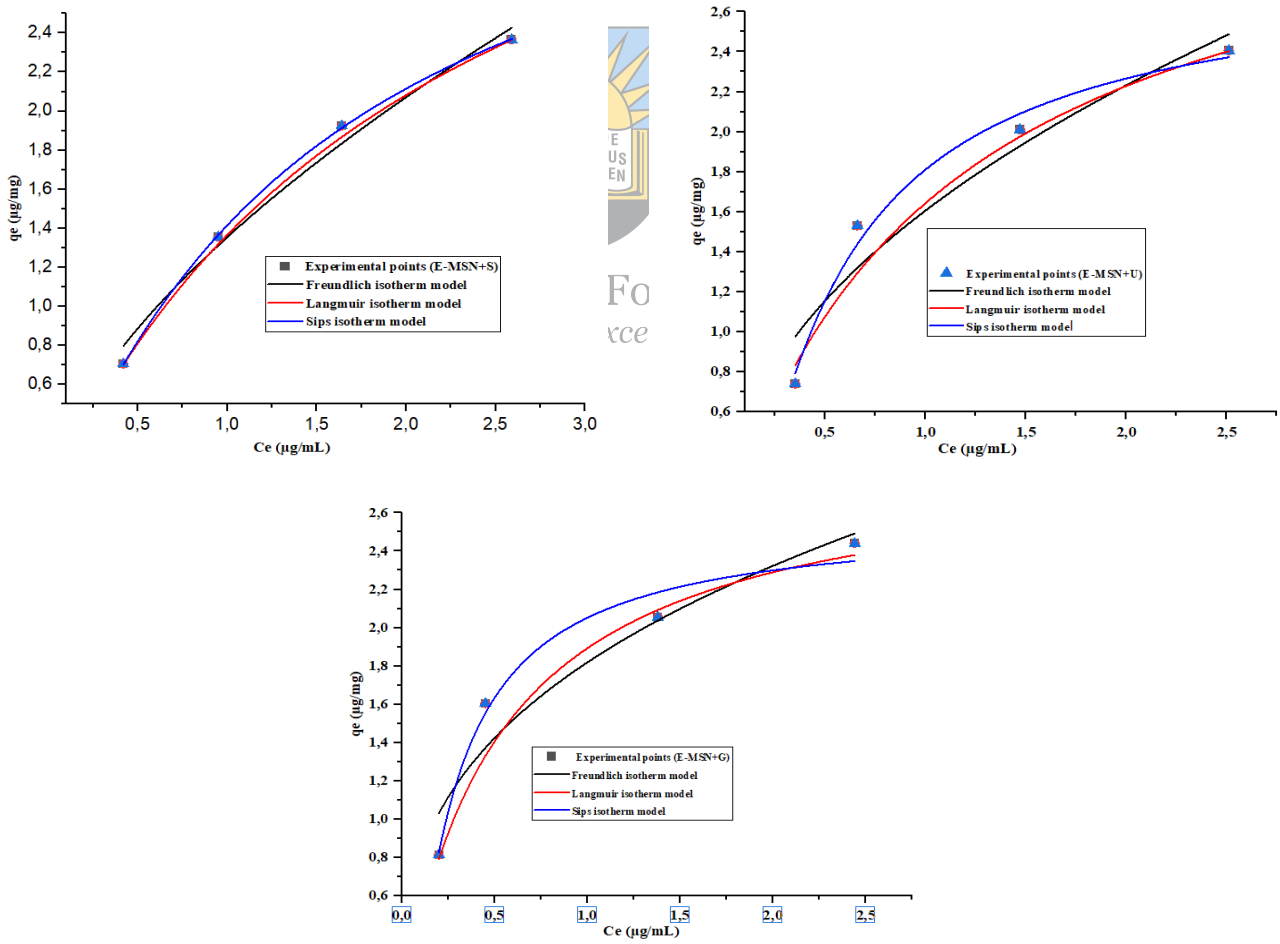


Figure 5.10: (A) Effects of DNA initial concentration on adsorption process; (B) nonlinear plots of Langmuir, Freundlich, and Sips isotherm model

5.8. Proof of concept (removal of bacterial DNA from hospital wastewater)

To ascertain the effectiveness of E-MSN+S, E-MSN+U, and E-MSN+G in a real-life scenario, real hospital wastewater was collected from Cofimvaba Han district Queenstown, Eastern Cape province of South Africa. The physicochemical parameters obtained before the commencement of the study are presented in Table 5. The result of the physicochemical analysis showed that hospital effluent is in a toxic condition with moderate total dissolved solids. The concentration of bacteria DNA after spiking to obtain different initial working concentrations was subjected to a batch adsorption study. At optimum conditions, the maximum % adsorption efficiency obtained at different initial concentrations (2.3, 4.13, and 5.96 µg/mL) were in the range of 60 to 71%, 63 to 73%, and 76 to 94% for E-MSN+S, E-MSN+U and E-MSN+G respectively (Fig. 11). It was observed that the % adsorption efficiency obtained from real hospital wastewater is lower than the simulated water. This may be attributed to other anionic interference present in the real wastewater. The anionic interference may cause repulsion effects between DNA molecules and adsorbents at the adsorptive site. The result obtained indicated that E-MSN+G showed high removal efficiency of bacteria DNA conveying ARGs from hospital wastewater.

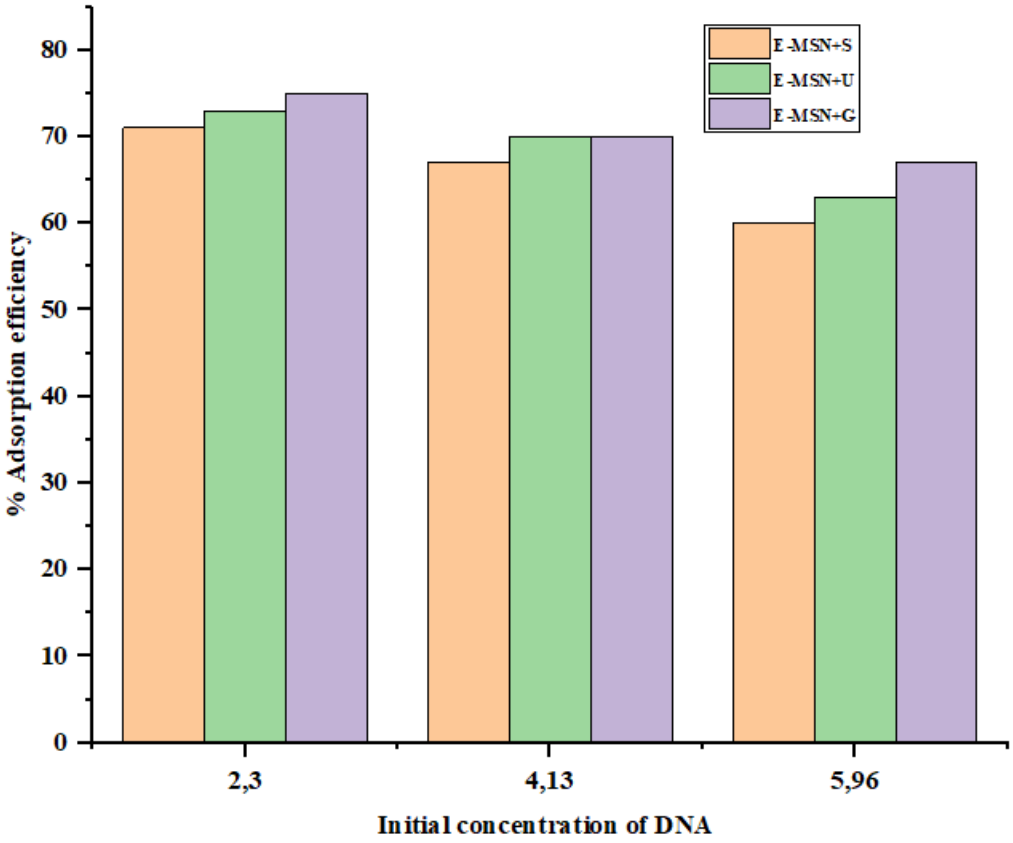


Figure 5.11: The removal of bacteria DNA conveying ARGs from hospital wastewater

Table 5.5: Physicochemical parameter of hospital wastewater

pH	Temperature	Total dissolved solids	Dissolved oxygen	Turbidity	Initial DNA concentration in wastewater (µg/mL)
7.79	13.35	30	7.29	15.9	0.474

5.9 Conclusion

The etched mesoporous silica nanoparticles were successfully synthesized via chemical etching techniques. The synthesized material was enhanced with different chaotropic salts to obtain E-MSN+S, E-MSN+U, and E-MSN+G, and the applications were extended to hospital wastewater to remove bacteria DNA conveying ARGs. The result obtained showed that DNA can bind onto the mesopore of silica nanoparticles with the help of different chaotropic salts. E-MSN+G exhibited high removal efficiency in the adsorption of bacteria DNA conveying ARGs in both the simulated water and hospital wastewater. Moreover, the adsorbents

exhibited high adsorption capacities and dispersibility and were easily recovered from an aqueous solution. The study batch adsorption parameters (effects of solution pH, contact time, adsorbent dosage, and initial concentrations) contributed significantly to the uptake of DNA from water/wastewater. The kinetics and isotherm studies of the experimental data fitted well into pseudo-second order and Sips models respectively. The adsorption isotherm model suggests heterogenous chemisorption for DNA uptake onto E-MSN+S, E-MSN+U, and E-MSN+G. Therefore, synthesized material aided by the different chaotropic salts (E-MSN+S, E-MSN+U, and E-MSN+G) showed high performance and can be an effective material for removing bacteria DNA from hospital wastewater.

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Chapter 6

Removal of bacteria DNA conveying antibiotic resistance genes from Ndevana Buffalo River by adsorption onto AgNPs@Fe_3O_4 nanocomposite

Submitted to Journal of Environmental pollution.

Chapter 6

Removal of bacteria DNA conveying antibiotic resistance genes from Ndevana Buffalo River by adsorption onto AgNPs@Fe_3O_4 nanocomposite

Abstract

In this study, magnetic properties were introduced onto the surface of nano silver (AgNPs@Fe_3O_4) for the removal of bacteria DNA conveying antibiotic resistance genes from water was synthesized and characterized. The elemental composition, surface morphology, functional group, and phase crystallinity of the AgNPs@Fe_3O_4 nanocomposite were analysed using energy dispersive spectroscopy (EDX), scanning electron microscopy (SEM), Fourier transformed infrared spectroscopy (FTIR), and x-ray diffraction spectroscopy (XRD). The result confirmed that the functionalization of AgNPs with magnetite was successfully achieved. The bacteria deoxyribonucleic acid (DNA) conveying antibiotic resistance genes (ARGs) used as a contaminant in this study was extracted from antibiotic-resistant *Enterococcus Faecium* and *Vibrio Parahaemolyticus. T*he molecular characterization result showed that the two-bacteria harbours tetracycline (*tetA* and *tetM*) and macrolides (*ermB*) resistance genes at 210,158 and 320 base pair (bp) with purity between 1.7 and 1.9, respectively. The application of AgNPs@Fe_3O_4 nanocomposite was subjected to DNA removal from aqueous solution, and the influence of some operating parameters (effect of solution pH, adsorbent dose, contact time, and initial concentration) was investigated and optimized. The results depicted that at neutral solution pH (pH 7.2), AgNPs@Fe_3O_4 nanocomposite has excellent adsorption efficiency of above 75.96 % from the polluted water.

In addition, kinetic and isotherm equilibrium studies confirmed that adsorption experimental data fitted best for Elovich kinetic (R^2 = 0.9999, X^2 0.00001) and Freundlich isotherm (R^2 = 0.94317, X^2 = 0.01996) model respectively. Adsorption studies conducted on river water recorded high adsorption efficiency of 96.7 %, suggesting field applicability of AgNPs@Fe_3O_4 nanocomposite.

6.1 Introduction

Rivers, streams, and lakes are contaminated with bacteria DNA conveying antibiotic resistance genes (ARGs) due to untreated effluents directly discharged from the agricultural industries. Antibiotics are organic substances produced either by natural microorganisms or industrial synthesis and are generally meant to prevent or treat infections in humans and animals (Iwu *et al.*, 2020; Wang *et al.*, 2015). Besides, many tones of antibiotics produced each year are mainly used for agricultural purposes. For example, about 90% of antibiotics produced in the United States of America (USA) are primarily used as animal feeding additives for growth promotion and to improve fruit quality and yield (Vladimir, 1967; Kirchhelle, 2018). Also, effective treatment of bacterial infections in livestock and crops is achieved by antibiotics drugs. Continuous applications of these antibiotics promote resistance mechanisms in bacteria, which may be acquired through mutation in the cell's DNA (deoxyribonucleic acid) during replication and horizontal gene transfer (Jiang *et al.*, 2018; Roy *et al.*, 2020). Most bacteria DNA has been reported to harbours antibiotic resistance genes (ARGs), and infections caused by ARGs are significant to public health (Ezeuko *et al.*, 2021a; McInnes *et al.*, 2020). Also, most of these bacteria DNA conveying ARGs are found in the animal guts, which can be excreted as active metabolites (Gudda *et al.*, 2020). Many bacteria DNA conveying ARGs are released during the routine evacuation of generated wastewater into rivers and lakes. Thus, animal waste is the primary source of bacteria DNA conveying ARGs into the natural water environment, while untreated effluents from households, hospitals, and sewages are the additional source (Amarasiri *et al.*, 2020; Hubeny *et al.*, 2021). Since the contamination of bacteria DNA conveying ARGs is rising, there is a global concern about stopping the unruly discharging of untreated effluents and eradicating the existing ones from natural water environments.

According to literature, several treatment methods have been employed to clean up these contaminants from rivers to reduce the toxic and hazardous effects caused by these contaminants. Such treatment methods include coagulation, chemical precipitation, advanced oxidation, reverse osmosis, adsorption, ion exchange, and membrane processes.(Bairán *et al.*, 2020; Barancheshme and Munir, 2018; Ezeuko *et al.*, 2021c). Adsorption seems to have a compelling potential to clean up these contaminants from the water environment. The advantages of adsorption in water purification include its simplicity in design, sludge-free, economic feasibility, ability to decontaminate and applicability in a large-scale industry (He *et al.*, 2020; Mousavi *et al.*, 2019). Various nano-sorbents such as metal oxide and magnetic nanoparticles have been reported to be effective nanoparticles for the decontamination of bacteria DNA conveying ARGs in water/wastewater. As reported in chapter 4, metallic nanoparticles, including silver nanoparticles (AgNPs), have been a promising adsorbent for bacteria DNA removal. The high removal efficiency was recorded due to their large surface area and volume ratio, nano-size effect, and affinity to bind negatively charged contaminants (Shokry *et al.*, 2019; Yap *et al.*, 2020). To increase the percentage removal efficiency, 1.0 M of AgNPs was functionalized with Fe_3O_4 NPs (magnetite) and the adsorbent was investigated for the removal of bacteria DNA conveying ARGs from aqueous solution and real water sample.

Generally, magnetic nano-sorbent nanoparticles have been used in clinical applications such as drug and gene delivery systems, sensing, binding agent, and protein immobilization (Eivazzadeh-keihan *et al.*, 2021; Ekinci *et al.*, 2021). Also, it has been fabricated on the sorbent surface for packaging duplex DNA during wastewater application, and excellent behaviour has been reported in literature (Zhang *et al.*, 2012). Magnetic nanoparticle, in particular iron (III) oxide (Fe_3O_4), is widely used for in science due to their unique properties. Such properties include superparamagnetic, low toxicity, high surface-to-volume ratio, easy

synthesis, and biocompatibility (Ganapathe *et al.*, 2020; Yew *et al.*, 2020). A study reported that silica functionalization achieved with magnetite on the surface shows high adsorption capacity towards DNA molecules, and the material was easy to recover from treated water (Zhang *et al.*, 2012). Another study reported an excellent adsorption capacity by using magnetite grafted onto the surface of titanium oxide nanoparticles to remove DNA from an aqueous solution (Mousavi *et al.*, 2019). The high DNA adsorption capacity recorded is due to their high porosity, greater surface area, increased surface energy, good magnetic character, and hydrophobicity of the material (Ghaemi and Absalan, 2014; Nizamuddin *et al.*, 2018). Due to their hydrophobicity nature, the functionalization of AgNPs with Fe_3O_4 would increase the adsorption capacity of the adsorbent. Also, surface functionalization gave the AgNPs a new surface property such as reactivity, dispersibility, high binding affinity, and new electronic structure without altering the nature of the original material (Ojemaye *et al.*, 2017; Tamayo *et al.*, 2018; Xu *et al.*, 2018). Therefore, Fe_3O_4 (magnetite) introduced to the silver matrix through surface functionalization are considered sites for adsorption of DNA molecules and, consequently, are expected to enhance the adsorption capacity of the sorbent. In this study, an adsorbent, AgNPs@Fe_3O_4, was synthesized and evaluated to remove bacteria DNA conveying ARGs from water sample obtained from the Ndevana Buffalo River.

6.2. Experimental

6.2.1. Reagents and chemical

Nitric acid, iron oxide, and sodium hydroxide were purchased from Sigma Aldrich, United State of America and 1.5 M silver nanoparticles were synthesized in the laboratory according to the method reported in Chapter 4. Nuclease-free water and DNA kit were purchased from Thermo Fischer, South Africa. All the chemicals were of analytical grade and used as purchased.

6.2.2. Instrumentation

The Fourier transform infrared spectroscope (FTIR) (Perkin-Elmer Universal ATR 100) was determined by the surface functional group in 4000 to 350 cm^{-1}. Determination of surface morphology and elemental composition of the adsorbents were conducted using the Scanning electron microscope (SEM) and energy dispersive x-ray spectroscope (EDX) (JOEL JSM-6390LVSEM). Average crystallinity and phase compositions were determined with X-ray diffraction (XRD) (Bruker D8). Residual bacteria DNA conveying ARGs was quantified using a dsDNA assay kit (Q32850) specific to the Qubit 1.0 Fluorometer (Thermofischer) during the adsorption experiment.

6.2.3. Molecular identification of antibiotic resistance genes

Antibiotic-resistant *Enterococcus faecium* and *Vibrio parahaemolyticus* used in this study were obtained from our laboratory archives isolated from the beach water located in East London and wastewater treatment plant around the University of Fort Hare, Alice, province of Eastern Cape, South Africa. Antibiotic susceptibility testing (AST) on the bacteria isolates was conducted according to the disk diffusion method, following the procedure of Kirby-Bauer recommended by CLSI (Humphries *et al.*, 2021). The bacteria isolates were resistant to antibiotics such as linezolid, erythromycin, ampicillin, tetracycline, vancomycin, tetracycline, PB 300, meropenem, amikacin, and ciprofloxacin. Extraction of genomic DNA was conducted by boiling method (Ribeiro *et al.*, 2016), and molecular characterization for the determination of antibiotic resistance genes they harbor was evaluated according to the method previously discussed in chapters 4 (2.3) and 5 (2.2). The stock DNA was stored at -20 ^{o}C for further analysis. Absorbance ratio at 260 nm, 280 nm, and 320 nm was measured using Multiparameter HACH DR 6000 Ultraviolet Spectroscopy, and it is in the range of 1.7 and 1.9. This range confirmed the removal of interfering compounds. Polymerase chain reaction (PCRs) assay confirming the presence of resistance genes in the bacteria isolates was prepared using the primers presented in Table 6.1. The amplicons were examined using 1.5 %

(w/v) agarose gel, stained with 10 µL of ethidium bromide at a processing time of 45 mins (Han *et al.*, 2015). Then, it was pictured using the Alliance BioDoc-It system.

Table 6.1: PCR primers, sequences, and protocols used in this study

Class of Antibiotic	PCR primers	Primer sequences	Product size (bp)	PCR protocols	References
Tetracyclines	*tetA*	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	94 °C – 5 min; 35[94 °C – 1 min; 55 °C – 1 min; 72 °C 1½ min]; 72 °C – 5 min.	(Titilawo *et al.*, 2015)
	tetM	F AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	94 °C – 5 min; 35[94 °C – 1 min; 55 °C – 1 min; 72 °C 1:30 min]; 72 °C – 5 min.	(Adeniji *et al.*, 2020; Osode and Okoh, 2009)
Macrolides	*ermB*	BN1: CGAGTGAAAAAGTACTCAACA BN2: CGGTGAATATCCAAGGTACG	320	94 °C – 3 min; 35 [94 °C – 1 min; 55 °C – 1 min; 72 °C 1 min]; 72 °C – 10min.	

6.3. Procedure for synthesis of AgNPs and Fe3O4

The molar concentration (1.5M) of Silver nanoparticles (AgNPs) was prepared according to the method discussed previously in Chapter 4 (Section 2.2) (Wang *et al.*, 2005). Magnetic nanoparticles (Fe_3O_4) were prepared according to the literature method (Rashidi Nodeh *et al.*, 2017) with slight modifications. The equation of the reaction is shown in equation 6.1. A stock solution of 25 mmol of ferrous ammonium sulphate hexahydrate [Fe $(NH_4)_2(SO_4)_2.6H_20$] and 50 mmol of iron (III) chloride hexahydrate ($FeCl_3.6H_20$) were mixed and added drop by drop into 20 mmol of NaOH. The solution was vigorously stirred under a magnetic stirrer for 24 h at room temperature. The residue obtained was separated by centrifuge. 0.01 M of HCl was added to neutralize the anionic charge on the materials. The

recovery particles were washed severally with deionized water and separated by an external magnet. The particles were dried in the oven, calcined for 3 h at 550 ºC, and marked as Fe_3O_4 NPs.

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \quad \ldots\ldots\ldots (6.1)$$

6.3.1. Procedure for the synthesis of AgNPs-Fe_3O_4 composite

AgNPs with magnetite (Fe_3O_4) composite material was prepared as described in the literature (Mousavi *et al.*, 2019; Muraro *et al.*, 2020). The proposed chemical structure of the synthesized AgNP@Fe_3O_4 nanocomposite are illustrated in Fig. 6.1. 4 g of synthesized AgNPs and 2 g of Fe_3O_4 were dissolved in 60 mL of ammonia and 40 mL of acetonitrile. The mixture was sonicated for 1 h. Then, 4mL of ammonia and water was added drop by drop, and the mixture was allowed to stir for 24 h under a magnetic stirrer at 1500 rpm at room temperature. The mixture was washed with deionized water, separated using an external magnet, calcined for 3 h at 550 ºC, and marked as AgNP@Fe_3O_4 nanocomposite.

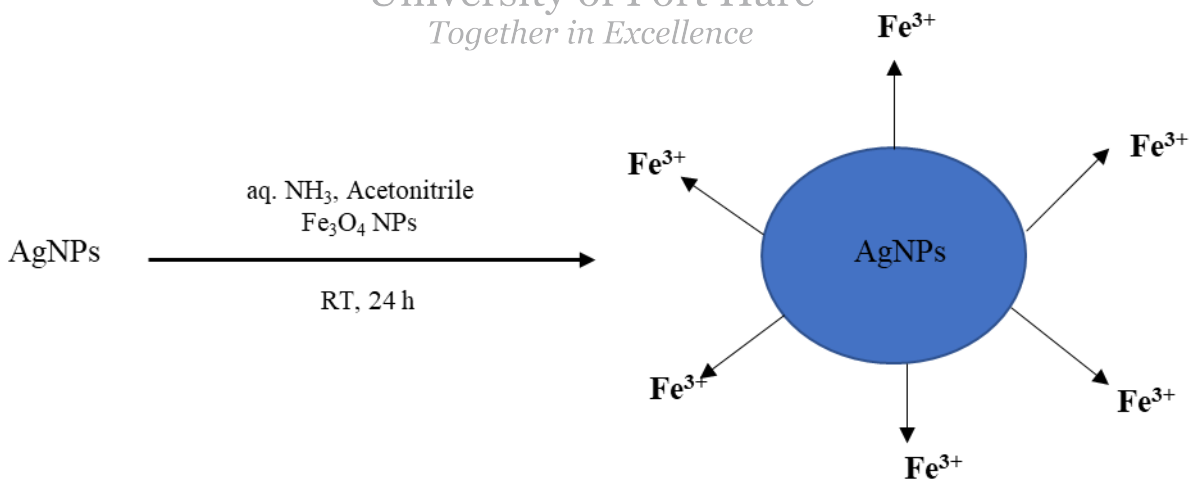


Figure 6.1: The chemical structure of the prepared adsorbent (AgNPs@Fe3O4 NPs)

Adsorption experiment of bacteria DNA conveying ARGs was conducted using batch reactor experiment investigating the effects of operating parameters such as solution pH (2-9), contact time 0-90 mins), DNA initial concentrations (3.64, 7.28, and 10.98 µg/mL prepared

from DNA stock solution) and effect of adsorbent doses (10-40 mg). Nuclease-free sterile water was used in all the batch sample preparation. In general, 20 mg of the synthesized AgNPs@Fe_3O_4 was added to a 25 mL Erlenmeyer flask containing 6 mL of nuclease-free water (NFW) polluted with 2 mL of genomics DNA extracted from antibiotic *Enterococcus faecium* and *Vibrio parahaemolyticus*. The batch experiments were conducted at a fixed solution pH of 7.2 and room temperature on a KS260 control orbital shaker agitated from 0-360 mins at 300 rpm. After the equilibration time, the adsorbent loaded with bacteria DNA molecules was quickly recovered by centrifugal force. The residual DNA concentration left in the supernatant solution was quantified using a dsDNA assay kit (Q32850) Qubit 1.0 Fluorometer (Thermofischer). For the time-concentration profile, samples were collected at various time intervals (t) at a different initial concentration (3.64, 7.28, and 10.98 µg/mL) under a fixed adsorbent dose of 20 mg. Effects of the adsorbent dose were also investigated by increasing the doses from 10-40mg at the same fixed solution pH and room temperature. The number of DNA molecules adsorbed at time interval *t* (*qt*) and equilibrium (*qe*) onto AgNPs@Fe_3O_4 was calculated using the following Equations (6.2) and (6.3), respectively (Fu *et al.*, 2015).

$$qt\ or\ qe = \frac{C_i(\mu gmL) - Cf\ or\ Ct(\mu gmL)}{m\ (mg)} \times V(mL) \quad \text{..... (6.2)}$$

$$\%R = \frac{C_i(\mu gmL) - Cf\ or\ Ct(\mu gmL)}{C_i(\mu gmL)} \times 100 \quad \text{..... (6.3)}$$

Where V = volume of adsorbate solution (mL), m = adsorbent mass (mg), C_i (µg/mL), and C_f or C_t (µg/mL) are the initial and final DNA concentrations after the time (t) and equilibrium respectively, and % R is the removal efficiency, and qe or qt are adsorption capacity recorded at the time (t) and equilibrium.

The adsorption model, such as kinetic and isotherm models, were fitted into the experimental data to determine the adsorption design or mechanism. The kinetic models, equations, and parameters include:

- Natarajan and Khalaf first order = $\boldsymbol{log}(\frac{\boldsymbol{Ci}}{\boldsymbol{Ct}}) = \frac{\boldsymbol{K1}}{\boldsymbol{2.303\ t}}$.....(6.4)
- $Pseudo\ First\ order = \log(qe\ - qt) = -\frac{K_1}{2.303t}$ ... ... (6.5)
- $Pseudo\ second\ order\ \frac{t}{qt} = \frac{1}{K_2 qe^2} + \frac{1}{qe}t$ ... ... (6.6)

$$Elovich\ model\ (qt) = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \text{ ... ... (6.7)}$$

Ci and ***Ct*** (µg/mL**)** are the initial concentration, and the final amount of DNA adsorbed at contact time ***t*** (mins), respectively. $\mathbf{K_1}$ (min^{-1}) and $\mathbf{K_2}$ (g/ (µg mins)) are the adsorption rate constant for the Natarajan and Khalaf first order, Pseudo first order and second-order kinetic, **qe** and **qt = (**µg/mg) is the amount of DNA adsorbed at equilibrium and at time interval ***t***.

Then, the adsorption isotherm, equations, and parameters used in this study are:

- $Freundlich\ isotherm\ (qe) = KFCe^{1/n}$..... (6.8)
- $Langmuir\ isotherm\ (qe) = \frac{KLqmCe}{1 + KLCe}$ ... ... (6.9)

Where ***qe*** (µg/g) and ***qm*** (µg/g) are the amount of DNA adsorbed per unit mass of adsorbent at equilibrium and theoretical maximum adsorption capacity, respectively. $\boldsymbol{K_f}$ (µg/g) and $\boldsymbol{K_L}$ (mL/µg) are the Freundlich, and Langmuir isotherm constant related to the adsorption energy, **Ce**: equilibrium concentration, and ***n*** = empirical constant, showing that adsorption occurred on heterogeneous surfaces through a multilayer adsorption mechanism.

6.5. DNA adsorption onto AgNPs@Fe_3O_4 nanocomposite from a water sample collected from the Ndevana River

Three water samples from the Ndevana River containing bacteria DNA conveying ARGs were spiked with DNA solution in the ratio of 1:1, 1:2, and 1:3 to obtain initial concentrations of 1.48, 2.14, and 3.35 µg/mL. The real water sample was collected in November 2021 and was confirmed to have bacteria DNA conveying ARGs. Meanwhile, the water sample were collected aseptically in a sterile 1 Litre glass bottles along the river courses by midstream dipping of sample bottles at 25-30 cm down the water column, with mouth titling against the flow of the river. All the samples were transported on ice to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory and processed within 4-6 h of collection. Before the commencement of the adsorption study, physicochemical parameters were conducted using a Multiparameter device (HANNA H19829) on the sample site. Adsorbent dose of 40 mg, at pH of 7.02, contact time of 90 mins were employed, and the experiment was conducted at room temperature. All the experimental data were obtained in triplicate, and the average result was used for data analysis. Meanwhile, the Ndevana River is situated East of King Williams Town, under Buffalo City Metropolitan Municipality in Eastern Cape, Province of South Africa as shown in the map below:

Figure 6.2: The map of Ndevana Buffalo river situated at Eastern Cape, South Africa

6.6. Data analysis

The data obtained from this study were plotted and analysed using OriginPro Graphing and

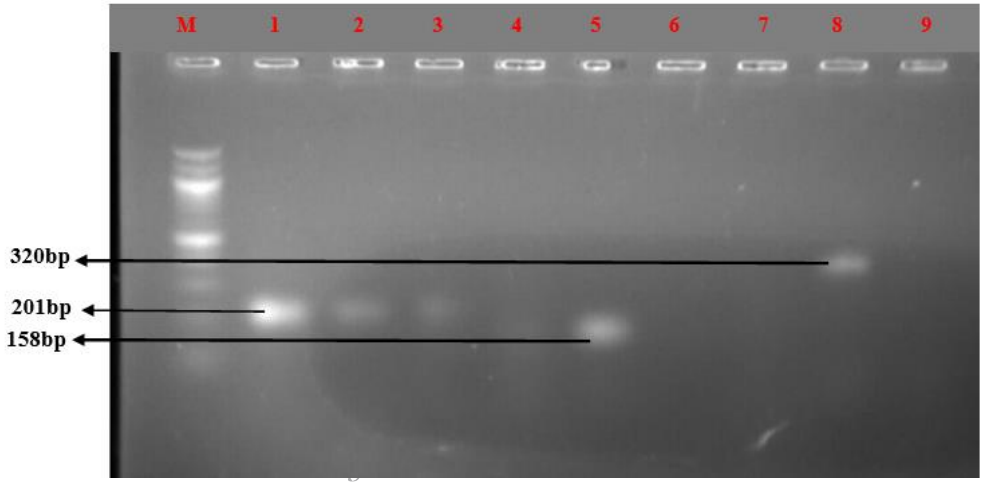
Analysis 2021 (v.9.8.0 200), Microsoft Excel 2019, and Image J software.

6.7. Results and discussion

6.7.1. Molecular characterization of bacteria DNA from Enterococcus faecium and Vibrio Parahaemolyticus

The PCR product amplified at 320bp, 201bp, 210pb, and 158bp for *ermB*, *tetA*, and *tetM* genes is presented in Figure 6.3A and B. The gel image shows that ***Enterococcus faecium*** **and** ***Vibrio parahaemolyticus*** convey antibiotic resistance genes at different base pairs.

(A)



(B)

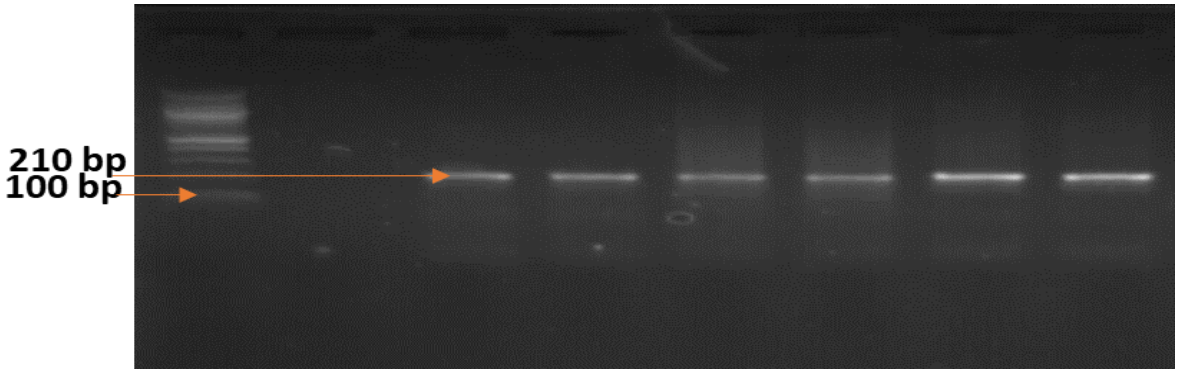


Figure 6.3. (A) Gel electrophoresis representing resistance genes for erythromycin (ermB) amplified at 320 bp and tetracycline (*tetA, tetM*) amplified at 201 and 158bp for Enterococcus faecium, (B) Resistance genes for tetracycline (tetA) amplified at 210bp.

6.8 Adsorbent ($AgNPs@Fe_3O_4$) characterization

6.8.1 FTIR analysis

FTIR determines the presence of distinct groups in the synthesized adsorbent based on their vibrational mode and specific wavelength from the infrared range (Kędzierska *et al.*, 2021). The FTIR spectrum of $AgNPs@Fe_3O_4$ nanocomposite presented in Fig. 6.4 showed that five (5) peaks were visible, and they are 3284 cm^{-1}, 1538 cm^{-1}, 1372 cm^{-1}, 1036 cm^{-1} and 583 cm^{-1}. The vibrational peaks at 3284 cm^{-1} and 1538 cm^{-1} are assigned to the stretching vibration of N-H of primary amine and O-H groups. The peaks at 1372 cm^{-1} and 1036 cm^{-1} represent C-O stretching vibration and N-H bending vibration, respectively. The band at 583 cm^{-1} indicates the Fe-O vibration in the magnetite phase. Other peaks at 1250 cm^{-1}, 654 $cm^{-1,}$ and 517 cm^{-1} are assigned to the hydroxyl group or molecules of water. The peaks 3284 cm^{-1} and 583 cm^{-1} confirmed the successful synthesis of $AgNPs@Fe_3O_4$ nanocomposite. The result is similar to published literature on the synthesis of AgNPs grafted with magnetite nanoparticles (Kędzierska *et al.*, 2021; Mahmodi *et al.*, 2022).

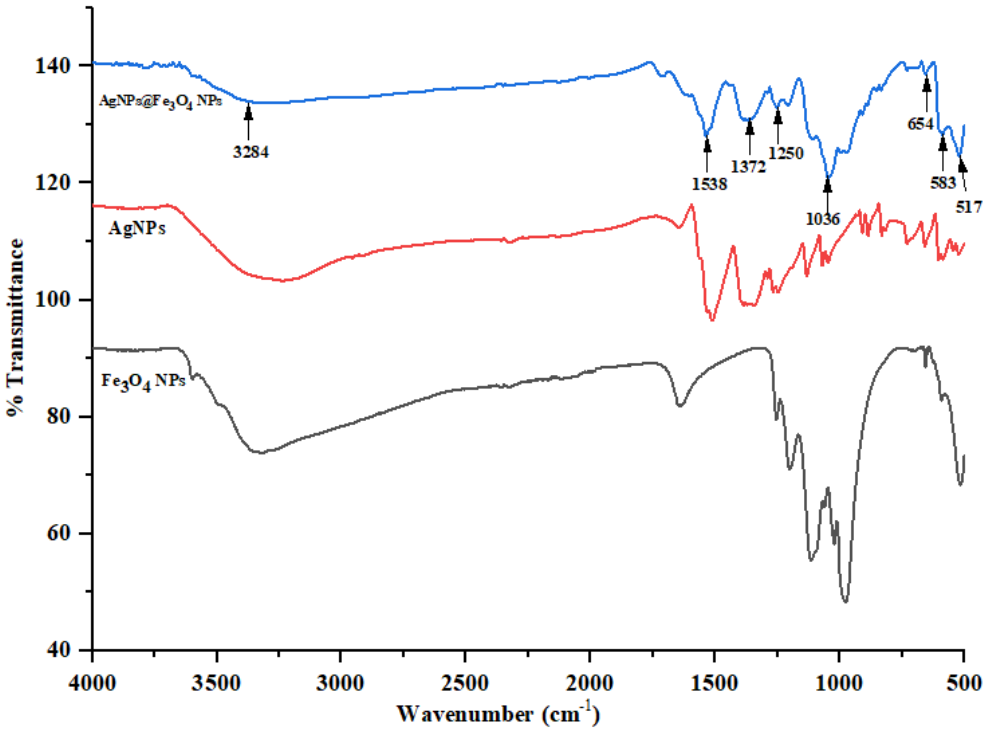


Figure 6.4: FTIR spectra of the synthesized AgNPs@Fe3O4 nanocomposite

6.8.2 SEM analysis

Scanning electron microscopy (SEM) analysed the surface morphology and microstructure. Figures 6.4A and B represent SEM images of the AgNPs@Fe_3O_4 nanocomposite captured at 20 µm and 50 µm after synthesis. The morphology obtained from nano-silver (AgNPs) coated with magnetite (Fe_3O_4) showed a nearly spherical shape with a rough surface, this result has been reported in the literature (Ansari *et al.*, 2020; Chen *et al.*, 2018; Manyi-Loh *et al.*, 2018). The AgNPs-Fe_3O_4 composite material provided a slight regular and uniform size with the appearance of microscopic particles. It is claimed that small particles can increase the surface area, reduce diffusion resistance, and enhance mass transfer (Pang *et al.*, 2015). Also, the Fe-O group from magnetite prevented the AgNPs@Fe_3O_4 from aggregation. The image depicts the successful grafting of AgNPs by Fe_3O_4.

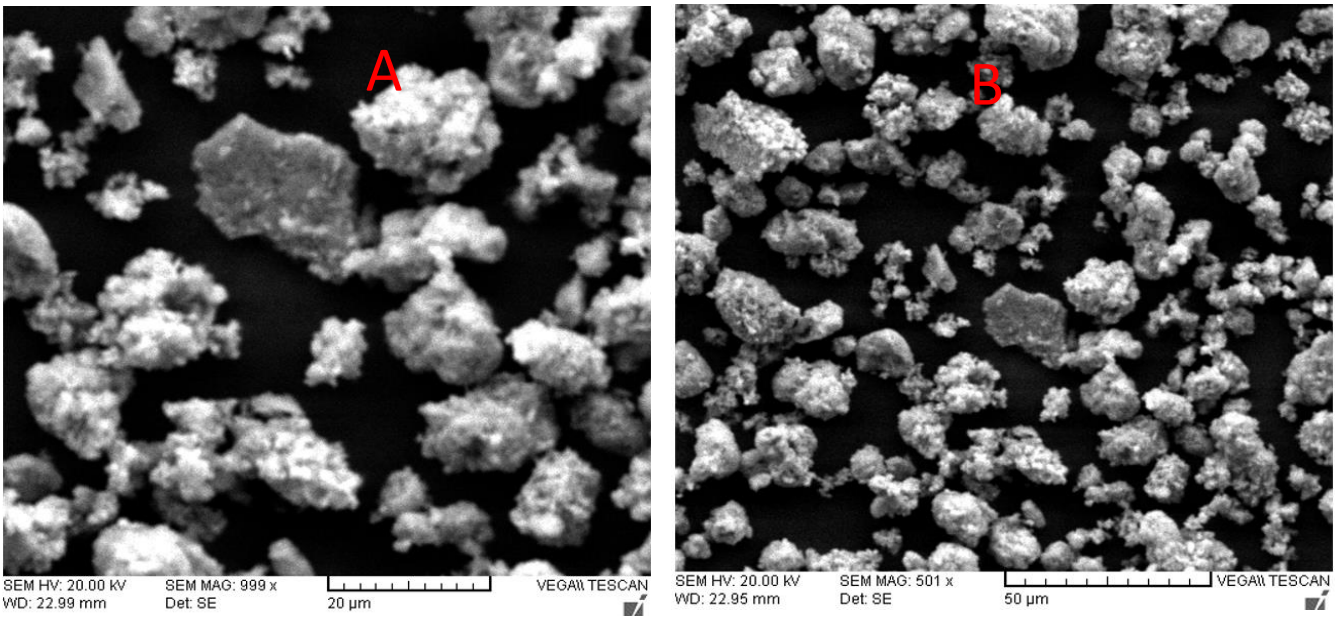


Figure 6.5 A and B: SEM micrograph of nano-silver functionalized magnetite (AgNPs@Fe3O4 nanocomposite) captured at different magnifications

6.8.3. EDX analysis

The elemental composition of AgNPs@Fe_3O_4 nanocomposite was determined through EDX analysis. The functionalized AgNPs@Fe_3O_4 showed a signal in Fe (34.61%) and Ag (12.48%) region at approximately 6.2 Kev and 3.0 Kev (Fig. 6.5). Other optical peaks were seen at S, O, N, and C with calculated weight percentages (W%) of 2.42%, 4.88%, 5.98%, and 9.55%, respectively. This result is similar to an already published study on the elemental composition of AgNPs @Fe_3O_4 (Ghasemi *et al.*, 2021; Nasiri *et al.*, 2022). The appearance of multiple peaks representing Fe confirmed that Fe_3O_4 NPs were dispersed on the surface of AgNPs.

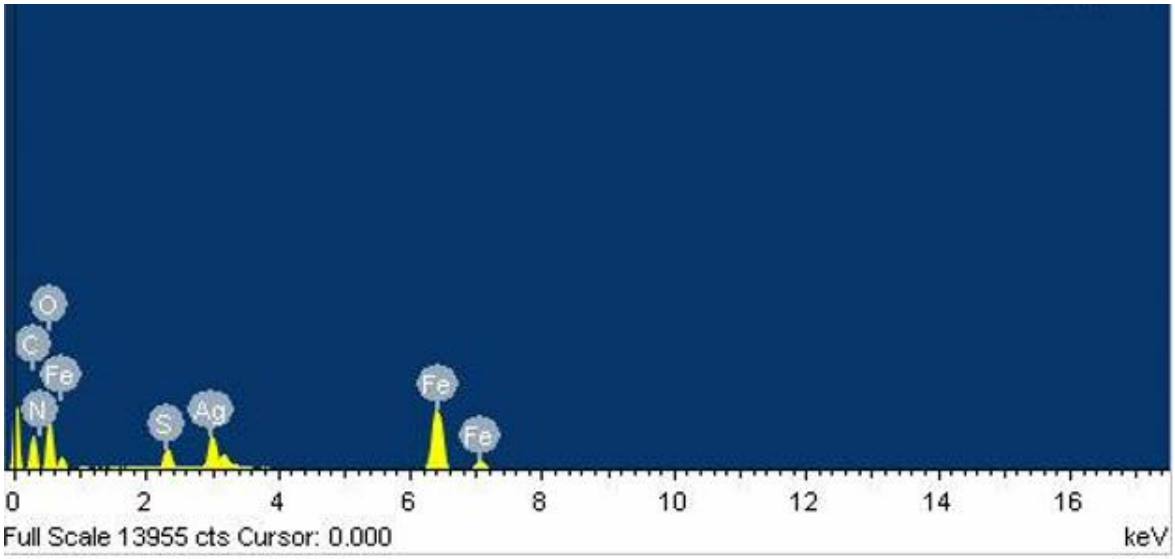


Figure 6.6: Energy-dispersive X-ray spectroscopy (EDX) of AgNPs@Fe3O4 nanocomposite showing the elemental composition of the nanocomposite

6.8.4. XRD analysis

X-ray diffraction techniques (XRD) examined the phase purity and crystallinity of functionalized AgNPs treated with Fe_3O_4 NPs to obtain AgNPs@Fe_3O_4 nanocomposite. The AgNPs, Fe_3O_4 NPs, and AgNPs@Fe_3O_4 nanoparticles were scanned in 5-80^0 but captured from 30-80^0. The characteristic peak in the diffraction pattern was detected, as illustrated in Fig. 6.6. The diffraction peaks seen at 2θ value of 33.1^0 {311}, 54.1^0 {511}, and 64.1^0 {440} agrees with the face-cantered cubic structure of Fe_3O_4 NPs (magnetite). Similarly, the diffraction peaks observed at 2θ value of 38.1^0 {111}, 44.2^0 {200}, 64.5^0 {220}, 77.5^0 {311} were also in agreement with AgNPs. These diffraction peaks correspond to JCPDS card no 00.001.1111 and 00-004-0783 for Fe_3O_4 NPs, and AgNPs. The diffraction peak of AgNPs@Fe_3O_4 observed at 2θ values of 64.1^0{440} and 64.5^0{220} confirmed the successful functionalization of AgNPs with Fe_3O_4 NPs. The diffraction peaks occurring on the same plane for AgNPs@Fe_3O_4 at 2θ value of 64.10{440} and Ag 64.5^0{220} for Fe and Ag, respectively, confirmed the successful functionalization of AgNPs with Fe_3O_4 NPs. The average crystallinity size of AgNPs@Fe_3O_4 is 19 nm, and it was calculated using the Scherrer equation (Equation 10):

$$D = \frac{K\lambda}{\beta cos\theta} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.10)$$

Where D = Average crystallite size (nm), K = Scherrer constant of 0.94 for spherical crystallites with cubic symmetry, ⅄ = X-ray wavelength, CuKα = 1.5406 Å, β = line broadening at FWHM in radians, and θ = Bragg's angle in degree, half of 2θ.

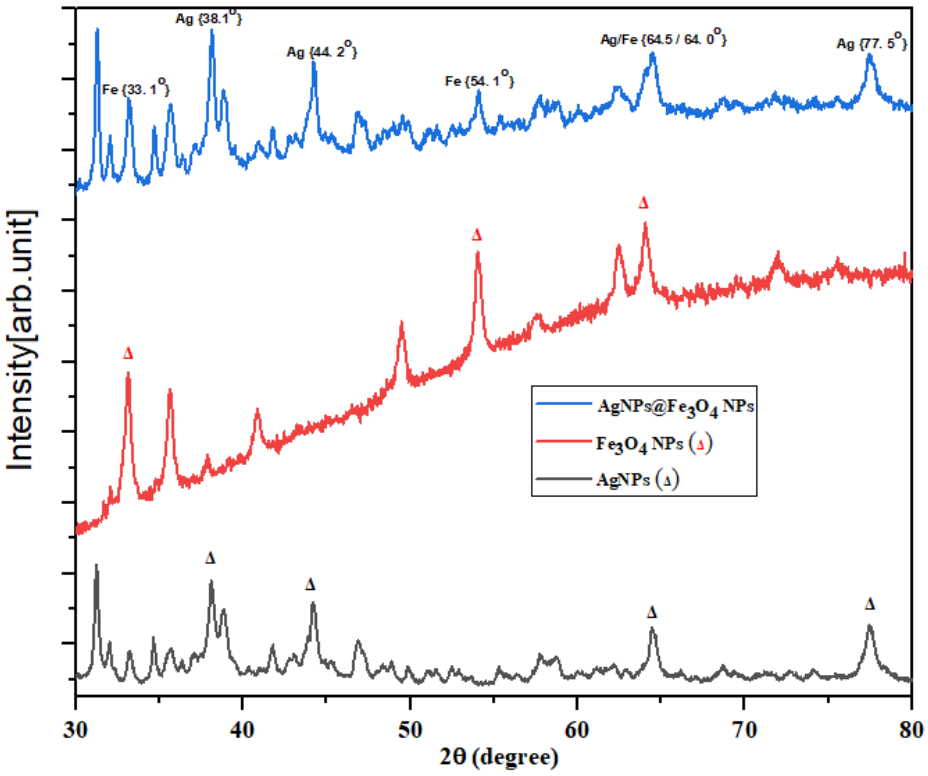


Figure 6.7. X-ray diffraction pattern of AgNPs@Fe3O4 nanocomposite

6.9. Operating parameters for the adsorption process

6.9.1. Effect of solution pH

The variation of solution pH plays a vital role in the DNA adsorption process because pH is a key parameter that contributes to high adsorption capacity due to its influence on the surface or binding site of the adsorbent and solution chemistry (Otalvaro *et al.*, 2019; Othman *et al.*, 2018). The effect of solution pH on DNA removal using functionalized AgNP@Fe_3O_4

nanocomposite was investigated by varying the initial pH of DNA solution in the range of 2-9 at a fixed DNA concentration (7.28 µg/mL), adsorbent dose of 20 mg for 360 mins. The experiment was conducted at room temperature. The addition of a few drops of either 0.1 M HCl or NaOH to adjust the pH of the solution can affect the adsorbent's surface, the binding site for DNA molecules through protonation and deprotonation transitions (Kamboh *et al.*, 2019). The effects of pH on removing bacteria DNA conveying ARGs are illustrated in Fig. 6.7. It was observed that DNA adsorption onto AgNP@Fe_3O_4 nanocomposite partially depends on solution pH because removal occurred even at neutral pH. High removal efficiencies were achieved when pH was high to moderate acidic (98 to 80%) and significantly dropped at basic pH (45 to 44%). The decrease in DNA basic pH solution is due to the abundance of the hydroxide ion (OH^-), adsorbent protonation of the hydroxyl group, causing a competition of hydrogen bonding on the adsorptive or binding site (He, Lin, *et al.*, 2016; Mousavi *et al.*, 2019). The high adsorption efficiency observed at acid pH (2-6) may be attributed to the cationic behaviour of the magnetic nanocomposite found at the surface of the adsorbent. At neutral pH (7.2), adsorption efficiency was 75.96%, which proves that AgNP@Fe_3O_4 nanocomposite can adsorb DNA through electrostatic interaction and without any pH influence. Consequently, to ensure no depurination of DNA resulting from the addition of acidic or basic pH, the solution pH 7.2 was adopted in all other adsorption studies.

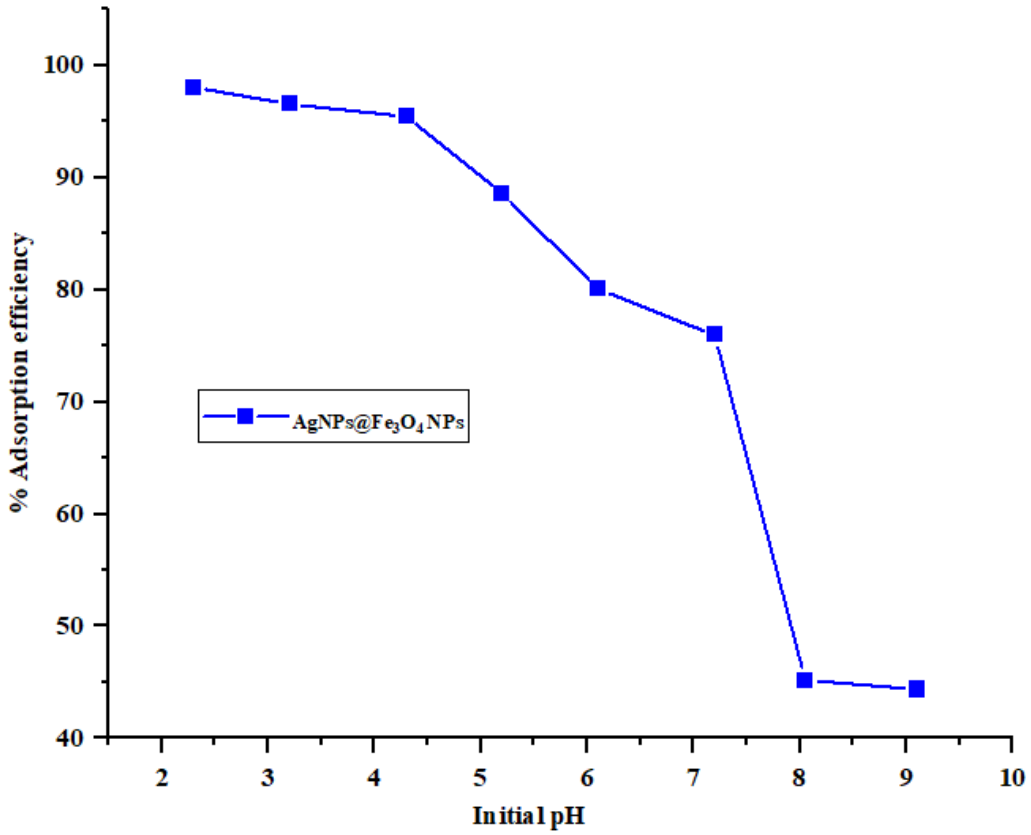


Figure 6.8. Effects of pH on DNA adsorption onto AgNPs@Fe3O4 nanocomposite

6.9.2. Effect of adsorbent doses

The adsorption of bacteria DNA conveying ARGs onto AgNPs@Fe_3O_4 nanocomposite was studied by decreasing and increasing adsorbent doses. The study measured 20 mg of AgNPs@Fe_3O_4 nanocomposite into a solution containing a DNA initial concentration of 7.28 µg/mL at fixed pH of 7.2 and agitated for 90 mins. Meanwhile, varying adsorbents during adsorption studies is an effective parameter that determines the adsorption capacity of a given adsorbent at a fixed initial concentration (Fu *et al.*, 2015; Zhou, Luo, *et al.*, 2018). The influence of varying AgNPs@Fe_3O_4 nanocomposite in the DNA solution is illustrated in Fig. 6.8, It was observed that adsorption capacity increased rapidly with an increase in adsorbent dose. The percentage adsorption capacity increased from 89.42 to 93.26 %, respectively, increasing adsorbent mass from 10 to 40 mg. The adsorbent was increased to 50 mg to reach a constant value, but no significant removal was observed. The highest adsorption capacity of

93.26% obtained at the increase in adsorbent mass of 40 mg provided a more adsorptive site that led to more DNA uptake by the adsorbent and this result agrees with already published literature (Xu *et al.*, 2019). Therefore, an adsorbent dose of 40 mg is an accepted economical dosage for DNA conveying ARGs removal from an aqueous solution.

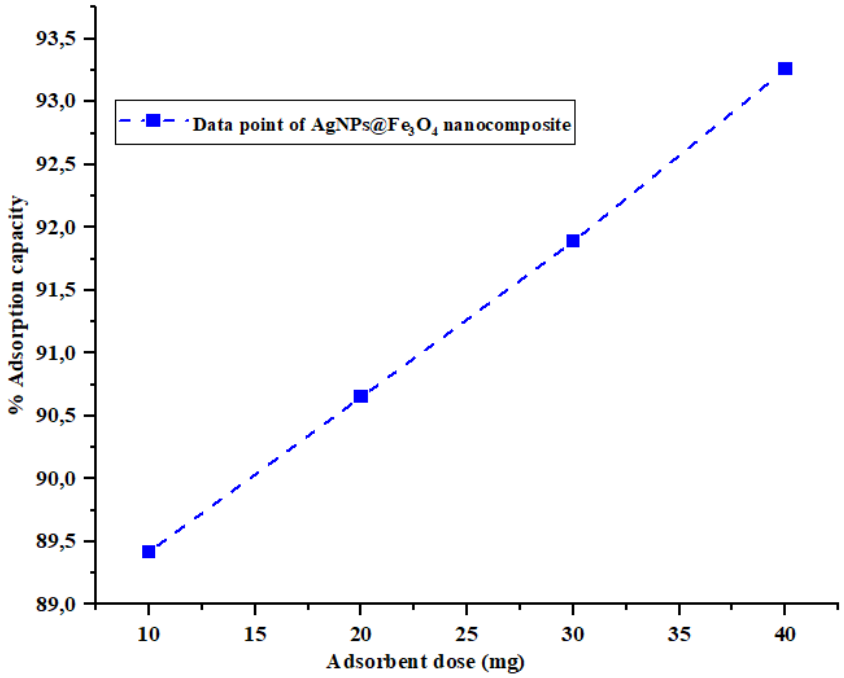


Figure 6.9: Effect of adsorbent dose on the adsorption of DNA onto AgNPs@Fe3O4 nanocomposite, adsorbate concentration = 7.28 µg/mL, reaction time = 60 mins; speed = 300 rpm, pH = 7.2, and at room temperature

6.9.3 Effect of contact time and adsorption kinetic

The adsorption of bacteria DNA conveying ARGs onto AgNPs@Fe_3O_4 nanocomposite as a function of contact time was conducted at different time intervals while maintaining the adsorbent dose of 40 mg, pH of 7.2, and at room temperature. It was observed that when the contact time was increased from 0-90 mins, percentage adsorption capacity at equilibrium time increased rapidly from 0-94.5%, 0-82.0%, and 0-79.6% at different initial concentrations of 3.64, 7.28, and 10.98 µg/mL respectively (Fig. 6.9A). The calculated equilibrium time (qt) was found to be 0-1.72, 0-2.99 and 0-4.37 for 3.64, 7.28 and 10.98 µg/mL. It was noticed that adsorption of DNA onto AgNPs@Fe_3O_4 nanocomposite was rapid, between 5-15 mins. This

observation may be due to the availability of a more active site at the initial stage (Ojemaye *et al.*, 2018). At an initial concentration of 3.64 µg/mL, the % adsorption capacity was high and reduced while the concentrations increased. This confirmed that the adsorption process in this study depends on the initial DNA concentration. Equilibration at 60 mins suggested that the adsorbent's removal affinity was more efficient than other adsorbents reported in this project (Chapters 4 and 5), and DNA adsorbed on the external surface of the nanocomposite without being diffused inside the nanocomposite materials. Beyond 90 mins, no significant DNA removal onto the nanocomposite was observed at different initial concentrations. Therefore, an equilibrium time of 90 mins was used as the optimum time for subsequent experimentation.

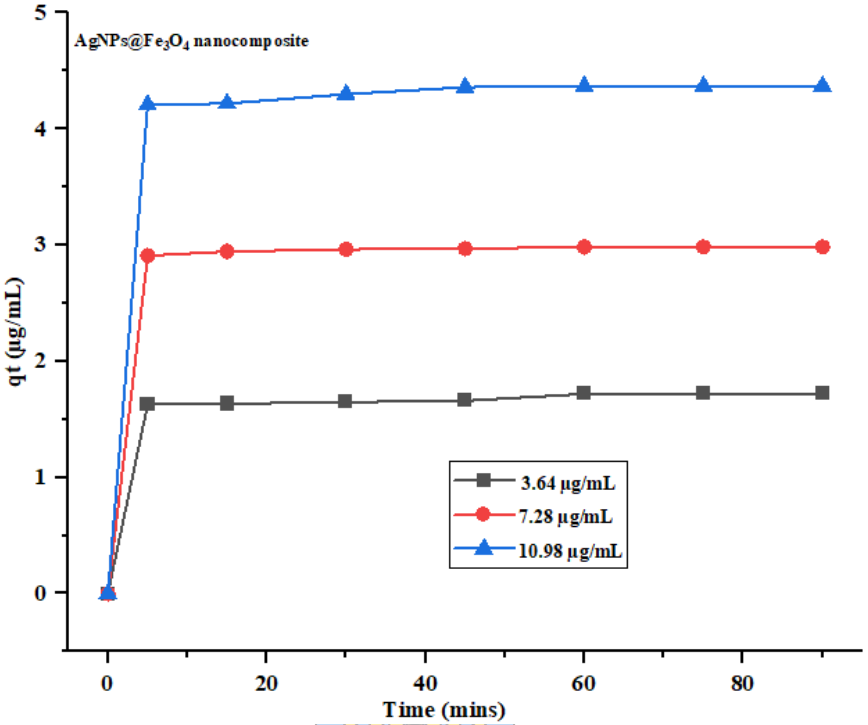
The adsorption kinetic is an imperative parameter for studying the contact time during the adsorption process. It was examined by the kinetic model such as Natarajan and Khalaf first order, Pseudo first order, pseudo second order, and Elovich models represented with equations 3,4,5, and 6 (Ansari *et al.*, 2020; Idris *et al.*, 2012; Yu *et al.*, 2017b). The kinetic results are illustrated in Fig. 9 (B&C), and the calculated parameters are presented in Table 6.2. According to Table 6.2 and Fig. 6.9 (B & C), the experimental data are best fitted to Pseudo second order and Elovich kinetic model with highest correlation coefficient of (R^2 = 0.9998) and (R^2 = 0.9999) than Natarajan & Khalaf first order (R^2 = 0.63415) and Pseudo first order (R^2 = 0.8784). The high correlation coefficient of these two models indicated that the adsorption of DNA onto AgNPs@Fe_3O_4 nanocomposite is based on the sharing of electrons between the DNA molecules and adsorbent, and the adsorption occurred on the heterogeneous surface of the nanocomposites. To further test the applicability of Pseudo second order and Elovich kinetic model, Chi-square (X^2) was employed to evaluate the experimental and calculated data difference. The model with a smaller X^2 indicated the best fit for the study. Among the two models, experimental data for Elovich kinetic model have an

excellent fit with a reduced Chi-square of (X^2 = 0.00001, 0.00006, and 0.00008) compared to pseudo-second order (X^2 = 0.2361, 0.0024 and 0.00463) indicating the rate of DNA adsorption onto AgNPs@Fe_3O_4 nanocomposite occurred on the heterogenous surface.

Table 6.2. Adsorption kinetic model and their parameter values for DNA uptake onto AgNPs@Fe_3O_4 nanocomposite

Kinetic model	Parameters	Initial concentration (µg/mL)		
		3.64	**7.28**	**10.98**
Natarajan and Khalaf First Order	K_1(^{1}/min)	0.00014	0.00091	0.00008
	R^2	0.63415	0.49836	0.53251
Pseudo First Order		**1.72**	**2.98**	**4.37**
	qe (^{Cal})(µg/mg)	0.0019	0.00003	0.0004
	K_1 (^{1}/min)	0.69	0.76	0.88
Pseudo Second Order	qe(^{exp})(µg/mg)	0.57064	0.73866	0.87841
	R^2	**1.72**	**2.98**	**4.37**
	qe (^{Cal})(µg/mg)	0.75924	0.56547	0.46095
	K_2 (g/µg min)	1.87	2.77	4.07
	qe(^{exp})(µg/mg)	0.58492	0.33533	0.22915
	h	0.9995	0.9998	0.9993
	R^2	0.2361	0.0024	0.0046
	X^2	0.9535	1.91123	1.07711
Elovich		3.33291	0.63142	1.0026
	α(mg^{-1}min^1)	0.9998	0.9999	0.9996
	β (µg/mg)	0.00006	0.00001	0.00008
	R^2	0.9998	0.9999	0.9996
	X^2	0.00006	0.00001	0.00008

(A)



(B&C)

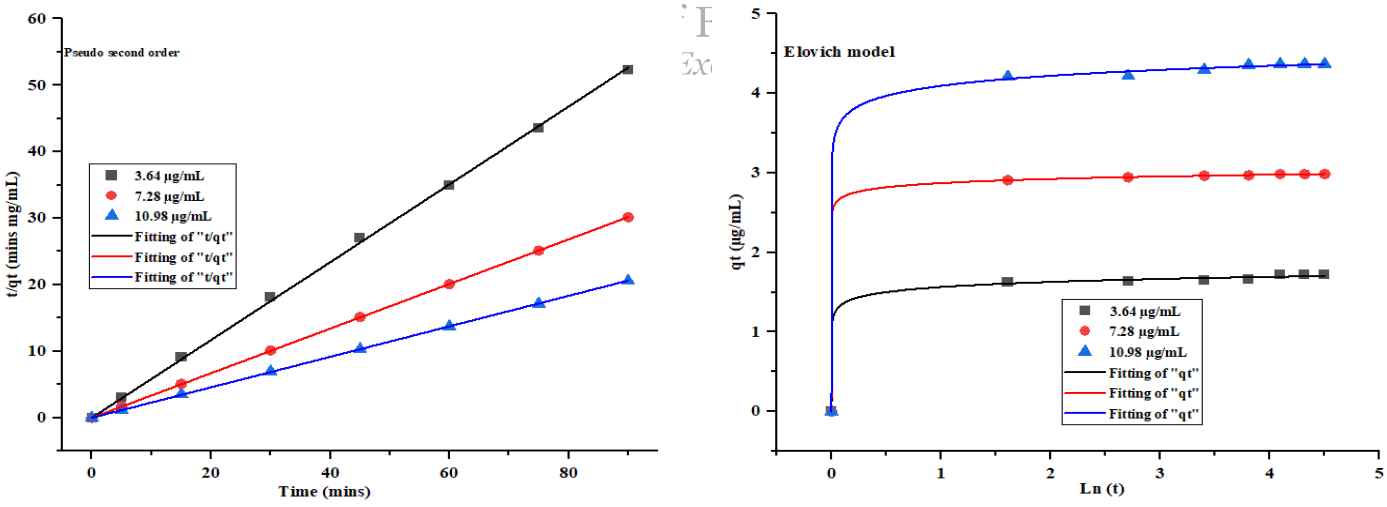


Figure 6.10 (A) Influence of contact time on DNA adsorption onto AgNPs@Fe_3O_4 nanocomposite and kinetic models of (B&C) pseudo second order and Elovich kinetic model

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6.9.4. Effect of initial concentration and adsorption isotherm

A vital parameter that affects the adsorption process is the initial concentration. This study investigated the effect of initial concentration by varying the DNA concentrations (3.64, 7.28, and 10.98 µg/mL) onto AgNPs@Fe_3O_4 nanocomposite at a constant time of 90 mins, pH of 7.2, adsorbent dose of 40 mg and at room temperature. From the given plot (Fig. 6.11 A), it was observed that adsorption capacity increased rapidly at DNA initial concentration of 3.64 µg/mL with the highest percentage adsorption capacity of 94.5 %. This is attributed availability of vacant sites on the sorbent surface. At the increase in DNA concentration to 7.28 (82.0%) and 10.98 µg/mL (79.5%), a slight decrease of % adsorption capacity at an equilibrium time of 60 mins was observed, indicating the saturation of the adsorptive site on the adsorbent surface. However, as initial DNA concentration increases, the mass transfer on the active site of adsorbents becomes smaller, leading to a decrease in DNA % adsorption capacity (Shen *et al.*, 2015). Therefore, to provide information on the relationship between the adsorbed DNA molecules and equilibrium residual concentrations in the solution, a validated mathematical equation of the Langmuir and Freundlich model was employed in the experimental data. Besides, the adsorption isotherm described the mechanisms of the adsorption process and the maximum adsorption capacity of the adsorbent (Kamboh *et al.*, 2019).

In addition, Langmuir is a non-linear equation describing the adsorbate's monolayer adsorption onto a homogenous adsorbent surface. i.e., it provides information on the optimum capacity and the surface properties of the adsorbent (Fu *et al.*, 2015; Guo and Wang, 2019). Freundlich described the heterogeneous surface nature of the adsorbent and multilayer type of adsorption best explained the model (Batool *et al.*, 2018). In this study, the result of fitted parameters is presented in Table. 6.3 and the plots are shown in Fig. 6.10 B. The isotherm model applied to the experimental data for DNA adsorbed best fitted in the Freundlich model

with the highest correlation coefficient (R^2 = 0.9517) than Langmuir (R^2 = 0.8462). The high coefficient exhibited by Freundlich indicated the adsorption of onto AgNPs@Fe_3O_4 nanocomposite occurred on a heterogeneous surface, and the reaction is governed by Van da Waals forces or weak electrostatic forces attracting the DNA molecules in a neutral solution onto the cation available at the sorbent surface (Mangla *et al.*, 2022). To further test the applicability of the Freundlich model to the experimental data, the value of Freundlich adsorption capacity (K_F) and adsorption intensity (n) were determined. K_F and n are constants obtained from calculating intercept and slope, and adsorption is favourable when K_F is in the range of 1-20 and n is above 1 (Hamad *et al.*, 2011; Pandey *et al.*, 2010). From the result, the value of K_F is 2.988, indicating the favourability of the model, and the value of n is 2.4569 indicating the favourability of the model.

Table 6.3: Calculated isotherm parameters for the bacteria DNA adsorption onto AgNPs@Fe_3O_4 nanocomposite

Isotherm model	**Parameters**				
Freundlich	K_F (µg/g)	n	X^2	R^2	
	2.9889	2.4569	0.1996	0.94317	
Langmuir	K_L (µg/g)	qm (µg/mL)	X^2	R_L	R^2
	2.3518	4.6977	0.54641	0.1045	0.8462

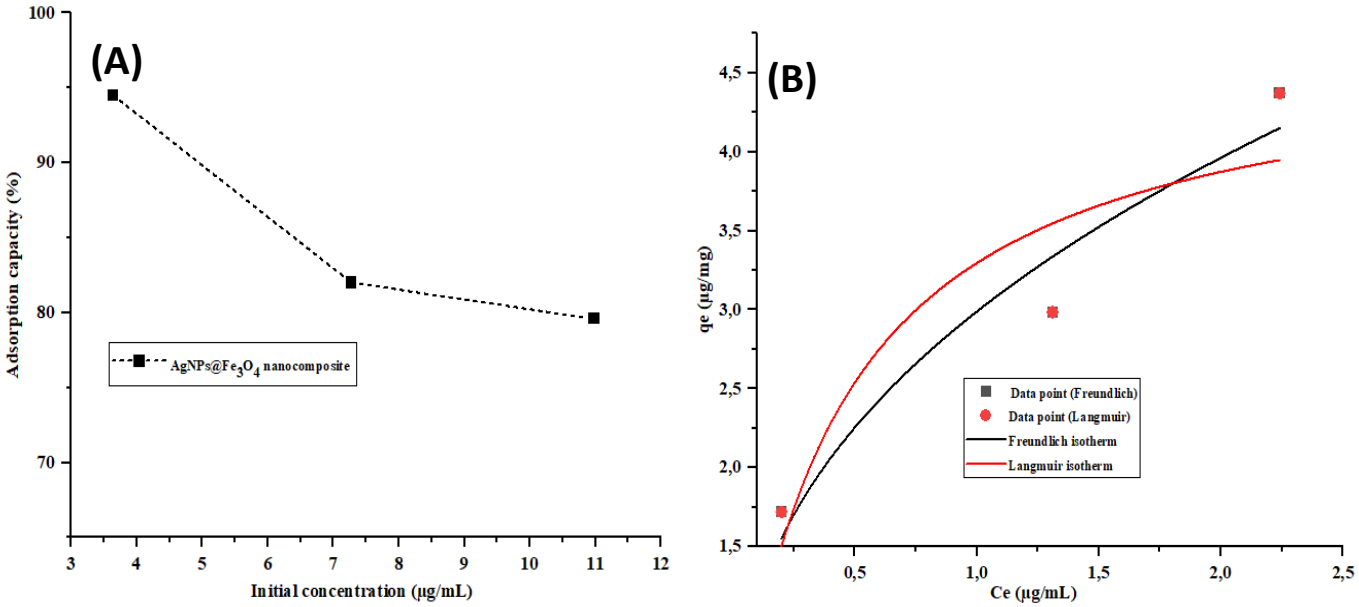

Figure 6.11: (A) Effect of initial concentration on DNA adsorption onto AgNPs@Fe_3O_4 nanocomposite and isotherm models of (B) Freundlich and Langmuir model

6.10. Adsorption of bacteria DNA from Ndevana water sample

Water sample was collected from the Ndevana River and was originally quantified to have bacteria DNA conveying ARGs in December 2021. Ndevana River is situated East of King William's town under Metropolitan Municipality in Eastern Cape, Province of South Africa. The efficiency and capability of the functionalized nanocomposite material (AgNPs@Fe_3O_4 nanocomposite) were tested. The water sample was spiked to obtain the initial concentrations of 1.48, 2.14, and 3.35 µg/mL. Before the commencement of the adsorption study, the physicochemical parameters were investigated, and the result is highlighted in Table 6.4. It was observed that the water sample was very toxic with high total dissolved solids and may be dangerous to aquatic lives situated in the river. The prepared water sample with different initial concentrations was subjected to a batch adsorption study. The optimum parameters were applied, such as pH of 7.2, adsorbent dose of 40 mg, and contact time of 90 minutes and at room temperature. After equilibration, the maximum percentage adsorption capacity was quantified at different initial concentrations. The result obtained showed that AgNPs@Fe_3O_4

nanocomposite was very effective in adsorbing DNA from natural waters. The % adsorption efficiency obtained were 96.76%, 95.93% and 93.13% for the DNA initial concentration of 1.48, 2.14 and 3.35 µg/mL respectively (Fig. 6.12). It was noticed that the % removal or adsorption efficiency on the real water samples was high compared to the simulated water. This may be that the simulated water's initial concentrations are higher than the real water sample. Also, it may be that water samples contain no anionic interference.

Table 6.4: Physicochemical parameter of water sample from Ndevana Buffalo River

pH	Temperature	Total dissolved solids	Dissolved oxygen	Turbidity	Initial DNA concentration in river water (µg/mL)
7.02	35.8	32.55	14.23	18.82	0.612

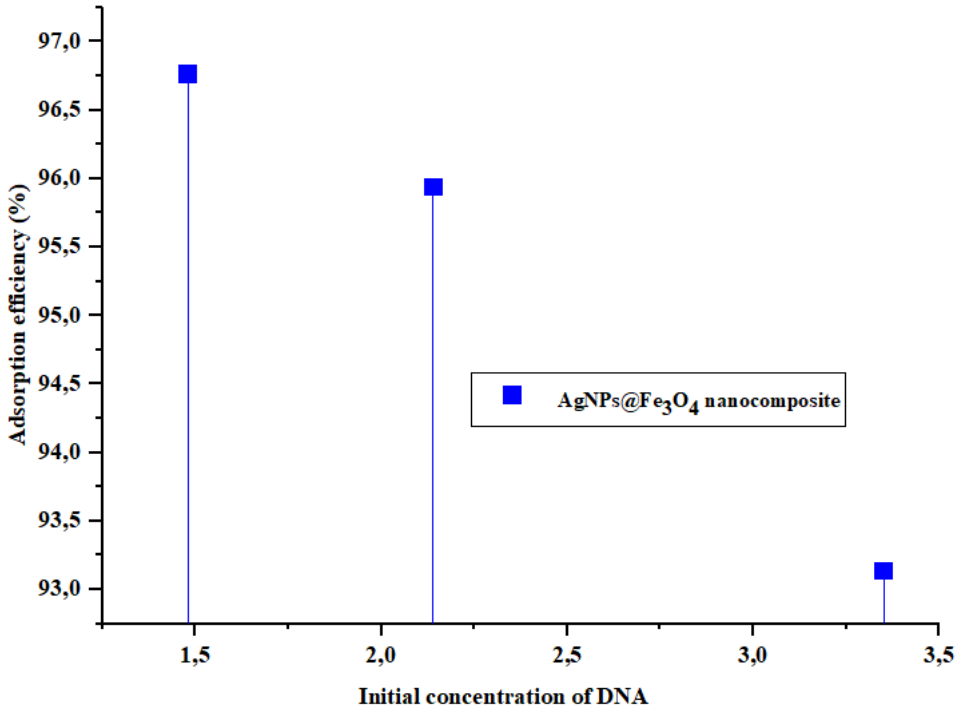

Figure 6.12: The removal of bacteria DNA conveying ARGs from Ndevana Buffalo river

6.11. Conclusion

In this study, the synthesis of AgNPs-Fe_3O_4 NPs (AgNPs@Fe_3O_4 nanocomposite) and its application to remove bacterial DNA conveying ARGs from simulated water and real water samples were successfully demonstrated. High adsorption efficiency (above 90% at pH of

7.2), the rapidness of adsorption within a short time interval, and are the most advantageous properties exhibited by AgNPs@Fe_3O_4 nanocomposite. The equilibrium experimental data were best fitted to Elovich kinetic (R^2 = 0.9999) and Freundlich isotherm (R^2 = 0.94317) compared to other models used. The Chi-square (X^2), an effective tool used to analyse the kinetic models aside from the R^2, was employed. It was observed that Elovich (X^2 = 0.00001) and Freundlich (X^2 = 0.1996) models had a reduced Chi-square. The value of K_F in Freundlich further confirmed that AgNPs@Fe_3O_4 nanocomposite has high affinity for DNA removal from river water and other water matrices. Therefore, AgNPs adsorbents with magnetite may be a promising material for achieving wholesome removal of bacteria DNA conveying ARGs from contaminated water.

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Chapter 7

Mesoporous silica nanoparticle as a superior adsorbent with high capacity: Synthesis, surface functionalization, and the removal of bacteria DNA conveying antibiotic resistance genes from Uitenhage wastewater treatment plant

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Abstract

Mesoporous silica nanoparticles functionalized with 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine (MSNPs@TPPY) were introduced as a highly efficient adsorbent for the removal of bacteria DNA conveying antibiotic resistance genes from aqueous solution and wastewater. MSNPs@TPPY was subjected to characterization using Fourier Transform Spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDX), and point of zero charges (PZC). The adsorption study was conducted in a batch reactor step. Different operating parameters such as the effect of solution Ph, adsorbent dose, contact time, and initial DNA concentration were optimized. The equilibration time of 90 mins was recorded, displaying a maximum adsorption capacity of 90 and 97.72 % for simulated water and actual wastewater sample. The experimental data fitted to adsorption models showed that the adsorption rate and equilibrium obeyed Elovich kinetic model with a high correlation coefficient and reduced Chi-square (R^2 = 0.9949 to 0.9986 and X^2 = 0.0003-0.0014), and Langmuir isotherm (R^2 = 0.9887 and X^2 = 0.0032). Finally, the adsorbent proved its effectiveness and applicability for DNA removal with the advantages of high adsorption capacity, ease of synthesis, and functionalization steps.

7.1 Introduction

Lately, wastewater treatment plants (WWTPs) have been recognized as a prime hotspot for the proliferation and spread of bacteria DNA conveying antibiotic resistance genes (ARGs). Reports have shown that bacteria DNA conveying ARG contamination is rapidly increasing due to extensive antibiotic usage worldwide (Ezeuko *et al.*, 2021c; Kraemer *et al.*, 2019).

Receiving untreated effluents from households, hospitals, and agricultural farm facilitates the evolution and dissemination of antibiotic bacteria DNA harbouring ARGs in the WWTPs (Rodriguez-Mozaz *et al.*, 2015b; Singh, Singh, *et al.*, 2019). According to World Health Organization (WHO), bacteria DNA conveying ARGs poses a severe health risk to the public, and its detection above the maximum limit in WWTPs and other water environments increases the rate of morbidity and mortality recorded yearly (Brealey *et al.*, 2021; Some *et al.*, 2021). The widespread dissemination of these contaminants to other water matrices is linked to inappropriate treatment options, and the consequences to global public health are increasing at an alarming rate. Therefore, adequate treatment strategies are urgently needed to stop the rapid increase of these emerging contaminants.

Several treatment options have been reported for removing bacterial DNA conveying ARGs in WWTPs (Foroughi *et al.*, 2022a; Karaolia *et al.*, 2018; Zhang, Zhuang, *et al.*, 2016). Such treatment options are membrane bioreactor, advanced oxidation, adsorption, and conventional treatment processes. Adsorption processes seem to be very efficient due to their low cost, simplicity sludge free, low energy consumption, flexibility, and ability to regenerate and recover an adsorbent after the treatment process (Kubra *et al.*, 2021; Shrestha *et al.*, 2021). Inorganic-based nanomaterials as adsorbents are highly considered due to their hydrophobic nature, surface functionalization/modification, easy synthesis, ability to remove genetic components from heterotrophic bacteria, and high adsorption capacity (Ezeuko *et al.*, 2021a; Ojemaye *et al.*, 2017; Ray *et al.*, 2013). Mesoporous silica nanoparticle (MSNPs), an inorganic nanomaterial, is interestingly considered for the removal due to their unique features such as ordered mesoporous structure, large specific surface area, tuneable pore size, and morphology which can be controlled during the synthesis (Huang *et al.*, 2020; Rizzi *et al.*, 2021). In addition, the surface of MSNPs possesses a negative charge with the ability of

ion exchange (Huang *et al.*, 2020; Lee, Lo, *et al.*, 2008). The negative surface can either be removed during synthesis or balanced by adding cations.

Currently, the adsorption of bacteria DNA onto the original surface of MSNPs through electrostatic interaction would be complex because DNA molecules possess a strong negative charge in their backbone. To achieve this, surface functionalization of MSNPs would assist in the adsorption of DNA onto the mesopore. Besides, surface functionalization means adding chemical properties such as functional group, electronic properties, etc., onto the surface of nanomaterials without altering their core components (Ojemaye *et al.*, 2017; Zhu *et al.*, 2019). It provides uniform surfaces and enhances the efficacy of integrated moieties and nanomaterials (Wang *et al.*, 2022). In the adsorption process, surface functionalization improves the stability and adsorption capacity of the adsorbents. Functionalization of surface materials can be successfully achieved via direct (in situ and condensation) and indirect methods (grafting) (Mahajan *et al.*, 2021). Many authors have reported high adsorption capacities on the adsorption of DNA onto the surface functionalized MSNPs with either acidic or basic solution pH at different pore sizes. Studies conducted by the various research group on the successful DNA adsorption onto MSNPs functionalized surfaces at different solution pH and pore sizes, obtaining different adsorption capacities, are presented in Table 7.1.

Table 7.1: Comparison of adsorption capacities of some surface functionalized MSNPs as adsorbents on the adsorption of DNA.

Type of functionalized MSNPs	Pore size (nm)	DNA	Qe (mg/mL)	pH	Source
MSNPs@Mg^{2+} MSNPs@Ca^{2+}	5.7 5.7	Calf thymus DNA	5.71 2.17	8.0	(Solberg and Landry, 2006)
MSNPS@APTES	5.7	Escherichia coli plasmid DNA	2.22	2.0	(Gao *et al.*, 2009)
APTES@MSNPs	2.6	Plasmid DNA from a mammalian host	15.7	3.5	(Yang *et al.*, 2012)
M-MSNPs	2.7	Salmon DNA	12.2	5.2	(Li *et al.*, 2012)
MNSPs@Au^{2+}	2.5	ssDNA	1.87	7.6	(He *et al.*, 2020)

MSNPs@Ag^{+}	2.8		1.99	3.0	
MSNPs@PEI	8.7	Calf thymus DNA	17.3	8.0	(Zolghadrnasab *et al.*, 2021)

Mg^{2+} = magnesium cation; Ca^{2+} = calcium cation; APTMS = 3-(Aminopropyl) triethoxysilane; M = magnetic; Au^{2+} = gold cation; Ag^{+} = silver cation; PEI = poly-ethyleneimine; ssDNA = single stranded deoxyribonucleic acid.

Recently, a broad range of ligands based on the concept of coordination chemistry has been used extensively for surface functionalization/modification of nanoparticles. Such ligands are monodentate, bidentate, tridentate, and tetradentate ligands. Terpyridine base complexes such as 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine, a tridentate ligand, have attracted much interest due to their high binding affinity, stabilization (i.e., non-covalent linkage via pyridine moieties) and functionalization (i.e., covalent linkage via lateral substitution) of nanostructure (Winter *et al.*, 2011, 2012). In this study, MSNPs were prepared by chemical etching techniques. Surface functionalization was conducted using a synthesized 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine via esterification method and denoted as MSNPs@Tppy. The structural and textural properties of the synthesized MSNPs, terpyridine ligand, and MSNPs@Tppy were characterized by FTIR, SEM, EDX, and PZC. The MSNPs@Tppy was used as a high-capacity adsorbent to remove bacteria DNA conveying ARGs from an aqueous solution and actual water sample from Uitenhage WWTPs. The influence of operating parameters (solution pH, time-concentration profile, adsorbent dosage, and salt effect) on the DNA adsorption onto MSNPs@Tppy was studied. The possible adsorption mechanism and design were investigated using kinetic and isotherm models.

7.2. Materials and methods

7.2.1. Materials

Nitric acid (HNO_3), 3-aminopropyltriethoxysilane (APTES), ethanol, dry dimethylformamide (DMF), Succinic anhydride, dicyclohexyl carbodiimide (DCC), 4-dimethyl aminopyridine (DMAP), Tetraethyl orthosilicate (TEOS), Urea, guanidine HCl, 4-hydroxybenzaldehyde, 2-acetylpyridine, sodium hydroxide (NaOH), aqueous ammonia (NH4), sodium chloride

(NaCl), and sodium dodecyl sulphate (SDS) were purchased from Sigma Aldrich, South Africa. Nuclease-free water and DNA kit were purchased from Thermo Fischer. All the chemicals used in this study were of analytical grade and used as purchased.

7.2.2. Instrumentation

The Fourier transform infrared spectroscope (FTIR) (Perkin-Elmer Universal ATR 100) was determined by the surface functional group in 4000 to 350 cm^{-1}. Determination of surface morphology and elemental composition of the adsorbents were conducted using the Scanning electron microscope (SEM) and energy dispersive x-ray spectroscope (EDX) (JOEL JSM-6390LVSEM). Average crystallinity and phase compositions were determined with X-ray diffraction (XRD) (Bruker D8). During the adsorption experiment, residual bacteria DNA conveying ARGs was quantified using a dsDNA assay kit (Q32850) specific to the Qubit 1.0 Fluorometer (Thermofischer).

7.3. Mesoporous silica nanoparticles (MSNPs) and 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine synthesis

Mesoporous silica nanoparticles (MSNPs) were prepared according to the method previously discussed in Chapter 5 (5.3) (Salehtash *et al.*, 2018). 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine, a ligand, was synthesized according to the method described in published literature (Ojemaye and Okoh, 2019) with slight modifications. 2.423 mg, 0.02 mol/L of 2-acetylpyridine was added to a solution containing 15 mL of ethanol and water mixed in the ratio of 2:1 (v/v) and 0.228 mg, 0.01mol/L of 4-hydroxybenzaldehyde. The mixture was allowed to stir vigorously for 20 mins. Afterward, 1.458mg of 0.25 mol/L of NaOH and 30 mL of aqueous ammonia (NH_3) were added and stirred vigorously at room temperature for 24 h (Fig. 7.1). The cream precipitate obtained was filtered, washed four (4) times with 10 mL of ethanol and water, and dry overnight at room temperature. The off-white powder product

obtained was denoted as OH-TPPY. The reaction yields of 64.2 % were calculated using the Equation (7.1):

$$\textbf{Reaction yield}(\%) = \frac{\textbf{Actual yield}}{\textbf{Therotical yield}} \times 100 \quad \text{..... (7.1)}$$

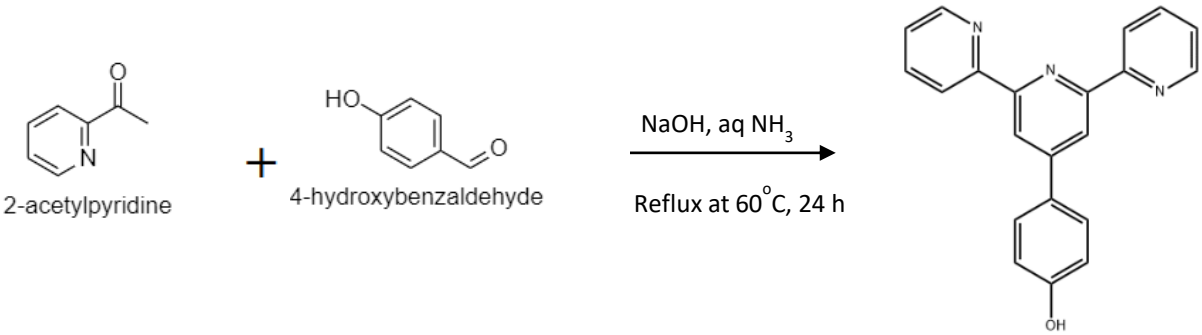


Figure 7.1: Synthesis of 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine

In this study, the process of MSNPs surface functionalization was achieved by three practical steps and according to the method described in the literature (Ojemaye and Okoh, 2019). The proposed chemical structure is illustrated in Fig. 7.2 and 7.3.

7.3.1. Amine functionalization

Mesoporous silica nanoparticles (MSNPs) surface was converted to amine-functionalized mesoporous silica nanoparticles (MSNPs-NH_2) by dissolving 4 g of MSNPs in 40 mL of absolute ethanol. The solution was ultrasonicated for 40 mins to homogenize the mixture. Then, 5 mL of 3-aminopropyltriethoxysilane (APTES) was added slowly to the already stirring mixture and allowed to reflux at 40°C for 24 h. The residue was centrifuged, washed with distilled water, and oven-dried at 55°C. The particle obtained was marked as MSNPs-NH_2.

7.3.2. Carboxylic functionalization

The amine-functionalized mesoporous silica nanoparticle (MSNPs-NH_2) surface was converted to carboxylic functionalized by dispersing 9.22 g of MSNPs-NH_2 in dry DMF into the solution of succinic anhydride already dissolved in DMF. The solution was allowed to stir vigorously for 48 h at 15 ^oC. The off-white crude obtained was washed with ethanol and dried overnight, and the products were labelled as MSNPs-COOH.

7.3.3. Ligand functionalization

In the esterification process, the MSNPs-COOH were converted to 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine functionalized mesoporous silica nanoparticles. It was achieved by replacing the carboxylic group on the surface of MSNPs-COOH with a terpyridine ligand. 4.7 g of MSNPs-COOH was dissolved in 10 mL of dry DMF. 0.1 g of DCC was added to accelerate the reaction rate. The mixture was stirred continuously in the dark for 24 h. Then, 1.988g of OH-TPPY dissolved in 10 ml of dry DMF was added drop by drop in the presence of DMAP. The yellowish suspension was stirred for another 24 h, separated by centrifuged, washed severally with distilled water, and allowed to oven-dry overnight at 55 ^oC. The final product was denoted as MSNPs@TPPY.

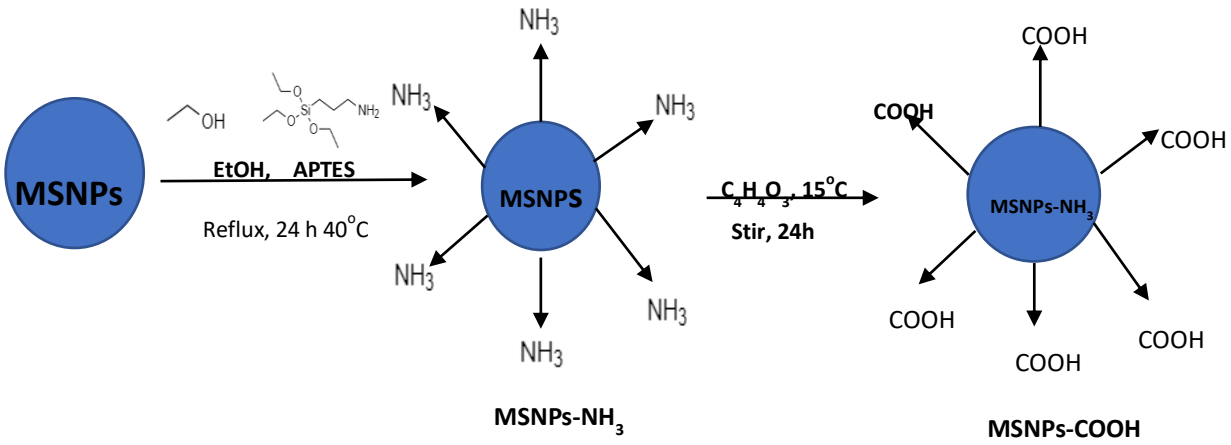


Figure 7.2: Surface functionalization procedure of MSNPs from amine (MSNPs-NH3) and carboxylic (MSNPs-COOH) acid

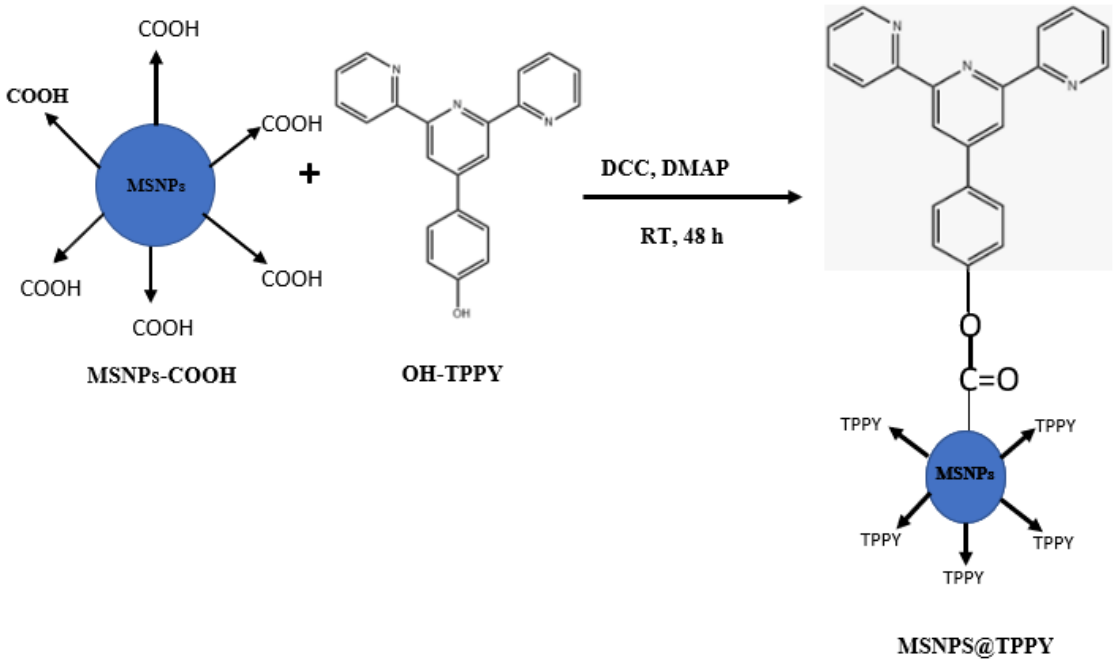


Figure 7.3: Synthesis procedure on the conversion of carboxylic acid functionalized mesoporous silica nanoparticles (MSNPs-COOH) to 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine functionalized MSNPs surface

7.4. Adsorbent characterization

The functional group identification, elemental compositions, morphologies, crystallinity, and phase compositions of the adsorbents were determined using Fourier transform infrared spectroscope (FTIR) (Perkin-Elmer Universal ATR 100), a scanning electron microscope (SEM) coupled with an energy dispersive x-ray spectroscope (EDX) (JOEL JSM-6390LVSEM) respectively. Adsorbent net charge (PZC) was determined using batch reaction techniques.

7.5. Bacteria DNA extraction and molecular identification

Antibiotic-resistant *Enterococcus faecium* and *Vibrio parahaemolyticus* used in this study were obtained from our laboratory archives isolated from the beach water located in East London and wastewater treatment plant around the University of Fort Hare, Alice, province of Eastern Cape, South Africa. Antibiotic susceptibility testing (AST) and identification of antibiotic resistance genes were conducted according to a method previously discussed in Chapter 6 (section 2.3). The genomic DNA was stored at -20 ºC for subsequent analysis. The PCR primers and conditions used during the extraction are previously listed in chapter 6 (Table 1). The DNA purity and concentration measuring absorbance at 260 nm, 280 nm, and 320 nm using Multiparameter HACH DR 6000 Ultraviolet Spectroscopy were in the range of 1.7 and 1.9, confirming the absence of inference compounds.

7.6. Adsorption studies

The adsorption experiment of bacteria DNA conveying ARGs was prepared by the batch adsorption process. First, a batch experiment containing 10 mL of bacteria DNA from the stock solution was prepared. Their pH values were adjusted by adding 0.1mol/L hydrochloric acid (HCl) and sodium hydroxide (NaOH). The bacteria DNA adsorption experiment was done by adding specific amounts of adsorbent (20 mg) at room temperature. The solution was placed on a KS260 control orbital shaker at a speed of 300 rpm. A portion was collected at specific intervals to determine optimum pH, contact time, concentration, and adsorbent dosage. Then, kinetic and isotherm models were investigated. All samples were centrifuged, and the residual DNA in the supernatant was measured using a dsDNA assay kit (Q32850) Qubit 1.0 Fluorometer (Thermofischer). The equilibrium adsorption capacity was calculated using Equations 7.2 and 7.3.

$$qt\ or\ qe = \frac{C_i(\mu gmL) - Cf\ or\ Ct(\mu gmL)}{m\ (mg)} \times V(mL) \quad \text{..... (7.2)}$$

$$\%R = \frac{C_i(\mu gmL) - Cf \text{ or } Ct(\mu gmL)}{C_i(\mu gmL)} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7.3)$$

Where V = volume of adsorbate solution (mL), m = adsorbent mass (mg), C_i (µg/mL), and C_f or C_t (µg/mL) are the initial and final DNA concentrations after the time (t) and equilibrium respectively, and % R is the removal efficiency, and qe or qt are adsorption capacity recorded at the time (t) and equilibrium.

The kinetic models such as Natarajan and Khalaf first order (Idris *et al.*, 2012), Pseudo first order (Aarab *et al.*, 2020; Yusuff *et al.*, 2019), Pseudo second-order (Al Bsoul *et al.*, 2021; Yusuff *et al.*, 2019) and Elovich models (Pezoti *et al.*, 2016; Wu *et al.*, 2009)were fitted into the experimental data to determine the adsorption design or mechanism. The kinetic models, equations, and parameters include:

- Natarajan and Khalaf first order = $\boldsymbol{log}(\frac{Ci}{Ct}) = \frac{K1}{2.303\ t}$…… ……(7.4)
- $Pseudo\ First\ order = \log(qe - qt) = \frac{K_1}{2.303t} \ldots\ldots\ldots\ldots (7.5)$
- $Pseudo\ second\ order\ \frac{t}{qt} = \frac{1}{K_2 qe^2} + \frac{1}{qe}t \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7.6)$
- $Elovich\ model\ (qt) = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \ldots\ldots\ldots\ldots (7.7)$

Ci and ***Ct*** (µg/mL**)** are the initial concentration, and the final amount of DNA adsorbed at contact time ***t*** (mins), respectively. $\mathbf{K_1}$ (min $^{-1}$) and $\mathbf{K_2}$ (g/ (µg mins)) are the adsorption rate constant for the Natarajan and Khalaf first order, Pseudo first order and second-order kinetic, **qe** and **qt = (**µg/mg) is the amount of DNA adsorbed at equilibrium and at time interval ***t***.

To determine the maximum equilibrium adsorption capacity and the underlying mechanism

for the adsorption of bacteria DNA onto the proposed adsorbent, isotherm models such as Freundlich (Aarab *et al.*, 2020; Yusuff *et al.*, 2019), Langmuir (Dawodu *et al.*, 2019; Motahari *et al.*, 2015) and Sips (Siqueira *et al.*, 2020; Yang *et al.*, 2021) model were fitted into the experimental data. The adsorption isotherm, equations, and parameters used in this study are:

- $Freundlich\ isotherm\ (qe) = KFCe^{1/n}$ ..... (7.8)
- $Langmuir\ isotherm\ (qe) = \frac{KLqmCe}{1 + KLCe}$ ..... (7.9)
- $Sips\ (qe) = \frac{qm\ (KLCe)\frac{1}{n}}{1 + (KLCe)\frac{1}{n}}$ ..... (7.10)

Where ***qe*** (µg/g) and ***qm*** (µg/g) are the amount of DNA adsorbed per unit of adsorbent at equilibrium and theoretical maximum adsorption capacity, respectively. K_f (µg/g) and K_L (mL/µg) are the Freundlich, and Langmuir isotherm constant related to the adsorption energy, **Ce**: equilibrium concentration, and ***n*** = empirical constant, showing that adsorption occurred on heterogeneous surfaces through a multilayer adsorption mechanism.

7.7. Adsorption of bacteria DNA conveying ARGs from an actual wastewater sample

An actual wastewater sample was collected from WWTPs located at Uitenhage, Port Elizabeth, Eastern Cape Province of South Africa, around October 2021. The sample was initially quantified to have 0.447 µg/mL of bacteria DNA conveying ARGs. Batch experiments were prepared by spiking the actual water sample in 1:1, 1:2, and 1:3 with DNA from the stock solution to obtain new initial concentrations of 1.45. 2.47 and 3.46 µg/mL. Physicochemical parameters were conducted using a Multiparameter device (HANNA H19829) on the sample site. The water was transported to the laboratory in an ice pack and

stored in the refrigerator for the study. Optimum parameters such as adsorbent dose of 40 mg, pH of 7.01, and contact time of 90 mins were employed on the different initial DNA concentrations (1.45. 2.47, and 3.46 µg/mL). The experiment was conducted at room temperature. All the experimental data were obtained in triplicate, and the average result was used for data analysis.

7.8. Data analysis

The data obtained from this study were plotted and analysed using OriginPro Graphing and Analysis 2021 (v.9.8.0 200), Microsoft Excel 2019, and Image J software.

7.9. Result and discussion

7.9.1. Characterization

7.9.1.1. FTIR analysis

The successful synthesis of TPPY-OH is confirmed by FTIR spectra (Fig. 7.4). Nine (9) functional groups were identified. They are a weak band from O-H stretching vibrations (3536 cm^{-1}, alcohol), C-H stretching (3084 cm^{-1}, alkene), C-H stretching (2809 cm^{-1}), C=N stretching (1583 cm^{-1}, imine), C=C stretching (1520 cm^{-1}, cyclic alkene), C-N stretching (1227 cm^{-1}, aromatic amine), C=C bending, (835 cm^{-1}, alkene), pyridine ring (790 cm^{-1}), and C=H bending (569-516 cm^{-1}, alkene). The result is similar to the FTIR results of synthesized 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine reported in the literature (Ojemaye and Okoh, 2019; Targhan *et al.*, 2019; Tella *et al.*, 2019).

The FTIR spectrum of MSNPs has the following functional groups: Si-O bending mode at 462 cm^{-1}, asymmetrical and symmetric stretching of Si-O-Si bridge at 800 cm^{-1} and 1067 cm^{-1}. The result corresponds to the result reported in the literature (Jabir *et al.*, 2018; Salehtash *et al.*, 2018)

In the FTIR of MSNPs@TPPY, the following functional groups comprised of MSNPs and TPPY-OH were observed. The bands at 492 cm^{-1}, 798 cm^{-1}, 1212 cm^{-1}, 1537 cm^{-1}, and 1685 cm^{-1} are attributed to Si-O bending mode, pyridine ring, and C-N stretching (aromatic amine), C=N stretching frequency (imine) and C-H stretching vibration of aromatic associated with pyridine ring. This result confirmed the successful integration or functionalization of terpyridine ligand onto the surface of mesoporous silica nanoparticles.

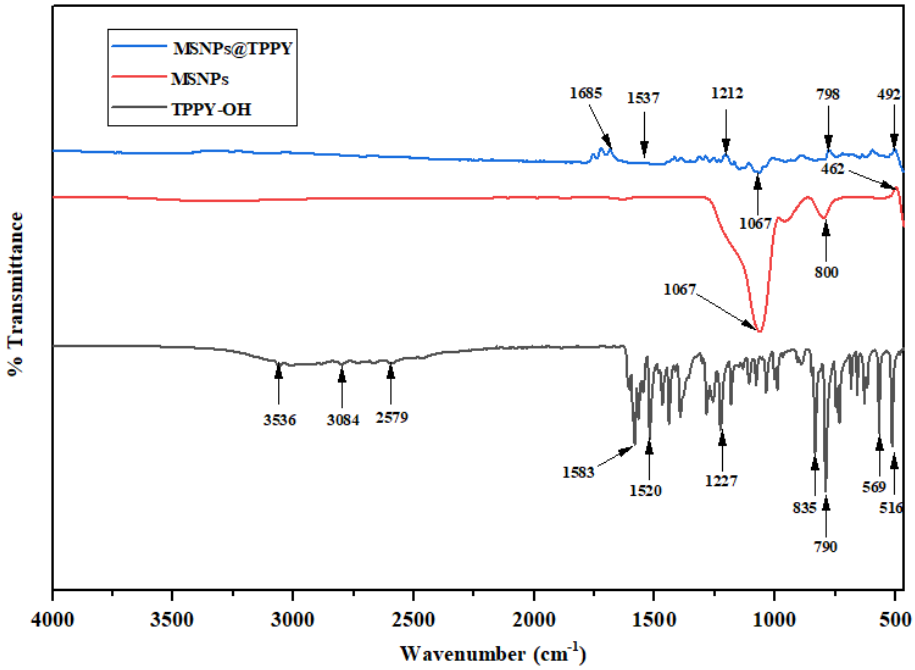


Figure 7.4: FTIR spectra of mesoporous silica nanoparticles (MSNPs), 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine (TPPY-OH) and functionalized mesoporous silica nanoparticles (MSNPs@TPPY)

7.9.1.2. EDX analysis

EDX analysis was used to identify the elemental composition of the synthesized ligand, mesoporous silica nanoparticle and functionalized adsorbents. For the synthesized 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine (TPPY-OH), the EDX spectrum shows the presence of C (weight = 86.56%, atomic weight = 89.56%) and O (weight = 13.44%, atomic weight = 10.44%) at 0.4 and 0.5 Kev respectively (Fig. 7.5A). EDX spectrum of mesoporous silica nanoparticles (MSNPs) indicated the presence of Si (weight = 31.20%, atomic weight = 19.11%), O (weight = 46.88%, atomic weight = 50.40%), N (weight = 4.47%, atomic weight

= 5.50%) and C (17.45%, atomic weight =24.99%) at approximately 2.0, 0.3,0.2, and 0.2 Kev respectively (Fig. 7.5B). Also, the functionalized MSNPs@TPPY has sharp peak at 2.0 Kev represent the presence of Si (weight = 31.20%, atomic weight = 9.11%) and other element such as O (weight = 46.88%, atomic weight = 50.40%), N (weight = 4.47%, atomic weight = 5.50%), and C (weight = 17.45%, atomic weight = 24.99%) (Fig. 7.5C). This result is similar to previously studies (Cookson and Stirk, 2019; Ghasemi *et al.*, 2017). The presence of nitrogen with atomic of 5.50% revealed that TPPY is attached to the surface of MSNPs.

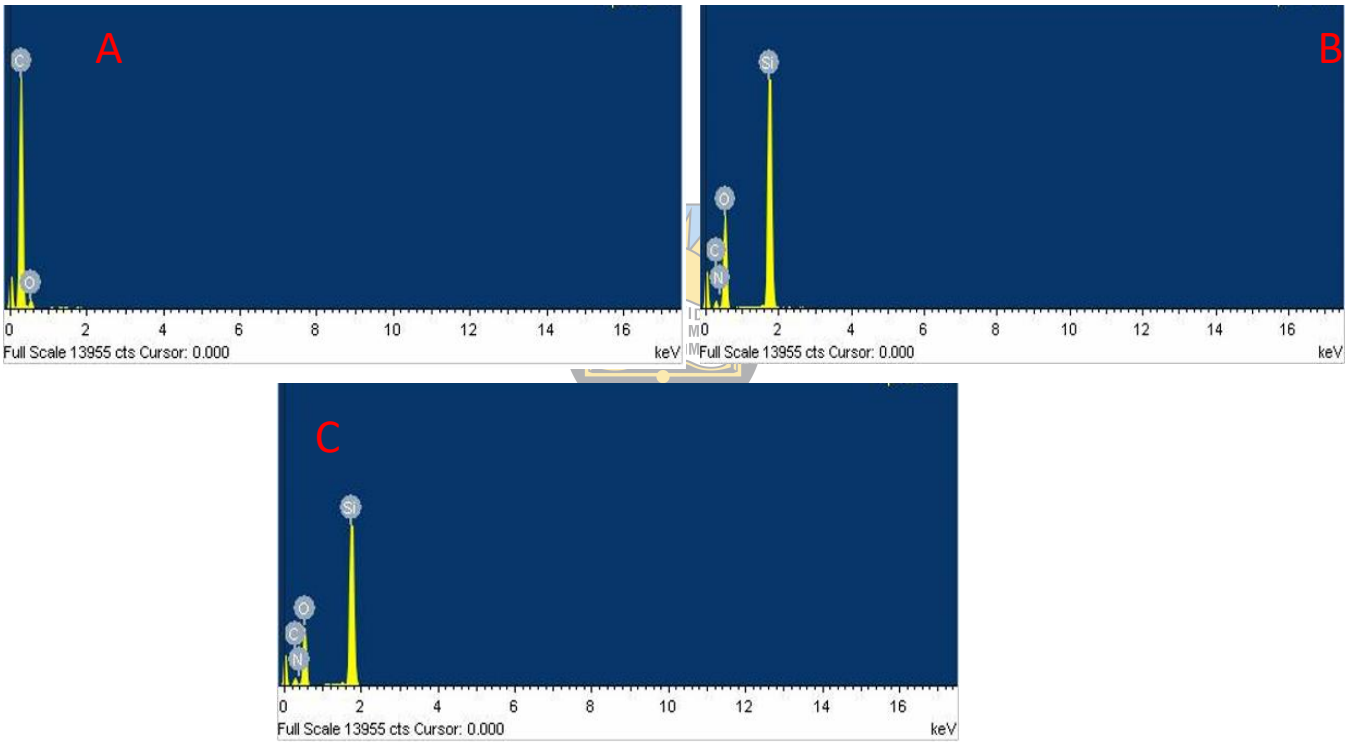


Figure 7.5: EDX spectra of (A) TPPY-OH, (B) MSNPs, and (c) MSNPs@TPPY

7.9.1.3 SEM analysis

The surface morphologies of TPPY-OH, MSNPs, and functionalized MSNPs @TPPY were investigated by scanning electron microscopy. The micrograph of the three materials was captured in different magnifications (20µm -50µm). Fig. 7.6A and B revealed flat wire-like morphology with nearly uniform size (Winter *et al.*, 2011). The MSNPs exhibited a non-spherical shape with a rough surface attributed to surface pore formation (Jabir *et al.*, 2018).

Then, MSNPs@TPPY revealed a nearly spherical and glass-like structure with aggregation showing the coating of TPPY onto the surface of MSNPs without altering its original functionality (Li, *et al.*, 2019).

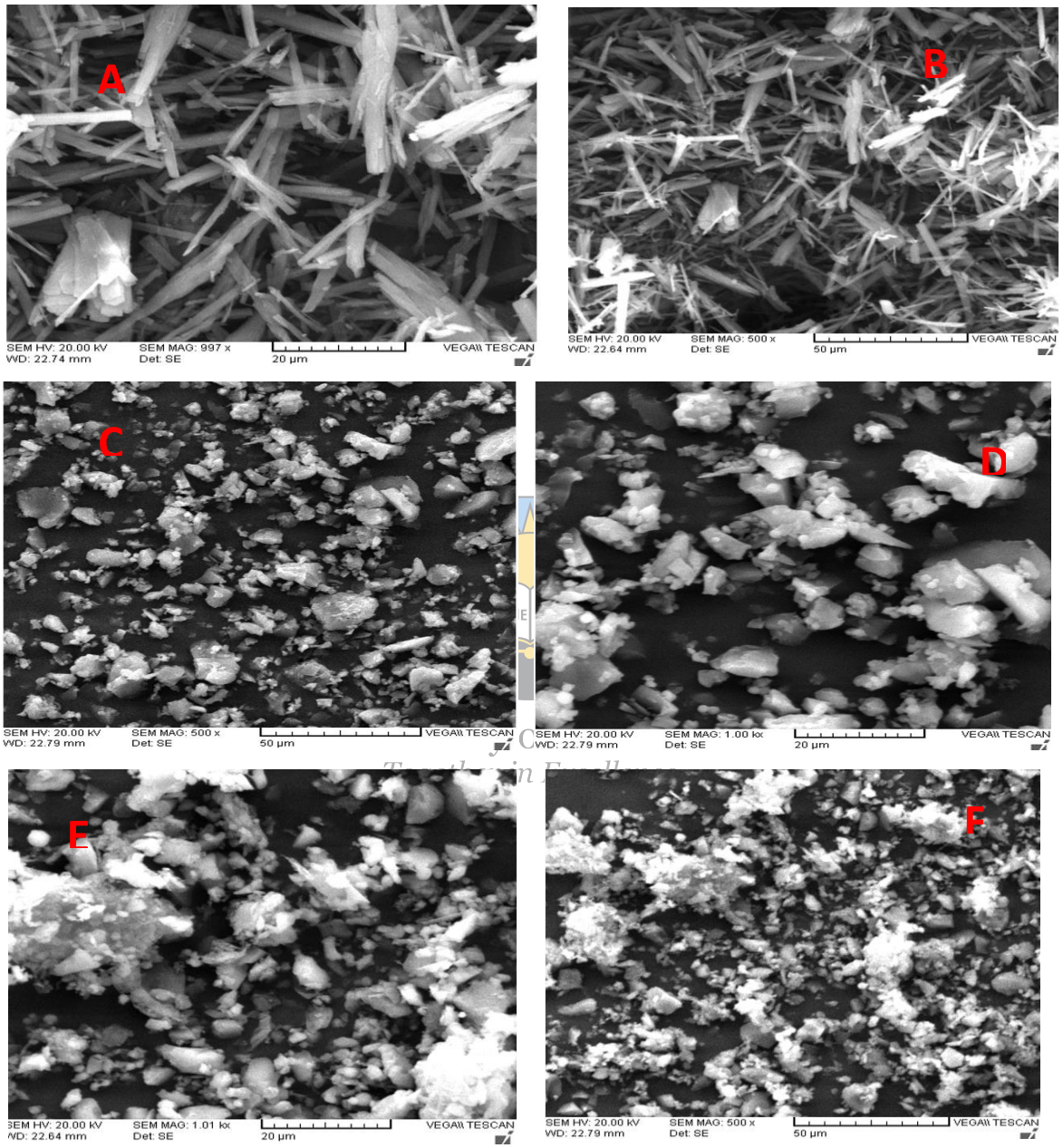


Figure 7.6: SEM images captured at 20 µm and 50 µm magnification; (A, B) TPPY-OH, (C, D) MSNPs, and (E, F) MSNPs@TPPY

7.9.1.4. PZC analysis

Point of zero charges of the MSNPs @TPPY (adsorbent) was conducted in a batch reactor, adjusting the pH of the solution containing 0.1g of adsorbent from 2-12. Meanwhile, PZC determines the net charge on the surface of the adsorbent (Miyittah *et al.*, 2016; Pezoti *et al.*,

2016). Fig. 7.7 presents the plot of pH_{PZC} of MSNPs@TPPY, and the value is 7.9. It means that at pH below 7.9, the adsorbent surface possesses a positive and negative charge at pH above the pH_{PZC}. It implies that during the adsorption of DNA, the % removal efficiency would be high when pH < pH_{PZC} (electrostatic attraction) and low at pH >pH_{PZC} (repulsion effect). Also, the bacteria DNA possesses a negative charge in its backbone.

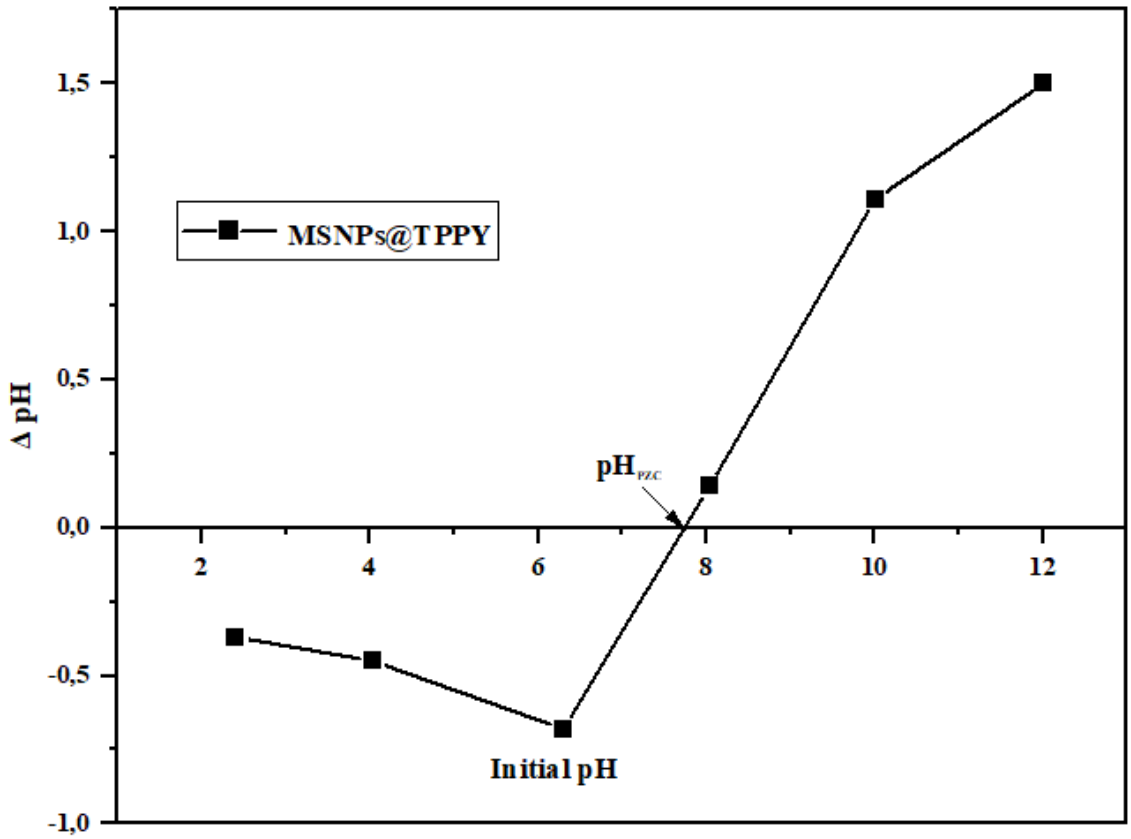


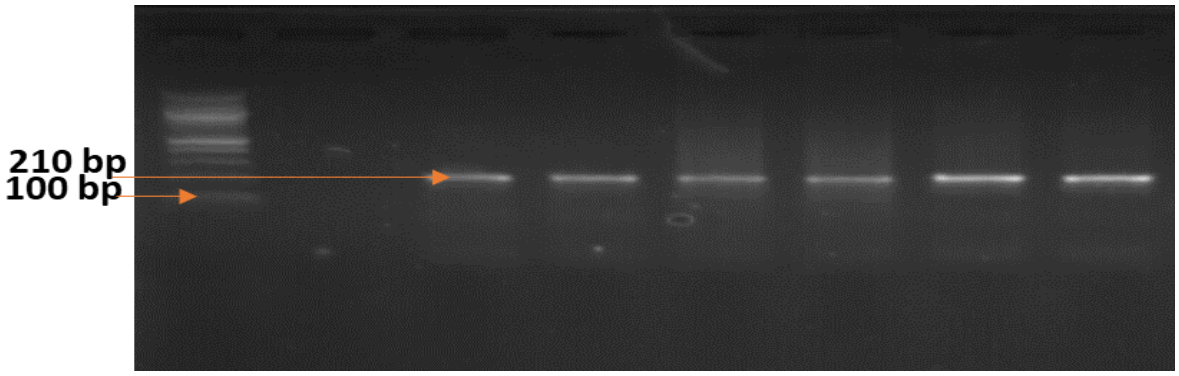
Figure 7.7: Point of zero charge (pHPZC) of MSNPs@TPPY (adsorbent)

7.10. Molecular characterization of bacteria DNA from *Enterococcus faecium* and *Vibrio Parahaemolyticus*

Antibiotic susceptibility testing (AST) shows that antibiotic-resistant bacteria are resistant to linezolid, erythromycin, ampicillin, tetracycline, vancomycin, PB 300, meropenem, and amikacin. The amplicon amplified at 210 bp, 320 bp, 201bp, and 158bp represent the *ermB*, *tetA*, and *tetM* genes, and the gel image is presented in Fig. 7.8 A and B. The gel image confirmed that ***Enterococcus faecium*** **and** ***Vibrio parahaemolyticus*** were conveying

antibiotic resistance genes at different base pairs. Absorbance ratio measured at 260 nm, 280 nm, and 320 nm using Multiparameter HACH DR 6000 Ultraviolet Spectroscopy, shows that it is in the range of 1.7 and 1.9. This range confirmed the removal of interfering compounds.

(A)



(B)

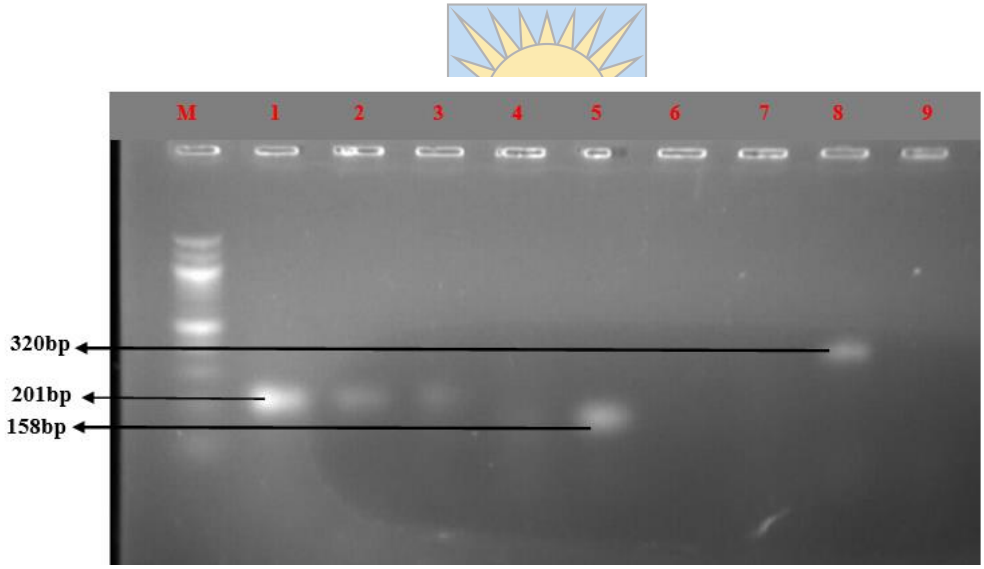


Figure 7.8. Gel electrophoresis represents (A) resistance genes for tetracycline (tetA) amplified at 210 bp; (B) resistance genes for erythromycin (*ermB*), *tetA*, and *tetM* amplified at 320, 201 and 158 bp

7.11. Effect of operating parameters on the adsorption

7.11.1 Solution pH

The adsorbent surface charge and solution pH are essential tools that determine the adsorbent's capacity to remove contaminants from water/wastewater (Foroutan *et al.*, 2019; Hasanpour and Hatami, 2020). The solution pH was studied over a 2.3 to 9.02, and the data

plot was presented in Fig. 7.9. The adsorption of bacteria DNA conveying ARGs onto MSNPs@TPPY at an adsorbent dose of 20 mg recorded the optimum percentage adsorption capacity (% qe (µg/mL)) of 93.71% at pH 2.3 (lower pH). Adsorption capacity at high pH (8.2 and 9.02) and neutral pH (7.01) values were 69.37, 58.39%, and 78.79 %, respectively. Note, below pH_{PZC} values (7.9) of adsorbent; the adsorbent possesses a net positive charge, responsible for the maximum adsorption capacity at 2.3 due to the anionic nature of the DNA. At solution pH > 7.0, the $O.H.^-$ competes with the available sites with the negative charge of the DNA, thus reducing the DNA uptake by MSNPs@TPPY (repulsion effect). The electrostatic attraction between the positively charged MSNPs@TPPY and negatively charged DNA molecules enhanced the removal efficiency of DNA from the solution (He *et al.*, 2020). To ensure that DNA bases are intact without the acidic or basic pH interference and the adsorbent's potency, the pH of 7.01 was considered for further experimentation.

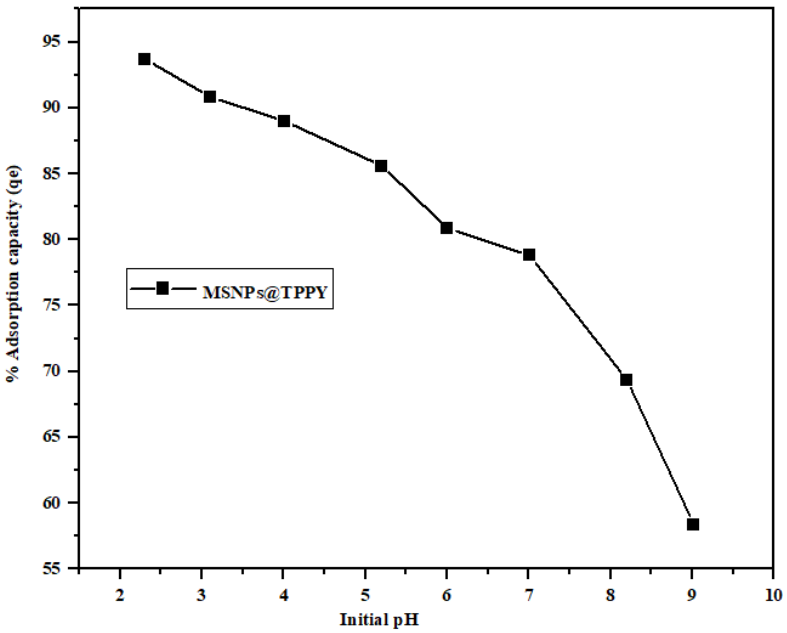


Figure 7.9: Effect of solution pH on the removal of bacteria DNA by the functionalized MSNPs@TPPY; adsorbent dose = 20 mg, DNA concentration = 3.82 µg/mL, reaction time = 360 mins and speed = 300 rpm and at room temperature

7.11.2 Adsorbent dose

In this study, the effects of adsorbent dose on the DNA uptake by the functionalized MSNPs@TPPY were investigated by maintaining the pH of 7.01 and the initial adsorbent concentration of 3.82 µg/mL, contact time of 360 mins, and at room temperature. An increase in the % adsorption efficiency (from 77.74 to 96.33 %) was observed when loading adsorbent from 10 mg to 40 mg; the result is shown in Fig. 7.10. The high adsorption efficiency of DNA may be attributed to an increase in the surface area and adsorptive site available on the MSNPs@TPPY surface (Duan *et al.*, 2021; Wang *et al.*, 2008). Other studies reported increased DNA uptake when the adsorbent increased at acidic pH (Saoudi *et al.*, 2AD; Wu *et al.*, 2011). The acidic pH contributed more to the increase in the adsorptive site. High loading of MSNPs@TPPY in the solution contributed to high DNA uptake by the adsorbent during the adsorption process. Therefore, the adsorbent dosage of 40 mg as the optimal dosage would be adopted in the subsequent studies.

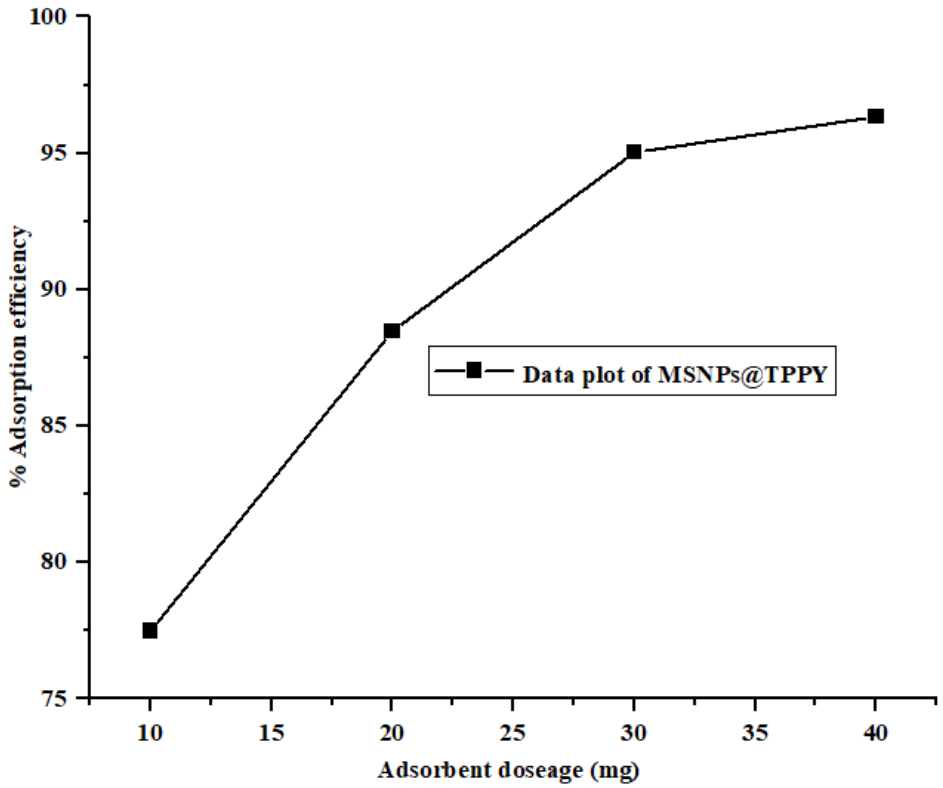


Figure 7.10: Effect of adsorbent dose on the removal of bacteria DNA onto MSNPs@TPPY, DNA concentration = 3.82 µg/ml, reaction time = 360 mins; speed = 300 rpm, pH = 7.01, and at room temperature

7.11.3 Initial DNA concentration and isotherm study

The plots of MSNPs@TPPY adsorption capacity against the initial concentration of DNA molecules of 1.91, 3.82, 5.73, and 7.64 µg/mL maintaining the adsorbent dose of 40 mg, contact time of 360 mins, pH 7.01, and at room temperature are presented in Fig. 7.11 A. As the initial DNA concentration increases, the amount of DNA adsorbed on the surface of MSNPs@TPPY decreases. The decrease in the DNA uptake by the adsorbent may be due to the increase in initial adsorbate concentration leading to saturation of the adsorptive site, which may reduce the driving force of mass transfer leading to the adsorbent (Shattar *et al.*, 2020; Shen *et al.*, 2015). This suggests that the adsorption of DNA onto MSNPs@TPPY is dependent on the initial concentration.

Isotherm models such as Freundlich, Langmuir, and Sips were employed to study the adsorption systems. Meanwhile, the Freundlich model is based on the adsorption of the heterogeneous layer (Obijole *et al.*, 2021; Tan *et al.*, 2021). Langmuir explains the adsorption that occurs on the monolayer surface of the adsorbent (Araújo *et al.*, 2018; Togue Kamga, 2019), while Sips is a three-parameter model that explains adsorption equilibrium data in the combination of Freundlich and Langmuir model (Al-Asheh *et al.*, 2000; Yang *et al.*, 2021). From Fig. 7.11B and the data presented in Table 7.2, it was confirmed that adsorption of bacteria DNA onto MSNPs@TPPY obeyed Langmuir model with highest correlation coefficient (R^2) = 0.9887 and a reduced Chi-square (X^2) = 0.0032 compared to Freundlich (R^2 = 0.92607 and X^2 =0.0991) and Sips (R^2 = 0.92605 and X^2 =0.1981). The result showed that the adsorption of bacteria DNA conveying ARGs is a monolayer that occurs on the adsorbent's homogenous surface site through electrostatic interaction (Ajeng *et al.*, 2022; Ukhurebor *et al.*, 2021).

Table 7.2: Calculated isotherm parameters for the bacteria DNA adsorption onto MSNPS@TPPY

Isotherm model	**Parameters**				
Freundlich	K_F (µg/g)	*n*	X^2	R^2	
	2.39852	1.90623	0.09902	0.92607	
Langmuir	K_L (µg/g)	qm (µg/mL)	X^2	R_L	R^2
	1.28626	4.31793	0.0032	0.289289	0.9887
Sips	qm	Ks	n	X^2	R^2
	6.53753	0.00185	0.52519	0.1981	0.92605

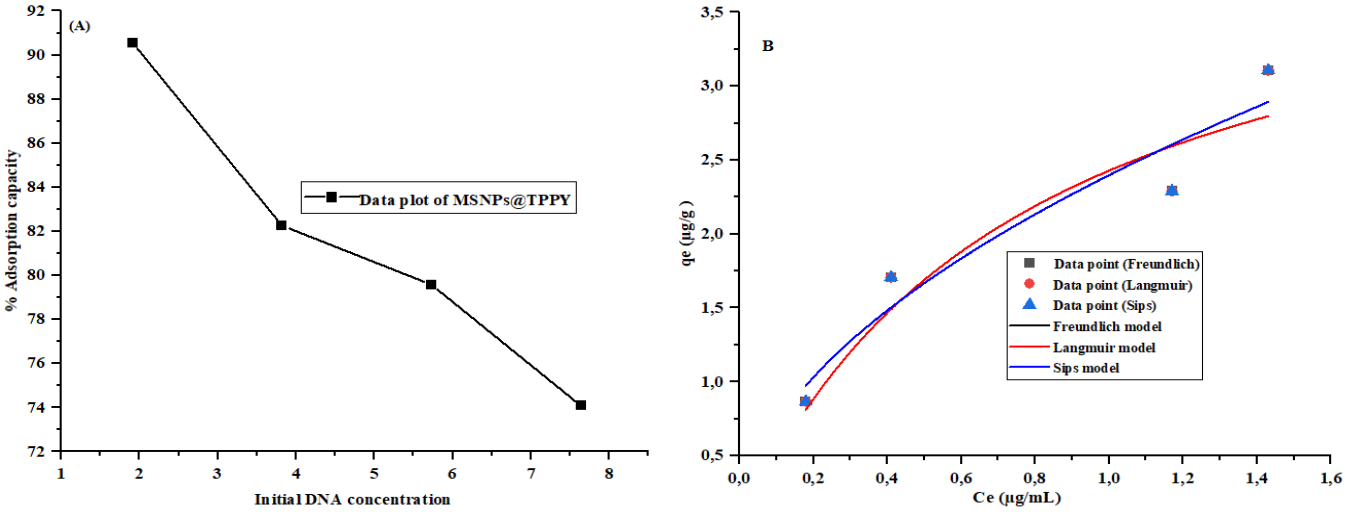


Figure 7.11: (A) Effect of initial concentration on DNA adsorption onto functionalized MSNPs@TPPY and isotherm models of (b) Freundlich, Langmuir, and Sips model

7.11.4 Contact time and adsorption kinetic

Fig. 7.12 A shows the effect of contact time at different DNA initial concentrations (1.91, 3.82, 5.73, and 7.64 µg/mL), maintaining the optimum parameters such as solution pH of 7.01, adsorbent dose of 40 mg, and at a different time interval. It was observed that the adsorption capacity of DNA onto MSNPs@TPPY in all the initial concentrations increased rapidly between 5-15 mins and gradually stagnated at an equilibrium time of 90 mins. This behaviour showed the availability of vacant sites at the initial stage of the adsorption. A gradual process signified that repulsive force took place, making a vacant site difficult to adsorb more DNA (Fu *et al.*, 2015). At equilibration, the % removal efficiencies obtained are 90.57, 89.26, 79.58, and 74.08% for DNA initial concentration 1.91, 3.82, 8.73 and 7.64 µg/mL respectively. Also, the calculated adsorption equilibrium time (qt) recorded are 0.87, 1.71, 2.13, and 2.83 for 1.91, 3.82, 8.73 and 7.64 µg/mL respectively. The equilibrium time at 90 mins showed that adsorption occurred on the external surface of the adsorbent.

Adsorption kinetics on the removal of DNA via functionalized MSNPs@TPPY was studied

as a function of time investigated at different adsorbent doses. The kinetic data on the adsorption of bacteria DNA onto the functionalized adsorbent via Natarajan and Khalaf first order (NKF), pseudo-first order (PFO), pseudo-second order (PSO), and Elovich kinetic models (EKM). The parameters are presented in Table 7.3, and the plot of the Elovich model (best fitted) is illustrated in Fig. 7.12B. Interestingly, the correlation coefficient for Elovich model (R^2 = 0.9949 to 0.9986) was high with a reduced Chai-square (X^2 = 0.0003-0.0014) compared to NKF (R^2 = 0.65046 to 0.8997; X^2 = 0.08 to 0.1), PFO (R^2 = 0.88059 to 0.0.9197; X^2 = 0.60 to 1.08), PSO (R^2 = 0.9661 to 0.9988; X^2 = 0.27 to 0.63). The highest R^2 obtained by the Elovich indicates that the rate of DNA adsorption onto MSNPs@TPPY is based on chemisorption via electrostatic attraction between the DNA molecules and adsorbent heterogeneous surface. Also, it is assumed that the adsorption rate decreases exponentially as the DNA initial concentration increases (Grunenwald *et al.*, 2014).

Table 7.3. Adsorption kinetic model and their parameter values for DNA uptake onto functionalized MSNPs@TPPY

Kinetic model	Parameters	Initial concentration (µg/mL)			
		1.91	**3.82**	**5.73**	**7.64**
Natarajan and Khalaf First Order	K_1(^{1}/min)	0.000127	0.000126	0.00008	0.00009
		0.8997	0.8078	0.65046	0.8082
	R^2	0.08	0.14	0.13	0.08
	X^2	**0.865**	**1.705**	**2.28**	**2.83**
Pseudo First Order		-0.00023	-0.00022	-0.00021	-0.00025
	qe (^{Cal})(µg/mg)	0.84925	0.90507	1.00304	1.42773
		0.9128	0.9197	0.8805	0.9078
	K_1 (^{1}/min)	0.69	0.67	0.60	1.08
		0.865	**1.705**	**2.28**	**2.83**
Pseudo Second Order	qe(^{exp})(µg/mg)	1.08170	0.76331	0.65519	0.55624
	R^2	1.04083	0.87986	0.82154	0.76458
	X^2	1.17184	0.59091	0.44220	0.32517
		0.9661	0.9986	0.9979	0.9949
		0.63	0.54	0.27	0.28
	qe (^{Cal})(µg/mg)	1.7056	2.0278	3.0708	1.43103
		3.9979	3.35903	3.78238	1.47178
	K_2 (g/µg min)	0.9965	0.9986	0.9979	0.9949
		0.0003	0.0005	0.0014	0.0006

	qe(^{exp})(µg/mg)	1.7056	2.0278	3.0708	1.43103
	h	3.9979	3.3590	3.7823	1.47178
Elovich	R^2	0.9965	0.9986	0.9979	0.9949
	X^2	0.0003	0.0005	0.0014	0.0006
	$\alpha(mg^{-1}min^{1})$				
	β (µg/mg)				
	R^2				
	X^2				

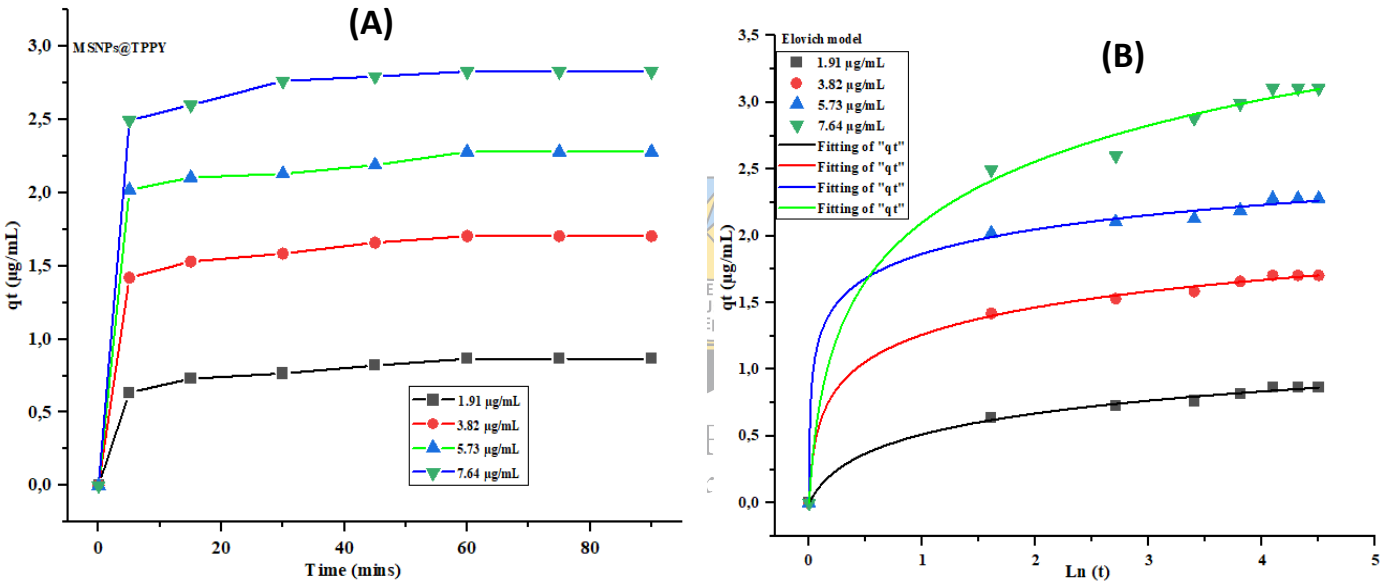


Figure 7.12: (A) Effect of contact time on DNA adsorption onto functionalized MSNPs@TPPY and kinetic models of (B), Elovich kinetic model

Adsorbent's potency was confirmed by its application into real wastewater to remove bacteria DNA conveying ARGs. The actual water sample collected from Uitenhage WWTPs was quantified, and physicochemical parameters obtained are presented in Table 7.4. Three DNA initial concentrations were obtained by spiking water samples with the stock DNA in the 1:1, 1:2, and 1:3 to obtain new initial concentrations of 1.45. 2.47 and 3.46 µg/mL. From Fig. 13, it was observed that MSNPs@TPPY adsorbed more DNA compared to simulated water with the effect of optimum parameters such as solution pH of 7.01, adsorbent dose of 40 mg,

contact time of 90 mins, and at room temperature. The % removal efficiency was 97.72, 96.59, and 91.61 for 1.45. 2.47 and 3.46 µg/mL, respectively. The removal efficiency may be attributed to the low initial concentration of the actual water samples and no anionic interference in the natural wastewater. These values confirmed that high removal efficiency depends on the initial concentration of DNA.

Table 7.4: Physicochemical parameter of water sample from Uitenhage WWTPs

pH	Temperature	Total dissolved solids	Dissolved oxygen	Turbidity	The initial DNA concentration in river water (µg/mL)
7.33	17.38	25.67	16.23	15.6	0.447

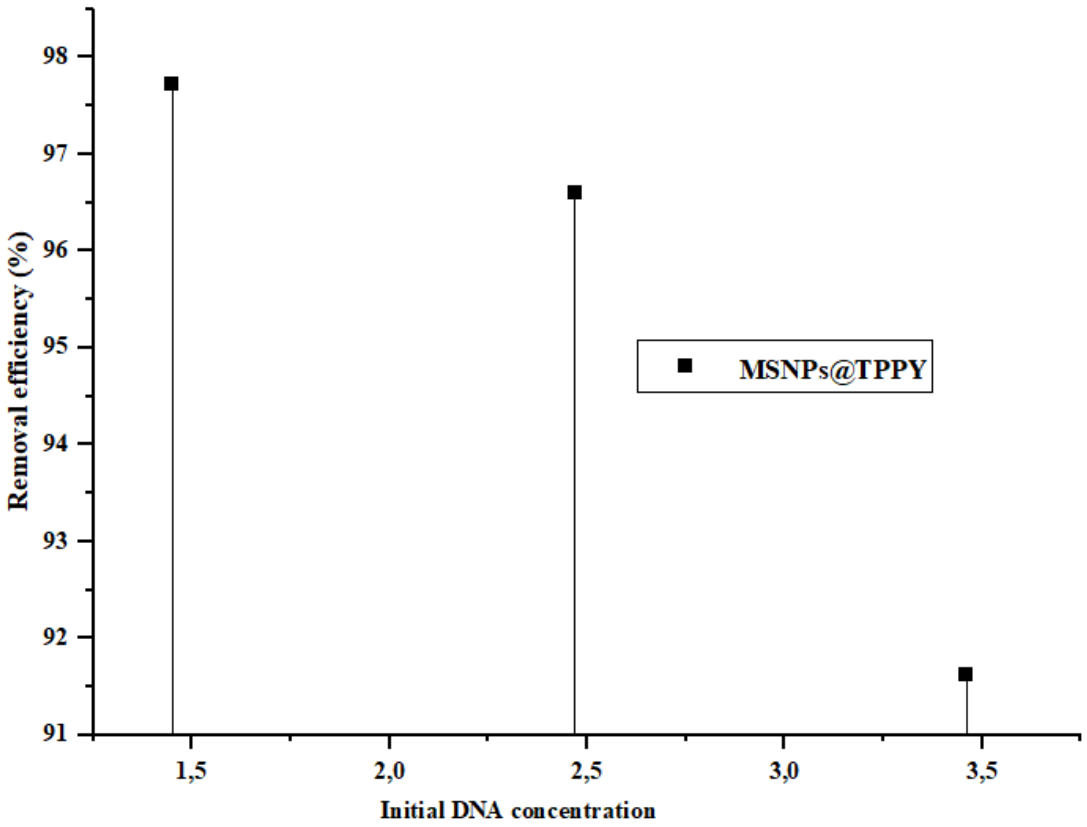


Figure 7.13: The removal of bacteria DNA conveying ARGs from Uitenhage WWTPs

7.13. Conclusion

Herein, mesoporous silica nanoparticles were synthesized and functionalized its surface with

4(4-hydroxyphenyl)-2,2;6",2-terpyridine and denoted as MSNPs@TPPY. The structural characterization of the sample was investigated by FTIR, SEM, EDX, and PZC. The experimental parameters on the removal of bacteria DNA by the MSNPs@TPPY included the effect of solution pH, contact time, initial DNA concentrations, and adsorbent loading/dose. The adsorption of DNA onto MSNPs@TPPY obeyed the Langmuir isotherm and Elovich kinetic model. The maximum adsorption capacity obtained during the effect of contact time and initial DNA concentration was 90.57% at 90 mins. When the adsorbent dose was raised to 40 mg, the adsorption capacity increased to 96%. The application of MSNPs@TPPY to natural wastewater recorded a maximum adsorption capacity of 97.72%, indicating that the removal of bacteria DNA conveying ARGs was spontaneous and favourable.

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Chapter 8

General conclusion and recommendations

Bacteria deoxyribonucleic acid (DNA) conveys antibiotic resistance genes (ARGs) which are serious water contaminant of the 21st century that threatens public health. This is because of the extensive use of antibiotics by humans and animals, which accelerates the proliferation and dissemination of bacteria DNA conveying ARGs in different water matrices such as rivers, lakes, and wastewater treatment plants (WWTPs). The menace of bacteria DNA conveying ARGs is on the rise worldwide because of the release of untreated effluents from households, hospitals, agricultural, and pharmaceutical industries into water sources without proper or adequate treatment. Stringent control of the release of effluents containing bacteria DNA conveying ARGs and the application of adequate treatment materials and techniques that would assist in eradicating the existing ones present in water are the solutions that may stop the consequences of consuming ARGs infected water. Various treatment techniques and materials have been employed for the eradication of these contaminants from water/wastewater, and adsorption techniques with nanomaterials seem to be efficient due to their low cost, simplicity sludge free, low energy consumption, flexibility, and ability to regenerate and recover an adsorbent after the treatment process. In addition, nanomaterials gained much interest as efficient adsorbents due to their excellent properties, including large surface

area, high reactivity, dependable size properties, degree of functionalization, affinity to target contaminant, and ability to regenerate and recover after the treatment process.

This study synthesized metallic nanomaterials such as silver nanoparticles (AgNPs) and mesoporous silica nanoparticles (MSNPs). Their surface functionalization was achieved by incorporating magnetite (Fe_3O_4 NPs) and a tridentate ligand (4-(4-hydroxyphenyl)-2,2;6",2-terpyridine) onto the surface of AgNPs and MSNPs. These materials were used as adsorbents for removing bacteria DNA conveying ARGs from simulated aqueous solution and real effluents from the hospital, river, and wastewater treatment plants.

The surface functionalization of these nanoparticles was achieved by attaching Fe_3O_4 nanocomposites to the silver surface (AgNPs@ Fe_3O_4) and 4-(4-hydroxyphenyl)-2,2;6",2-terpyridine to silica mesopore (MSNPs@TPPY). These adsorbents were subjected to characterization analysis using FTIR, SEM, EDX, UV spectroscopy, and PZC. The adsorption capacities of the functionalized materials were compared to their as-synthesized materials to obtain the adsorbents with the highest removal efficiency.

Meanwhile, the bacteria used in this study were antibiotic-resistant *Enterococcus faecium* and *Vibrio parahaemolyticus* isolated from the beach water located in East London and wastewater treatment plant around the University of Fort Hare, Alice, province of Eastern Cape, South Africa. Before the commencement of genomic DNA extraction, antibiotic susceptibility testing (AST) was conducted on the bacteria isolates using the disk diffusion method, following the procedure of Kirby-Bauer recommended by CLSI, and the result confirmed that bacteria isolates are resistant to linezolid, erythromycin, ampicillin, tetracycline, vancomycin, PB 300, meropenem, and amikacin. Polymerase chain reaction (PCRs) assays were prepared. The amplicons pictured using the Alliance BioDoc-It system confirmed the presence of resistance genes at 201bp, 320bp, 210bp, and 158bp for *tetA*, *ermB*, *tetA*, and *tetM*.

The removal of bacteria DNA conveying ARGs by AgNPs, MSNPs, AgNPs@ Fe_3O_4, and MSNPs@TPPY were examined via batch adsorption experiments. The effects of operating parameters such as pH, contact time, initial DNA concentration, chaotropic salt, adsorbent dosage, and cationic and anionic salts were investigated for each adsorbent during the adsorption process.

The following conclusions were drawn based on the results obtained from this study

- The genomic DNA (contaminant) extracted from the antibiotic-resistant *Enterococcus faecium* and *Vibrio parahaemolyticus* was successfully achieved via the boiling method. The bacteria are resistant to different antibiotics, successfully achieved by AST using the disk diffusion method. Molecular characterization via gel electrophoresis confirmed that they harbor different resistance genes at different base pairs (158, 201, 210, and 320bp).
- The synthesis of AgNPs at different molar concentrations (0.1, 0.5, and 1.0 M) represented as BD1, BD2 and BD3 were successfully synthesized and confirmed by characterization techniques such as FTIR, SEM, EDX, and UV spectroscopy, XRD and PZC. FTIR analysis revealed Ag-O vibrational peaks around 410 cm^{-1} and UV-Visible absorption frequency at 420 nm and 550 nm, typical of the metallic nanoparticle. XRD, SEM, and EDX revealed that AgNPs is a crystalline face-centered cubic material having the elemental composition of pure silver (Ag) with weight and atomic percentage (%) of 90.50 and 54.60 at 3KeV. Removal of bacteria DNA conveying ARGs in aqueous solutions involving different parameters (effects of pH, time-concentration profile, adsorbent dose, cations) confirmed that bacteria DNA adsorbed onto BD1, BD2, and BD3 of different initial concentrations at pH 6.9 and BD3 being the one with the highest adsorption capacity of 88.55 % at 225 mins. Maximum equilibration time was achieved after 180, 195, and 225 mins. The adsorption models fitted into the experimental data obeyed Langmuir isotherm model (R^2 = 0.97625 and X^2 = 0.12142, R^2 = 0.96049 and X^2 = 0.24403, R^2 = 0.85108 and reduced X^2 =

1.00914 for BD1, BD2, and BD3) and pseudo-second-order with the highest R^2 values (R^2 = 0.90 to 0.98).

- Mesoporous silica nanoparticles were successfully synthesized via chemical etching techniques. FTIR spectra confirmed the presence of a peak at 462 cm^{-1}, indicating the Si-O bending mode at 805 $cm^{-1,}$ and 1069 cm^{-1} confirmed the asymmetric and symmetric stretching of the Si-O-Si bridge and the obscure band at 997 cm^{-1} corresponding to the Si-OH group. EDX confirmed the elemental composition by the appearance of solid peaks representing Si at approximately 2Kev. SEM captured at different magnifications (20µm - 50µm) showed a non-spherical shape with a nearly uniform size forming agglomeration. Adsorption studies were conducted by adding different chaotropic salts alongside the MSNPs (E-MSN+S, E-MSN+U, and E-MSN+G) as adsorbents into the diluted DNA solution at pH 7.02. The chaotropic salts (2 M guanidine HCl, urea, and sodium chloride) reduced the repulsion effects between DNA and MSNPs because both the adsorbent (MSNPs) and contaminants (DNA) possess a negative charge. These chaotropic salts assisted in mediating bacteria DNA onto silica mesopore, which aids in the removal of bacteria DNA by MSNPs from aqueous solution and hospital wastewater. Among the different chaotropic salts used to compliment the silica nanoparticles, 2 M guanidine HCl exhibited the highest percentage (%) removal efficiency (80%) compared to urea (75%) and sodium chloride (70%) in both simulated aqueous solution and hospital wastewater. Maximum equilibration time was achieved after 180, 195, and 210 mins. The adsorption data obtained from all the three adsorbents were best fitted into the Sips isotherm model and pseudo-second-order kinetic model. The reaction is governed by electrostatic interactions that occur on heterogeneous surfaces.
- The synthesis and surface functionalization of AgNPs@Fe_3O_4 nanocomposite was confirmed using FTIR, SEM, EDX, and XRD techniques. The application of the

nanocomposite in removing bacteria DNA from a simulated aqueous solution and an actual water sample from the river was successfully demonstrated. High adsorption efficiency (above 90% at pH of 7.2) the rapidness of adsorption within a short time interval and are the most advantageous properties exhibited by AgNPs@Fe_3O_4 nanocomposite. The adsorption of bacteria DNA conveying ARGs was spontaneous and fast compared to as-synthesized AgNPs. The experimental equilibrium data fit the Elovich kinetic and Freundlich isotherm model.

- The surface functionalization of MSNPs via three steps which includes conversion to amine (MSNPs-NH_2), carboxylic (MSNPs-COOH), and finally to 4(4-hydroxyphenyl)-2,2;6",2-terpyridine (MSNPs@TPPY) were successfully achieved and confirmed by characterization techniques. FTIR confirmed the attachment of the functional group that aids the adsorption of DNA onto MSNPs@TPPY. SEM showed a nearly spherical and glass-like structure showing the coating of terpyridine (TPPY) onto the surface of MSNPs without altering its original functionality. EDX confirmed the elemental compositions. The application of MSNPs@TPPY for removing bacteria DNA from an aqueous solution and a water sample from WWTPs exhibited a high adsorption capacity of 77.74 to 96.33% when the adsorbent dose increased from 20 to 40 mg at pH 7.01 and contact time of 90 mins. The maximum adsorption capacity on the actual water samples from WWTPs reached 97.72% at 90 mins. The removal process was spontaneous and favorable, confirming high adsorption capacity at a shorter contact time. The experimental data fitted into Elovich kinetic and Langmuir isotherm, indicating that adsorption of bacteria DNA conveying ARGs occurred via electrostatic interaction on the homogenous surface site.

Based on these reports, the following recommendations are provided for future work.

- An investigation on the incorporation or coating of the adsorbents with antibiotics to remove bacteria DNA conveying ARGs and ARB from water/wastewater.

- An investigation of the effects of different solvents such as ethanol, methanol, and isopropanol for removing bacteria DNA and other biological contaminants from WWTPs, river, and hospital effluents.
- Investigation of the ARB inactivation and removal from water/wastewater using functionalized silver (AgNPs@Fe_3O_4 NPs) and silica (MSNPs@TPPY).

Appendix 1

Data for different molar concentration of silver nanoparticles (BD1= 0.1 M, BD2 =0.5 M and BD3 =1.0 M)

Table A-I.1: Experimental data for the adsorption of bacteria DNA onto BD1 (0.1M), BD2 (0.5M) and BD3 (1.0M) as a function of pH (6.9) [conditions: contact time 360 mins, agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

Initial pH	C_i (µg/mL)	C_f(µg/mL)	Adsorbent weight	% Adsorption	Adsorption capacity
2,07	9,92	1,27	20	86,69	873,65
3,01	9,92	1,8	20	81,85	820,12
4,01	9,92	4,15	20	79,32	582,77
5,02	9,92	5,13	20	78,44	483,79
6,01	9,92	6,89	20	70,7	306,03
7,04	9,92	6,45	20	68,08	350,47
8,06	9,92	7,61	20	55,8	233,31
9,01	9,92	7,91	20	50,26	203,01

Initial pH	C_i (µg/mL)	C_f(µg/mL)	Adsorbent weight	% Adsorption	Adsorption capacity
Initial pH	C_i (µg/mL)	C_f(µg/mL)	Adsorbent weight	% Adsorption	Adsorption capac
Initial pH	C_i (µg/mL)	C_f(µg/mL)	Adsorbent weight	% Adsorption	Adsorption capacity

2,03	9,92	1	20	89,92	900,92
3,06	9,92	1,06	20	89,31	894,86

4,07	9,92	2,74	20	80,38	725,18
5,03	9,92	5,3	20	79,57	466,62
6,07	9,92	5,43	20	78,36	453,49
7,06	9,92	6,72	20	73,26	323,2
8,03	9,92	6,96	20	69,84	298,96
9,04	9,92	7,02	20	69,23	292,9

Table A-I.2: Experimental data for the adsorption of bacteria DNA onto BD1 (0.1M) as a function of contact time (180 mins) [conditions: pH (6.9), agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Weight of adsorbent (m**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	4,98	4,98	20	0	0	0
5	4,98	2,5	20	49,79919679	1,24	1,24
15	4,98	2,3	20	53,81526104	1,34	1,34
30	4,98	2,1	20	57,8313253	1,44	1,44
45	4,98	1,9	20	61,84738956	1,54	1,54
60	4,98	1,7	20	65,86345382	1,64	1,64
75	4,98	1,5	20	69,87951807	1,74	1,74
90	4,98	1,3	20	73,89558233	1,84	1,84
105	4,98	1,1	20	77,91164659	1,94	1,94
120	4,98	0,9	20	81,92771084	2,04	2,04
135	4,98	0,7	20	85,9437751	2,14	2,14
150	4,98	0,5	20	89,95983936	2,24	2,24
165	4,98	0,5	20	89,95983936	2,24	2,24
180	4,98	0,5	20	89,95983936	2,24	2,24

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	9,92	9,92	20	0	0	0
5	9,92	5,77	20	41,83467742	2,075	2,075
15	9,92	5,37	20	45,86693548	2,275	2,275
30	9,92	4,97	20	49,89919355	2,475	2,475
45	9,92	4,57	20	53,93145161	2,675	2,675
60	9,92	4,17	20	57,96370968	2,875	2,875
75	9,92	3,77	20	61,99596774	3,075	3,075
90	9,92	2,97	20	70,06048387	3,475	3,475
105	9,92	2,57	20	74,09274194	3,675	3,675
120	9,92	2,15	20	78,3266129	3,885	3,885
135	9,92	1,73	20	82,56048387	4,095	4,095
150	9,92	1,33	20	86,59274194	4,295	4,295
165	9,92	1,33	20	86,59274194	4,295	4,295
180	9,92	1,33	20	86,59274194	4,295	4,295

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	14,98	14,98	20	0	0	0
5	14,98	10,83	20	27,703605	2,075	2,075
15	14,98	10,33	20	31,041389	2,325	2,325
30	14,98	9,71	20	35,18024	2,635	2,635
45	14,98	9,08	20	39,385848	2,95	2,95
60	14,98	8,46	20	43,5247	3,26	3,26

75	14,98	7,83	20	47,730307	3,575	3,575
90	14,98	7,15	20	52,269693	3,915	3,915
105	14,98	6,66	20	55,540721	4,16	4,16
120	14,98	6,55	20	56,275033	4,215	4,215
135	14,98	5,65	20	62,283044	4,665	4,665
150	14,98	4,21	20	71,895861	5,385	5,385
165	14,98	4,21	20	71,895861	5,385	5,385
180	14,98	4,21	20	71,895861	5,385	5,385

Table A-I.3: Experimental data for the adsorption of bacteria DNA onto BD2 (0.5M) as a function of contact time (195 mins) [conditions: pH (6.9), agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	4,98	4,98	20	0	0	0
5	4,98	2,3	20	53,81526104	1,34	1,34
15	4,98	2,1	20	57,8313253	1,44	1,44
30	4,98	1,9	20	61,84738956	1,54	1,54
45	4,98	1,7	20	65,86345382	1,64	1,64
60	4,98	1,5	20	69,87951807	1,74	1,74
75	4,98	1,3	20	73,89558233	1,84	1,84
90	4,98	0,9	20	81,92771084	2,04	2,04
105	4,98	0,6	20	87,95180723	2,19	2,19
120	4,98	0,59	20	88,15261044	2,195	2,195
135	4,98	0,48	20	90,36144578	2,25	2,25
150	4,98	0,37	20	92,57028112	2,305	2,305
165	4,98	0,34	20	93,17269076	2,32	2,32
180	4,98	0,34	20	93,17269076	2,32	2,32
195	4,98	0,34	20	93,17269076	2,32	2,32

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with**

						respect to time (qt)
0	9,92	9,92	20	0	0	0
5	9,92	5,23	20	47,27822581	2,345	2,345
15	9,92	4,63	20	53,3266129	2,645	2,645
30	9,92	4,03	20	59,375	2,945	2,945
45	9,92	3,43	20	65,4233871	3,245	3,245
60	9,92	2,83	20	71,47177419	3,545	3,545
75	9,92	2,23	20	77,52016129	3,845	3,845
90	9,92	1,63	20	83,56854839	4,145	4,145
105	9,92	1,03	20	89,61693548	4,445	4,445
120	9,92	0,94	20	90,52419355	4,49	4,49
135	9,92	0,9	20	90,92741935	4,51	4,51
150	9,92	0,86	20	91,33064516	4,53	4,53
165	9,92	0,85	20	91,43145161	4,535	4,535
180	9,92	0,85	20	91,43145161	4,535	4,535
195	9,92	0,85	20	91,43145161	4,535	4,535

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	14,98	14,98	20	0	0	0
5	14,98	10,63	20	29,038718	2,175	2,175
15	14,98	9,83	20	34,379172	2,575	2,575
30	14,98	9,03	20	39,719626	2,975	2,975
45	14,98	8,23	20	45,06008	3,375	3,375
60	14,98	7,43	20	50,400534	3,775	3,775
75	14,98	6,63	20	55,740988	4,175	4,175
90	14,98	5,83	20	61,081442	4,575	4,575
105	14,98	5,03	20	66,421896	4,975	4,975
120	14,98	4,23	20	71,76235	5,375	5,375
135	14,98	3,43	20	77,102804	5,775	5,775
150	14,98	3,42	20	77,169559	5,78	5,78

165	14,98	3,4	20	77,303071	5,79	5,79
180	14,98	3,4	20	77,303071	5,79	5,79
195	14,98	3,4	20	77,303071	5,79	5,79

Table A-I.4: Experimental data for the adsorption of bacteria DNA onto BD3 (1.0 M) as a function of contact time (225 mins) [conditions: pH (6.9), agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	4,98	4,98	20	0	0	0
5	4,98	1,99	20	60,04016064	1,495	1,495
15	4,98	1,94	20	61,04417671	1,52	1,52
30	4,98	1,93	20	61,24497992	1,525	1,525
45	4,98	1,87	20	62,4497992	1,555	1,555
60	4,98	1,78	20	64,25702811	1,6	1,6
75	4,98	1,63	20	67,26907631	1,675	1,675
90	4,98	1,13	20	77,30923695	1,925	1,925
105	4,98	1,59	20	68,07228916	1,695	1,695
120	4,98	0,63	20	87,34939759	2,175	2,175
135	4,98	0,45	20	90,96385542	2,265	2,265
150	4,98	0,25	20	94,97991968	2,365	2,365
165	4,98	0,23	20	95,3815261	2,375	2,375
180	4,98	0,2	20	95,98393574	2,39	2,39
195	4,98	0,18	20	96,38554217	2,4	2,4
210	4,98	0,18	20	96,38554217	2,4	2,4
225	4,98	0,18	20	96,38554217	2,4	2,4

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with**

						respect to time (qt)
0	9,92	9,92	20	0	0	0
5	9,92	4,88	20	50,8064516	2,52	2,52
15	9,92	4,56	20	54,0322581	2,68	2,68
30	9,92	4,24	20	57,2580645	2,84	2,84
45	9,92	3,92	20	60,483871	3	3
60	9,92	3,6	20	63,7096774	3,16	3,16
75	9,92	3,28	20	66,9354839	3,32	3,32
90	9,92	2,92	20	70,5645161	3,5	3,5
105	9,92	2,42	20	75,6048387	3,75	3,75
120	9,92	2,07	20	79,1330645	3,925	3,925
135	9,92	1,57	20	84,1733871	4,175	4,175
150	9,92	1,25	20	87,3991935	4,335	4,335
165	9,92	0,93	20	90,625	4,495	4,495
180	9,92	0,63	20	93,6491935	4,645	4,645
195	9,92	0,33	20	96,6733871	4,795	4,795
210	9,92	0,33	20	96,6733871	4,795	4,795
225	9,92	0,33	20	96,6733871	4,795	4,795

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	14,98	14,98	20	0	0	0
5	14,98	10,21	20	31,842457	2,385	2,385
15	14,98	9,41	20	37,182911	2,785	2,785
30	14,98	8,61	20	42,523364	3,185	3,185
45	14,98	7,81	20	47,863818	3,585	3,585
60	14,98	7,01	20	53,204272	3,985	3,985
75	14,98	6,16	20	58,878505	4,41	4,41
90	14,98	5,36	20	64,218959	4,81	4,81
105	14,98	4,56	20	69,559413	5,21	5,21
120	14,98	3,76	20	74,899866	5,61	5,61

135	14,98	3,54	20	76,368491	5,72	5,72
150	14,98	3,45	20	76,969292	5,765	5,765
165	14,98	3,3	20	77,970628	5,84	5,84
180	14,98	3,25	20	78,304406	5,865	5,865
195	14,98	2,95	20	80,307076	6,015	6,015
210	14,98	2,95	20	80,307076	6,015	6,015
225	14,98	2,95	20	80,307076	6,015	6,015

Table A-I.5: Experimental data for the adsorption of bacteria DNA onto BD1 (0.1 M), BD2 (0.5M), BD3 (1.0M) for the effects of adsorbent dose (20 mg) [conditions: pH (6.9), agitation speed 300 rpm, contact time (180, 195, 225 mins for BD1, BD2, and BD3 and at room temperature]

Vol. of adsorbate(ml)	**Adsorb. Dose (mg)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**
2,02	10	9,92	2,9	225	70,766129	3,51
2,02	20	9,92	2,4	225	75,806452	3,76
2,02	30	9,92	1,97	225	80,141129	3,975

Vol. of adsorbate(ml)	**Adsorb. Dose (mg)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**
2,02	10	9,92	2,8	225	71,77419	3,56
2,02	20	9,92	1,8	225	81,85484	4,06
2,02	30	9,92	0,99	225	90,02016	4,465

Vol. of adsorbate(ml)	**Adsorb. Dose (mg)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**
2,02	10	9,92	2,3	225	76,81452	3,81
2,02	20	9,92	1,48	225	85,08065	4,22
2,02	30	9,92	0,75	225	92,43952	4,585

Table A-I.6: Experimental data for the adsorption of bacteria DNA onto BD1 (0.1 M), BD2 (0.5M), BD3 (1.0M) for the effects of cation [conditions: pH (6.9), agitation speed 300 rpm, adsorbent dose (20 mg), contact time (180, 195, 225 mins for BD1, BD2, and BD3 and at room temperature]

Vol. of adsorbate(ml)	Adsorb. Dose (mg)	Cation dose (g)	C_i (µg/mL)	C_f (µg/mL)	Time	% Adsorption	Cations used
2,02	30	0,075	9,92	6,9	225	50,44	Na+
2,02	30	0,3	9,92	1,25	225	87,39	Ca2+
2,02	30	0,097	9,92	1,06	225	79,23	Mg2+
Vol. of adsorbate(ml)	**Adsorb. Dose (mg)**	**Cation dose (g)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Cations used**
2,02	30	0,075	9,92	6,25	225	56,99	Na+
2,02	30	0,3	9,92	1,19	225	88	Ca2+
2,02	30	0,097	9,92	1,4	225	85,89	Mg2+
Vol. of adsorbate(ml)	**Adsorb. Dose (mg)**	**Cation dose (g)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Cations used**
2,02	30	0,075	9,92	6,09	225	58,6	Na+
2,02	30	0,3	9,92	1,18	225	88,1	Ca2+
2,02	30	0,097	9,92	1,35	225	89,94	Mg2+

Table A-I.7: Experimental data for the adsorption of bacteria DNA onto BD1 (0.1 M), BD2 (0.5M), BD3 (1.0M) for the effects of anions [conditions: pH (6.9), agitation speed 300 rpm, adsorbent dose (20 mg), contact time (180, 195, 225 mins for BD1, BD2, and BD3 and at room temperature]

Vol. of adsorbate (ml)	Adsorb. Dose (mg)	Anion dose (g)	C_i (µg/mL)	C_f (µg/mL)	Time	% Adsorption	Anion used
2,02	30	0,59	9,92	8,82	225	11,08	CO_3^{2-}
2,02	30	0,73	9,92	8,43	225	15,02	NO_3^-
2,02	30	0,61	9,92	8,14	225	17,94	Cl^-
Vol. of adsorbate (mL)	**Adsorb. Dose (mg)**	**Anion dose (g)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Anion used**
2,02	30	0,59	9,92	8,61	225	13,21	CO_3^{2-}
2,02	30	0,73	9,92	8,31	225	16,23	NO_3^-
2,02	30	0,61	9,92	8,06	225	18,75	Cl^-
Vol. of adsorbate (ml)	**Adsorb. Dose (mg)**	**Anion dose (g)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Anion used**
2,02	30	0,59	9,92	8,53	225	14,01	CO_3^{2-}
2,02	30	0,73	9,92	8,28	225	16,53	NO_3^-
2,02	30	0,61	9,92	8,03	225	19,05	Cl^-

Table B-I.1: Experimental data for the adsorption of bacteria DNA onto MSN+G MSN+S and MSN+U as a function of pH (7.2) [conditions: contact time 360 mins, agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

Initial pH	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity
2,01	3,66	0,42	20	88,52459016	1,62
3,2	3,66	0,49	20	86,61202186	1,585
4,2	3,66	0,55	20	84,9726776	1,555
5,1	3,66	0,78	20	78,68852459	1,44
6,3	3,66	0,83	20	77,32240437	1,415
7,2	3,66	0,9	20	75,40983607	1,38

8,1	3,66	1,45	20	60,38251366	1,105
9,1	3,66	1,66	20	54,64480874	1

Initial pH	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity
2,01	3,66	1,1	20	69,945355	1,28
3,2	3,66	1,23	20	66,393443	1,215
4,2	3,66	1,24	20	66,120219	1,21
5,1	3,66	1,33	20	63,661202	1,165
6,3	3,66	1,39	20	62,021858	1,135
7,2	3,66	1,4	20	61,748634	1,13
8,1	3,66	2,01	20	45,081967	0,825
9,1	3,66	2,16	20	40,983607	0,75

Initial pH	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity
2,01	3,66	0,68	20	81,420765	1,49
3,2	3,66	0,71	20	80,6010929	1,475
4,2	3,66	0,75	20	79,5081967	1,455
5,1	3,66	0,86	20	76,5027322	1,4
6,3	3,66	0,91	20	75,136612	1,375
7,2	3,66	0,99	20	72,9508197	1,335
8,1	3,66	1,95	20	46,7213115	0,855
9,1	3,66	2	20	45,3551913	0,83

Table B-I.2: Experimental data for the adsorption of bacteria DNA onto MSN+G MSN+S and MSN+U as a function of contact time (210, 195, 180 mins for MSN+G, MSN+U, MSN+S respectively) and initial DNA concentration [conditions: pH of 7.2, agitation speed 300 rpm, adsorbent dose of 20 mg and at room temperature]

(MSN+G)

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	1,83	1,83	20	0	0	0
5	1,83	0,85	20	53,55191257	0,49	0,49
15	1,83	0,77	20	57,92349727	0,53	0,53
30	1,83	0,75	20	59,01639344	0,54	0,54

45	1,83	0,67	20	63,38797814	0,58	0,58
60	1,83	0,61	20	66,66666667	0,61	0,61
75	1,83	0,58	20	68,30601093	0,625	0,625
90	1,83	0,46	20	74,86338798	0,685	0,685
105	1,83	0,25	20	86,33879781	0,79	0,79
120	1,83	0,24	20	86,8852459	0,795	0,795
135	1,83	0,23	20	87,43169399	0,8	0,8
150	1,83	0,22	20	87,97814208	0,805	0,805
165	1,83	0,21	20	88,52459016	0,81	0,81
180	1,83	0,2	20	89,07103825	0,815	0,815
195	1,83	0,2	20	89,07103825	0,815	0,815
210	1,83	0,2	20	89,07103825	0,815	0,815

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	3,66	3,66	20	0	0	0
5	3,66	1,44	20	60,655738	1,11	1,11
15	3,66	1,35	20	63,114754	1,155	1,155
30	3,66	1,15	20	68,579235	1,255	1,255
45	3,66	0,97	20	73,497268	1,345	1,345
60	3,66	0,89	20	75,68306	1,385	1,385
75	3,66	0,63	20	82,786885	1,515	1,515
90	3,66	0,62	20	83,060109	1,52	1,52
105	3,66	0,56	20	84,699454	1,55	1,55
120	3,66	0,55	20	84,972678	1,555	1,555
135	3,66	0,54	20	85,245902	1,56	1,56
150	3,66	0,5	20	86,338798	1,58	1,58
165	3,66	0,48	20	86,885246	1,59	1,59
180	3,66	0,45	20	87,704918	1,605	1,605
195	3,66	0,45	20	87,704918	1,605	1,605
210	3,66	0,45	20	87,704918	1,605	1,605

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**

0	5,49	5,49	20	0	0	0
5	5,49	2,82	20	48,63387978	1,335	1,335
15	5,49	2,53	20	53,91621129	1,48	1,48
30	5,49	2,23	20	59,38069217	1,63	1,63
45	5,49	1,99	20	63,75227687	1,75	1,75
60	5,49	1,85	20	66,30236794	1,82	1,82
75	5,49	1,83	20	66,66666667	1,83	1,83
90	5,49	1,77	20	67,75956284	1,86	1,86
105	5,49	1,73	20	68,48816029	1,88	1,88
120	5,49	1,68	20	69,3989071	1,905	1,905
135	5,49	1,66	20	69,76320583	1,915	1,915
150	5,49	1,57	20	71,40255009	1,96	1,96
165	5,49	1,43	20	73,95264117	2,03	2,03
180	5,49	1,38	20	74,86338798	2,055	2,055
195	5,49	1,38	20	74,86338798	2,055	2,055
210	5,49	1,38	20	74,86338798	2,055	2,055

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	7,32	7,32	20	0	0	0
5	7,32	3,95	20	46,0382514	1,685	1,685
15	7,32	3,61	20	50,6830601	1,855	1,855
30	7,32	3,33	20	54,5081967	1,995	1,995
45	7,32	3,25	20	55,6010929	2,035	2,035
60	7,32	3,2	20	56,284153	2,06	2,06
75	7,32	3,19	20	56,420765	2,065	2,065
90	7,32	3,05	20	58,3333333	2,135	2,135
105	7,32	2,77	20	62,1584699	2,275	2,275
120	7,32	2,69	20	63,2513661	2,315	2,315
135	7,32	2,61	20	64,3442623	2,355	2,355
150	7,32	2,59	20	64,6174863	2,365	2,365
165	7,32	2,49	20	65,9836066	2,415	2,415
180	7,32	2,44	20	66,6666667	2,44	2,44
195	7,32	2,44	20	66,6666667	2,44	2,44
210	7,32	2,44	20	66,6666667	2,44	2,44

(MSN+U)

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	1,83	1,83	20	0	0	0
5	1,83	0,87	20	52,45901639	0,48	0,48
15	1,83	0,85	20	53,55191257	0,49	0,49
30	1,83	0,77	20	57,92349727	0,53	0,53
45	1,83	0,69	20	62,29508197	0,57	0,57
60	1,83	0,65	20	64,48087432	0,59	0,59
75	1,83	0,59	20	67,75956284	0,62	0,62
90	1,83	0,55	20	69,94535519	0,64	0,64
105	1,83	0,45	20	75,40983607	0,69	0,69
120	1,83	0,41	20	77,59562842	0,71	0,71
135	1,83	0,38	20	79,23497268	0,725	0,725
150	1,83	0,37	20	79,78142077	0,73	0,73
165	1,83	0,35	20	80,87431694	0,74	0,74
180	1,83	0,35	20	80,87431694	0,74	0,74
195	1,83	0,35	20	80,87431694	0,74	0,74

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	3,66	3,66	20	0	0	0
5	3,66	1,54	20	57,923497	1,06	1,06
15	3,66	1,5	20	59,016393	1,08	1,08
30	3,66	1,38	20	62,295082	1,14	1,14
45	3,66	1,2	20	67,213115	1,23	1,23
60	3,66	1,15	20	68,579235	1,255	1,255
75	3,66	1,01	20	72,404372	1,325	1,325
90	3,66	0,98	20	73,224044	1,34	1,34
105	3,66	0,87	20	76,229508	1,395	1,395
120	3,66	0,77	20	78,961749	1,445	1,445
135	3,66	0,79	20	78,415301	1,435	1,435
150	3,66	0,65	20	82,240437	1,505	1,505
165	3,66	0,6	20	83,606557	1,53	1,53
180	3,66	0,6	20	83,606557	1,53	1,53

195	3,66	0,6	20	83,606557	1,53	1,53

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	5,49	5,49	20	0	0	0
5	5,49	2,88	20	47,54098361	1,305	1,305
15	5,49	2,59	20	52,82331512	1,45	1,45
30	5,49	2,43	20	55,73770492	1,53	1,53
45	5,49	2,11	20	61,56648452	1,69	1,69
60	5,49	2,01	20	63,38797814	1,74	1,74
75	5,49	1,95	20	64,48087432	1,77	1,77
90	5,49	1,88	20	65,75591985	1,805	1,805
105	5,49	1,83	20	66,66666667	1,83	1,83
120	5,49	1,79	20	67,39526412	1,85	1,85
135	5,49	1,68	20	69,3989071	1,905	1,905
150	5,49	1,59	20	71,03825137	1,95	1,95
165	5,49	1,47	20	73,22404372	2,01	2,01
180	5,49	1,47	20	73,22404372	2,01	2,01
195	5,49	1,47	20	73,22404372	2,01	2,01

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	7,32	7,32	20	0	0	0
5	7,32	3,97	20	45,7650273	1,675	1,675
15	7,32	3,88	20	46,9945355	1,72	1,72
30	7,32	3,69	20	49,5901639	1,815	1,815
45	7,32	3,55	20	51,5027322	1,885	1,885
60	7,32	3,33	20	54,5081967	1,995	1,995
75	7,32	3,2	20	56,284153	2,06	2,06
90	7,32	3,06	20	58,1967213	2,13	2,13
105	7,32	2,87	20	60,7923497	2,225	2,225
120	7,32	2,76	20	62,295082	2,28	2,28
135	7,32	2,69	20	63,2513661	2,315	2,315
150	7,32	2,55	20	65,1639344	2,385	2,385
165	7,32	2,51	20	65,7103825	2,405	2,405

180	7,32	2,51	20	65,7103825	2,405	2,405
195	7,32	2,51	20	65,7103825	2,405	2,405

MSN+S

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	1,83	1,83	20	0	0	0
5	1,83	0,77	20	57,92349727	0,53	0,53
15	1,83	0,76	20	58,46994536	0,535	0,535
30	1,83	0,75	20	59,01639344	0,54	0,54
45	1,83	0,69	20	62,29508197	0,57	0,57
60	1,83	0,67	20	63,38797814	0,58	0,58
75	1,83	0,62	20	66,12021858	0,605	0,605
90	1,83	0,58	20	68,30601093	0,625	0,625
105	1,83	0,55	20	69,94535519	0,64	0,64
120	1,83	0,47	20	74,31693989	0,68	0,68
135	1,83	0,43	20	76,50273224	0,7	0,7
150	1,83	0,42	20	77,04918033	0,705	0,705
165	1,83	0,42	20	77,04918033	0,705	0,705
180	1,83	0,42	20	77,04918033	0,705	0,705

Time	C_i (µg/mL)	C_f (µg/mL)	Adsorbent weight (mg)	% Adsorption	Adsorption capacity (qe)	Adsorption capacity with respect to time (qt)
0	3,66	3,66	20	0	0	0
5	3,66	1,66	20	54,644809	1	1
15	3,66	1,64	20	55,191257	1,01	1,01
30	3,66	1,6	20	56,284153	1,03	1,03
45	3,66	1,54	20	57,923497	1,06	1,06
60	3,66	1,46	20	60,10929	1,1	1,1
75	3,66	1,33	20	63,661202	1,165	1,165
90	3,66	1,21	20	66,939891	1,225	1,225
105	3,66	1,19	20	67,486339	1,235	1,235
120	3,66	1,09	20	70,218579	1,285	1,285
135	3,66	0,99	20	72,95082	1,335	1,335

150	3,66	0,95	20	74,043716	1,355	1,355
165	3,66	0,95	20	74,043716	1,355	1,355
180	3,66	0,95	20	74,043716	1,355	1,355

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	5,49	5,49	20	0	0	0
5	5,49	3,56	20	35,15482696	0,965	0,965
15	5,49	3,19	20	41,89435337	1,15	1,15
30	5,49	2,42	20	55,91985428	1,535	1,535
45	5,49	2,38	20	56,64845173	1,555	1,555
60	5,49	2,22	20	59,56284153	1,635	1,635
75	5,49	2,16	20	60,6557377	1,665	1,665
90	5,49	1,89	20	65,57377049	1,8	1,8
105	5,49	1,85	20	66,30236794	1,82	1,82
120	5,49	1,77	20	67,75956284	1,86	1,86
135	5,49	1,68	20	69,3989071	1,905	1,905
150	5,49	1,64	20	70,12750455	1,925	1,925
165	5,49	1,64	20	70,12750455	1,925	1,925
180	5,49	1,64	20	70,12750455	1,925	1,925

Time	**C_i (µg/mL)**	**C_f (µg/mL)**	**Adsorbent weight (mg)**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorption capacity with respect to time (qt)**
0	7,32	7,32	20	0	0	0
5	7,32	4,89	20	33,1967213	1,215	1,215
15	7,32	4,35	20	40,5737705	1,485	1,485
30	7,32	3,56	20	51,3661202	1,88	1,88
45	7,32	3,43	20	53,1420765	1,945	1,945
60	7,32	3,22	20	56,010929	2,05	2,05
75	7,32	3,01	20	58,8797814	2,155	2,155
90	7,32	2,78	20	62,0218579	2,27	2,27
105	7,32	2,7	20	63,1147541	2,31	2,31
120	7,32	2,65	20	63,7978142	2,335	2,335
135	7,32	2,64	20	63,9344262	2,34	2,34
150	7,32	2,59	20	64,6174863	2,365	2,365

165	7,32	2,59	20	64,6174863	2,365	2,365
180	7,32	2,59	20	64,6174863	2,365	2,365

Table B-I.3: Experimental data for the adsorption of bacteria DNA onto MSN+G, MSN+U, MSN+S for the effects of adsorbent dose (20 mg) [conditions: pH (7.2), agitation speed 300 rpm, contact time (210, 195, 180 mins for MSN+G, MSN+U, and MSN+S, and at room temperature]

Vol. of adsorbate (mL)	Adsorb. Dose (mg)	C_i (µg/mL)	C_f (µg/mL)	Time	% Adsorption	Adsorption capacity (qe)
3,01	10	3,66	0,87	210	76,2295082	2,79
3,01	20	3,66	0,38	210	89,6174863	1,64
3,01	30	3,66	0,25	210	93,1693989	1,13666667
3,01	40	3,66	0,2	210	94,5355191	0,865
Vol. of adsorbate (mL)	**Adsorb. Dose (mg)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**
3,01	10	3,66	0,93	195	74,5901639	2,73
3,01	20	3,66	0,45	195	87,704918	1,605
3,01	30	3,66	0,36	195	90,1639344	1,1
3,01	40	3,66	0,27	195	92,6229508	0,8475
Vol. of adsorbate (mL)	**Adsorb. Dose (mg)**	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**
3,01	10	3,66	1,01	180	72,4043716	2,65
3,01	20	3,66	0,74	180	79,7814208	1,46
3,01	30	3,66	0,43	180	88,2513661	1,07666667
3,01	40	3,66	0,39	180	89,3442623	0,8175

Table B-I.4: Experimental data for the adsorption of bacteria DNA onto MSN+G, MSN+U, MSN+S from hospital effluent [conditions: pH (7.2), adsorbent dose (45 mg) agitation speed 300 rpm, contact time (210, 195, 180 mins for MSN+G, MSN+U, and MSN+S, and at room temperature]

Adsorbent Dose/Salt (mg)	C_i (µg/mL)	C_f (µg/mL)	Time	% Adsorption	Adsorption capacity (qe)	Adsorbent sample

45	2,3	0,56	210	75,6521739	0,38666667	HF + MSN+G
45	2,3	0,6	195	1	0,37777778	HF + MSN+U
45	2,3	0,65	180	73,9130434	0,36666667	HF + MSN+S
				8		
				71,7391304		
				3		

Adsorbent Dose/Salt (mg)	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorbent sample**
45	4,13	1,2	210	70,9443099	0,65111111	HF + MSN+G
45	4,13	1,23	195	3	0,64444444	HF + MSN+U
45	4,13	1,33	180	70,2179176	0,62222222	HF + MSN+S
				8		
				67,7966101		
				7		

Adsorbent Dose/Salt (mg)	**C_i (µg/mL)**	**C_f (µg/mL)**	**Time**	**% Adsorption**	**Adsorption capacity (qe)**	**Adsorbent sample**
45	5,96	1,95	210	67,2818791	0,89111111	HF + MSN+G
45	5,96	2,2	195	9	0,83555556	HF + MSN+U
45	5,96	2,35	180	63,0872483	0,80222222	HF + MSN+S
				2		
				60,5704698		

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ETHICAL CLEARANCE CERTIFICATEAREC-150311-008

Certificate Reference Number:	**OKO021SEZE01/19/E**
Project title:	**Effectiveness of functionalizes silver and silica nanoparticles for the removal of the nucleic acidsfrom the wastewater**
Nature of Project:	**PhD in Chemistry**
Principal Researcher:	**Adaora Stella Ezeuko**
Supervisor:	**Prof. O Okoh**

On behalf of the University of Fort Hare's Animal Research Ethics Committee

(AREC)I hereby give ethical approval in respect of the undertakings contained in the above- mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the AREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
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- The Principal Researcher must report to the AREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

- The AREC retains the right to
- • Withdraw or amend this Ethical Clearance Certificate if ○ Any unethical principal or practices are revealed or suspected;
- ○ Relevant information has been withheld or misrepresented;
- ○ Regulatory changes of whatsoever nature so require;
- ○ The conditions contained in the Certificate have not been adhered to.

  • Request access to any information or data at any time during the course or after completion of the project.
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- • In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office.

Yours sincerely

Dr. Craig Tambling
AREC Chairperson

1 November 2019