SCREENING AND BIOSYNTHESIS OF SILVER NANOPARTICLE PRODUCING BIOFLOCCULANT AND ITS APPLICATION IN THE TREATMENT OF WASTEWATER SAMPLE

BY

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DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university. All citations and sources of this information are clearly acknowledged by means of references.

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CERTIFICATION

This is to certify that the content of this project entitled "**Screening and Biosynthesis of Silver Nanoparticles Producing Bioflocculant and its Application in the Treatment of Wastewater Samples**" was prepared and submitted by **ABRIFOR FAVOURED ELOHOR** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MICROBIOLOGY.** The original research work was carried out by him under my supervision and is hereby accepted.

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DEDICATION

I dedicate this work to God Almighty for his divine strength, wisdom and his guidance and also to my parents Engr. & Mrs. Abrifor for their love and support.

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ABBREVIATIONS

Ag	Silver
AgNPs	Silver Nanoparticles
$AgNO_3$	Silver nitrate
Bacillus sp	*Bacillus* specie
BPB	Bioflocculant producing broth
DNA	Deoxyribose nucleic acid
FT IR	Fourier transform infrared spectroscopy
G	Gram
L	Litre
KWN	Karra Wastewater on Nutrient agar
RNA	Ribonucleic acid
RWN	Restaurant Wastewater on Nutrient agar
NPs	Nanoparticles
Nm	Nanometer
OD	Optical density
UV	Ultraviolet

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ABSTRACT

Bioflocculants are biopolymers secreted by microbes like bacteria and fungi. They are capable of causing a phenomenon called; bioflocculation which is the clumping together of fine, dispersed and organic particles. The use of bacteria species for the purpose of bioflocculant production and synthesizing metallic NPs is due to high flocculation efficiency even at low concentrations and its ability to survive at higher concentration of metallic ions. In this study, a total of eleven (11) morphologically different bacteria were isolated. Morphological and biochemical characteristics of the selected bacteria isolate were investigated. The bacteria isolates were screened in kaolin suspension to determine their flocculating activities. Isolate RWN2 showed the highest flocculating activities, follow in order by isolate KWN4 and was identified as *Bacillus* sp. The effects of the physico-chemical parameters such as carbon sources, nitrogen sources and cations on bioflocculant production by isolate KWN4 and RWN2 at different incubation periods were evaluated. The visual observation, UV spectrum analysis, Fourier transform infrared spectroscopy (FTIR) analysis and the flocculating activities of the bacteria mediated nanoparticle was evaluated. Silver nanoparticle synthesized by *Bacillus* sp. KWN4 and *Bacillus* sp. RWN2 was confirmed by a color change from yellowish to brown, the highest absorbance peak in the UV spectrum analysis was recorded after 72 hrs showing a slight peak at 400 nm and the FTIR spectrum analysis showed 15 peaks which were present between 3762 cm^{-1} to 360 cm^{-1}. The bioflocculants and biosynthesized silver nanoparticles was applied as a flocculating agent in the treatment of wastewater sample. This study has shown that *Bacillus* sp. KWN4 and *Bacillus* sp. RWN2 isolated from wastewater is a potential bioflocculant and nanoparticle producer which can be applied in the treatment of wastewater sample.

Keywords: bioflocculants, *Bacillus* sp., nanoparticles, Ultraviolet spectrum, FT-IR spectroscopy

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Since the beginning, water has been utilized for domestic purposes, animal husbandry, and irrigation. The natural generation of groundwater is still enigmatic, but successful methods have been developed to bring it up to the surface (Mathew *et al*., 2014). Surface water has various constituents that needs to be removed from the water supply system. These removed components can be classified into: colloidal solids, settle able suspended solids, and dissolved solids (Kannj and Achi, 2011). Since human daily activities are primarily dependent on water, discharging waste into water can make people sick and harm the environment (Amoatey *et al*., 2011). Wastewater has distinct sources and characteristics from industrial, domestic and stormwater runoff (Amoatey *et al*., 2011). Wastewater is water whose physical, chemical and biological properties have been altered as a result of the introduction of specific substances that render it unsafe for some purposes such as drinking (Amoatey *et al*., 2011). Treatment of drinking water mostly consists of: flocculation, coagulation, filtration and disinfection processes. Administration of a coagulant in water destabilizes the negatively charged particles, creating large flocs through an aggregation process known as coagulation (Dlamini *et al*., 2017). This study provides insight into new and safer ways to protect these contaminated waters by utilizing biosynthesized AgNPs for the treatment of wastewater samples. Flocculation is an crucial parameter used for the removal of suspended solids in domestic and industrial wastewater treatment (Agunbiade *et al*., 2016). Flocculants have a variety of biotechnological applications, including in wastewater and drinking water treatment, dye solution removal, inorganic solid suspensions and industrial downstream processing (Singha, 2012).

Flocculation happens with the help of flocculants, which are natural, organic and inorganic substances that promote the aggregation of particles to form flocs (Agunbiade *et al*., 2016). Natural occurring flocculants are usually in a form of extracellular polymers (EPS) of proteins, glycoproteins, lipids, glycolipids, polysaccharides (such as cellulose) and nucleic acids (Cong-Liang *et al.,* 2012). Bioflocculants are biopolymers secreted by microbes like

bacteria and fungi. They are capable of causing a phenomenon called; bioflocculation which is the clumping together of fine, dispersed and organic particles (Adebayo *et al*., 2020). The main components of extracellular macromolecules such as polysaccharides, proteins and nucleic acid which are biodegradable, safe and environmentally friendly (Adebayo *et al*., 2020).

Nanoparticles (NPs) are particles with a size range of 0.1-100 nm and vary in thickness, active surfaces with essential catalytic properties, and different energy levels that allow adjustment of important electronic properties and shows complete characteristics. Nanospheres, nanorods, nanocubes, nanoplates, nanoribbons, nanotetrapods, and nanoprisms are examples of nanoparticles (Tiquia-Arashiro and Rodrigues, 2018). Silver nanoparticles (Ag-NPs) have been extensively studied for their use as disinfectants in medical institutions, and an increasing amount of research on silver nanoparticles applications in the food industry, drinking water treatment and distribution systems, it has become the most widely used nanoparticles in consumer products (Zheng *et al*., 2011). Given the high availability, the release of silver nanoparticles (AgNPs) into domestic and industrial waste streams is inevitable. These Ag-NPs can adversely affect microbial communities in biological wastewater treatment systems due to their antimicrobial properties (Zheng *et al*., 2008). Various synthetic methods have been employed to meet the requirements of AgNPs. In general, traditional physical and chemical methods seem too expensive and dangerous. Interestingly, biologically-produced AgNPs exhibit high yield, solubility, and high stability (Zhang *et al*., 2005). Silver nanoparticles made from bioflocculants have been reported to have strong antibacterial activity in wastewater and wastewater treatment. Bioflocculant stabilized-AgNPs cthat can reduce bacterial population in wastewater have also been reported (Adebayo *et al*., 2020). Some efforts have been made to combat the pollution and restore water bodies using chemicals such as chlorine (Cl), potassium aluminum sulphate $KAl(SO_4)_2$ and the likes, but the problem is with the use of these chemicals that they can be persistent in nature and in the body system, causing irritation and adverse side effects to the body (Adebayo *et al*., 2020). For this reason, there is a need for safer water treatment alternatives; one that will not persist after use, is harmless, and has the same effect as traditional chemical water purifiers.

1.2 Statement of Problem

Chemical methods are commonly used for water treatment. However, some chemical flocculant are carcinogenic and not biodegradable, and utilization of such chemical flocculants will have an important environmental impact, such as producing toxic sludge containing metal hydroxides which causes problems with their disposal and increasing metal concentration which have an impact on the sustainability of human health. Nanotechnology is a rapidly developing technology. And several methods are currently available to improve industrial production. The most commonly used methods for nanoparticles synthesis are physical and chemical. Both of these methods are known to be efficient and reliable, but they both have some shortcomings, as it requires a lot of energy consumption such as laser ablation while the other may require the use of expensive reducing and oxidizing agents to stabilize the nanoparticles.

1.3 Justification

Synthesis of nanoparticles for the treatment of wastewater requires methods that are essentially eco-efficient with margin of safety to human and animal health. Different chemicals have been applied to synthesize nanoparticles. Although some of these methods are effective, they often resulted in environmental hazards and health threats. The damaging effect of wastewater as well as the physico-chemical treatments used, do not only impose bad impact to the environment and humans but also to the economic viability of any country. Therefore, to ensure the safety of the ecosystem and humans, synthesis of nanoparticles, need novel, effective use of other materials with different or same mechanism of action. Therefore, the utilization of biological methods such as biosynthesis of nanoparticles using a flocculant produced from microorganisms could be a promising approach to replace chemical flocculants as these bioflocculants are derived from polysaccharides or natural polymers which are very attractive because of their eco-friendly products. Compared to chemical flocculants, bioflocculants are safe and stable shear polymers that are sufficiently biodegradable, easy availability of reproducible resources and does not produce side effects from the waste which has continued to increase water and wastewater treatment. Additionally, since silver nanoparticles have been researched to be a good

antimicrobial agents (Oghyanous, 2021), using it as flocculating agent makes it more useful to human health as it can be used to prevent pathogenic microorganisms.

1.4 Aim of the study

The aim of this study is to synthesis silver nanoparticles from a bioflocculant producing bacteria.

1.5 Objectives of the study

- To isolate, screen and select bioflocculant-producing bacteria from wastewater samples
- To identify the selected isolates using morphological and biochemical characterizations.
- To produce bioflocculant, and determine the effect of physico-chemical parameters on production.
- To biosynthesis silver nanoparticle using the selected bioflocculant producing bacteria.
- To apply the bioflocculant produced and biosynthesized silver nanoparticles in the treatment of wastewater samples.

CHAPTER TWO

LITERATURE REVIEW

A survey in literature presents a quite enormous and various body of research on nanoparticles synthesis. Most of this literature is ardent to areas of nanoparticles application in medicine, diagnostic tools, and types of method used for nanoparticles synthesis. Major factors affecting the type of nanoparticle produced are also discussed. Furthermore, characterization and application of nanoparticles in different biotechnological fields are reviewed.

2.1 Screening

Screening may be a strategy in microbiology where the nearness and area wanted by microorganisms is decided among different microorganisms (Yanling *et al*., 2006).

2.2 Flocculants

Flocculants are utilized for the accumulation of colloidal substances and cellular materials and in this way are broadly connected in numerous mechanical forms, counting wastewater treatment, downstream handling, nourishment and maturation forms (Adebami *et al*., 2013).

2.3 Flocculation

Flocculation is the method by which destabilized particles combine into bigger totals so that they can be isolated from the wastewater (Adebami *et al*., 2017).

2.4 Types of Flocculants

Flocculants are classified into three groups: organic, inorganic and naturally occurring flocculants (Sedar *et al*., 2006). Natural occurring flocculants are more often than in a frame of extracellular polymers (EPS) of proteins, glycoproteins, lipids, glycolipids, polysaccharides and nucleic acids (Cong-Liang *et al.*, 2012). Depending on the charge of the flocculant they can either be anionic, cationic, and neutral.

2.4.1 Organic Flocculants

Organic flocculants such as polyacrylic acid, polydiallylmethyl ammonium chloride and polyacrylamide (PAM) have been the most part utilized completely different mechanical forms due to their high viability and low taken a toll, inspite of the fact that they are exceptionally destructive to both people and environment (Kwon *et al*., 1996). The monomers of acrylamide are known to be neurotoxic as well as carcinogenic to people while aluminum salts have been detailed to cause Alzheimer's disease (Shadia *et al*., 2011)

2.4.2 Inorganic Flocculants

This class of flocculants is widely used in water treatment and fermentation industries due to their excellent flocculating rate and low cost (Piyo *et al*., 2009). Available polyvalent metal salts such as aluminum sulphate and ferric chloride fall into this class and have been reported evidence of their ability as efficient flocculants (Piyo *et al*., 2009). However, these flocculants have their own usage limitations:

- Required or needed in large amounts
- They are very sensitive to pH fluctuations (Brostow *et al*., 2009)
- They are not applicable to all types of dispersing systems
- They are not effective at flocculating very fine particles

2.4.3 Naturally occurring Flocculants

This group includes chitosan, sodium alginate and microbial flocculants (Xia *et al*., 2008; Yim *et al*., 2007). Within this group, bioflocculants are of great interest due to the following advantages:

- They are nontoxic, harmless and non-cross contamination (Xia *et al*., 2008).
- They are biodegradable (Lachhawani, 2005)
- They form strong and larger flocs without affecting the pH of the medium, causing a faster sedimentation process than those of simple solidifying electrolytes.
- They are cost-effective and require lower amounts (1-5 ppm).

2.5 Bioflocculation process

The natural process of flocculating, settling, and removing particles, suspended solids, and color in wastewater using bioflocculants is called bioflocculation (Tsao *et al.,* 2006). Macromolecules that microorganisms excrete into the environment as a result of substrate metabolism, microbial growth, and microbial degradation are potential bioflocculants (Carlos *et al*., 2011). Both prokaryotic and eukaryotic microbes can secrete micro flocculants (Wingender *et al*., 1999). Microorganisms, especially bacteria are short-lived, versatile, and mostly produce bioflocculants (Adewale *et al.,* 2012). Microbial flocculants perform fundamental bacterial capacities such as clumping bacterial cells, adherence to surfaces, shaping flocs and biofilms, and holding water to

diminish cell drying up. Bioflocculants absorb both organic and inorganic compounds (Wingender *et al.*, 1999; More *et al*., 2014).

Bioflocculants also knowns as biocoagulants are defined as encapsulated, slimy, loosely bound or tightly bound depending on the method used to bind to or extract and separate them from cells. (Hendricks, 2006). Microbial strains from algae, actinomyces, bacteria, and fungi have been shown to produce bioflocculants (He *et al*., 2010). Over a 100 species of microorganisms that produce bioflocculants have been described and characterized for their bioflocculant properties or activities (Ahmad *et al*., 2015). Bioflocculants show high flocculation efficiency even at low concentrations (Lin *et al*., 2011). This is due to the microbial flocculant matrix, a special microbial flocculant component with that show adsorption capacities, hydrophobicity, hydrophilicity and biodegradability. Bioflocculant is much safer to use than synthetic flocculants. Some microorganisms are useful in various biotechnological applications. However, some may be opportunistic and pathogenic via secondary metabolites (Spellman *et al*.,2014). Toxic compounds can damage to immune cell function, causing cell lysis or even cell-death. This may directly or indirectly lead to an increase in human infections and diseases (Mims *et al.*, 2004).

Therefore, for biosafety reasons, only bioflocculants from bacterial strains can be used for food, feed production or wastewater treatment. These bioflocculants should be tested for toxicity before use (Zhong *et al*., 2014). In this study, the cytotoxicity and potential genotoxicity of the harvested, purified bioflocculants are determined using the diphenyl-tetra-zolium bromide (MTT) assay and Ames test, respectively (Zhong *et al*., 2014).

2.6 Factors affecting bioflocculation production

A variety of factors such as inoculum size, carbon sources, nitrogen sources, temperature, initial pH, shaking speed, and incubation period are likely to affect bioflocculant production.

2.6.1 Effect on Inoculum size

Inoculum size is an important parameter in the production of a bioflocculants. (Mabinya *et al*., 2011). Inoculum size prolongs the plateau of bacteria growth and the niche of a large inoculum

sizes, inhibiting bioflocculant production (Zhang *et al.*, 2002). Thus, insufficient inoculum size prolongs the lag phase of microbial growth there by retarding bioflocculant production. Bioflocculants are produced during the late stages of exponential growth. However, larger inoculum sizes leads to faster nutrient depletion due to competition between microorganisms (Yokoi *et al.*, 1997).

2.6.2 Effect of carbon sources on bioflocculant production

Organic carbon sources include glucose, sucrose, fructose, maltose, starch, lactose, xylose and molasses. All microorganisms require nutrients as their energy source for growth (Liu *et al.*, 2009). Different carbon sources affect bioflocculant production by different microorganisms to different extents (Deng *et al*., 2005). Bioflocculant produced by the consortium of *Provivencia rettger*i and *Bacillus megaterium*, prefer glucose, xylose, maltose, starch, lactose, fructose and sucrose as their sources of carbon.

The impact of various carbon reassessment at the fabricating of a bioflocculant delivered with the help of utilizing *Bacillus* sp. affirmed that sucrose ended up the first-rate carbon supply for each preparations of bioflocculant and cellular boom in any case within the nearness of maltose and ethanol decently low bioflocculant gotten to be accomplished. A study by Mabinya *et al*. (2012) identified Arthrobacter species. Raats produced the highest flocculating activities of 75.4% and 73.4%, respectively, but lactose and sucrose were applied because in the presence of fructose and starch the carbon was restored, respectively, and no bioaggregation interest was detected. The highest flocculant activity (97.15%) is achieved with a bioflocculant produced from Serratia ficaria, while lactose was the optimal carbon source. Glucose and ethanol have also been shown to be beneficial as carbon sources.

The new carbon source, palm jaggery and maltose have been located to be each beneficial for bioflocculant manufacturing through *Bacillus subtilis* MSBN17 in addition to growth of the cell. Palm jaggery become the maximum desired and most inexpensive carbon supply than industrial sugar and it resulted in the maximum flocculating activity of up to 92.07%.

2.6.3 Effect of nitrogen sources on bioflocculant production

Zaki *et al.* (2014) studies showed that nitrogen sources are important trophic nutrient factors driving the production of bioflocculants when microorganisms use organic and inorganic nitrogen sources or both to produce bioflocculant. Examples of organic nitrogen sources included peptone, urea, yeast extract and casein. Inorganic nitrogen sources include ammonium chloride, ammonium sulphate and sodium nitrate. Liu *et al.* (2009) reported that *Chryseobacterium daeguense* W6 uses an organic nitrogen source, with tryptone being the most preferred, to produce a bioflocculant, resulting in over 90% flocculating activity, while all inorganic nitrogen sources reported poor accumulation activity. According to Piyo *et al*. (2009) observed that *Bacillus* sp. Gilbert made good use of inorganic nitrogen sources such as ammonium chloride to produce a bioflocculant with a flocculating activity of 91% of his, while organic nitrogen sources such as urea and peptone were rarely included. When organic nitrogen sources have been utilized, bioflocculant fabricating ended upmore reasonable in comparison to inorganic nitrogen sources with yeast extricate since the specified nitogen supply helping the leading flocculating charge of 78% (Zheng *et al*., 2008). *Arthrobacter* sp. Raats become capin a position to make use of each natural (urea)was able to utilize both organic (urea) and inorganic (ammonium sulphate) nitrogen sources with urea being greater efficaciously used and ensuing in the maximum flocculating activity.

Bioflocculant manufacturing through *S. ficaria* now become no longer favoured withinside the presence of urea, peptone and ammonium sulphate as nitrogen sources. Although each both beef/pork and yeast extracts have been personally supportive of bioflocculant manufacturing, the very best flocculating activity become performed whilst a combination of the 2 nitrogen reassests (pork extract and urea) become utilized (Gong *et al*., 2008). From the numerous nitrogen sources investigated, inorganic nitrogen supply (ammonium sulphate) become a desired supply for each manufacturing and cell growth, at the same time as all natural nitrogen sources examined caused terrible bioflocculant manufacturing and cell growth (Sathiyanarayanan *et al*., 2013).

2.6.4 Effect of Temperature on Bioflocculant production

Temperature plays an important role in bioflocculant production because different microorganisms prefer different growth temperatures (Gao *et al.*, 2009). It has been reported that the flocculation rate increases with increasing growth temperature (Yang, *et al.*, 2007). The

metabolism of microorganisms has a direct relationship with the culture temperature (Kurane and Nakata, 1991). The literature reports that most of the bioflocculanting microorganisms prefer a temperature range of 25^{o}C to 35 ℃ for bioflocculant production. The enzymatic reaction is directly affected by optimal temperature used, which in turn affects the production of a bioflocculant (Kurane and Nakata, 1991). In another study, bioflocculant production by some microbial populations was reported to be optimal at 30 ^{o}C (Zhang *et al*., 2007). The fungi *Aspergillus parasiticus*, *Arthrobacter* sp. Raats, *Cobetia* sp., induced their bioflocculants at 28 ℃ in a study reported by Deng *et al*. (2005) and Ugbenyen *et al*. (2012).

2.6.5 Effect of initial pH on growth medium on bioflocculant production

The culture medium pH and oxidation potential determine cell charge, which can affect nutrient uptake an enzymatic reactions (Xia *et al*., 2008). The TJ-1 bioflocculant produced by *Proteus mirabilis* reaches its optimum pH when the initial pH is adjusted to neutral pH 7, resulting in large amounts of acid or base being used to adjust the pH, was saved reducing the cost of producing this bioflocculant quantities of acids or alkali used to adjust the pH, therefore reducing the production cost of this bioflocculant. Microorganisms have different initial pH values for bioflocculant production. For *Aspergillus parasiticus*, lower pH was found to be favourable for fungal growth and bioflocculant production (Deng *et al*., 2005). The optimal pH ranged from 5 to 6 and bioflocculant growth and production decreased above pH 7 (Deng *et al*., 2005). The optimal pH for production of bioflocculant produced by *Penicillium purpurogenum* was achieved by adjusting the initial average pH to5.5. When the initial pH was adjusted to 5-8, this bioflocculant was able to generate several pH regulators as a pH change of 5.5 occurred after 1 day of culture and remained constant through day 4 (Liu *et al*., 2010).

2.6.6 Effect of shaking speed on bioflocculant production

The stirring rate enhances the flocculation activity increasing the aeration process where the nutrients are uniformly distributed in the growth medium (Maliehe *et al.,* 2015). The bioflocculant generation is proportional to and dependent on the dissolved oxygen tension (DOT) of the culture medium. The increase or decrease of microbial respiration is strongly dependent on the increase or decrease in DOT of the culture medium, the nutrient uptake and enzymatic reactions are dependent on DOT (Salehizadeh and Shojaosadati, 2001). Choi *et al.* (1998) reported that production of bioflocculant from different microorganisms requires different rates.

Improved bioflocculation production by *Paecilomyces sp.* at 200 revolutions per minute (rpm). In another study Kurane and Nokata (1991) observed that the by *Alcaligenes latus* bioflocculant was produced at 300 rpm. Unlike these, the bioflocculating agent TJ1 was produced at 130 rpm by *Proteus mirabilis* (Zhang *et al.*, 2010).

2.6.7 Effect of incubation period on bioflocculant production

Agreeing to discoveries by Deng *et al*. (2005), flocculation increment with the time of incubation. Between the incubation period of 24 to 60 hrs, *Aspergillus parasiticus* was in its exponential development stage and the flocculating action expanded from 12 to 95% respectively. Past a development period of 72 hrs, the fungus comes to its stationary stage in which the most elevated flocculating rate for Kaolin suspension was 98.1% (Deng *et al*., 2005). *P. mirabilis* TJ1 showed a growth curve in which bioflocculant generation was nearly parallel with cell development amid the logarithm stage and the pH of culture medium diminished strongly. This bioflocculant comes to its most noteworthy flocculating activity of 93.13% at stationery stage (Xia *et al*., 2008). A study carried out by Liu *et al*. (2010) on bioflocculant generation by *Chryseobacterium daeguense* W6, appeared a sharp increment in cell development for the first 8 h of incubation whereas the culture pH diminished marginally. In any case the flocculating action was exceptionally low. During the death phase of the growth curve, the flocculating rate increased to 90% after 54hrs when the OD600 was below 0.3 and it can be concluded that MBF-W6 was an intracellular bioflocculant.

2.7 Bioflocculation process and the contributing factors in flocculating efficiency

2.7.1 Effect of bioflocculant dosage on flocculation process

To attain most extreme flocculating activity of the bioflocculant, measurement estimate is one of contributing components which should be taken into thought (Zufarzaana *et al.*, 2012). Analysts are inquisitive about finding a fetched viable bioflocculant that must be compelling at low measurement. Diverse measurement sizes of diverse microorganisms have been reported within the past decades. Takeda *et al.* (1991) detailed on a bioflocculant which required 20 mg/l dose to realize the most noteworthy flocculating activity, delivered by *Rhodoccus erythropolis* and

Enterobacter sp. In 2010. Lee *et al*, proposed that a bioflocculant created by *Arcualendron* sp. require 2 mg/l to reach the most noteworthy flocculating activity.

A consortium bioflocculant created by *Pestalotiopsis* sp., *Bacillus* sp and *Gyrodinium impudicum* KG03 was compelling at a low measurement of 1 mg/l for high flocculating activity (Lu *et al*., 2005). In another study Zhang *et al.* (2002) documented that the ideal dose which was required to achieve maximum flocculating action was at 30 mg/l from *Sarongium cellusum*. Opposite to this discoveries, 0.1 mg/l bioflocculant was adequate to realize most noteworthy flocculating activity when a bioflocculant from *Bacillus mucilaginosus* was utilized (Deng *et al.,* 2003). Lu *et al.* (2005) reported the most noteworthy dose of 90 mg/l when a bioflocculant by *Enterobacter aerogenes* was observed with kaolin clay.

2.7.2 Cations effect on bioflocculation

Cations invigorate the flocculating activity of microorganisms. (Okaiyeto *et al.,* 2014). Microorganisms utilize distinctive cations, a few favour trivalent, divalent and other monovalent. Cations play a crucial part within the flocculation forms by neutralizing and settling the remaining negative charge of both useful groups and the surface charge of the suspended particles. This debilitate electrostatic repugnance between particles, and upgrading flocculation activity (Yang *et al*., 2007).

2.7.3 Thermal stability of the bioflocculant

In a study conducted by Kurane *et al.* (1994) and Salehizadeh *et al.* (2001), *Rhodococcus erythropolis* and *Bacillus firmus* created bioflocculant, after it was subjected to boiling (bubbling) water for 15 mins. the flocculating activity diminish to 50%. He *et al*. (2008) expressed that the bioflocculant REA-11 did not have any noteworthy diminish in flocculating activity after it was heated at 80 ℃ for 1 h but advance increment in temperature up to 100 ℃ radically diminished the flocculating action. Gong *et al.* (2008) documented that the bioflocculant produced by *Aeromonas sp.* diminished by only 9.2% after it was heated at 100 ℃ for 1 hr. A bioflocculant produced from *Serratia ficaria* decreased flocculating activity by 15% after it was heated at 100 ℃ for 15 min and 20% reduction in flocculating activity after it was subjected to 50 ℃ for 30 mins (Gong *et al*., 2008). A bioflocculant mainly polysaccharide produced from a consortium of *Rhozobium radiobacter* and *Bacillus sphaeicus* retained 90% flocculating activity after heated at 100 ℃ for 30 mins (Wang, 2011). Thermostable

bioflocculant was reported to have maintain 78% flocculating activity after heated for 25 min. at 100 ℃.

2.7.4 Molecular weight effect on flocculating activity of the bioflocculant

Bioflocculants with diverse molecular weights have been reported in literature (Li *et al.*, 2009). Molecular weight of the bioflocculant contributes to its flocculating proficiency (Shojaosadati, 2001). Filtered bioflocculant processed from *B. subtilis* was 1.5 x 105 Da in the study reported by Yokoi *et al.* (1997). A bioflocculant TKF04 with a molecular weight of 3.2 x 105 Da was created from *Citrobacter* sp. (Fujita *et al*., 2000). A thermal and antacid stable biopolymer with a molecular weight of 8.1× 104 Da was created from *Agrobacterium* sp. M-503. Molecular weight could be a critical figure in supporting the bridging instrument in flocculation of kaolin suspension (Zhang *et al*., 2010 and Li *et al*., 2008).

2.8 Nanoparticles

Nanoparticle or ultrafine particle is a particle of matter with at least one dimension smaller than 1 micron and potentially as small as atomic and molecular length scales (~ 0.2 nm) (Vert *et al*., 2012). Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Biodegradable polymeric nanoparticles have been made in recent years, and used for drug delivery devices, particularly those coated with hydrophilic polymer such as poly ethylene glycol (PEG). Furthermore, their ability to circulate for a prolonged time and target particular organs, carry DNA in gene therapy, and having the ability to deliver proteins, has great benefit (Lee *et al*., 2010).

Nanoparticles interact with one another in a variety of ways, they may stay free or establish communities depending on the attractive or repulsive contact forces between them. It is still difficult to categorize these interactions. Nanoparticles suspended in gas appear to bind to each other more readily than those dispersed in liquids. Nanoparticles have sparked a lot of attention due to their unique features and applications that outperform their bulk counterparts. Metal oxide nanopowders such as silicon oxide (SiO_2), titanium oxide (TiO_2), aluminum oxide (Al_2O_3), iron oxide (Fe_3O_4, Fe_2O_3) are of commercial importance. Compound semiconductors (cadmium telluride, CdTe or gallium arsenide, GaAs, etc.), metals [especially precious metals such as silver (Ag), gold (Au), alloys, etc.] are also steadily more used industrially. Nanoparticles have unique biological properties from large surfaces-area-volume ratio and compact size allow efficient

binding, absorption and transport of molecules such as small drug, protein, DNA, RNA, probes (Khan *et al*., 2015).

2.9 Classification of Nanoparticles

Organic, inorganic and carbon-based Nanoparticles are the three sorts of nanoparticles. (Ealias *et al*., 2017). Micelles, dendrimers, liposomes, hybrid and compact polymeric NPs make up the first group, the second group includes Metal based Nanoparticles, Metal oxides-based Nanoparticles and the third group includes fullerenes, graphene, carbon Nano tubes (CNT), carbon nanofibers and carbon black (Ealias *et al*., 2017).

2.9.1 Organic Nanoparticles

- **Micelles:** Micelles are amphipathic molecules formed into nanostructures such as polymers and lipids. When exposed to the aquatic environment, it hides hydrophobic groups in the structure and reveals hydrophilic groups. On the other hand, in a lipid-rich environment, their structure can be reversed. For amphipathic drugs, the polar group of the drug aligns with the amphipathic micelle structure close to the hydrophilic group of the micelle, while less water soluble drugs can be loaded into the hydrophobic core of the micelle (Ealias *et al*., 2017).
- **Dendrimers:** A dendrimer is an artificial polymer with a tree-like structure, the atoms of which are located on a number of branches originating from the central center (Singh *et al*., 2017).
- **Liposomes:** Liposomes composed of lipids surrounding the water core are the most basic types of nanoparticles (hollow or solid-structured nanoparticles that can load anti-cancer drugs, target substances, and tracers) (Park *et al*., 2002).
- **Compact polymeric NPs**: Nanostructures constructed entirely of natural or synthetic polymers are called Compact Polymer NPs. In most cases, they are more stable than liposomes. Finally, the therapeutic material can be covalently bound, allowing continuous topical drug delivery over several weeks while suppressing drug leakage in these polymer nanostructures (Orive *et al*., 2009). It can also be adsorbed on the surface of NPs, dissolved or trapped in NP structures (nanospheres), or encapsulated in polymer shells (nanocapsules) (Cartaxo *et al*., 2015).

2.9.2 Inorganic Nanoparticles:

Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide based nanoparticles are generally categorized as inorganic nanoparticles.

- **Metal based Nanoparticles:** Are nanoparticles made from metals, either destructively or constructively to nanometric sizes. Aluminum (Al), cadmium (Ca), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn) are the most used metals for the synthesis of nanoparticles (Ealias *et al*., 2017).
- **Metal oxides-based Nanoparticles**: Metal oxide-based nanoparticles are made to change the characteristics of metal nanoparticles. The commonly synthesized are Aluminum oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), Silicon dioxide (SiO_2), Titanium oxide (TiO_2), Zinc oxide (ZnO). In comparison to their metal counterparts, these nanoparticles exhibit an extraordinary characteristic. (Ealias *et al*., 2017).

2.9.3 Carbon-based Nanoparticles:

Nanoparticles that are completely made of carbon are knows as carbon-based nanoparticles (Bhaviripudi *et al*., 2007). They can be classified into fullerenes, graphene, carbon nano tubes (CNT), carbon nanofibers and carbon black and sometimes activated carbon in nano size.

- **Fullerenes:** Fullerenes are like soccer-like structures made of nanostructured carbon atoms. Nanotubes which are a cylindrical accumulation of carbon atoms, can also be formed using fullerenes. Fullerenes have been used in many sensor systems. (Singh *et al*., 2017).
- **Graphene:** Graphene is an allotrope of carbon. It is a hexagonal honeycomb lattice of carbon atoms on a flat two-dimensional surface. Generally, the graphene sheet is about 1 nm thick. (Ealias *et al*., 2017).
- **Carbon Nano Tubes (CNT):** Carbon nanotubes are nanometer-sized cylinders made of more than one layers of graphite. Carbon nanotubes can be classified into two types: single-walled carbon nanotubes and multi-walled carbon nanotubes. (Ealias *et al*., 2017). Carbon nanotubes offer new technologies for drug and gene delivery due to their unique electrical, thermal, and structural properties (Tanaka *et al*., 2004).

- **Carbon Nanofiber**: Carbon nanofibers are made from the same graphene nanosheets as CNTs, but are more conical or cup-shaped than traditional cylindrical tubes (Ealias *et al*., 2017).
- **Carbon black:** A carbon-based amorphous material in the range of 20-70 nm in diameter, spherical in shape. The interaction of the particles is so strong that they bind to each other as aggregates to form aggregates at about 500 nm (Ealias *et al*., 2017).

2.10 Properties of Nanoparticles

Nanoparticles possess either physical or chemical properties.

2.10.1 Physical properties of Nanoparticles

Physical properties include optical properties such as nanoparticle color, light transmittance, absorption and reflectivity, and UV absorption and reflectivity when applied in solution or on a surface. It also includes mechanical properties such as elasticity, ductility, tensile strength and flexibility that are important for use (Ealias *et al*., 2017). Other properties such as hydrophilicity, hydrophobicity, buoyancy, diffusion and sedimentation are incorporated into many modern everyday lives. Magnetic and electrical properties such as conductivity, semi-conductivity, and resistivity have paved the way for use of nanoparticles with state-of-the-art electron thermal conductivity in renewable energy applications (Ealias *et al*., 2017).

2.10.2 Chemical properties of Nanoparticles

Their use depends on chemical properties such as the reactivity of the nanoparticles with the target and stability and sensitivity to variables such as humidity, atmosphere, heat and light. Disinfection of the antimicrobial, antifungal, toxicological skills of nanoparticles is used for biomedical and ecological applications. Nanoparticles corrosive, anti-corrosion, oxidation, reductive and flammability determine their corresponding uses. The amount of air or gas required for the operation is calculated by measuring the concentration of nanoparticles in the gas phase. The power or efficiency of the system is defined by the concentration, size and variance of the nanoparticles in the air or gas region. Condensed particle counter (CPC) is typically used to quantify concentrations (Ealias *et al*., 2017).

2.11 Synthesis of Nanoparticles

Various methods are available for the production of nanoparticles, including chemical, physical, and biological protocols. Chemical synthesis is convenient because it produces a large number of nanoparticles in a short time. However, this technique requires capping chemicals to stabilize the size of the nanoparticles. In addition, the chemical reagents commonly used to produce and stabilize nanoparticles are dangerous and produce environmentally harmful by-products. The demand for environmentally safe and non-toxic nanoparticle manufacturing processes has increased interest in biological systems that avoid the use of harmful chemicals as by-products. Plants, fungi, yeasts, actinomycetes, bacteria, and other natural resources can all be used to produce nanoparticles. Intracellular and extracellular inorganic nanoparticles can be produced by unicellular and multicellular organisms (Vithiya and Sen, 2011).

2.11.1 Physical synthesis of Nanoparticles

Two of the more noteworthy physical approaches for nanoparticles synthesis are laser ablation and evaporation-condensation (Kruis *et al.,* 2000). The physical methods of nanoparticles production includes the use of force to crush, reduce larger bulk materials into nanoparticles. The physical methods are mostly top-down methods, i.e. bulk material-to- nanoparticles. They are: mechanical milling, nanolithography, sputtering, laser ablation, physical vapor deposition (PVD). These various methods cannot be employed in industrial or large-scale production because they require the utilization of high amount of energy, and requires time to achieve thermal stability (Jung *et al.,* 2006). Generally, they are nor very efficient. However, these processes typically require sophisticated equipment, chemicals, radiant heat, and high power consumption, which increases operating costs. (Khandel *et al*., 2018).

The preferences of physical synthesis methods are the absence of solvent contamination in the prepared thin films and the consistency of nanoparticles (NPs) dispersion in differentiation to chemical synthesis. However, physical synthesis of NPs requires In addition, devices such as a tube furnace consume energy and preheating time is fundamental before it reaches a steady working temperature (Magnusson *et al.,* 1999). A exhibit uncovered that silver NPs could be synthesized through a little ceramic heater with a restricted heating area (Jung *et al.,* 2006).

2.11.1.1 Mechanical Milling

Mechanical milling, milling or attrition, is a top-down production method for nanoparticles, in which different elements are ground in a non-reactive atmosphere. It synthesizes amorphous or crystalline nanoparticles. This process is performed using a planetary ball, tumble mill, or high energy vibrator. Nanoparticles are produced by the shearing effect of the balls during grinding (Algarasi *et al*., 2011). Oxidation of powdered nanoparticles should be handled in a vacuum system or glovebox. The size, number and velocity of the balls all affect the energy transferred to the nanoparticles to prevent this (Algarasi *et al*., 2011).

2.11.1.2 Nanolithography

Nanolithography is a method of manufacturing nanoscale materials using a variety of processes. Optical, e-beam, multiphoton, nanoimprint, and scanning probe lithography (Ealias *et al*., 2017). In this process, the desired shape is printed onto a photosensitive material and portions of the material are carefully removed to create the desired shape and structure (Ealias *et al*., 2017). It is expensive to operate and maintain.

2.11.1.3 Laser Ablation

Laser ablation is a commonly used method to produce nanoparticles from solvents (Ealias *et al*., 2017). When a laser beam hits a metal while immersed in a liquid solution, a plume of plasma that forms nanoparticles condenses (Amendola *et al*., 2009). It serves as an alternative to chemical reduction of metals to form nanoparticles, producing nanoparticles stably in organic solvents and without the need for chemicals (Ealias *et al*., 2017).

2.11.1.4 Sputtering

This is the deposition of nanoparticles on a surface by expulsion of particles from the surface by collision with ions (Shah *et al*., 2006). A thin layer of nanoparticles is deposited and then strengthened. Some factors that affect nanoparticle size and shape are layer thickness, annealing temperature, annealing time, and substrate type (Lugscheider *et al*., 1998).

2.11.1.5 Physical Vapor Deposition

Physical vapor deposition is a combination of a nanoparticle manufacturing process and a thin layer deposition process with sizes ranging from a few nanometers to a few micrometers. This method is environmentally friendly and consists of three main steps: evaporation of material from a powerful source, transport of pulverized material, nucleation and development to produce a film of nanoparticles (Ma *et al*., 2014). Other techniques used in physical vapor deposition include sputtering, electron beam evaporation, and pulsed laser deposition (Prabrakaran *et al*., 2021).

2.11.2 Chemical synthesis of Nanoparticles

Most common approach for nanoparticles synthesis is decrease by organic and inorganic decreasing operators (Wiley *et al.,* 2005). Examples of reducing agents which are utilized in metal ions decrease are as follows: elemental hydrogen, ascorbate, sodium borohydride ($NaBH_4$), sodium citrate, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block. Diminishment of metal ions such as silver (Ag+) in aqueous or non-aqueous solutions comes approximately into a course of action of metallic silver (Ag0), which is taken after by cluster course of action. In expansion, the formed clusters definitely result into metallic colloidal silver particles formation (Evanoff *et al*., 2004).

It is basic to utilize defensive amid nanoparticle arrangement to stabilize and ensure the NPs that can be ingested on or tie onto nanoparticle surface (Oliveira *et al.,* 2005). The stabilize molecule development, and ensure particles from sedimentation, agglomeration, or losing their surface or losing their suface properties properties (Evanoff *et al*., 2004).

2.11.2.1 Chemical Reduction

Chemical diminish by reducing and oxidizing administrators is the preeminent common way for synthesizing nanoparticles. Cases of reducing agents which are utilized for nanoparticles synthesis include sodium citrate, sodium borohydride ($NaBH_4$), ascorbate and elemental hydrogen. Diminishing and oxidizing agents are included to stabilize the nanoparticles, the utilization of these agents in the midst of nanoparticles synthesis is outstandingly essential as they in addition prevent the happening of agglomeration (Merga *et al.,* 2007). In expansion,

surfactants which comprises of functionalities such as amines, acids, alcohol and thiols, secure the nanoparticles from losing their surface properties.

Compounds that are polymeric, example: poly (vinylpyrrolidone), poly (vinyl alcohol), and poly (methacrylic acid) have been documented to be successful defensive agents in stabilization of NPs. Synthesis of few metallic NPs can be done at room temperature, by mixing metal ion solution with diminished polyoxometalates which helps as a stabilizing and decreasing agents (Wiley *et al.,* 2005).

2.11.2.2 Micro emulsion Techniques

The technique is based on two-phase aqueous system, which involves the initial spatial separation of reactants (reducing agents and metal precursor) into two immiscible phase. Formed metal clusters at the interface are stabilized, as the results of their surface being coated with stabilizer molecules occurring in the non-polar aqueous medium, and transferred to the organic medium by the inter-phase transporter (Evanoff *et al.,* 2004). The use of highly toxic organic solvents is one of the major shortcomings of this technique. Therefore, huge amount of organic solvents and surfactant must be separated and removed from the final product.

2.11.2.3 Ultraviolet-initiated photo reduction

UV-initiated photoreduction may be a straightforward and viable strategy, which has been recorded for synthesizing NPs with the help of citrate, poly acrylic acid, collagen and polyvinylpyrrolidone. For instance, in a study conducted by Huang *et al*. (2007) NPs were synthesized through photoreduction of silver nitrate in the nearness of citrate which served as stabilizing agent for avoidance of NPs aggregation.

The properties of synthesized NPs were considered as a function of UV irradiation time. The bigger NPs were achieved when irradiated beneath UV for 3 hrs. Disintegrated NPs to smaller sizes come about when further irradiation happened (Merga *et al.,* 2007). Poly (vinylalcohol) has been utilized to synthesize NPs at room temperature by UV irradiation photoreduction. Both the concentration of poly (vinylalcohol) and silver nitrate engage in recreation major part in the development of the dendrites and nanorods (Evanoff *et al* 2004).

2.11.2.4 Electrochemical synthetic method

Synthesis of NPs utilizing electrochemical synthetic strategy makes it conceivable to control particle size by altering electrolysis parameters and by changing the composition of electrolytic solution homogeneity of the synthesized NPs can be accomplished. Electrochemical diminishment brought about in a polyphenylpyrrol coated nanospheroids with measure range between (3-20 nm) (Johans *et al.,* 2002). Nanospheroids (1-18 nm) were gotten in another study by electrochemical reduction inside or outside zeolite crystals concurring to silver exchange degree of compact zeolite film modified electrodes (Zhang *et al.,* 2002). Spherical NPs with size range (10-20 nm) with limit estimate disseminations were suitably arranged in aqueous solution by an electrochemical method (Ma *et al.,* 2004). Poly N-vinylpyrrolidone made a difference to avoid agglomeration as it helps as a stabilizer.

2.11.2.5 Irradiation methods

Production of NPs can be accomplished through the utilization of variety of irradiation methods. A well-defined shape and estimate conveyance NPs were synthesized utilizing laser irradiation of an aqueous solution (Abid *et al.,* 2002). Besides, NPs were synthesized utilizing laser in a photo-sensitization synthetic method. At brief irradiation times, 20 nm NPs were synthesized while 5 nm NPs were synthesized when irradiation time was increased.

2.11.3 Biosynthesis of Nanoparticles

The process of producing nanoparticles from suitable microorganisms such as bacteria, fungi, yeasts, algae, and actinomycetes is called nanoparticle biosynthesis (Li *et al*., 2011). Antibacterial activity is widespread in these bacteria and actinomycetes. Actinomycetes are Gram-postive, aerobic, spore-forming bacteria. They exhibit branched filamentous growth and contain high guanine plus cytosine content in their nucleic acid -DNA (Chamikara, 2016). They are Gram-positive mycelial bacteria capable of producing various compounds important to the industry and medical field such as: fungicides, immunosuppressants and antibiotics (Kodzius and Gojobori, 2015). They possess the ability to produce antibiotics and other important therapeutic compounds. The spore-forming characteristic of actinomycetes supports the production of nanoparticles.

Microorganisms produce bioactive metabolites (enzymes, proteins) when exposed to metal ions. The presence of these bioactive metabolites is essential for the reduction of nanoparticles. In addition, the proteins released by the microorganism's act as capping agents, ensuring stable nanoparticle production. Each microorganism has its own metabolic processes and enzymatic activity, and not all microorganisms can produce nanoparticles. In this sense, the selection of appropriate microorganisms is essential for the formation of nanoparticles (regardless of enzyme activity or metabolic pathways) (Li *et al*., 2011). Nanoparticles are formed when microorganisms attack ions in the environment and use enzymes provided by cellular processes to convert metal ions into detailed metals. It is possible to distinguish between intracellular and extracellular synthesis depending on the origin of the nanoparticles (Mann *et al*., 2001). The intracellular synthesis of nanoparticles consists of the transport of ions to microbial cells to form nanoparticles in the presence of enzymes (Zhang *et al*., 2011). During extracellular synthesis of nanoparticles, metal ions are trapped on the cell surface and reduced in the presence of enzymes (Zhang *et al*., 2011). Compared to the extracellular pathway, extracellular production is cheaper and has been widely used. It can be used primarily for the synthesis of large quantities of material requires simple downstream treatments that avoid many synthetic processes and it is easy to separate and industrialize. While obtaining nanoparticles by intracellular synthesis is due to the fact that additional steps are needed, such as: the cell biomass is centrifuged and subjected to multiple ultrasonic cycles to destroy the cells to obtain purified nanoparticles. However, the exact underlying mechanism has not yet been fully elucidated (Markus *et al*., 2016).

Biological processes are of great interest in the synthesis of metals and metal oxide nanoparticles due to their low use of toxic chemicals, environmental friendliness and low energy consumption. Nanoparticles are biosynthesized using biologically active products derived from plants and microorganisms such as bacteria, fungi and yeast. This method is promising due to its effectiveness, environmentally friendly technology, low cost, simplicity and mass productivity (Kasi *et al*., 2014). In addition, the biological production of metals and metal oxide nanoparticles uses metal precursors normally present as soluble salts that precipitate in suspensions containing extracts of biological components from microbial cells or microorganisms is required. The synthetic reaction takes minutes or hrs, depending on the growth conditions, and the white suspension or suspension color changes to the deposits in the lower flask (Mohd *et al*., 2019). As a result, this indicates a successful change. Temperature, pH, metal precursor concentration, and

reaction time are also important factors in determining nanoparticle formation rate, yield, and morphology. The properties of the nanoparticles produced are physico-chemically investigated, including shape, size, functional groups, surface charge and purity (Król *et al*., 2017). The production of metals and metal oxide nanoparticles requires the ability of microorganisms to withstand heavy metals. In addition, it is well known that increased metal exposure can affect a variety of microbial activity. When stressed, microorganisms tend to break down ions into their respective metals. This allows them to function as natural nanofactories. Metal adsorption and chelation by intracellular and extracellular proteins results in high metal resistance to microorganisms that live in metal-rich ecological niches. Therefore, mimicking the natural biomineralization process could be a promising strategy for the synthesis of metals and metal oxide nanoparticles. Various metal-reducing microorganisms have been isolated for the production of metal nanoparticles (Mohd *et al*., 2019).

Compared to other eukaryotic microorganisms, bacteria have been used in the synthesis of nanoparticles due to their ease of handling and genetic engineering properties (Velusamy *et al*., 2016). Due to their non-pathogenicity and high production of various enzymes, reproducible bacteria such as lactic acid bacteria have received a great deal of attention in the production of nanoparticles through the bacteria. Lactic acid bacteria are also known as dietary health-promoting microorganisms (Prasad *et al*., 2009). In addition, lactic acid bacteria are facultative anaerobic bacteria, and because of their negative electrodynamic potential, they are easily attracted to metal ions to form nanoparticles under redox conditions (Prasad *et al*., 2009). Previous studies have shown that fungi can bind to the surface of nanoparticles to produce large amounts of extracellular proteins that stabilize and prevent aggregation (Sarkar *et al*., 2014). As a result, it is advantageous to use fungi for nanoparticle synthesis because they are efficient in secreting extracellular enzymes and proteins. Yeast is highly resistant to toxic metals and has been shown to synthesize fungal-like metal nanoparticles (Mohd *et al*., 2019). Nanoparticle size, shape and dispersion have been shown to be affected by reaction time. Micro-mediated synthesis of nanoparticles appears to be environmentally friendly and safe because the synthetic process does not use harmful or dangerous chemicals. This method is superior to the traditional method. In addition, fungal-mediated synthesis appears to be a potential competitor to synthesis, as it produces more physiologically active chemicals than other microorganisms. Nevertheless, microorganisms are promising in terms of cell proliferation activity compared to other options.

In addition, the mechanism of biosynthesis of nanoparticles varies from microorganism to microorganism and is not fully understood, so further research is needed (Mohd *et al*., 2019).

Table 2.1: Biosynthesis of Nanoparticles (Vithiya and Sen 2011)

Sources	Names of the organisms	Localization	Types of Nanoparticles Produced	Size Ranges (nm)
Plant	*Azadirachta indica* (Neem)	Extracellular	Ag, Au	50-100
	Geranium leaves plant extract	No	Ag	16-40
	Avena sativa (Oat)	Extracellular	Au	5-85
	Aloe vera	Extracellular	Au	50-350
Fungi	*Fusarium oxysporum*	Intracellular	Au	20-40
	Verticillium sp.	Intracellular	Ag	25-12
	Aspergillus fumigatus	Intracellular	Ag	5-25
	Schizosaccharomyce pombe	Intracellular	CdS	200
	Fusarium oxysporum and *Verticillium sp.*	Intracellular	Magnetite	20-50
Yeast	Yeast strain MKY3	Extracellular	Ag	2-5
	Candida glabrata	Intracellular	CdS	200
	Schizosaccharomyce pombe	Intracellular	CdS	200
Bacteria	*Pseudomonas stutzeri*	Intracellular	Ag	200
	LactoBacillus strains	Intracellular	Ag, Au	No
	Escherichia coli	Intracellular	CdS	2-5
	Klebsiella pneumonia	Extracellular	Au	5-32

2.11.3.1 Biosynthesis of metallic Nanoparticles using bacteria

Frequent exposure of bacteria to distinctive and sometimes harsh natural conditions in nature requires them to have a survival mechanism. Continuance in these extraordinary conditions is dependent on their capacity to withstand natural stresses (Dhillon *et al*., 2012). To resist assortment of stresses such as high poisonous quality within the environment as a results of high metallic ions concentrations, natural defense mechanisms exist in bacteria to support overcome these toxic conditions. Mechanisms such as changes in metal concentration via redox responses offer assistance to microorganisms to deal with environmental conditions biologically. Besides, systems such as efflux, intracellular accumulation, and precipitation of metals and extracellular formation of complexes offer assistance to microorganisms to survive in high metal concentrated environment (Dhillon *et al*., 2012).

Bacterial species which are primarily utilized for metallic nanoparticles synthesis comprises of *Alcaligenes faecalis, LactoBacillus spp., Actinobacter spp*., *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Corynebacterium spp*., and *Pseudomonas spp*. (Iravani, 2014; Sunkar *et al*., 2012; Tollamadugu *et al*., 2011). The biological decrease of Ag+ ions to Ag nanoparticles includes an electron transfer process (Ahmed *et al.*, 2003). Au ions (Au+ and Au3+) were decreased to Au nanoparticles by *Pseudomonas aeruginosa* in a study conducted by (Husseiny *et al*., 2007). Be that as it may, non-involvement of biological enzymes has also been demonstrated. For example, in a study by Liu *et al.* (2005) Ag nanoparticles were synthesized from dried cells of *Bacillus megaterium.* (Sneha *et al.*, 2005). They were also able to prove that non-enzymatic reactions did not happen when *Corynebacterium sp* was used in Au particles formation.

Different components such as appropriate pH, few organic functional groups presence at the cell wall, and temperature are accepted to actuate nanoparticle reduction (Lin *et al.,* 2001). Fu *et al.* (2000), reported that *LactoBacillus sp*. A09 and *Bacillus megaterium* D01 dried biomass uncovered that Ag ions reduction to produce nanoparticle can come about as a results of the interaction of functional groups found in cell wall of the bacteria. Physical parameters such as temperature and pH changes can moreover essentially have impact on size, shape, and composition of a nanoparticle (Hulkoti *et al.,* 2013). Therefore, optimization of synthesis parameters amid nanoparticle formation is important to move forward general properties of the formed particle. Size and morphology of nanoparticles were shown to have impact on both pH

and metallic salt concentration. At a pH of 6, spherical Au nanoparticles with size range between 10-20 nm were sythnesized at low concentration of $AuCl_4$, when *Rhodopseudomonas capsulata* was utilized (He *et al.*, 2008). When the salt concentration was increased above pH 6, Ag nanowires came about (He *et al.*, 2008). Alteration of the pH brought about in comparable results when the pH was changed to 4, both spheres and triangular nanometre scale plates were synthesized by altering the pH in the solution He *et al.*, 2008). The studies indicate that medium's pH should be controlled to urge nanoparticles with desired morphology.

2.12 Different Factors which affect Nanoparticle Synthesis

A number of controlling components such as pH, reactant concentrations, reaction time, and temperature have impact on biological synthesis of metallic nanoparticles

2.12.1 Effect of pH on nanoparticles Formation

The pH of the reaction medium accounts for a large part of nanoparticle formation (Gardea-Torresdey *et al*., 1999). Recent studies have shown that nanoparticles have different sizes and shapes due to changes in the pH of the reaction medium. Low (acidic) pH values resulted in the formation of larger nanoparticles compared to high (alkaline) pH values (Sathishkumar *et al*., 2010). For example, Avena sativa (oat) biomass at pH 2 produced rod-shaped Au nanoparticles with larger sizes (25–85 nm). Small particles ranging in size from 5 to 20 nm were observed at pH 3 and 4 (Armendariz *et al*., 2004). This indicates that between pH 3 and 4, the extract contained more functional groups accessible for the formation of Au nanoparticles. At pH 2, particle aggregation occurred due to fewer functional groups present in plant extracts.

In a similar study, Cinnamon zeylanicum bark extract produced an increase in nanoparticle numbers with increasing bark extract concentration. The shape of the nanoparticles became spherical above pH 5 (Armendariz *et al*., 2004). In the synthesis of palladium (Pd) nanoparticles from Zeilanicum cinnamon bark extract, the particle size did not increase with increasing pH. Below pH 5, the particle size ranged from 15 to 20 nm and at higher pH values from 20 to 25 nm (Sathishkumar *et al*. 2009).

2.12.2 Effect of reactant concentration on nanoparticles formation

The concentration of biomolecules in plant extracts can affect the formation of metal nanoparticles. A study using extracts of sun-dried camphor (camphor) leaves found that the

amount of camphor extract changed the shape of Au and Ag nanoparticles synthesized in the reaction medium (Huang *et al*., 2007). For example, using chloroauric acid as a precursor and adding concentrations of the extract changed the shape of the resulting nanoparticles from spherical to tetrahedral pyramidal. Similarly, the amount of Aloe vera leaf extract in reaction media containing spherical gold nanoparticles chlorella varied from triangular platelets (Chandran *et al*., 2002). The presence of carbonyl compounds in the extract helps form particle growth. Particle size was adjusted by extract concentration between 50 and 350 nm. Similarly, varying the concentration of Plectranthus amboinicus leaf extract in the medium yielded Ag nanoparticles with different shapes (Narayanan *et al*., 2010).

2.12.3 Effect of reaction time on nanoparticles formation

Reaction time is an important factor in the synthesis of spherical Ag nanoparticles (Ahmad *et al*., 2012). Ananas comosus (pineapple) extract showed a rapid color change observed within 2 minutes. (Ahmad *et al*., 2012). Nanoparticles were observed within 2 minutes in the reaction medium after rapid reduction of aqueous $AgNO_3$. Only a slight color change was observed after a reaction time of 5 minutes. Spherical nanoparticles with a size of about 12 nm were formed.Dwivedi and Gopal (2007) synthesized Ag and Au nanoparticles using pigweed album leaf extracts. Synthesized nanoparticles appeared within 15 minutes and continued to form for up to 2 hrs. Very few nanoparticles were produced after 2 hrs (Prathna *et al*., 2011). Prathna *et al*. (2011) showed that increasing the reaction time in the combination of azadirachta indica leaf extract and his AgNO3 led to an increase in nanoparticle size. Particle sizes range from 10 to 35 nm with a time variation of 30 minutes to 4 hrs (Prathna *et al*., 2011).

2.12.4 Effect of reaction temperature on nanoparticles formation

Reaction temperature is well known as a critical factor in any synthesis. Temperature has been shown to be an important factor in determining the size, shape and yield of nanoparticles formed from plant extracts (Sathishkumar *et al*., 2010). For example, using Citrus sinensis (sweet orange) at 25 °C synthesized his Ag nanoparticles with an average size of ~35 nm. However, an increase in reaction temperature is caused by shrinking the particle size of the nanoparticles to 10 nm at 60 °C (Kaviya *et al*., 2011). Like one by Song *et al*. (2005) Synthesis of highly stable Ag nanoparticles in the reaction temperature range of 25 - 95°C. In a study using Avena sativa (oat) biomass, thermal modification of the reaction conditions altered the size and shape of the

synthesized Au nanoparticles (Armendariz *et al*., 2004). According to Gericke (2006), the high formation rate of Au nanoparticles is promoted by high temperature. The formation of spherical Au nanoparticles occurs at low temperatures and the formation of plate-like Au nanoparticles occurs at high temperatures (Gericke, 2006). The rate of reaction and particle formation appears to increase with higher or higher temperatures of reaction, while the average particle size decreases and the number of particles increases amid the reaction. This all depends on the nucleation process (Armendariz *et al.*, 2004).

2.13 Characterization of Nanoparticles

The physicochemical properties of nanoparticles are vital for their behavior, bio-distribution, security, and viability. In this manner, characterization of AgNPs is vital in arrange to assess the useful perspectives of the synthesized particles. Characterization is performed employing an assortment of explanatory procedures, including UV-vis spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) (Ealias *et al*., 2017).

2.14 Applications of Nanoparticles

Below are some of the significant applications of Nanoparticles

2.14.1 In Medicine

The use of nanoparticles in drug delivery has benefited medicine thanks to nanotechnology. The fundamental challenge in designing and developing an innovative drug delivery system is the accurate and safe delivery of the drug to the target area at the right time to achieve controlled release and maximize the therapeutic effect. To reach the target cells, the target nanocarriers must cross the barriers of blood tissue. They must cross the cellular barrier and enter the target cells via specific endocytosis and transcytosis transport processes in order to attack the cytoplasmic target (Fadeel *et al*., 2010). Nanoparticle drug carriers can cross the blood-brain barrier and the tight epithelial junction of the skin, usually preventing the drug from reaching its intended target. Second, due to its high surface area-to-volume ratio, nanocarriers have excellent pharmacokinetics and biodistribution of therapeutic agents, thus limiting toxicity by promoting

accumulation at the target site (Vaidyanathan *et al*., 2009). They make hydrophobic substances more soluble and more suitable for parenteral administration. In addition, they improve the stability of peptides and oligonucleotides, among other things. This procedure saves money and has fewer side effects (Li *et al*., 2011). Nanotechnology can be used to support the regeneration and repair of damaged tissue. Tissue engineering can replace traditional treatments such as artificial implants and organ transplants (Mudshinge *et al*., 2011). An example of this is the growth of carbon nanotube scaffolds in bone. Gold is not a new substance in medicine. Gold is used in several clinics in the Indian medical system Ayurveda. Using gold as a memory booster is a common recipe. To improve the mental health of babies, certain medicinal formulations contain gold (Ealias *et al*., 2017).

2.14.2 In Food

Nanoparticles aids in the improvement of food production, processing, protection and packaging. For example, in the food packaging process, a nanocomposite coating can directly introduce the anti-microbial substances onto the coated film surface (Laad *et al*., 2016). One of the examples is the canola oil sector, which uses nanodrops, a food ingredient meant to transfer vitamins and minerals (Ealias *et al*., 2017).

2.14.3 In Cosmetics and Sunscreens

Traditional ultraviolet (UV) protective sunscreens lack long-term stability in use. Sunscreens that use nanoparticles, such as titanium dioxide, have several advantages (Ealias *et al*., 2017). The UV protection properties of titanium oxide and zinc oxide nanoparticles because they are transparent to visible light and absorb and reflect UV rays, they have been incorporated into some sunscreens. As a pigment, iron oxide nanoparticles are used in some lipsticks (Wiechers *et al*., 2010).

2.14.4 In Catalysis

Nanoparticles have a large surface area which means they have a higher catalytic activity. Nanoparticles are effective catalysis in chemical production because of their exceptionally large surface to volume ratio (Crooks *et al*., 2001). One of the most important uses is the use of platinum nanoparticles in car catalytic converters, which reduce the amount of platinum required due to the nanoparticles extremely high surface area, lowering the cost and enhancing

performance. Nanoparticles are used in several chemical reactions, such as the reduction of nickel oxide to metal nickel (Ni) (Ealias *et al*., 2017).

2.14.5 In Construction

The building process has been improved by nanotechnology, making it faster, cheaper and safer. For example, when nanosilica (SiO_2) is added to regular concrete, nanoparticles can improve mechanical properties and longevity (Nazari *et al*., 2011). Adding hematite (Fe_2O_3) nanoparticles to concrete increases its strength. Steel is the most common and most used building material. The properties of steel can be improved by using nanotechnology in steel. For example, using nano-sized steel for bridge construction provides stronger steel cables (Nazari *et al*., 2011). Glass is another important design element.

The application of nanotechnology in the construction of glass is the subject of extensive research. Titanium dioxide (TiO_2) nanoparticles are antiseptic and antifouling, so they can be used to catalyze strong chemical reactions that decompose volatile organic compounds (VOVs) and organic pollutants, making them suitable for glazing coatings (Xu *et al*., 2007). Nanotechnology allows greater light and heat isolation from windows. Nanoparticles are added to the paint to provide self-healing, corrosion resistance and insulation. These colors improve performance by making them resistant to saltwater attacks. Nanoparticles in paints are lightweight and have excellent properties that improve performance (Machado *et al*., 2015). So, when used on aircraft for example, they reduce the overall weight and amount of paint required, benefiting both the environment and the company's bottom line. (Ealias *et al*.,2017).

2.14.6 In Electronics

The increased need for large-screen, high-brightness displays in computer monitors and televisions in recent years has prompted the usage of nanoparticles in display technologies. Nanocrystalline lead telluride, cadmium sulphide, zinc selenide and sulphide, for example, are utilized in current light emitting diodes (LED) of modern displays (Teng *et al*., 2008). The rise in popularity of portable consumer electronics such as cellphones, and laptop computers has resulted in a huge need for small, light and high capacity batteries. Separator plates in batteries should be made of nanoparticles. Due to its foam-like (aerogel) shape, they can store significantly more energy than standard batteries. Because of their huge surface area,

nanocrystalline nickel and metal hydride batteries require less recharging and last longer (Published *et al*., 2016). Nanoparticles with increased electrical conductivity are utilized to detect gases such as NO_2 and NH_3. This is due to the increase in nanoparticle pores caused by charge transfer from nanoparticles to NO_2 as gas molecules bind them together, making them a superior gas sensor (Ealias *et al*., 2017).

2.14.7 In treatment of wastewater

Most countries today are facing drinking water problems, more especially developing countries. These challenges faced by the world are as the results of depleting fresh water supply due to the following factors: (a) lengthy droughts, (b) growth of the population, (c) more stringent health based regulations and (d) demands from competing range of users (Laboratories, 2003; World Health Organization, 1996; US Environmental Protection Agency, 1996). US Environmental Protection Agency (2012), states that defense against possible chemical and biological terrorist acts of water treatment systems is also becoming a serious concern in water resources planning. Chemical and physical disinfection agents such as chlorine and its derivatives, ultraviolet light are techniques which are used in water treatment (Droste, 1997).

Additionally, the utilization of halogens such as chlorine (Cl) and bromine (Br) are widely applied as antibacterial agents. Coordinate utilization of halogens as bactericides in pure form causes issues as they are profoundly harmful and have high vapour pressure. NH4+ is the most common cation influencing human and animal health found in water. Expulsion of ammonia in drinking water is exceptionally imperative as it avoids oxygen depletion, algae bloom and it is extremely toxic to most fish species (Jung *et al.,* 2006). For this reason it is accepted that the application of bioflocculants in water treatment can resolve these antagonistic effects.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Location and Collection

Samples were collected from three different wastewater sources in western part of Nigeria; which are Industry effluent (from Eternal Plc, Shagamu, Ogun state); Abattoir wastewater (from Kara in Ogun state) and Kitchen effluent (from a Restaurant in Ogun state). The samples were collected using sterile containers and transported to the laboratory for further analysis.

3.2 Materials

The materials used includes; Distilled water, Sterile Petri dishes, Sterile containers, Cotton wool, Alcohol (70% ethanol), Inoculating loop, Slides, Test tubes, Measuring cylinder, Conical flask, Durham tubes, Beaker, Aluminum Foil, Dropper, medium bottles, micropipette.

3.3 Culture Media

The culture media used includes; Nutrient Broth, Starch hydrolysis medium, Methyl red /Vogues Proskauer Agar, Nutrient Agar for the isolation of bacteria, Bioflocculant producing broth.

3.4 Equipment and reagent

Equipment used includes; Oven, Incubator, Autoclave, Weighing Balance, Thermometer, water bath, Colony counter, UV Spectrophotometer, Fourier transform infrared spectrophotometer, Reagents used include; Gram iodine, Kovacs Reagent, Crystal violet, Barrit’s reagent, Methyl red.

3.5 Preparation of Nutrient Agar

Nutrient agar was prepared according to the manufacturer's instructions. Twenty-eight grams (28.0 g) of nutrient agar was weighed out on a balance and placed in a sterile Erlenmeyer flask. 1000 ml of distilled water was placed in an Erlenmeyer flask. Seven grams (7 g) of nutrient agar was measured into 250 ml of water. The solution in the Erlenmeyer flask was vortexed to dissolve the medium. The solution was then boiled in a water bath to ensure proper mixing and then homogenization. After boiling, the medium was autoclaved at 121°C for 15 minutes.

Immediately after autoclaving, medium was poured onto plates after serial dilutions were made. The medium was allowed to solidify in this position.

3.6 Isolation of Microorganisms from the Wastewater Samples

A 1 ml wastewater sample was taken and serially diluted to $10^{-6.}$ From this, His 0.1 ml aliquots of each diluted sample were taken, the samples were evenly spread over the surface of the plate, and nutrient agar was poured over them using the pour plate method (Athalye *et al*., 1981). The plates were then incubated at 37°C for 24 hrs and examined for the appearance of colonies. Upon observation, morphologically distinct colonies were subcultured on nutrient agar plates at 37°C for 24 hrs.

3.7 Pure Culture Technique

From the primary plates, different isolates were sub-cultured aseptically by streaking onto the prepared nutrient agar plates. The Plates were incubated at 37°C for 24 hrs. These resulted in pure culture of the isolated organism. Streaking of the pure culture of isolates was done on a prepared sterile set agar slant in McCartney bottles and kept in the refrigerator for further tests and identification.

3.8 Screening of Isolates for Bioflocculant Production

Isolates were screened using an adapted protocol from Xia *et al.* 2008 which included a bioflocculant producing broth consisting of 20 g glucose, 0.5 g urea, 0.5 g yeast extract, 0.2 g $AlSO_4$, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.2 g $MgSO_4$, $7H_2O$, and 0.1 g NaCl. Includes broth (BPB), all dissolved in 1 liter of deionized water. The pH of the broth was adjusted to 7.0 and the medium was sterilized in an autoclave at 121°C for 15 minutes. After cooling, a pure culture of the isolate was inoculated and incubated on a rotary shaker at 37 ºC and a rotation speed of 120 rpm for 3 days (Adebayo *et al*., 2020).

3.9 Determination of the Flocculation Activities

The flocculating activity was measured according to the method developed by Kurane and Matsuyama and reported by Gao *et al*. A modified method was determined. A suspension of kaolin clay is used as a test material for measuring flocculating activity. Kaolin clay was suspended in distilled water at a concentration of 5 g/l at pH 7 and used as a stock solution for

sample testing. 1% $CaCl_2$ was also prepared. To confirm flocculating activity, 0.1 ml of cell-free supernatant of each isolate was mixed with 9 ml of kaolin clay suspension and 0.25 ml of $CaCl_2$ solution. A control was also prepared containing the same ingredients, except that 0.1 ml of bacterial supernatant was replaced with 0.1 ml of normal BPB solution. After gentle mixing, the mixture was left at room temperature for 5 minutes. The optical density of the upper clarified phase of controls and isolates was then measured using a UV spectrophotometer set at a wavelength of 550 nm. The flocculating activity of the isolates was calculated using the following formula:

$$\text{Flocculating activity (\%)} = \left[\frac{A-B}{B}\right] x\ 100$$

Where A and B is the optical density of the control and isolate respectively (Adebayo *et al*., 2020). The isolates possessing the culture broth with the highest flocculating activity against a Kaolin suspension were selected and further identified.

3.10 Characterization of the Selected Isolates

The selected isolates were identified using morphological and Biochemical characterizations.

3.10.1 Morphological Characterization

Morphological characterizations were done using colonial, cellular and pigment appearances on culture plates (Olutiola *et al*., 2000).

3.10.2 Biochemical Characterization

Biochemical characterizations were done using Gram Staining, Catalase test, Methyl red/ Voges Proskauer test, Coagulase test, Starch hydrolysis test, Sugar fermentation Test, Simmons Citrate test.

3.10.2.1 Gram Staining procedure

The gram stain is fundamental to the phenotypic characterization of bacteria. A smear was made on a glass slide and allowed to air dry. The crystal violet which is the primary stain was flooded on the fixed culture for 1 minute; the stain was washed off with water. Iodine solution was added to the smear for 1 minute and was poured off; then was rinsed with water. A few drops of ethyl

alcohol (decolourizer) were added and rinsed with water immediately after 10 seconds and finally safranin which is the secondary stain was added for 45 seconds and washed off, then the smear was left to air dry. After the drying of the slide, it was observed under the microscope using 100x magnification lens. Gram staining was done to find reactions of the bacterial isolates to Gram reagents. Gram stain helps in distinguishing and classifying bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria (Olutiola *et al*., 2000).

3.10.2.2 Catalase Test

The catalase enzyme protects bacteria from the accumulation of hydrogen peroxide (H_2O_2) that occurs during aerobic metabolism. The catalase test is performed to identify organisms that produce the enzyme catalase, which converts hydrogen peroxide into water and oxygen bubbles. In this test, loops of test organisms were smeared onto slides from Petrich dishes. A drop of hydrogen peroxide was then added to the smear and mixed. The presence of air bubbles indicates that the organism is producing catalase. Absence of air bubbles indicates a negative result (Olutiola *et al*., 2000).

3.10.2.3 Methyl Red (MR) test

This is a qualitative test of acid production by bacteria grown in MR-VP broth. Tubes containing MR-VP broth were inoculated with each test culture and incubated at 37° C for 24-48 hrs. After incubation, 5 drops of 0.4% (w/v) methyl red indicator was added to each tube and the tubes were examined for color change. A bright red color indicated a positive result. A yellow or orange color indicates a negative result (Olutiola *et al*., 2000).

3.10.2.4 Voges-Proskauer (VP) test

VP-positive bacteria use the butanediol fermentation pathway and produce acetylmethylcarbinol or acetoin, which react with Barritt's reagents A and B to produce a red color. Tubes containing MR-VP broth were inoculated with each test culture and incubated at 37°C for 48 hrs. After incubation, 0.5 ml of Barritt's reagent A (6% α-naphthol solution) and 0.5 ml of Barritt's reagent B (KOH) were added to the test tube. The tube was shaken vigorously to mix and allowed to stand to observe the gradual formation of a red color indicating a positive test. Acid is produced and a yellow or brown color indicates a negative test (Olutiola *et al*., 2000).

3.10.2.5 Sugar Fermentation Test

Weigh 1 g of peptone, 0.1 g of NaCl, 1 g of fermentable sugars (glucose, sucrose, maltose, lactose, and galactose) and 1 pint of bromocresol purple into an Erlenmeyer flask and add 100 ml of distilled water. Additionally, it was homogenized. , divided into 9 test tubes. An inverted Durham tube was added to each tube, capped and sterilized for 15 minutes. Individual isolates were then inoculated into each tube and incubated at 37°C for 24 hrs. Results were observed 24 hrs later (Olutiola *et al*., 2000).

3.10.2.6 Starch hydrolysis Test

A 20 ml aliquot of dissolved starch agar was aseptically poured into each sterile petri dish, allowed to solidify. Microorganisms were streaked onto the surface of the agar plate and incubated at 37°C for 24-48 hrs. The plate was then soaked with a certain amount of gram iodine. Unhydrolyzed starch formed a black color, hydrolyzed starch appeared as clear zones, and reddish-brown zones around colonies indicated partial hydrolysis of starch (Olutiola *et al*., 2000).

3.10.2.7 Coagulase Test

The coagulase test is a biochemical test used to distinguish Staphylococcus aureus (coagulase negative) from other staphylococcal species (coagulase negative) based on their ability to produce the coagulase enzyme. This enzyme clots plasma by converting fibrinogen to fibrin. The test was performed by placing a drop of plasma on the slide, adding the isolated organism, and mixing gently. Within 10 seconds, positivity was detected by agglutination of bacterial cells (Olutiola *et al*., 2000).

3.10.2.8 Citrate Utilization Test

A 2.14 g aliquot of Simmons citrate agar was dissolved in 500 ml distilled water and gently homogenized using a magnetic stirrer with gentle vortexing to completely dissolve the medium. The medium was then sterilized by autoclaving at 121° C. for 15 minutes, cooled to 50° C. and poured into sterile test tubes. A tube was then inserted into each test tube containing the loopful of each isolate, transferred to an incubator and incubated at 37 °C for 24 h. After 24 hrs, tubes were observed (Olutiola *et al*., 2000).

3.11 Effect of Physico-chemical Parameters on Bioflocculant Production

The effects of physico-chemical parameters such as carbon sources, nitrogen sources and cations on the bioflocculant produced by the isolate were investigated.

3.11.1 Effect of Carbon Source

The influence of sugars such as glucose, sucrose, fructose, galactose, lactose, inositol and maltose as carbon sources were examined on bioflocculant production. The production medium consist of 0.07 g of urea, 0.07 g of yeast extract, 0.028 g of $AlSO_4$, 0.7 g of K_2HPO_4, 0.028 g of $MgSO_4.7H_2O$, 0.014 g of NaCl and a pH of 7 in 140ml of distilled water in an Erlenmeyer's flasks and 20ml of the bioflocculant producing broth was separated into seven (7) big MacCartney bottles, to which 2.8g of each sugars were put inside the big MacCartney bottles and 10ml was then transferred into fourteen (14) small sized MacCartney bottles, for which seven bottles each were for the two isolates. After that, the medium was autoclaved at 121^oC for 15 mins. Afterwards the two isolates were inoculated into seven MacCartney bottles each and were cultured at 37^oC for 72 hrs.

3.11.2 Effect of Nitrogen source

The effect of nitrogen sources such as Sodium nitrate ($NaNO_3$), Ammonium nitrate (NH_4NO_3), peptone plus $NaNO_3$, Yeast extract plus $NaNO_3$ and peptone was investigated on bioflocculant production. The production medium consist of 2 g of glucose, 0.05 g of yeast extract, 0.02 g of $AlSO_4$, 0.2 g of KH_2PO_4, 0.5 g of K_2HPO_4, 0.02 g of $MgSO_4.7H_20$, 0.01 g of NaCl and a pH of 7 in 100ml of distilled water in an Erlenmeyer's flasks and 20ml of the bioflocculant producing broth was separated into seven (7) big MacCartney bottles, to which 0.05g of each nitrogen source were put inside the big MacCartney bottles and 10ml was then transferred into fourteen (14) small sized MacCartney bottles, for which seven bottles were for the two isolates each. Afterwards, the medium was autoclaved at 121^oC for 15 mins. Afterwards the two isolates were inoculated into seven MacCartney bottles each and was cultured at 37^oC for 72 hrs.

3.11.3 Effect of cations

The effect of cations, compounds which may be monovalent such as Potassium nitrate (KNO_3), Sodium acetate ($CH_3COONa.3H_2O$), divalent such as Copper(ii) sulfate ($CuSO_4$), Zinc sulfate

($ZnSO_4.7H_2O$), Ferrous chloride ($FeCl_2$), Iron(ii) sulphide (Fe_2S), Magnesium sulphate ($MgSO_4$ H_2O) and Calcium hydroxide ($Ca(OH)_2$) was investigated on bioflocculant production. The production media consist of 3.2 g of glucose, 0.08 g of urea, 0.8 g of yeast extract, 0.8 g of K_2HPO_4, 0.32 g of KH_2PO_4, 0.032 g of $MgSO_4.7H_2O$, 0.016 g of NaCl all in 160ml of a pH of 7 and 0.2 g of compounds which are either monovalent or divalent. Afterwards, the medium was autoclaved at 103 kPa for 20 mins. After inoculation, the cultures were incubated at 37ºC for 72 hrs.

3.12 Synthesis of Silver Nanoparticles

Each bacterial isolate was inoculated into 250 ml of sterile nutrient solution for biomass production. Erlenmeyer flasks were incubated at 37° C. on an orbital shaker and shaken at 120 rpm. (Gandhi *et al*., 2016). Silver nanoparticles were synthesized by mixing equal volumes (1:1) of 10 mM $AgNO_3$ solution and bacterial cell biomass in an Erlenmeyer flask. The mixture was incubated in the dark with stirring (100 rpm) at room temperature for 72 hrs. Content was monitored for visible color changes (Magdi *et al*., 2014).

3.13 Characterization of Silver Nanoparticles

Characterization is performed using various analytical techniques such as UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) (Zhang *et al*., 2016).

3.13.1 Ultraviolet (UV) spectrum analysis

UV spectroscopy was used to follow the reaction process and measure the absorbance of the silver nanoparticles. A blank was measured before carefully pouring the biosynthesized silver nanoparticle solution into a cuvette and loading it into a UV spectrophotometer. A blank sample was measured before each wavelength of the sample (Sandhu *et al*., 2017). The absorbance of silver nanoparticles was measured at different time intervals (24, 48, and 72 hrs, respectively).

3.13.2 Fourier transform infrared spectroscopy (FTIR)

FTIR was used to identify the nature of the chemical bonds and determine the chemical functional groups present on the silver nanoparticles. He put the dried and ground silver nanoparticles into the FTIR and recorded the FTIR spectrum (Vahabi *et al.*, 2017).

3.14 Applications of Bioflocculant and Biosynthesized Silver Nanoparticles

The wastewater samples used for this analysis were collected in Ogun State, southwestern Nigeria. Restaurant wastewater was collected from the Mountain Top University cafeteria and industrial wastewater was collected from Eternal Plc Shagamu in Ogun State. All effluents were stored at 4°C before flocculation. Physicochemical parameters of wastewater samples such as salinity, conductivity, temperature and pH were determined. Aggregation potential of bioflocculants produced by selected isolates and biosynthesized silver nanoparticles. Bioflocculants or biosynthesized silver nanoparticles at different concentrations (100-1000 mg/l), i. added to 1 mL. The compounds were thoroughly mixed and allowed to settle for 5 minutes before measuring the optical density (OD) of the upper phase clarified solution at 550 nm using a UV spectrophotometer to determine flocculating activity.(Adebami *et al*., 2017).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Colony count

Eleven (11) morphologically different bacteria were isolated from different sites located in the southwestern Nigeria. Table 4.1 shows the colony counts of the serial dilution plated for 10^{-3}, and 10^{-5} after at the end of 24 hrs of incubation period. For the first sampling, the colony count ranged from 120 x 10^3 - 66 x 10^5 cfu/mL for restaurant wastewater, 76 x 10^3 - 53 x 10^5 cfu/mL for karra wastewater, and 88 x 10^3 - 35 x 10^5 cfu/mL for Industry effluent. Second sampling ranged from 156 x 10^3 - 98 x 10^5 cfu/mL for restaurant wastewater, 102 x 10^3 - 69 x 10^5 cfu/mL for karra wastewater and 146 x 10^3 - 50 x 10^5 cfu/mL colony for Industry effluent respectively. The persistence of bacterial species in wastewater is due to the fact that bacteria begin to grow and multiply during the accelerated growth phase (Sood and Kumar., 2011). Growing bacteria migrate in search of nutrients and multiply rapidly (Sood and Kumar., 2011). They do not settle to form flocs and according to international standards, water contaminated to this degree can pose a hazard/threat to the environment as it is a favorable habitat for water-borne diseases. (Buthelezi *et al*., 2009). Ojidi and Oitayo. (2012), isolated bacterial species from different restaurants (sites A, B, C, and D) in Acre, Nigeria. All four sites were sampled over 24 hrs. Drainage sites with the highest number of colonies were site C, followed by site A and then site B, while the site with the lowest number of colonies was observed at site D.

Table 4.1: colony counts for wastewater samples

Sampling time	Sampling locations	Serial dilution factors/ colony counts	
		10^{-3} (cfu/mL)	10^{-5} (cfu/mL)
First sampling	Restaurant wastewater	120×10^3	66×10^5
	Karra wastewater	76×10^3	53×10^5
	Industry Effluent	88×10^3	35×10^5
Second sampling	Restaurant wastewater	156×10^3	98×10^5
	Karra wastewater	102×10^3	69×10^5
	Industry effluent	146×10^3	50×10^5

4.2 Morphological characterization of the isolates

Table 4.2 shows the morphological characteristics of the isolates obtained from the wastewater samples, including their shape, color, transparency, surface area, edges and elevation. Observed colors include white, opaque, milky, yellow. Shapes include: round, irregular and fibrous shapes. The observed margins include: wavy, whole, lobed and fibrous. The elevation includes: convex and flat. Surface cover: smooth and matte. Opacity includes: opaque and transparent. Garha *et al*. (2016) reported a similar result when morphological characterization of strains isolated from dairy wastewater samples, showing colors including: white, yellow, gray and milky white; Shapes include: irregular, thread-shaped and circular; opacity includes: opaque and transparent; and elevations include: flat, raised, convex and round. Mahesh *et al*. (2017) also reported a similar result when performing morphological characterization of municipal wastewater isolates, showing that the isolate's colors included: white, yellow and opalescent; shapes include: circles; Its opaques include: transparent; Surface covers: smooth: and its elevation covers: flat.

Table 4.2 Morphological Characteristics of the isolates

Isolates	Shape	Size	Color	Opacity	Elevation	Surface	Edge
RWN 1	Filamentous	Small	Milky	Opaque	Flat	Smooth	Filamentous
RWN 2	Irregular	Large	White	Opaque	Flat	Smooth	Undulate
RWN 3	Circular	Medium	Milky	Opaque	Flat	Smooth	Entire
RWN 4	Filamentous	Small	Milky	Opaque	Flat	Smooth	Filamentous
KWN 1	Circular	Medium	White	Opaque	Flat	Smooth	Entire
KWN 2	Circular	Small	Yellow	Transparent	Flat	Smooth	Entire
KWN 3	Irregular	Large	White	Transparent	Flat	Smooth	Entire
KWN4	Circular	Medium	Milky	Transparent	Flat	Smooth	Entire
EWN 1	Irregular	-	Milky	Opaque	Convex	Rough	Lobate
EWN2	-	-	Dark green	Opaque	-	Rough	-
EWN3	Irregular	small	White	Opaque	Flat	Dull	Undulate

4.3 Biochemical Characterization

Table 4.3 shows the biochemical characteristics of isolates obtained from wastewater samples, including; Gram stain, starch hydrolysis test, catalase test, MR-VP test, coagulase test, citrate utilization test and sugar fermentation for glucose, galactose, fructose and sucrose. Both positive and negative reactions to the test reagent were observed. The organisms observed were: *Bacillus* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Enterobacter* sp., *Actinomycetes* sp. These results are in agreement with the report of Mathias *et al*. (2017) who also quarantined *P. aeruginosa* and *Bacillus* sp. However, they also isolated *E. coli*, *S. aureus*, *Klebsiella* sp. and *Salmonella* sp. Was the predominant bacterium in wastewater samples from the Jimeta slaughterhouse in northern Nigeria (Adamawa State). In addition, Ulfat *et al*. (2021) also isolated *Pseudomonas* sp., *Bacillus* sp. However, they further isolated *Staphylococcus* sp., *Micrococcus* sp., *Klebsiella* sp., *Vibrio* sp., *Enterococcus* sp., *Salmonella* sp., *Escherichia coli* and *Proteus* sp. like bacteria in a wastewater sample taken from the Lahore Canal in Pakistan.

Table 4.3: Biochemical characterization of the isolates

Isolates	Gram staining	Cellular Morphology	Cell Arrangement	Catalase	Methyl Red	VP test	Citrate	Coagulase	Starch Hydrolysis	Glucose Fermentation	Galactose Fermentation	Fructose Fermentation	Sucrose Fermentation	Probable Organisms
RWN1	-	Bacilli	Clusters	+	-	-	+	+	+	-/-	-/-	A/-	A/-	*Staphylobacilli* sp.
RWN2	-	Bacilli	Chains	+	+	+	+	+	+	A/-	A/-	A/-	A/-	*Bacillus* sp.
RWN3	+	Bacilli	Clusters	+	+	+	-	+	+	A/-	A/-	-/G	A/-	*Corynebacterium* sp.
RWN4	-	Bacilli	Single	+	-	+	+	+	-	A/-	A/-	-/G	A/-	*Enterobacter* sp.
KWN1	-	Bacilli	Chains	+	+	-	+	+	+	A/-	A/-	A/G	A/-	*StreptoBacillus* sp.
KWN2	-	Bacilli	Singly	+	+	-	+	+	+	-/-	A/-	A/-	A/-	*Pseudomonas* sp.
KWN3	-	Bacilli	Clusters	+	+	-	+	+	-	-/-	A/-	-/-	A/-	*Staphylobacilli* sp.
KWN4	-	Bacilli	Clusters	+	+	+	+	-	-	A/-	A/-	-/G	-/-	*Bacillus* sp.
EWN1	+	Bacilli	Clusters	+	-	+	+	-	+	A/-	-/-	A/-	A/-	*Actinomycetes* sp.
EWN2	+	Bacilli	Clusters	+	+	+	+	-	+	A/G	A/G	A/G	-/-	*Actinomycetes* sp.
EWN3	+	Bacilli	Chains	+	+	-	+	+	+	A/-	A/-	A/-	A/-	*Streptobacilli* sp.

+ = Postive, - = Negative, A/G=Acid and Gas produced, A/- =Acid produced without Gas, -/G=Gas produced without Acid, -/- =Acid and Gas not produced.

4.4 Screening of the isolates

In this study, a total of eleven (11) bacteria were isolated from three different wastewater samples (restaurant wastewater collected from cafeterias, industrial wastewater collected from Eternal Plc, Shagamu and Abattoir processing wastewater from Karra in Ogun State, western Nigeria). The isolates were screened for bioflocculation production using kaolin solution (5g/L) as test material. Bacteria were screened for bioflocculation production as shown in Table 4.4. There was a significant difference ($P \geq 0.05$) in the flocculation ability of the isolates. The percentage of flocculating activity ranged from 8.035 - 29.68% with RWN2 isolate having the highest flocculating activity, followed by KWN4, EWN1, KWN1, RWN1, RWN3 and EWN3, KWN3 and. EWN2 while RWN4 isolate has at least . Based on the flocculation activities, the isolates with the highest flocculation activity were selected and characterized. These results are in contrast to that reported by Mathias *et al*. (2017) who reported that *P. aeruginosa* had the highest flocculation activity with 87.32%, while *Salmonella* spp. Had at least 13.5% flocculating activity while the flocculating activity of *E. coli*, *S. aureus*, *Klebsiella* sp. And *Bacillus* sp. are 35.76%, 47.87%, 56.6% and 69.54%, respectively. These results also contradict the report of Adebami *et al*. (2013) who reported that *Citrobacter* sp. (75.63%) had the highest flocculation activity, followed by *PaeniBacillus polymyxa* (69.54%), *Enterobacter asburiae* (69.54%) and *Streptococcus plurextorum* (69.14%) while *SoliBacillus silvestri* (68, 21%) have the least.

Table 4.4: Screening of bacterial isolates for flocculating activity

Isolates	Flocculating activity (%)
RWN1	22.99[e]
RWN2	29.68[a]
RWN3	22.54[f]
RWN4	8.035[i]
KWN1	23.21[d]
KWN2	22.32[f]
KWN3	21.42[g]
KWN4	27.23[b]
EWN1	26.97[c]
EWN2	12.27[h]
EWN3	22.54[f]

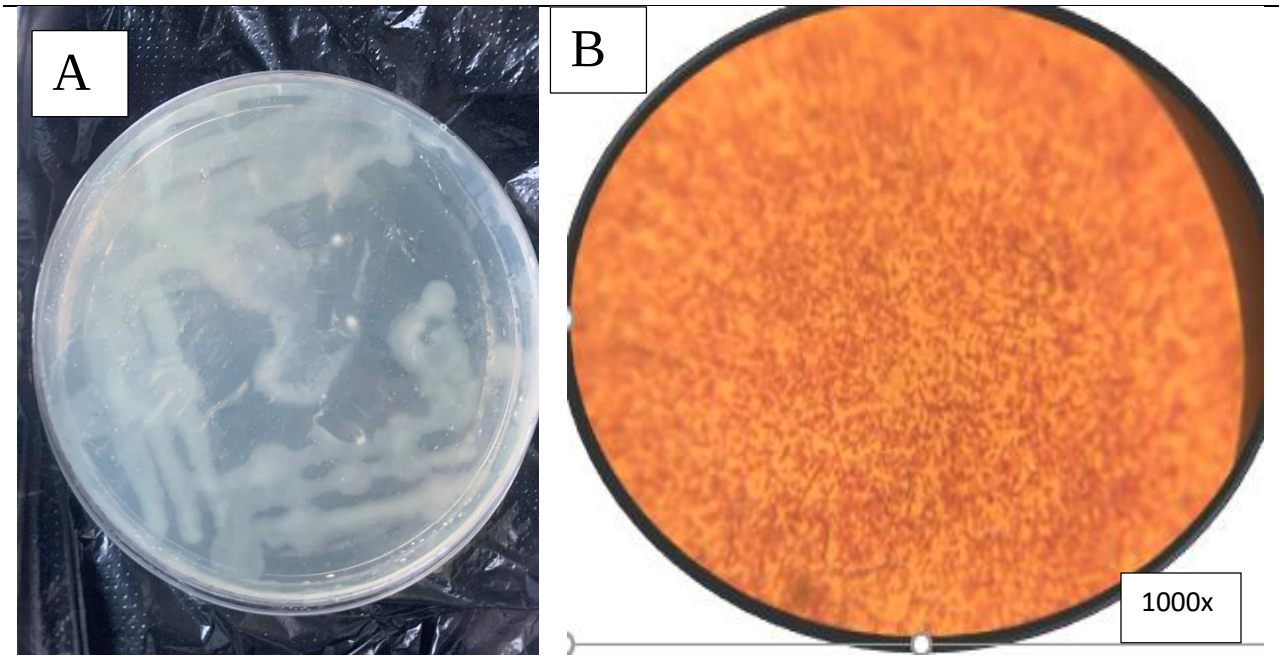

Plate 4.1: Morphological and Microscopic characterization of *Bacillus* sp. KWN4 (a) Isolate KWN4 on Nutrient agar plate (b) Microscopic appearance at 1000x magnification

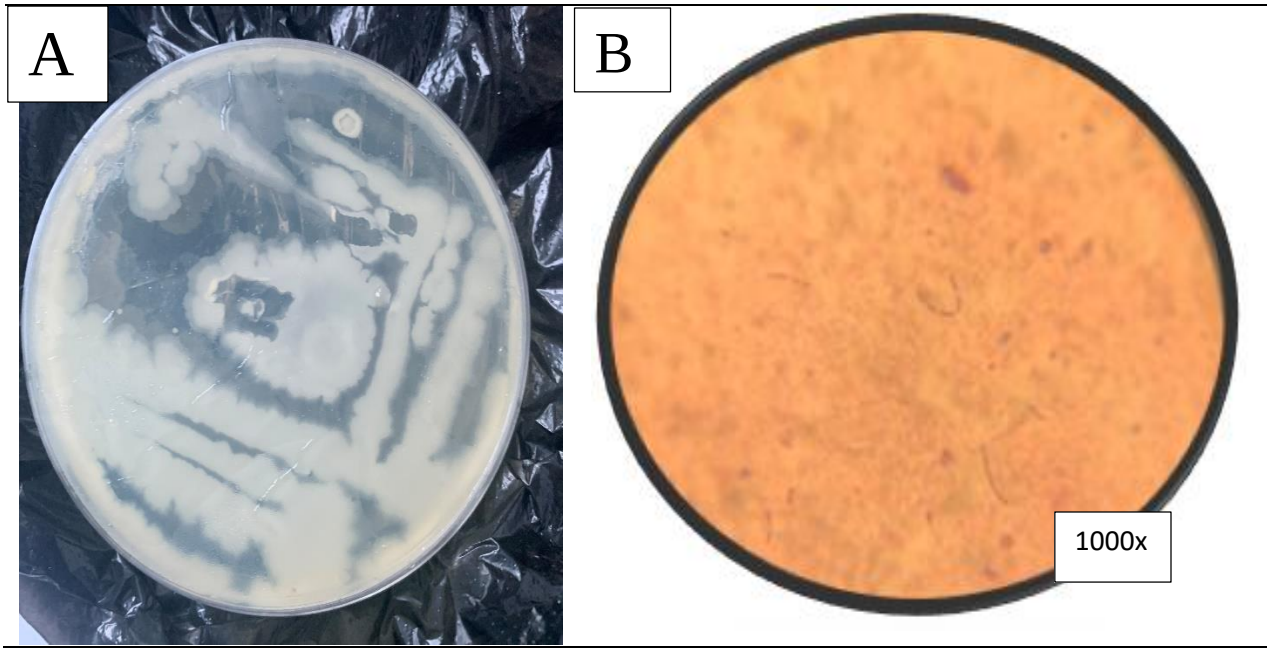

Plate 4.2: Morphological and Microscopic characterization of *Bacillus* sp. RWN2 (a)Isolate RWN2 on a Nutrient agar plate, (b) Microscopic appearance at 1000x

4.5 Effects of Physico-chemical parameters on bioflocculant produced from the selected isolates

The effects physico-chemical parameters such as carbon sources, nitrogen sources, and cations on the bioflocculant production by the isolate were investigated as shown below.

4.5.1 Effects of carbon sources on bioflocculant production

Figure 4.3 shows the effect of different carbon sources on bioflocculant production by Isolate KWN4. There was a significant difference (P≥0.05) in the flocculating activity of Isolate KWN4 at different carbon sources. At 12, 24, 48 and 72 hrs of incubation periods, the flocculating activity ranged from 12.14 - 61.75%, 26.36 - 47.34%, 0 - 10.92% and 21.84 – 29.52% respectively. At 12 hrs of incubation period, inositol (61.75%) supported the highest flocculating activities, follow in order by sucrose (46.16%), glucose (42.80%), galactose (40.82%), while fructose (10.33%) was the least. At 24 hrs of incubation period, sucrose (47.34%) supported the highest flocculating activities, follow in order by maltose (38.30%), lactose (35.40%), galactose (29.33%), while inositol (3.38%) was the least. At 48 hrs of incubation period, maltose (10.92%) supported the highest flocculating activities, follow in order by glucose (9.17%), galactose (8.39%), sucrose (7.81%), while lactose (0%) was the least. At 72 hrs of incubation period, inositol (14.67%) supported the highest flocculating activities, follow in order by lactose (28.49%), sucrose (26.96%), galactose (24.06%), while maltose (14.67%) was the least.

Figure 4.4 shows the effect of different carbon sources on bioflocculant produced by Isolate RWN2. There was a significant difference (P≥0.05) in the flocculating activity of Isolate RWN2 at different carbon sources. At 12, 24, 48 and 72 hrs of incubation periods, the flocculating activity ranged from 0 – 54.69%, 15.87 - 43.68%, 0.78 – 20.11%, and 0 – 33.27% respectively. At 12 hrs of incubation period, glucose (54.69%) supported the highest flocculating activities, follow in order by maltose (52.45%), inositol (41.34%), lactose (40.31%), while galactose (0%) was the least. At 24 hrs of incubation period, fructose (43.68%) supported the highest flocculating activities, follow in order by maltose (41.20%), glucose (29.05%), inositol (26.29%), while sucrose (15.87%) was the least. At 48 hrs of incubation period, maltose (20.11%) supported the highest flocculating activities, follow in order by fructose (13.47%), galactose (12.5%), lactose (11.91%), while sucrose (1%) was the least. At 72 hrs of incubation period, inositol (33.27%) supported the highest flocculating activities; follow in order by maltose

(30.71%), galactose and sucrose (20.13%), fructose (19.28%), while glucose (0%) was the least. The isolates were able to produce a reasonable level of bioflocculation in all carbon sources used. The production of bioflocculant is carried out on a medium containing glucose, sucrose and galactose as the main carbon sources. These two sugars appear to benefit cell growth as well as biofloc production by more than 60%, with glucose showing maximum activity (90%) as a carbon source. On the other hand, maltose, lactose and inositol were less appreciated by the test organism for bioflocculant production (Agunbiade *et al*., 2018). This result contradicts the report of Zhang *et al*. (2007) who reported that glucose inhibited cell growth in *Sorangium cellulosum* during bioflocculation production after 12 h incubation. This result contradicts the report of Liu *et al*. (2016) who reported that glucose was the preferred carbon source for Pseudomonas veronii as it produced the bioflocculating agent with the highest flocculation activity (90.8%) compared with other carbon sources after 24 h incubation. This result is similar to that reported by Sheng *et al*. (2006) who reported maltose as the preferred carbon source for *Klebsiella* sp. as it produced the bioflocculating agent with the highest flocculation activity (88.12%), compared with other carbon sources after 48 h of incubation. This result contradicts the report of Mabinya *et al*. (2012), who confirmed that the bioflocculating agent produced by *Arthrobacter* sp. Raats had the highest flocculation activity of 75.4% and 73.4% respectively when lactose and sucrose were used, and no bioagglutination was detected in the presence of other carbon sources.

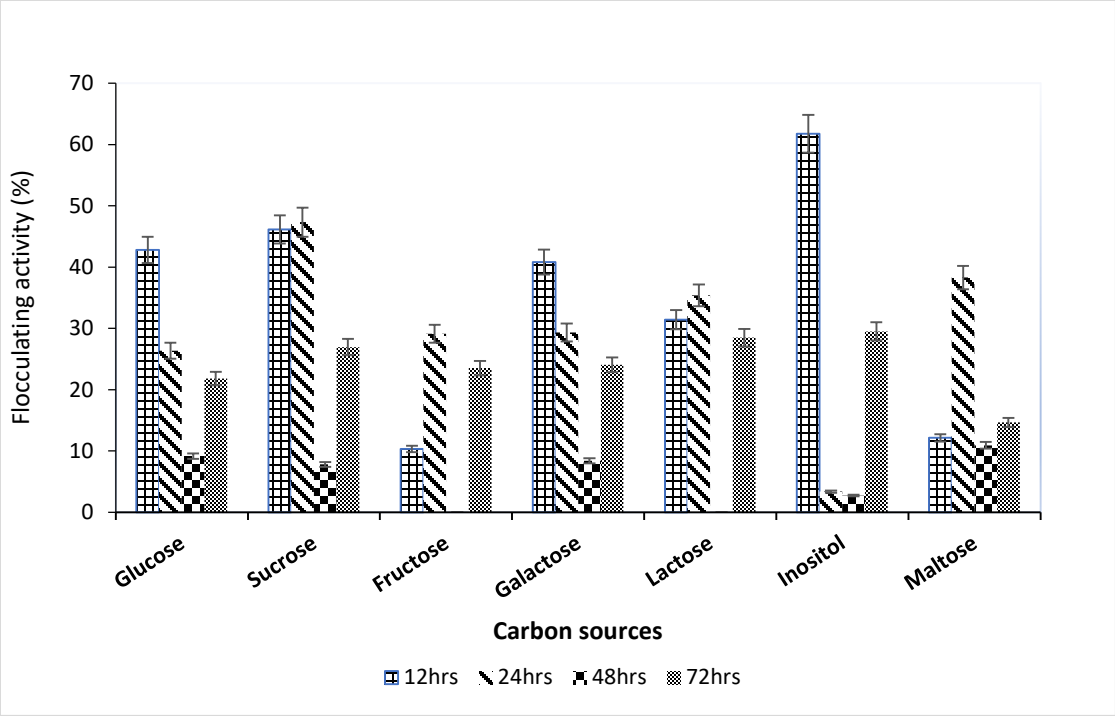

Figure 4.1: Effects of carbon sources on bioflocculant production by Isolate KWN4

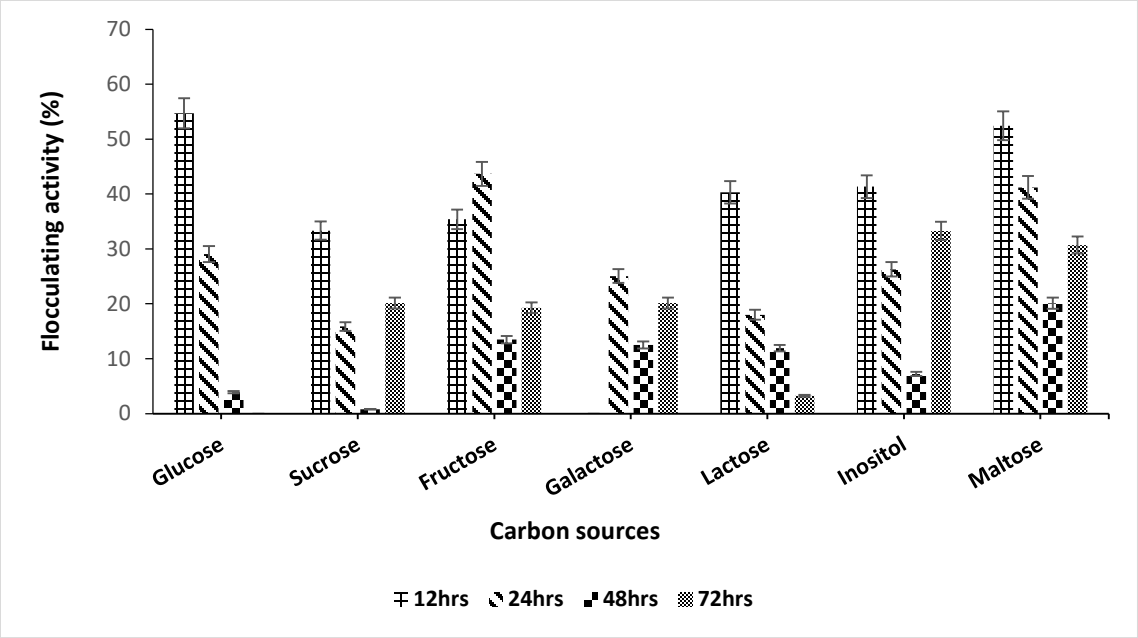

Figure 4.2: Effects of carbon sources on bioflocculant production by Isolate RWN2

4.5.2 Effects of nitrogen sources on bioflocculant production

From Figure 4.5 shows the effects of different nitrogen sources on bioflocculant produced by Isolate KWN4. There was a significant difference (P≥0.05) in the flocculating activity of Isolate KWN4 at different nitrogen sources. At 12, 24, 48, 72 hrs of incubation the flocculating activity ranged from 0.60 – 47.63%, 42.92 – 49.28%, 22.23 – 42.04% and 8.02 – 36.34% respectively. At 12 hrs of incubation period, ammonium nitrate (NH_4NO_3) (47.63%) supported the highest flocculating activities, follow in order by peptone (19.89%), peptone plus sodium nitrate ($NaNO_3$) (18.00%), yeast extract plus sodium nitrate ($NaNO_3$) (16.79%), while sodium nitrate ($NaNO_3$) (0%) was the least. At 24 hrs of incubation period, sodium nitrate (49.28%) supported the highest flocculating activities, follow in order by peptone plus sodium nitrate ($NaNO_3$) (68.66%), peptone (46.01%), ammonium nitrate (NH_4NO_3) (44.6%) while yeast extract plus sodium nitrate ($NaNO_3$) (42.92%) was the least. At 48 hrs of incubation period, yeast extract plus sodium nitrate (42.04%) supported the highest flocculating activities, follow in order by sodium nitrate ($NaNO_3$) (40.70%), ammonium nitrate (NH_4NO_3) (32.88%), peptone (27.35%), while peptone plus sodium nitrate ($NaNO_3$) (22.23%) was the least. At 72 hrs of incubation period, yeast extract plus sodium nitrate (36.34%) supported the highest flocculating activities, follow in order by sodium nitrate ($NaNO_3$) (35.49%), ammonium nitrate (NH_4NO_3) (29.86%), peptone (22.52%), while peptone plus sodium nitrate ($NaNO_3$) (8.02%) was the least.

Figure 4.6 shows the effects of different nitrogen sources on bioflocculant produced by Isolate RWN2. There was a significant difference (P≥0.05) in the flocculating activity of Isolate RWN2 at different nitrogen sources. At 12, 24, 48 and 72 hrs of incubation the flocculating activity ranged from 0 – 30.66%, 0.27 – 45.27%, 24.79 – 46.63%, and 0 – 30.54% respectively. At 12 hrs of incubation period, ammonium nitrate (NH_4NO_3) (30.66%) supported the highest flocculating activities, follow in order by yeast extract plus sodium nitrate ($NaN0_3$) (29.19%), peptone plus sodium hydroxide (9.21%), sodium nitrate (1.98%), while peptone (0%) was the least. At 24 hrs of incubation period, peptone (45.27%) supported the highest flocculating activities, follow in order by peptone plus sodium nitrate ($NaNO_3$) (19.73%), ammonium nitrate (NH_4NO_3) (14.35%) yeast extract plus sodium nitrate ($NaNO_3$) (5.59%), while ammonium nitrate (NH_4NO_3) was the least. At 48 hrs of incubation period, ammonium nitrate (NH_4NO_3) (46.63%) supported the highest flocculating activities, follow in order by yeast extract plus sodium nitrate ($NaNO_3$)

(44.33%), peptone plus sodium nitrate ($NaNO_3$) (43.39%), sodium nitrate ($NaNO_3$) (43.26%), while peptone (24.79%) was the least. At 72 hrs of incubation period, ammonium nitrate (NH_4NO_3) (30.54%) supported the highest flocculating activities, follow in order by sodium nitrate ($NaNO_3$) (29.01%), yeast extract plus sodium nitrate ($NaNO_3$) (21.50%), peptone plus sodium nitrate ($NaNO_3$) (17.74%), while peptone (0%) was the least. It has been well reported that nitrogen sources are imperative supplement components that upgrade bioflocculant generation (Ugbenyen *et al*., 2012). When natural nitrogen sources are been utilized, bioflocculant production gets to be more sensible in comparison to inorganic nitrogen sources with yeast extract since the required nitogen source making a difference the driving flocculating charge of 78% (Zheng *et al*., 2008). This result contradicts that of Cosa *et al*. (2013) reported that a complex nitrogen substrate composed of urea, yeast extract, and $(NH_4)_2SO_4$ promoted bioflocculant production in *VirgiBacillus* sp. Rob is compared to other nitrogen sources tested after 12 hrs of incubation. This result is similar to the findings of Liu *et al*. (2016) he identified *Pseudomonas veronii* as the preferred nitrogen source because the yeast extract produced the bioflocculant with the highest flocculating activity (91.9%) compared to other nitrogen sources after 24 hrs of incubation. This result contradicts the report by Cosa *et al*. (2011) Peptone shows the highest flocculating activity (70.4%) compared to other nitrogen sources after 48 hrs of incubation in *VirgiBacillus* sp. This result is similar to that reported by Suryani *et al*. (2011) he reported ammonium sulfate $(NH_4)_2SO_4$ as a preferred nitrogen source for *Chromobacterium violaceum* and *Citrobacter koseri*. This is because the bioflocculants with the highest flocculant activity compared to other nitrogen sources were produced after 72 hrs of incubation.

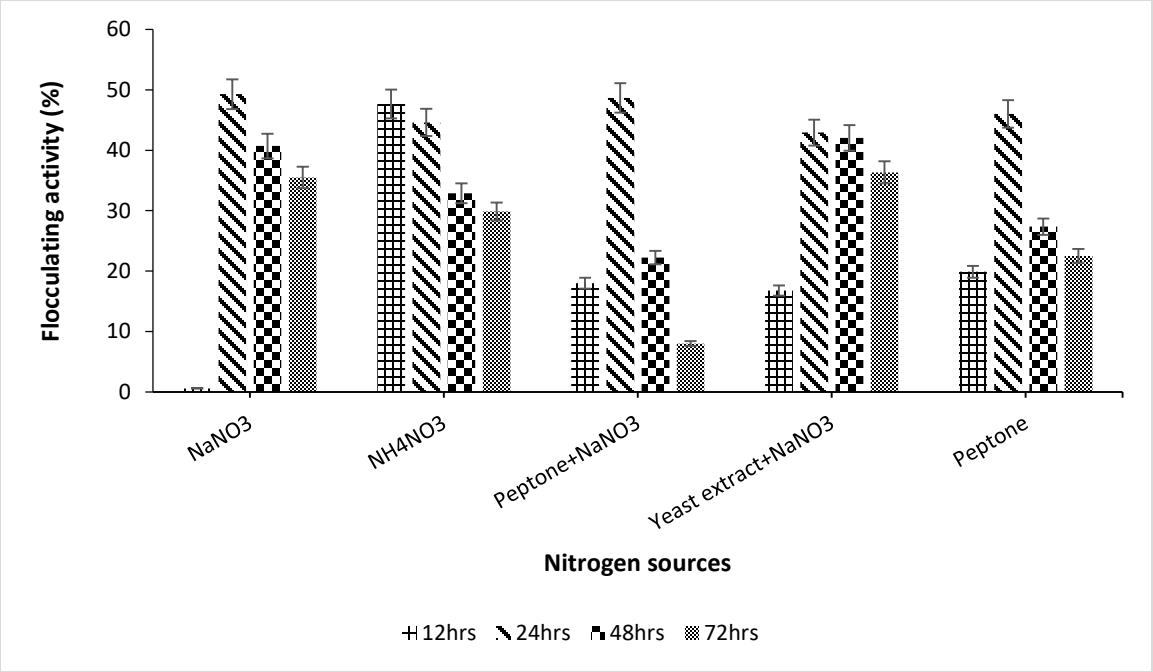

Figure 4.3: Effects of nitrogen sources on bioflocculant production by Isolate KWN4

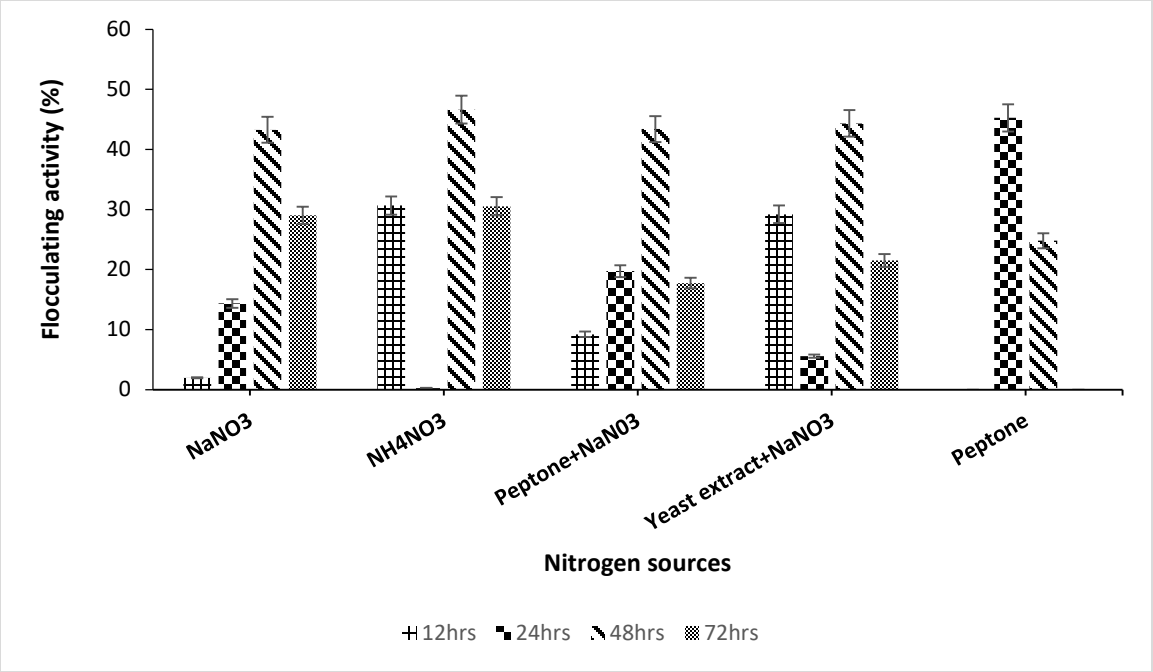

Figure 4.4: Effects of nitrogen sources on bioflocculant production by Isolate RWN2

4.5.3 Effects of cations on bioflocculant production

Figure 4.7 shows the effects of cations on bioflocculant production by Isolate KWN4. There was a significant difference (P≥0.05) in the flocculating activity of Isolate KWN4 at different cations. At 12, 24, 48, and 72 hrs of incubation periods, the flocculating activity ranged from 0 – 38.23%, 45.54 – 69.08%, 7.36 – 45.26%, and 0 – 50.34% respectively. At 12 hrs of incubation period, potassium nitrate (KNO_3) (38.23%) supported the highest flocculating activities of isolate KWN4, follow in order by iron (ii) sulphide (Fe_2S) (34.50%), zinc sulphate ($ZnSO_4.7H_2O$) (22.77%), calcium hydroxide ($Ca(OH)_2$) (19.80%), while ferrous chloride ($FeCl_2$) (0%) was the least. At 24 hrs of incubation period, potassium nitrate (KNO_3) (69.08%) supported the highest flocculating activities of isolate KWN4, follow in order by iron (ii) sulphide (Fe_2S) (68.04%), sodium acetate ($CH_3COONa.3H_2O$) (64.25%), calcium hydroxide ($Ca(OH)_2$) (60.04%), while ferrous chloride (FeCl) (45.54%) was the least. At 48 hrs of incubation period, calcium hydroxide ($Ca(OH)_2$) (45.26%) supported the highest flocculating activities of isolate KWN4, follow in order by copper (ii) sulphate ($CuSO_4$) (45.0%), zinc sulphate ($ZnSO_4.7H_2O$) (37.76%), sodium acetate ($CH_3COONa.3H_2O$) (37.5%), while ferrous chloride ($FeCl_2$) (7.36%) was the least. At 72 hrs of incubation period, calcium hydroxide ($Ca(OH)_2$) (50.34%) supported the highest flocculating activities of isolate KWN4, follow in order by magnesium sulphate ($MgSO_4$) (42.15%), zinc sulphate ($ZnSO_4.7H_2O$) (36.34%), sodium acetate ($CH_3COONa.3H_2O$) (35.66%), while ferrous chloride ($FeCl_2$) (0%) was the least.

Figure 4.8 shows the effects of Cations on bioflocculant production by Isolate RWN2. There was a significant difference (P≥0.05) in the flocculating activity of Isolate RWN2 at different cations. At 12, 24, 48, and 72 hrs of incubation the flocculating activity ranged from 0 – 13.59%, 54.10 – 69.63%, 10.92 – 54.07% and 0 – 68.25% respectively. At 12 hrs of incubation period, potassium nitrate (KNO_3) (13.59%) and sodium acetate ($CH_3COONa.3H_2O$) (13.59%) supported the highest flocculating activities of isolate RWN2, follow in order by calcium hydroxide ($Ca(OH)_2$) (13.52%), zinc sulphate ($ZnSO_4.7H_2O$) (12.28%), while ferrous chloride ($FeCl_2$) (0%) was the least. At 24 hrs of incubation period, sodium acetate ($CH_3COONa.3H_2O$) (69.63%) supported the highest flocculating activities of isolate RWN2, follow in order by potassium nitrate (KNO_3) (69.15%), calcium hydroxide ($Ca(OH)_2$) (68.94%), iron (ii) sulphide (Fe_2S) (63.0%), while

copper (ii) sulphate ($CuSO_4$) (54.10%) was the least. At 48 hrs of incubation period, calcium hydroxide ($Ca(OH)_2$) (54.07%), supported the highest flocculating activities of isolate RWN2, follow in order by magnesium sulphate ($MgSO_4$) (41.57%), zinc sulphate ($ZnSO_4.7H_2O$) (41.31%), iron (ii) sulphide (Fe_2S) (40.39%), while sodium acetate ($CH_3COONa.3H_2O$) (10.92%) was the least. At 72 hrs of incubation period, calcium hydroxide ($Ca(OH)_2$) (68.25%) supported the highest flocculating activities of the isolate RWN2, follow in order by zinc sulphate ($ZnSO_4.7H_2O$) (50.85%), potassium nitrate (KNO_3) (48.46%), sodium acetate ($CH_3COONa.3H_2O$) (47.78%), while copper (ii) sulphate ($CuSO_4$) (0%) was the least. The role of cations is to neutralize and stabilize the negative charge of both the functional groups of the kaolin particles and the bioflocculants in solution (Salehizadeh *et al*., 2004; Wu *et al*., 2007). According to a study by Levy *et al*. (1992) stated that trivalent, divalent, and monovalent cations are effective in stimulating the adsorption of bioflocculants to suspended kaolin particles by reducing the negative charge of both the polymer and the particles. It is This result contradicts the report by Ntozonke *et al*. (2017) reported that $Ca^{2+,}$ Mn^{2+}, and Al^{3+} promoted the production of bioflocculants in his *Bacillus* sp. Following activity: 62.3%, 65.1% after 12 hrs incubation, 69.8%. This result is similar to that reported by Sathiyanarayanan *et al*. (2013) he reported sodium chloride (NaCl) as the preferred cation of *Bacillus subtilis* MSBN17. This is due to the production of bioflocculants with the highest flocculant activity compared to other cations after 24 hrs of incubation. This result contradicts the report of Sheng *et al*. (2006) and Li *et al*. (2008), Kosa *et al*. (2011) reported that the presence of ferrous sulfate increased the production of bioflocculants in her *VirgiBacillus* sp. After 48 hrs of incubation. This result is similar to that reported by Manivasagan *et al*. (2015) reported that calcium chloride (CaCl) is the preferred cation for his *Streptomyces* sp. formation of bioflocculant with highest flocculating activity (84.8%) compared to other cations after 72 hrs of incubation.

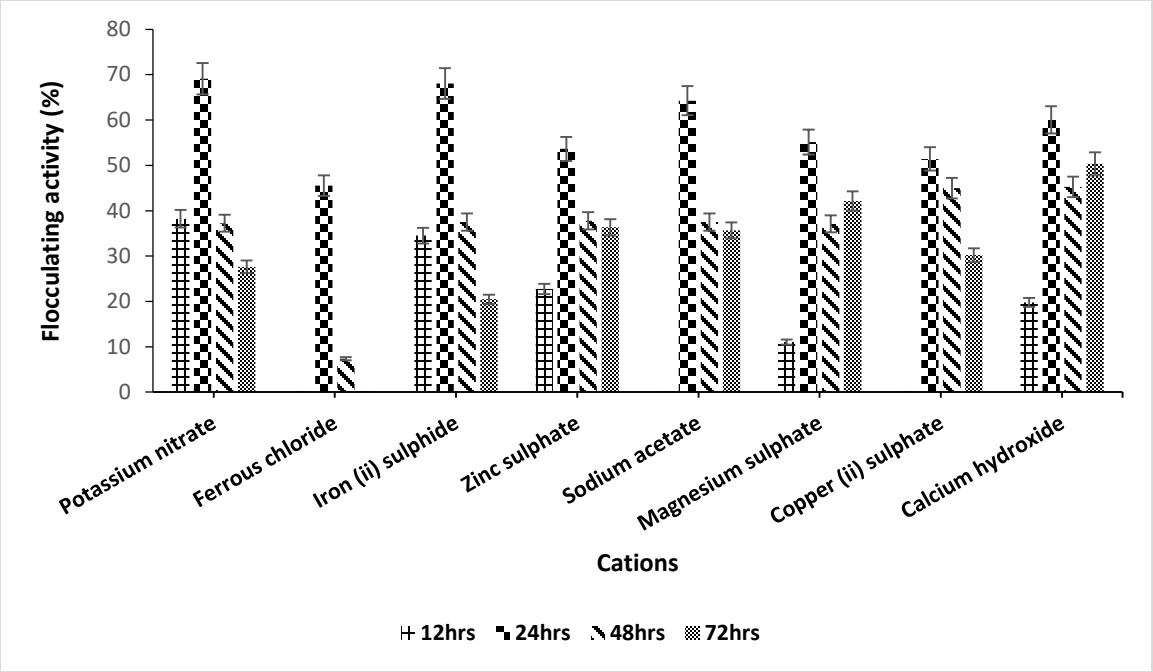

Figure 4.5: Effects of cations on bioflocculant produced by Isolate KWN4

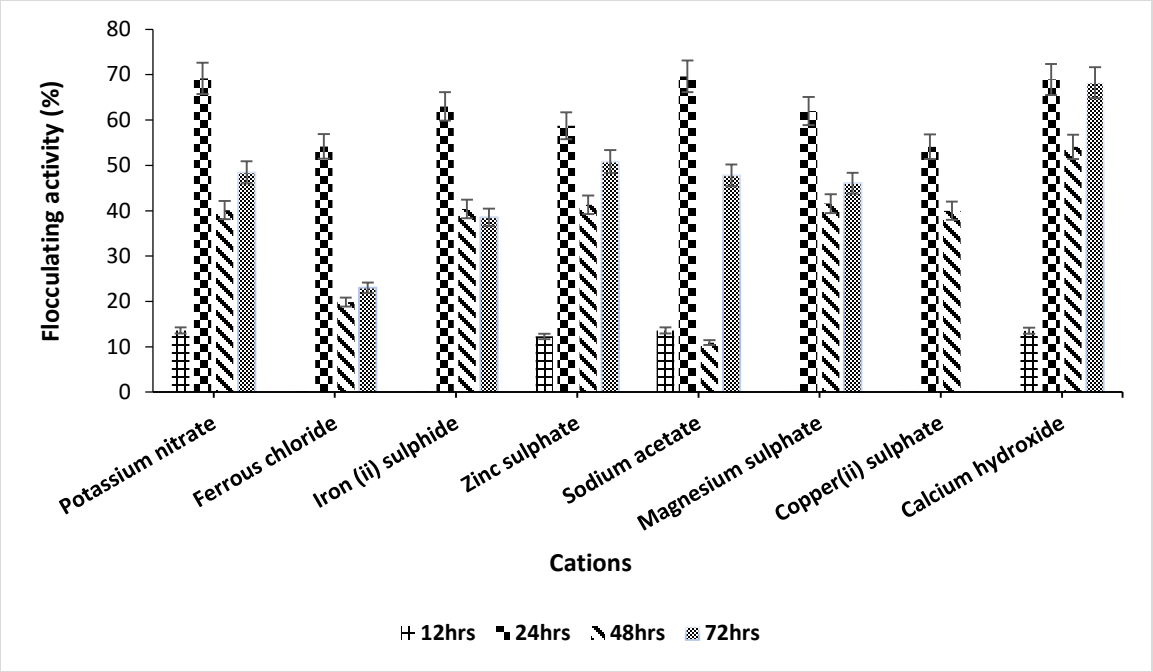

Figure 4.6: Effects of cations on bioflocculant production by Isolate RWN2

4.6 Biosynthesis of Nanoparticles

At present, attention has been paid to the development of nanoparticles, in particular through the use of microorganisms such as bacteria species which are capable of mobilization and immobilization of metals and in some cases, bacteria which can reduce metal ions show the ability to precipitate metals at nanometer (Iravani, 2014). Bacteria naturally secrets extracellular polymer during their growth, resulting in the formation stable flocs and it has different composition (Suryanp *et al*., 2011). Two of the isolates with the highest flocculating activity were used in the biosynthesizing of silver nanoparticles. Silver nanoparticles (AgNPs) are attracting increasing attention due to their unique physical, biological, and chemical properties. AgNPs are known to have potent antimicrobial activity against various microorganisms such as bacteria, viruses, and fungi due to their smaller size and large surface area (Franci *et al*., 2015). Silver nanoparticles have been successfully synthesized from other sources such as plant roots, stems, microorganisms such as *Bacillus* sp., Pseudomonas, etc. (Elbeshehy *et al*., 2015; Wan Mat Khalir *et al*., 2018; Khanal *et al*., 2022).

4.6.1 Visual observation

Plate 4.3 and Plate 4.4 shows the result of the isolate in broth after 72 hrs of incubation, $AgNO_3$ solution and color change of the bacterial biomass after biosynthesis. At the end of incubation period, a yellowish to brownish colouration was observed indicating the synthesis of silver nanoparticles which is regarded as the preliminary detection for formation of AgNPs. The color change varied at different hrs of agitation. Yellowish brown colour was observed after 24 hrs of agitation which seems lighter compared to brownish colour obtained after 72hrs of agitation, which is primarily due to excitation of surface plasmon resonance is typical of AgNPs (Sathiyanarayanan *et al*., 2013; Ahmad *et al.*, 2003). Biosynthesized silver nanoparticles were further confirmed by UV spectra and FTIR analysis (Gajbhiye *et al*., 2009). This result contradict the report carried out by Silva-Vinhote *et al*. (2017), who reported that the biosynthesized silver nanoparticles from Streptomyces sp. DPUA 1747 presented a light-yellow coloration under the same duration of incubation periods.

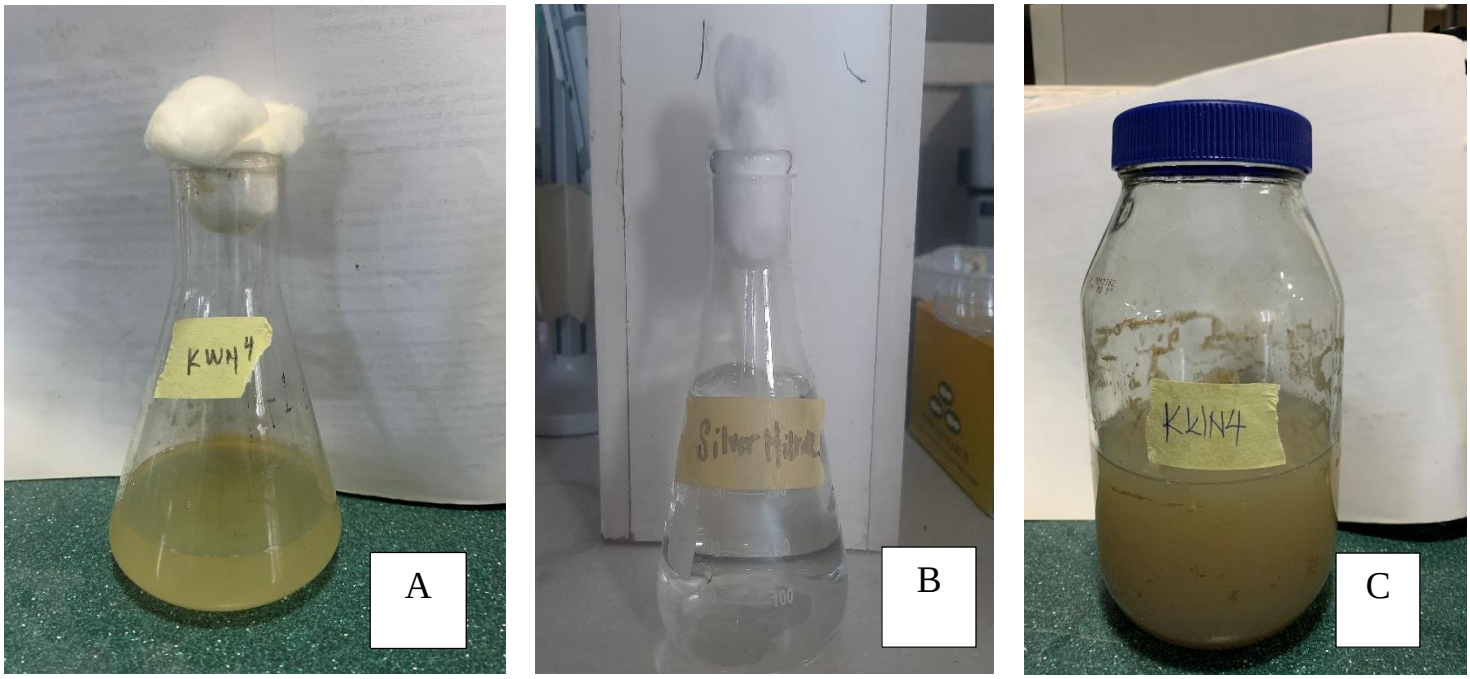

Plate 4.3: Biosynthesis of Silver nanoparticles. (a) Isolate KWN4 culture (b) Silver nitrate solution (c)Brown color of the biosynthesis of silver nanoparticle by the isolate KWN4

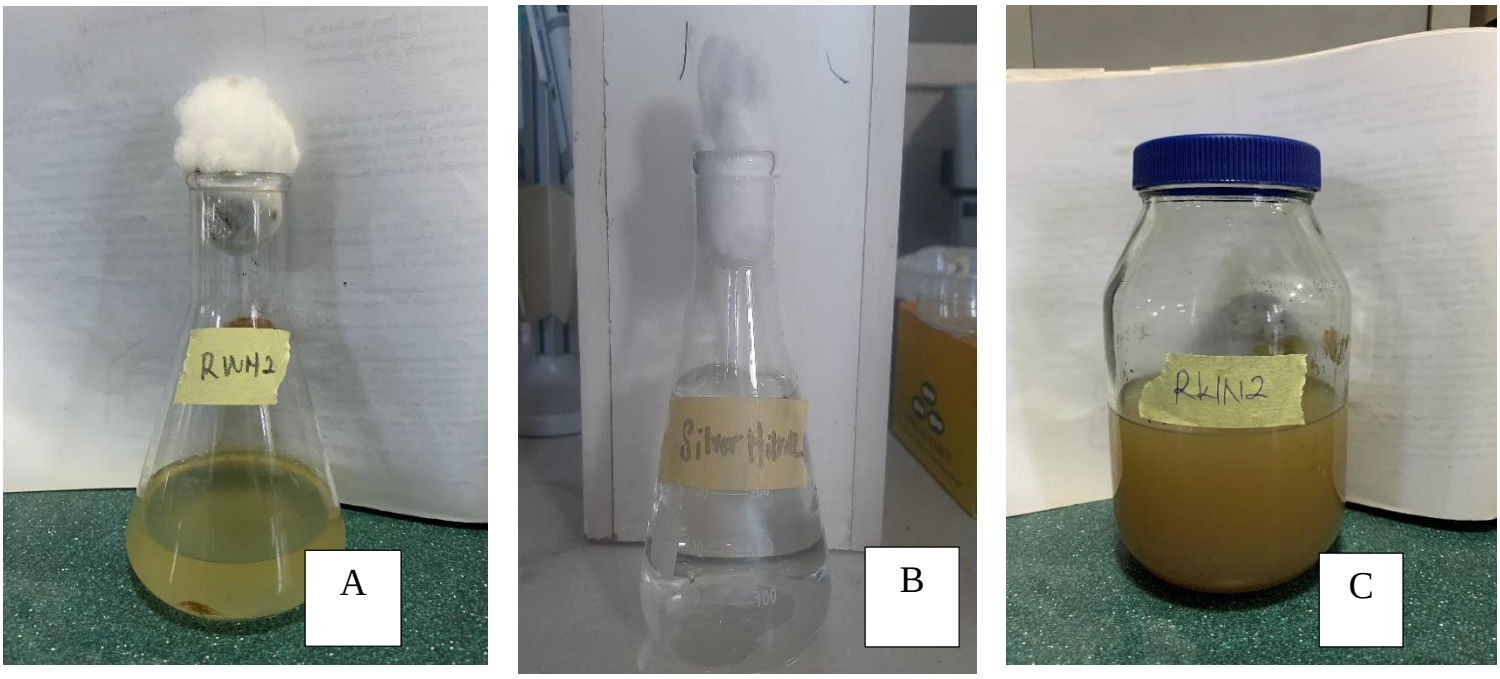

Plate 4.4: Biosynthesis of Silver nanoparticles. (a) Isolate RWN2 culture (b) Silver nitrate solution (c)Brown color of the biosynthesis of silver nanoparticle by the isolate RWN2

4.7 Characterization of Silver Nanoparticles

4.7.1 Ultraviolet spectroscopy

The UV spectrophotometer clearly shows an increase in the intensity of the silver solution over time, indicating the formation of an increased amount of silver nanoparticles in the solution (Devi *et al*., 2014). Figure 4.7 below represents UV-visible spectrum of the bacteria mediated synthesis of silver nanoparticles after 24, 48, and 72 hrs at the wavelength of 200 nm to 800 nm. At 24 hrs, an absorbance Peak was observed between 300 nm and 400nm. At 48 hrs, a slight absorbance peak was observed at 400 nm wavelength range indicating the reduction of silver nitrate in the culture broth and at 72 hrs a slight absorbance was observed at 400nm wavelength range indicating the reduction of silver nitrate in the culture broth respectively.

Figure 4.8 below represents UV-visible spectrum of the bacteria mediated synthesis of silver nanoparticles after 24, 48, and 72 hrs at the wavelength of 200 nm to 800 nm. At 24 hrs, an absorbance Peak was observed between 300 nm and 400nm. At 48 hrs, a slight absorbance peak was observed at 400 nm wavelength range indicating the reduction of silver nitrate in the culture broth and at 72 hrs a slight absorbance was observed at 400nm wavelength range indicating the reduction of silver nitrate in the culture broth respectively. This result is not in agreement with the report of Sukanya *et al*. (2013) who reported that the synthesized silver nanoparticles from Streptomyces sp. displayed its maximum absorbance at 450 nm. A slight peak was observed at 350 nm for 24 hrs UV spectrum analysis. At 48 hrs, the UV spectrum analysis showed a slight absorbance at 500 nm. At 72 hrs, the UV spectrum analysis showed no peak, it declined due to the slow production of the nanoparticle. Compared to the result of the UV analysis recorded after 48 hrs a peak could be observed indicating the gradual formation of the silver nanoparticle (Verma *et al*., 2010).

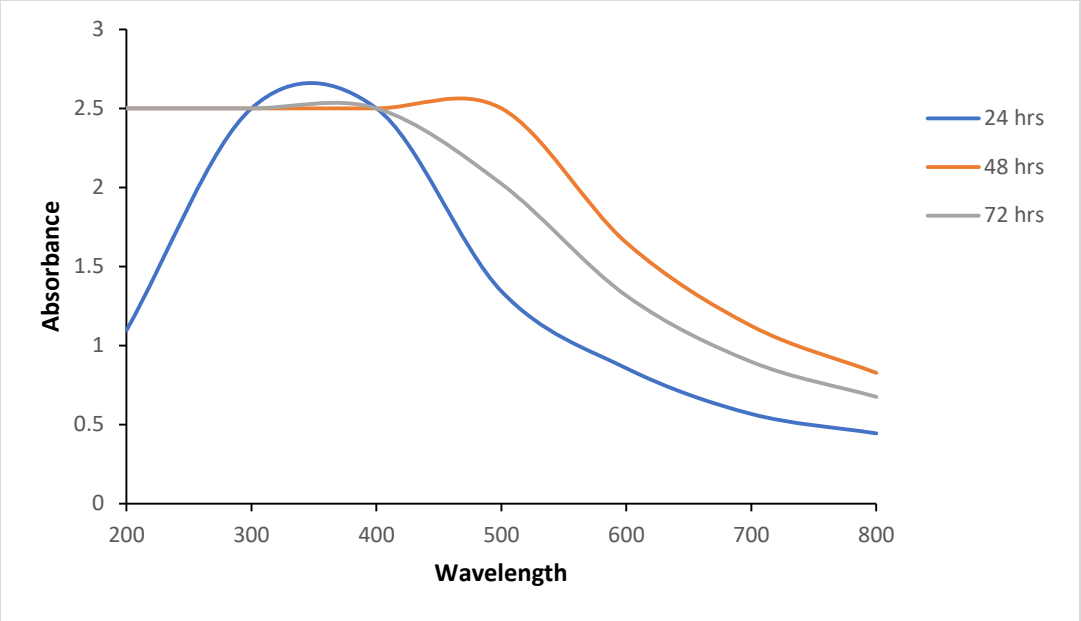

Figure 4.7: UV-visible spectra of the biosynthesized silver nanoparticle by isolate KWN4 at different incubation periods

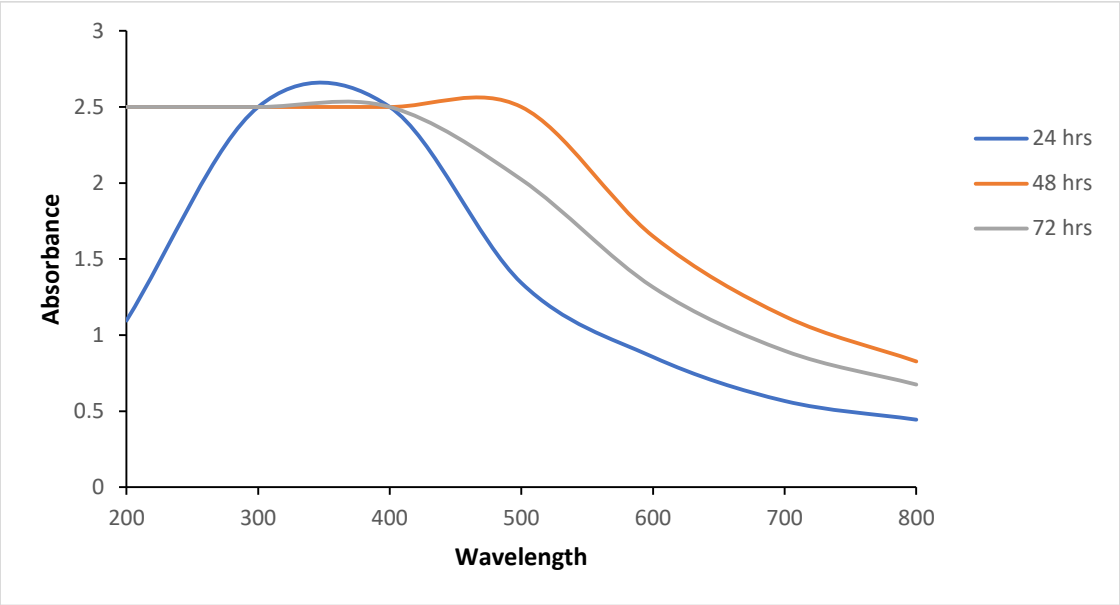

Figure 4.8: UV-visible spectrum of the biosynthesized silver nanoparticle by isolate RWN2 at different incubation periods

4.7.2 FTIR analysis of *Bacillus* sp. KWN4-AgNPs and *Bacillus* sp. RWN2-AgNPs

The Fourier transformation infrared spectroscopy (FTIR) spectrum of the greenly synthesized Silver Nanoparticle of *Bacillus* sp. KWN4-AgNPs and *Bacillus* sp. RWN2-AgNPs is shown in Figure 4.9 and 4.10 respectively. 15 peaks were present between 3762 cm^{-1} to 360 cm^{-1}. The FTIR spectrum showed a broad peak at 3438 cm^{-1} which corresponds to the broad intermolecular bonding of the alcoholic OH band. The peak at 2926.29 cm^{-1} indicates an alkane C-H stretching vibration. The peak at 1728.45 cm^{-1} indicate the strong aldehyde C=O stretching. The peak at 1637.57 cm^{-1} show the medium C=C stretching of the alkene group. Furthermore, the peak at 1514.38 cm^{-1} indicates a strong nitro compound group from the N-O stretching. The peak at 1381.79 cm^{-1} indicates medium aldehyde C-H bending. The peak at 1329.00 cm^{-1} shows the presence of alcohol group indicating the medium O-H bending. The peak at 1256.29 cm^{-1} indicates the strong aromatic ester bond of the C-0 stretching. The presence of a strong sulfoxide group with S=O stretching is indicated in the 1039.46 cm^{-1} peak and a peak at 605.00 cm^{-1} indicates the presence of a halo compound with C-I stretching. This result negates the report of Manivasagan *et al*. (2015) who detailed that the FTIR ghastly investigation of Streptomyces sp. uncovers the nearness of assimilation crests at 3428, 2071, 1634, 1067 and 687 cm^{-1}.And that the biosynthesized silver nanoparticles were observed in the presence of bands due to O–H stretching (3428 cm^{-1}), C O stretching (2071 cm^{-1}), N–H bend (1634 cm^{-1}), C–N stretching (1067 cm^{-1}) and C–Br stretching (687 cm^{-1}). The obtained FT-IR results confirmed the presence of various bioactive molecules such as aldehyde, alcohol, alkenes, carboxylate, sulfoxide, and amino acids that have been reported previously as a potential reducing agent for the biosynthesis of metal and metal oxide NPs (Salem *et al*., 2021). These result were in agreement with other reports found that the proteins play key role in the formation of silver nanoparticles and act as capping and stability agents in the synthesis of AgNPs (Jaidev *et al*., 2010).

Table 4.5: FTIR spectral positions with their corresponding vibration modes for biomass filtrate and Ag-NPs synthesized by *Bacillus* sp. KWN4-AgNp and *Bacillus* sp. RWN2-AgNP.

Absorption frequency (cm^{-1})	Chemical groups	Compound class
3762.00	-	-
3438.00	O-H stretching	Alcohol
2926.29	C-H stretching	Alkane
2369.33	-	-
1728.45	C=O stretching	Aldehyde
1637.57	C=C stretching	Alkene
1514.38	N-O stretching	Nitro compound
1427.00	-	-
1381.79	C-H bending	Aldehyde
1329.00	C-H bending	Alcohol
1256.29	C-O stretching	Aromatic ester
1039.46	S=O stretching	Sulfoxide
605.00	C-I stretching	Halo compound
459.76	-	-
360.52	-	-

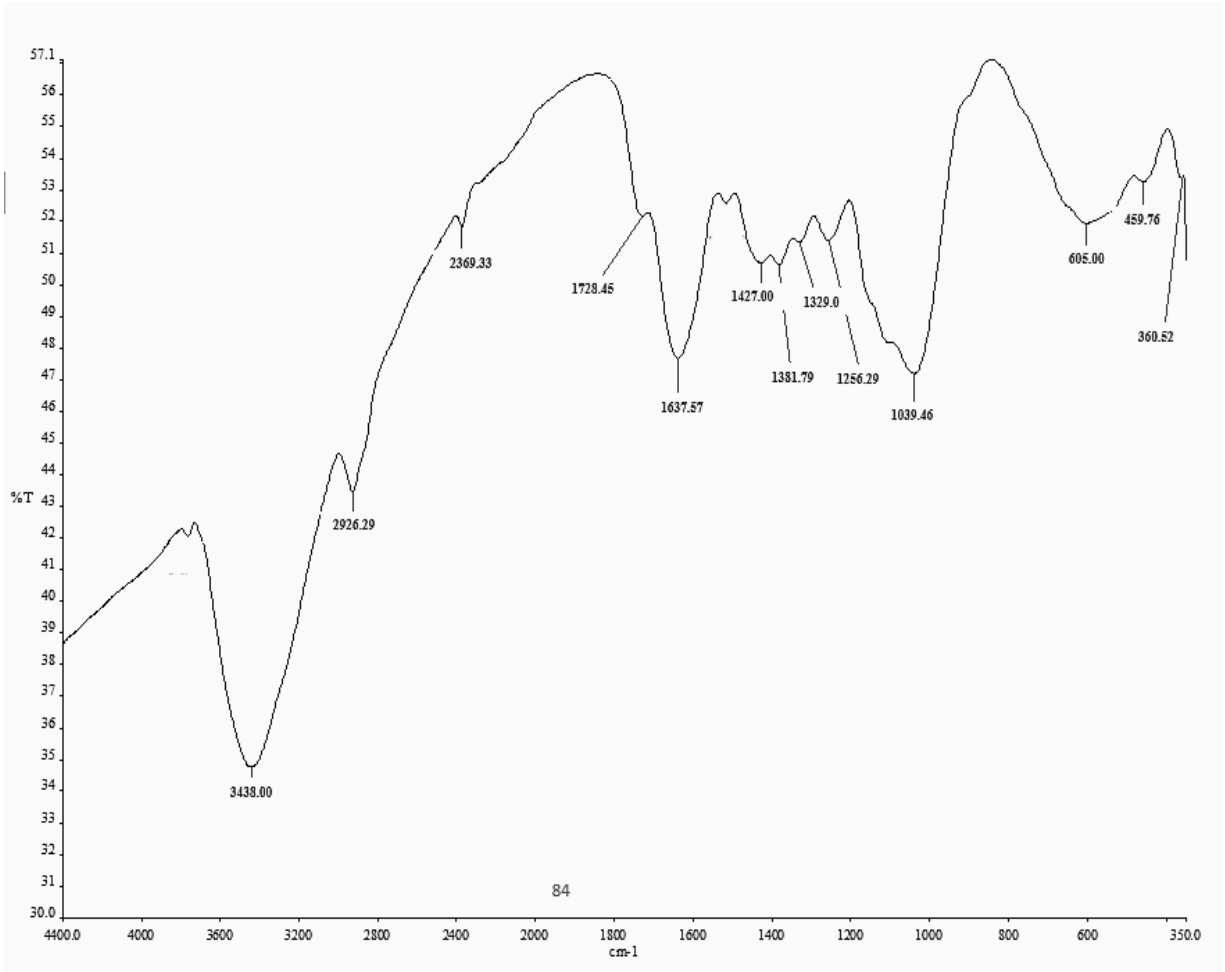

Figure 4.9: Fourier transformation infrared spectroscopy (FTIR spectra) of *Bacillus* sp. KWN4–AgNP

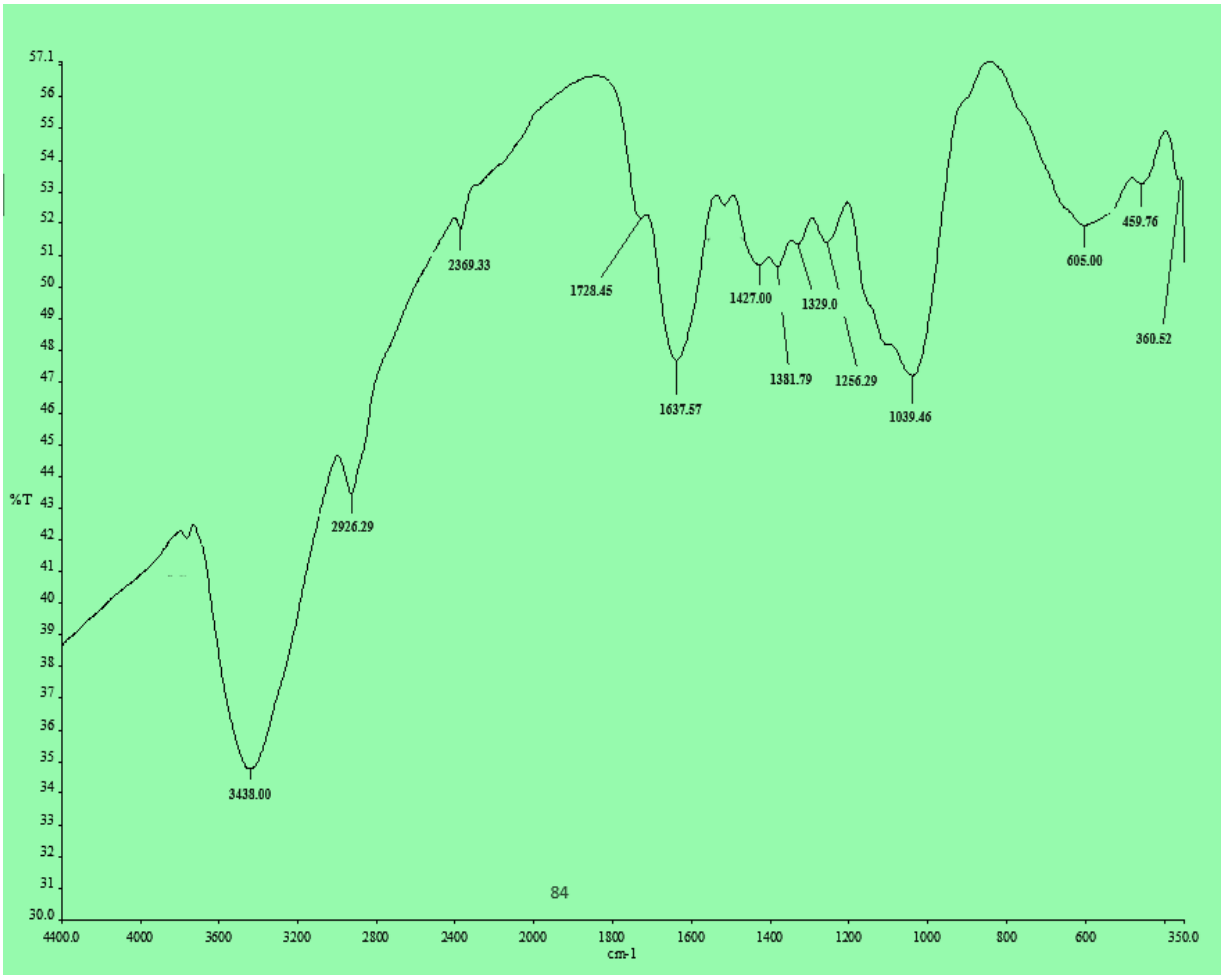

Figure 4.10: Fourier transformation infrared spectroscopy (FTIR spectra) of *Bacillus* sp. RWN2–AgNP

4.8 Application of Bioflocculants and Biosynthesized Silver Nanoparticle Produced by the selected strains on Industry effluent and Restaurant wastewater

The temperature, pH, conductivity and salinity of the wastewaters used for this analysis were determined. Table 4.5 presents the characteristics of the wastewater samples.

Table 4.6 shows the flocculating activities of bioflocculants and biosynthesized silver nanoparticle in Industry effluent and Restaurant wastewater. It was observed that as the concentration of bioflocculants and biosynthesized silver nanoparticles increases, the flocculating activities of bioflocculants and biosynthesized silver nanoparticles also increased to a certain level.

At concentration of 600mg/L bioflocculant of isolate KWN4 and RWN2 shows the highest flocculating activity of 96.57% and 91.43% respectively in industry effluent. While at the concentration of 800mg/L, the biosynthesized AgNPs of KWN4 and biosynthesized AgNPs of RWN2 shows the highest flocculating activity of 88.92% and 89.30% respectively in industry effluent. This result contradicts the report of Adebami *et al*. (2017) who reported that at the concentrations of 600 mg/L and 800 mg/L was needed by bioflocculant of *A. aquatilis* AP4 and *B. clausii* NB2 respectively to produce an optimum flocculating activity of 79.49% and 74.43% respectively. This result is agreed to the report of Sheng and Yang. (2011), who reported that there is no significant difference in the community profile was detected between samples with and without Ag-NP (200 mg/L) treatment. The slightly higher intensities of several bands in untreated samples may indicate a slight decrease in the viability of these genera under Ag-NP treatment.

Bioflocculant concentrations of 600 mg/L and 800 mg/L for isolates KWN4 and RWN2 show the highest flocculating activities of 86.07% and 89.20%, respectively, in restaurant wastewater. At concentrations of 800 mg/L and 1000 mg/L, biosynthesized KWN4 AgNPs and biosynthesized RWN2 AgNPs exhibit the highest flocculating activities of 85.42% and 86.60%, respectively, in restaurant wastewater. This result was consistent with that reported by Adebami *et al*. (2017) they found that bioflocculants of *A. aquatilis* AP4 and *B. clausii* NB2 at concentrations of 600 mg/L and 800 mg/L, respectively, to achieve optimal flocculant activity of 79.49% and 74.43%. Reported as required. Moreover, this result is consistent with the report of

Sheng and Yang. (2011) reported that no significant difference was found in community profiles between samples treated with Ag-NPs (200 mg/L) and untreated. The slightly higher intensity of some bands in untreated samples may indicate a slight decrease in viability of these species under Ag NPs treatment. When both the bioflocculant and the biosynthesized silver nanoparticles were tested on industrial wastewater, both showed the ability to flocculate wastewater, although they exhibited a higher percentage of flocculating activity compared to restaurant wastewater.

Table 4.6: Characteristics of the wastewater used for the analysis

Wastewater	Temperature (º C)	pH	Conductivity (mS/cm)	Salinity (mg/L)
Industry Effluent	26.5	7.3	0.26	270
Restaurant wastewater	26.5	6.5	0.64	516

Table 4.7: Flocculating activity of bioflocculant and biosynthesized silver nanoparticle in wastewater

Test materials	Flocculants	% Flocculating activity					
		100 (mg/L)	200 (mg/L)	400 (mg/L)	600 (mg/L)	800 (mg/L)	1000 (mg/L)
Industry Effluent	KWN4	75.39^{f}	81.02^{e}	88.57^{d}	96.57^{a}	94.23^{b}	93.30^{c}
	RWN2	77.43^{d}	69.35^{f}	78.54^{e}	91.43^{a}	85.06^{c}	89.35^{b}
	KWN4 AgNPs	53.84^{f}	53.90^{e}	65.23^{d}	67.53^{c}	88.92^{a}	73.84^{b}
	RWN2 AgNPs	62.02^{f}	70.94^{c}	68.78^{d}	66.54^{e}	89.30^{a}	76.88^{b}
Restaurant Wastewater	KWN4	57.98^{e}	63.89^{d}	75.02^{b}	86.07^{a}	67.69^{c}	63.89^{d}
	RWN2	56.90^{f}	67.98^{e}	69.95^{d}	84.27^{b}	89.20^{a}	73.69^{c}
	KWN4 AgNPs	57.50^{e}	66.90^{d}	70.82^{c}	79.56^{b}	85.42^{a}	79.56^{b}
	RWN2 AgNPs	70.86^{d}	64.36^{e}	70.86^{d}	81.63^{c}	84.36^{b}	86.60^{a}

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The wastewater evaluated in this study is a rich habitat containing a wide variety of microorganisms, including bioflocculant-producing bacteria. Among the 11 bacteria isolated in this study, the KWN4 and RWN2 isolates identified using morphological and biochemical characteristics were *Bacillus* sp. showed the highest flocculation activities of 29.68% and 27.23 screening endpoints, and were selected for further study. *Bacillus* sp. KWN4 and *Bacillus* sp. RWN2 is used in the production of bio coagulants and the factors affecting the bioflocculant produced from it have been studied at different time intervals (12, 24, 48 and 72 hrs). The results showed that for KWN4 isolate, at 12 hrs, inositol was its best carbon source, ammonium nitrate (NH_4NO_3) was the best nitrogen source, and the monovalent cation K^+ had a high synergistic effect on the compound. bioflocculation is generated. At 24 hrs, sucrose was the best source of carbon, sodium nitrate ($NaNO_3$) was the best nitrogen source, and the monovalent cation K^+ had a high synergistic effect on the bioflocculation produced. At 48 hrs, maltose was the best carbon source, yeast extract plus sodium nitrate ($NaNO_3$) was the best nitrogen source, and the divalent Ca^{2+} cation had a high synergistic effect on the bioflocculant produced. At 72 hrs, inositol was the best source of carbon, yeast extract plus sodium nitrate ($NaNO_3$) was the best source of nitrogen, and the divalent Ca^{2+} cation had a high synergistic effect on the bioflocculant produced. The results showed that for the RWN2 isolate, at 12 hrs, glucose was its best carbon source, ammonium nitrate ($NH_4NO_{3)}$ was its best nitrogen source, and the monovalent cation K^+ had a high synergistic effect on the bioflocculant produced. At 24 hrs, fructose was the best carbon source, peptone was the best nitrogen source, and the monovalent Na^+ cation had a high synergistic effect on the bioflocculant produced. At 48 hrs, maltose was the best source of carbon, ammonium nitrate (NH_4NO_3) was the best source of nitrogen, and the divalent Ca^{2+} cation had a high synergistic effect on the bioflocculant produced. At 72 hrs, inositol was the best source of carbon, ammonium nitrate (NH_4NO_3) was the best source of nitrogen, and the divalent Ca^{2+} cation had a high synergistic effect on the bioflocculant produced. The isolates used in the production of biological coagulants can reduce $AgNO_3$ for the biosynthesis of nanoparticles. The synthesized nanoparticles were characterized using a UV-Visible spectrometer, Fourier transform infrared (FTIR) spectroscopy, confirming the synthesis of the

nanoparticles. Bioflocculating agent and *Bacillus* sp. biosynthesis. Nanoparticles (BSNPs) have been applied in wastewater treatment and showed good flocculation efficiency for two selected wastewater samples (industrial wastewater and restaurant wastewater). The bioflocculation of KWN4 and RWN2 isolates showed good flocculation efficiency of 96.57% and 91.43%, respectively, when a concentration of 600 mg/L was added to industrial wastewater and AgNps produced total The combination of KWN4 and RWN2 showed good flocculation efficiency of 88.92% and 89.30 % respectively, when a concentration of 800 mg/L is added to industrial wastewater. The biological coagulant isolates KWN4 and RWN2 showed good flocculation efficiency of 86.07% and 89.20%, respectively, when concentrations of 600 mg/L and 800 mg/L were added to restaurant wastewater. and the biosynthetic AgNps of KWN4 and RWN2 showed good flocculation efficiency of 85.42% and 86.60% respectively when concentrations of 800 mg/L and 1000 mg/L were added to restaurant wastewater. Therefore, this study shows that the bioflocculating agent of the isolates KWN4 and RWN2 exhibits moderate antibacterial activity, implying that it has potential to be used as a coagulant and disinfectant in the treatment of wastewater samples compared with conventional flocculants and this study provides useful information on the development of novel biocide materials for wastewater treatment and other biotechnological processes.

5.2 Recommendations

Based on the findings, the following recommendations were made:

- Bioflocculant produced by *Bacillus* sp. KWN4 and *Bacillus* sp. RWN2 can be used as flocculating agent in the treatment of wastewater.
- Nanoparticle produced by *Bacillus* sp. KWN4 and *Bacillus* sp. RWN2 can be used as flocculating agent in the treatment of wastewater.

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APPENDIX 1

STATISTICAL ANALYSIS

Effect of Carbon sources on bioflocculant Production by KWN4 for 12hrs

Carbon Sources	**Bioflocculant Production**
Glucose	42.80[c]
Sucrose	46.16[b]
Fructose	10.33[g]
Galactose	40.82[d]
Lactose	31.43[e]
Inositol	61.75[a]
Maltose	12.14[f]

Effect of Carbon Sources on bioflocculant production by RWN2 for 12hrs

Carbon Sources	**Bioflocculant Production**
Glucose	54.69[a]
Sucrose	33.33[f]
Fructose	35.40[e]
Galactose	-34.36[g]
Lactose	40.31[d]
Maltose	41.34[c]
Inositol	52.45[b]

Effect of Nitrogen Sources on bioflocculant Production by KWN4 for 12hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	0.60^{e}
Ammonium nitrate (NH_4NO_3)	47.63^{a}
Peptone + $NaNO_3$	18.00^{b}
Yeast extract +$NaNO_3$	16.79^{d}
Peptone	19.89^{c}

Effect of Nitrogen Sources on bioflocculant production by RWN2 for 12hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	1.98^{d}
Ammonium nitrate (NH_4NO_3)	30.66^{a}
Peptone + $NaNO_3$	9.21^{c}
Yeast extract +$NaNO_3$	29.19^{b}
Peptone	-40.82^{e}

Effect of Cations on bioflocculant production by KWN4 for 12hrs

Cations	**Bioflocculant Production**
Potassium nitrate	38.23[a]
Ferrous chloride	-41.20[h]
Iron (ii) sulphide	34.50[b]
Zinc sulphate	22.77[d]
Sodium acetate	-2.62[g]
Magnesium sulphate	11.04[e]
Copper (ii) sulphate	-0.13[f]
Calcium hydroxide	19.80[c]

Effect of Cations on bioflocculant production by RWN2 for 12hrs

Cations	**Bioflocculant Production**
Potassium nitrate	13.59[a]
Ferrous chloride	-60.66[f]
Iron (ii) sulphide	-6.28[d]
Zinc sulphate	12.28[c]
Sodium acetate	13.59[a]
Magnesium sulphate	-72.53[h]
Copper (ii) sulphate	-7.03[e]
Calcium hydroxide	13.52[b]

Effect of carbon sources on bioflocculant production by KWN4 for 24hrs

Carbon Sources	**Bioflocculant Production**
Glucose	26.36[f]
Sucrose	47.34[a]
Fructose	29.12[e]
Galactose	29.33[d]
Lactose	35.40[c]
Inositol	3.38[g]
Maltose	38.30[b]

Effect of carbon sources on bioflocculant production by RWN2 for 24hrs

Carbon Sources	**Bioflocculant Production**
Glucose	29.05[c]
Sucrose	15.87[g]
Fructose	43.68[a]
Galactose	25.05[e]
Lactose	18.01[f]
Inositol	26.29[d]
Maltose	41.20[b]

Effect of nitrogen sources on bioflocculant production by KWN4 for 24hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	49.28[a]
Ammonium nitrate (NH_4NO_3)	44.60[c]
Peptone + $NaNO_3$	48.68[b]
Yeast extract +$NaNO_3$	42.92[e]
Peptone	46.01[d]

Effect of nitrogen sources on bioflocculant production by RWN2 for 24hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	14.35[c]
Ammonium nitrate (NH_4NO_3)	0.27[e]
Peptone + $NaNO_3$	19.73[b]
Yeast extract +$NaNO_3$	5.59[d]
Peptone	45.27[a]

Effect of cations on bioflocculant production by KWN4 for 24hrs

Cations	**Bioflocculant Production**
Potassium nitrate	69.08[a]
Ferrous chloride	45.54[h]
Iron (ii) sulphide	68.04[b]
Zinc sulphate	53.62[f]
Sodium acetate	64.25[c]
Magnesium sulphate	55.14[e]
Copper (ii) sulphate	51.41[g]
Calcium hydroxide	60.04[d]

Effect of cations on bioflocculant production by RWN2 for 24hrs

Cations	**Bioflocculant Production**
Potassium nitrate	69.15[b]
Ferrous chloride	54.17[g]
Iron (ii) sulphide	63.00[d]
Zinc sulphate	58.73[f]
Sodium acetate	69.63[a]
Magnesium sulphate	61.97[e]
Copper (ii) sulphate	54.10[h]
Calcium hydroxide	68.94[c]

Effect of carbon sources on bioflocculant production by KWN4for 48hrs

Carbon Sources	Bioflocculant Production
Glucose	9.17[b]
Sucrose	7.81[d]
Fructose	-1.36[f]
Galactose	8.39[c]
Lactose	-4.68[g]
Inositol	2.73[e]
Maltose	10.93[a]

Effect of carbon sources on bioflocculant production by RWN2 for 48hrs

Carbon Sources	Bioflocculant Production
Glucose	3.90[f]
Sucrose	0.78[g]
Fructose	13.47[b]
Galactose	12.5[c]
Lactose	11.91[d]
Inositol	7.22[e]
Maltose	20.11[a]

Effect of nitrogen sources on bioflocculant production by KWN4 for 48hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	40.70^{b}
Ammonium nitrate (NH_4NO_3)	32.88^{c}
Peptone + $NaNO_3$	22.23^{e}
Yeast extract +$NaNO_3$	42.04^{a}
Peptone	27.35^{d}

Effect of nitrogen on bioflocculant production by RWN2 for 48hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	43.26^{d}
Ammonium nitrate (NH_4NO_3)	46.63^{a}
Peptone + $NaNO_3$	43.39^{c}
Yeast extract +$NaNO_3$	44.33^{b}
Peptone	24.79e

Effect of cations on bioflocculant production by KWN4 for 48hrs

Cations	**Bioflocculant Production**
Potassium nitrate	37.23[e]
Ferrous chloride	7.36[g]
Iron (ii) sulphide	37.5[d]
Zinc sulphate	37.76[c]
Sodium acetate	37.5[d]
Magnesium sulphate	37.10[f]
Copper (ii) sulphate	45.0[b]
Calcium hydroxide	45.26[a]

Effect of cations on bioflocculant production by RWN2 for 48hrs

Cations	**Bioflocculant Production**
Potassium nitrate	40.13[e]
Ferrous chloride	19.86[g]
Iron (ii) sulphide	40.39[d]
Zinc sulphate	41.31[c]
Sodium acetate	10.92[h]
Magnesium sulphate	41.57[b]
Copper (ii) sulphate	40.0[f]
Calcium hydroxide	54.07[a]

Effects of carbon sources on bioflocculant production by KWN4 for 72hrs

Carbon Sources	**Bioflocculant Production**
Glucose	21.84[f]
Sucrose	26.96[c]
Fructose	23.54[e]
Galactose	24.06[d]
Lactose	28.49[b]
Inositol	29.52[a]
Maltose	14.67[g]

Effects of carbon sources on bioflocculant production by RWN2 for 72hrs

Carbon Sources	**Bioflocculant Production**
Glucose	-1.87[g]
Sucrose	20.13[c]
Fructose	19.28[d]
Galactose	20.13[c]
Lactose	3.24[e]
Inositol	33.27[a]
Maltose	30.71[b]

Effects of nitrogen sources on bioflocculant production by KWN4 for 72hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	35.49[b]
Ammonium nitrate (NH_4NO_3)	29.86[c]
Peptone + $NaNO_3$	8.02[e]
Yeast extract +$NaNO_3$	36.34[a]
Peptone	22.52[d]

Effects of nitrogen sources on bioflocculant production by RWN2 for 72hrs

Nitrogen Sources	**Bioflocculant Production**
Sodium nitrate ($NaNO_3$)	29.01[b]
Ammonium nitrate (NH_4NO_3)	30.54[a]
Peptone + $NaNO_3$	17.74[d]
Yeast extract +$NaNO_3$	21.50[c]
Peptone	0[e]

Effects of cations on bioflocculant production by KWN4 for 72hrs

Cations	**Bioflocculant Production**
Potassium nitrate	27.64[f]
Ferrous chloride	-0.17[h]
Iron (ii) sulphide	20.47[g]
Zinc sulphate	36.34[c]
Sodium acetate	35.66[d]
Magnesium sulphate	42.15[b]
Copper (ii) sulphate	30.20[e]
Calcium hydroxide	50.34[a]

Effects of cations on bioflocculant production by RWN2 for 72hrs

Cations	**Bioflocculant Production**
Potassium nitrate	48.46[c]
Ferrous chloride	23.03[g]
Iron (ii) sulphide	38.56[f]
Zinc sulphate	50.85[b]
Sodium acetate	47.78[d]
Magnesium sulphate	46.07[e]
Copper (ii) sulphate	-11.26[h]
Calcium hydroxide	68.25[a]

UV-VIS Spectrophotometry for Silver Nanoparticles- KWN4

Wavelengths(nm)	Absorbance (24 hrs)	Absorbance (48 hrs)	Absorbance(72 hrs)
200	1.096	2.500	2.500
300	2.500	2.500	2.500
400	2.500	2.500	2.500
500	1.343	2.500	2.023
600	0.856	1.650	1.315
700	0.567	1.124	0.897
800	0.444	0.827	0.675

UV-VIS Spectrophotometry for Silver Nanoparticle - RWN2

Wavelengths(nm)	Absorbance (24 hrs)	Absorbance (48 hrs)	Absorbance(72 hrs)
200	2.066	1.418	1.914
300	2.500	2.500	2.500
400	1.271	2.500	2.500
500	0.703	2.208	1.932
600	0.474	1.778	1.286
700	0.311	1.445	0.901
800	0.266	1.191	0.683

APPENDIX 2

COMPOSITION OF MEDIUM

Nutrient Agar

Peptones	5 g
Beef extract	1 g
Yeast extract	2 g
Sodium chloride (NaCl)	5 g
Agar	15 g
Distilled water	1 litre

Nutrient Broth

Peptones	10 g
Beef extract	1 g
Yeast extract	2 g
Sodium chloride	5 g
Distilled water	1 litre

Simmons citrate Agar

Sodium chloride (NaCl)	**5 g**
Sodium citrate (dehydrate)	2 g
Ammonium Dihydrogen Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate (heptahydrate)	0.2 g
Bromothymol Blue	0.08 g
Agar	15 g
Distilled water	1 litre

MR-VP Broth

Buffered Peptone	7 g
Dextrose	5 g
Dipotassium Phosphate	5 g
Distilled water	1 litre

Bioflocculant producing Broth

Glucose	20 g
Urea	0.5 g
Yeast extract	0.5 g
Aluminum sulphate ($AlSO_4$)	0.2 g
Dipotassium hydrogen phosphate (K_2HPO_4)	5 g
Potassium dihydrogen phosphate (KH_2PO_4)	2 g
Magnesium Sulphate ($MgSO_4.7H_2O$)	0.2 g
Sodium Chloride (NaCl)	0.1 g
Distilled water	1 litre