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Antibacterial Activity of Iron Oxide Nanoparticles Synthesized by *Bacillus coagulans* Toward Clinical *Escherichia coli* Isolates

A Thesis

Submitted to the Council of the Faculty of Science University of Babylon in Partial Fulfillment of the Requirements for the Doctorate Philosophy Degree in Science /Biology

By

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رَبِّ أَوْزِعْنِيٓ أَنْ أَشْكُرَ نِعْمَتَكَ
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وَأَنْ أَعْمَلَ صَالِحًا تَرْضَىٰهُ
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صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سورة النمل الاية (19)

Certification

I certify that the preparation of this thesis entitled "**Antibacterial Activity of Iron Oxide Nanoparticles Synthesized by *B. coagulans* Toward Clinical *E. coli* Isolates** " was prepared by " Qasim Abood Falyh AL-Maliki " under my supervision at University of Babylon/College of Science / Department of Biology as a partial fulfillment of the requirement for the Degree of Doctorate of Science in Biology- Microbiology .

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In view of the available recommendation , I forward this thesis for debate by the examining committee .

Professor

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Dedication

To my brother

the martyr Abu Haider

accept my effort to remain a witness to your memory

to the my supported

my dear family

my friends

Qasim

Summary

Upon emergence of resistant bacteria to almost various traditional antibiotics, metal oxides nanoparticles are favored choice subject to researchers as alternative antibacterial agent due to efficiency against resistant bacteria, biosynthesis of nanoparticles take more attention than chemical and physical methods because of the low cost , ecofriendly and biocompatibility

The aims of this study to confirmed the ability of *Bacillus coagulans* bacteria , one of famous biologically active spore forming gram positive probiotic bacillus bacteria to biosynthesis of iron oxide(Fe_3O_4) nanoparticles as well as evaluating the antibacterial and antibiofilm activity of nanoparticles against clinical *Escherichia coli* bacteria isolates .The bacteria was isolated from 120 urine specimens of urinary tract infection patients of external clinical laboratory in Al-Hakeem General Hospital in Baghdad during period from 10/10 to 1/12/2020 .The isolates diagnostic through cultured and various biochemical tests in addition to morphological identification by microscopic examination and confirm diagnostic by VITIC-2 system , where it was obtained of (72) *E. coli* isolates from all of urine samples The *B. coagulans* was identification in same diagnostic steps.

Cell free supernatant of *Bacillus coagulans* was used to biosynthesized and stabilized of Iron oxide nanoparticles with iron salt precursor $Fecl_3.6H_2O$(2.53g) and $Fecl_2.4H_2O$(0.99g) in ratio 2:1 , the color changing and black precipitant appear indicated formation of Fe_3O_4 Nanoparticles . Characterization of biogenic Iron oxide nanoparticles was obtained through many techniques such as Uv-Vis spectroscope , Fourier transform infra-red

spectrograph , X-Ray diffraction ,Scanning electron microscope and Atomic force microscope. The Uv-Vis spectroscopic spectrum recorded absorbance among expected beak of magnetic nanoparticles at 250.5 nm ,while Fourier transform infrared spectrum of Fe_3O_4 showed the characteristic peaks at 586 and 632 cm^{-1}.The average of biosynthesis nanoparticles by X-ray diffraction for mean crystalline size was 15 nm . Scanning electron microscope was used to reveal shape ,size and distribution of iron oxide nanoparticles ,the shape was mostly irregular cubic and homogenous with average size between 4-33 nm ,at last the Atomic force microscope was used to detected the three dimensional images structure and average diameter of Fe_3O_4 nanoparticles which has been 40-60 nm in diameter. Together ,these results indicated the ability of *B. coagulans* bacteria to biosynthesis Iron oxide nanoparticles.

The next step was to reveal the activity of biosynthesis Iron oxide nanoparticles to act as an antibacterial agent against *E.coli* uropathogenic bacteria , once alone and in synergy with polyhydroxybutyrate(PHB) biopolymer second , to compare the antibacterial activity with the preference for the effected on uropathogenic bacteria ,were largest inhibition zone appeared in concentration of 200µg/ml for the biosynthesis nanoparticles at 31.8 mm . The synergistic of Fe_3O_4 nanoparticles with PHB biopolymer as antibacterial agent was recorded in concentration of 200 µg/ml at 33.6 mm as largest inhibition zone that indicated the advantage of the synergistic effect. The iron oxide nanoparticles in concentration of 150 µg/ml showed satisfactory result were imped and disrupt biofilm formation by *E. coli*.

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List of Abbreviations

Abbreviation	Meaning
AFM	Atomic Force Microscopy.
BHIA	Brain Heart Infusion Agar
CLSI	Clinical and Laboratory Standards Institute
EPS	Extracellular Polysaccharide
FTIR	Fourier-Transform Infrared Spectroscopy
GRAS	Generally Recognized as a Safe
IONPs	Iron Oxide Nanoparticles
MDR	Multi Drug Resistance
MHT	Magnetic Hyperthermal Therapy
MRI	Magnetic Resonance Imaging
NPs	Nanoparticles
NS	Nano Scale
PHB	Polyhydroxybutyrate
PHA	Polyhydroxyalkanoate
PDR	Pandrug –Resistance
PTT	Photothermal therapy
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscope
SPR	Surface Plasmon Resonance

TDA	Top-down approach
UTIs	Urinary Tract Infection
Uv-Vis	Ultra Violate-Visible
VER	Vancomycin Resistance Enterococcus
VFs	Virulence Factors
WHO	World Health Organization
XRD	X-Ray Diffraction

Chapter One
Introduction
and
Literature Review

1.Introduction

Nanotechnology is science and engineering deal with scale of atoms and molecules ,generally in nanorange between 1-100 nm this scale at which the basic function of the biological components operated . The materials in this size exhibit unusual chemical and physical properties due to increase in surface area compared to volume of particles and unusual control of quantum effect at atomic level . Progress accelerated with this science when built first Scanning Tunneling Microscope (STM) allowing scientists to see single atoms , the discover and develop other devices like Atomic Force Microscope (AFM) and Magnetic Resonance Imaging(MRI) that become more advance techniques dealing with atomic scale inspired the researchers to experiment new material in the different scientific , industrial and medical fields. There are three important methods used in produce nanomaterial's : chemical, physical and biological through two approach top-down and bottom-up techniques, a self-assembly in some nanomaterial such as nanowires have been shown in simple scale (Pareek *et al*.,2017).

The biological methods take more attention in synthesis and production of nanoparticles ,in terms of use biological molecules such as protein, DNA and others ,furthermore finding a ways to put viruses, bacteria, and others microorganism in biosynthesis of nanomaterial ,also employed the plants to work in building nanomaterial's, by using different extraction parts of leafs, fruits ,seeds and roots(Agarwal *et al*., 2017) .

The biological approaches have more advantageous for the synthesis of NPs than the physical and chemical methods due to eco-friendly, low

cost, less harmful and toxic to human health and environment that reduced using high temperature , high pressure toxic chemical and saving energy(Yan *et al* ., 2017) . Natural environment interference with microorganisms in the requirement of growth and metabolic activity that provided suitable nutrition ,pH and temperature , so the manipulation in such requirement resulted in possibility adjust the size and shape of NPs biological synthesis by microorganism and this advantage made it possible to obtain nanoparticles in certain shapes and sizes, moreover biosynthesis reduce the toxicity of NPs and more compatibility when use in *vivo*.(Singh *et al* ., 2016))

There are many different materials used to coating iron oxide NPs and most popular are the polymers such as dextran, starch , albumin, chitosan and poly ethylene glycol (PEG) . In addition to amino acids like tyrosine and fatty acids like oleic acid as well as metals like gold and silver and oxides like silica and TiO_2 .The surface modfaction of iron oxide NPs with biocompatible coating improvement the stabilized and biocompatibility of nanoparticles in biological media and to prevent them from possible oxidation ,there for surface modification of iron oxide NPs can be employed for medical application like Magnetic Hyperthermal Therapy (MHT) and Magnetic Resonance Imaging(MRI) as contrast agent ,immunotherapy, targeted delivery of drugs, antibodies , proteins, and nucleic acids, also in hyperthermia , biosensor , tissue repair and in cell separation (Seyed *et al* ., 2019). The others important fields of application of iron oxide NPs are the antibacterial and antibiofilm toward susceptible both Gram negative and Gram negative bacterial as well as fungal species susceptible to toxic action of iron oxide NPs (Sergey *et al* ., 2021).

Aim of Study

The current study aimed to detect the ability of *Bacillus coagulans* bacteria to biosynthesis of iron oxide nanoparticles and to investigated the activity of this NPs as antibacterial and antibiofilm against uropathogenic *E.coli* isolates, and synergistic with biopolymer polyhydroxybutyrate (PHB) to reinforced antibacterial activity.

To achieve the above aims the following objectives were conducted:

1. Biosynthesis of iron oxide NPs by *B. coagulans* supernatant
2. Characterization of the biosynthesis Iron oxide NPs by using FTIR, Uv-Vis spectrometer, XRD, SEM and AFM
3. Isolation and identification of *E. coli* bacteria from urinary tract infection
4. Study some application of Iron oxide NPs against *E. coli* isolated from urinary tract infection such as antibacterial and antibiofilm
5. Study the antibacterial activity of nanocomposite Fe_3O_4 NPs with poly β-hydroxybutyrate (PHB) toward *E. coli*.

1.2 Literature Review

1.2.1 Nanotechnology

In 1959 physicist Richard Feynman is known as the father of nanotechnology talk in meeting of American physical society about the concept and idea of Nanotechnology and Nano science in lecture entitled "There's plenty of room at bottom". An invitation to enter a new field of physics when explain the ability of manipulate and control individual atom and molecules (William *et al*., 2018).

Professor Norio Taniguchi in 1974 coined the term Nanotechnology" Nanos" Greek word means ´dwarf that refer to material of one billionth of meter (10^{-9}m) to describe semi-conductor process such as thin- film deposition that deal with control on order of nanometer , and his defended still stand as proper statement. "Nano-technology" meaning consist of processing of separation, consolidation, and deformation of material by one atom or one molecule". Later with his crow added new definition " *integrated processing system for ultra-precision and ultra- fine production* (Drexler ., 1992)

K. Eric Drexler used term of nanotechnology in his book "*Engines of creation: The coming of Era Nanotechnology*". early which suggest the idea of nonsocial "assembler" to build a copy of itself and of other item with atomic control. And founded institute to help public interest and understanding nanotechnology.(Drexler.,1986). Nanotechnology defined by U.S National Nanotechnology initiative (NNI). The understanding and control of matter in dimension between approximately 1 - 100 nanometers, where unique phenomena enabling novel nanotechnology application. Encompassing nanoscale science, engineering, and technology, nanotechnology involve imagining , measuring , modeling and manipulating matter at this length (Nano gov).

Two scientific events in eighties of 20th century accelerated the growth of nanotechnology in modern era the first event : physical which invention of scanning tunneling microscope which expand the vision and processing of atom and bounds (Binning and Rohrer.,1986).Second event : chemical which discovery of " fullerenes" the terms was used related next to work with graphen tubes (carbon nano tube) one of its application use in nanoscale electronics and devices .(Kroto *et al*., 1985).

Generally, nanotechnology deals with nanoparticles structure size between 1-100 nanometer in at least one dimension 1D. Nanoparticles exhibit significant unique interest chemical , physical and biological properties due to size , shape , and surface area where the smaller dimensional provided higher surface area compared with 3D nanoparticles, as well increased in surface-to-volume ratio offset by decrease of dimensional (Khan *et al* ., 2017). There are three methods to synthesis nanoparticles, chemical, physical, and biological (Luo *et al*.,2006). Although chemical and physical methods widely use in production and limits used in biomedical application duo to their possible toxicity or release hazard compounds more over consumption large amount of energy(Awwad *et al*., 2013).Hence, based on versatile application in biomedicine encouraged the researcher to improvement and development of Bio-assisted methods in nanomaterial production (Varshney *et al*.,2012) .Further, biosynthesis provided environmentally benign, cost-effective, low toxic, efficient protocol to synthesis and fabricated in size and shape of nanoparticles (Geethalakshmi *et al.*, 2010) . Biosynthesis method broadly using bacteria, algae yeast and viruses (Sintubin *et al*.,2009) and using plant extracted (Iravani *et al*.,2011).

1.2.2 Nanoparticles.

The reason behind this board interest in Nanoparticles wild ranges of use in different field based on portability to provide specific applications**,** and that comes back to its specific properties , different models of synthesis and different types of nanoparticles(Baig *et al*., 2021).

Nanoparticles can be classified according many different parameters such as their origin(natural or artificial) ,chemical composition (organic or inorganic). Formation (geogenic ,biogenic ,anthropogenic ,atmospheric), shape(morphology) and size Fig(1-1) (Borouman *et al*., 2015) .Beside chemical and physical characteristic , all of those classification may be associated by one or more feature consequently in different type of well-known nanoparticle such as ***carbon-based NPs*** :Beside two natural pure carbon crystalline form Diamond and graphite two major class fullerene(C_{60}) discovered by Kroto and his team in 1985 (Kroto *et al.*, 1985) and carbon Nanotubes first observe by Iijima in 1991 in soot (Khan *et al.*, 2017). Fullerene are globular hollow cage continent of nanomaterial of carbon used commercials because of their electric conductivity, high strength ,electron affinity and structure ,fullerene consider one of many allotropes of carbon in which carbon atoms connected by single or double bound formed hollow globular cage closed (Bucky balls) or partially closed mesh and take many other shape and size (Astefanei *et al* .,2015). Carbon nanotube elongation tube shape , single layer consider intermediate between fullerene cages and flat graphite or sheet mesh when called single- one layer graphite mesh rolling by itself such case the carbon nanotube walled NPs(SWNTs) (Iijima, 1991) .When CNPs formed from two layers or more represent double-walled

NPs (DWNTs) and multi-walled NPs(MWNTs) ,because of nanostructure and strength CNPs widely used in electrical conductivity (Ibrahim ., 2013).

Ceramic NPs : one of important type of nanoparticles is (Nano ceramic) fall under nonmetallic sold inorganic, heat resistant , and found in different forms , polycrystalline ,amorphous ,pours ,hollow, dense . They are synthesis by successively heating and cooling ,where they formed by processing sol-gel , which mixed NPs with solution and gel to produce NPs(Sigmuned *et al*., 2006).Many applications of Nano ceramic such as catalysis , imaging application and also in medical technology , construction, energy supply (Thomas *et al*.,2015)

Semiconductor NPs: use in different application because of properties between metals and nonmetals one of most characteristic posse band gap tuning qualify material in optical properties and electronic dives (Ali *et al*.,2017)

Polymeric NPs : organic ,synthetic or natural origin , have nanosphere nanocapsular shape ,because of biodegradable and biocompatible widely use in drug delivery (Mansha *et al*., 2017).

Lipid- based NPs: organic , spherical with rang from 10-1000 nm diameter, lipid NPs possess sold lipid core surrounded by matrix soluble lipophilic molecules , resulting in drug carriers and delivery(Puri *et al*.,2009 ; Rawat *et al*.,2011).

Metal Nanoparticles : Metals NPs are made of pure alkali or Nobel metal like(Ag ,Au and Cu etc..) or their compounds e.g.(oxide, sulfides , chloride etc.). Surface Plasmon resonance (SPR) ,electromagnetic optoelelctrical

,shape and size, properties made of metal nanoparticles used in many different application (Dreaden *et al*.,2012).

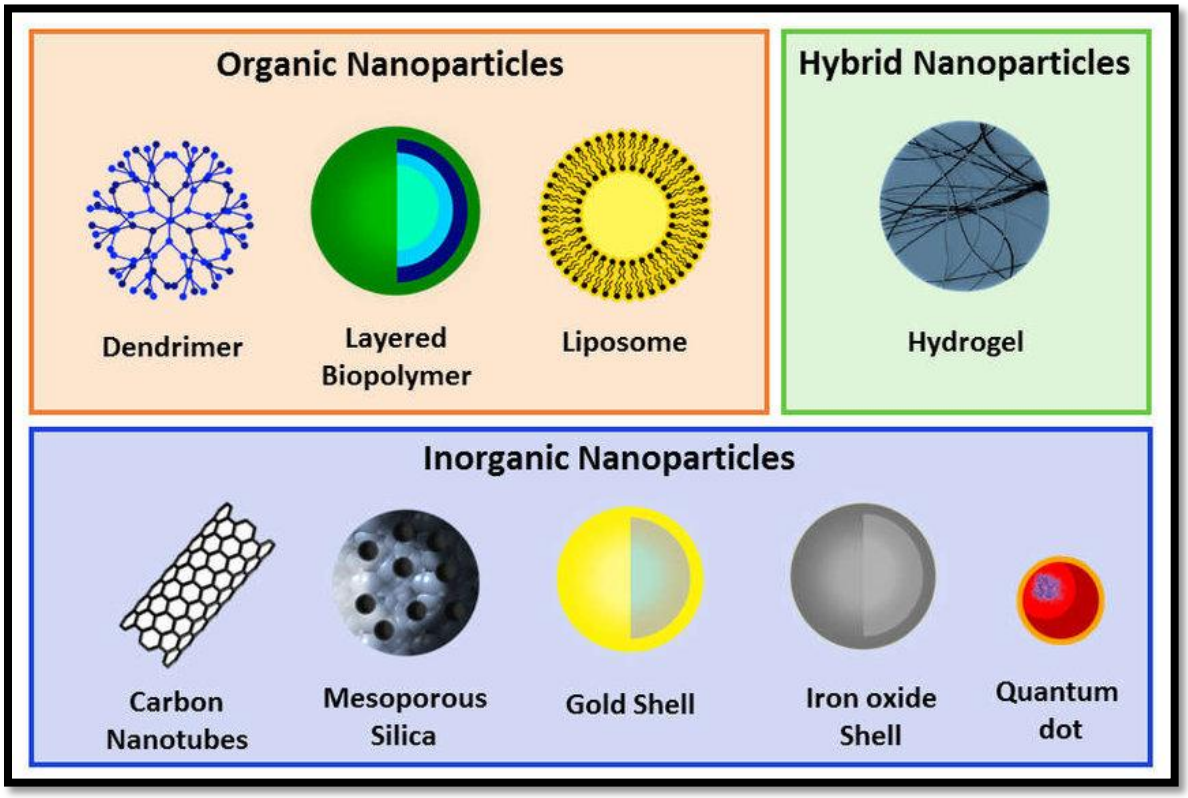


Figure (1-1) Schematic representation of different types of nanoparticles (NPs) divided into organic, hybrid and inorganic categories (Silva *et al*.,2019).

1.2.3 Synthesis of Nanoparticles

There are a variety methods categorized as chemical ,physical , and biological can be employed for the synthesis of nanoparticles. The basic of methods broadly divided into two main classes (1) Top-down approach and (2) Bottom-up approach these approaches as shown in Fig(1.2) can be divided into different subclass based on operations ,reaction conditions and protocol steps (Iravani ., 2011).

- Top-down approach synthesis of Nanoparticles via break down solid material to nanoscale through external force.
- Bottom-up approach synthesis of Nanoparticles via self-assembly of liquid or gas atoms or molecules through reaction precursors.

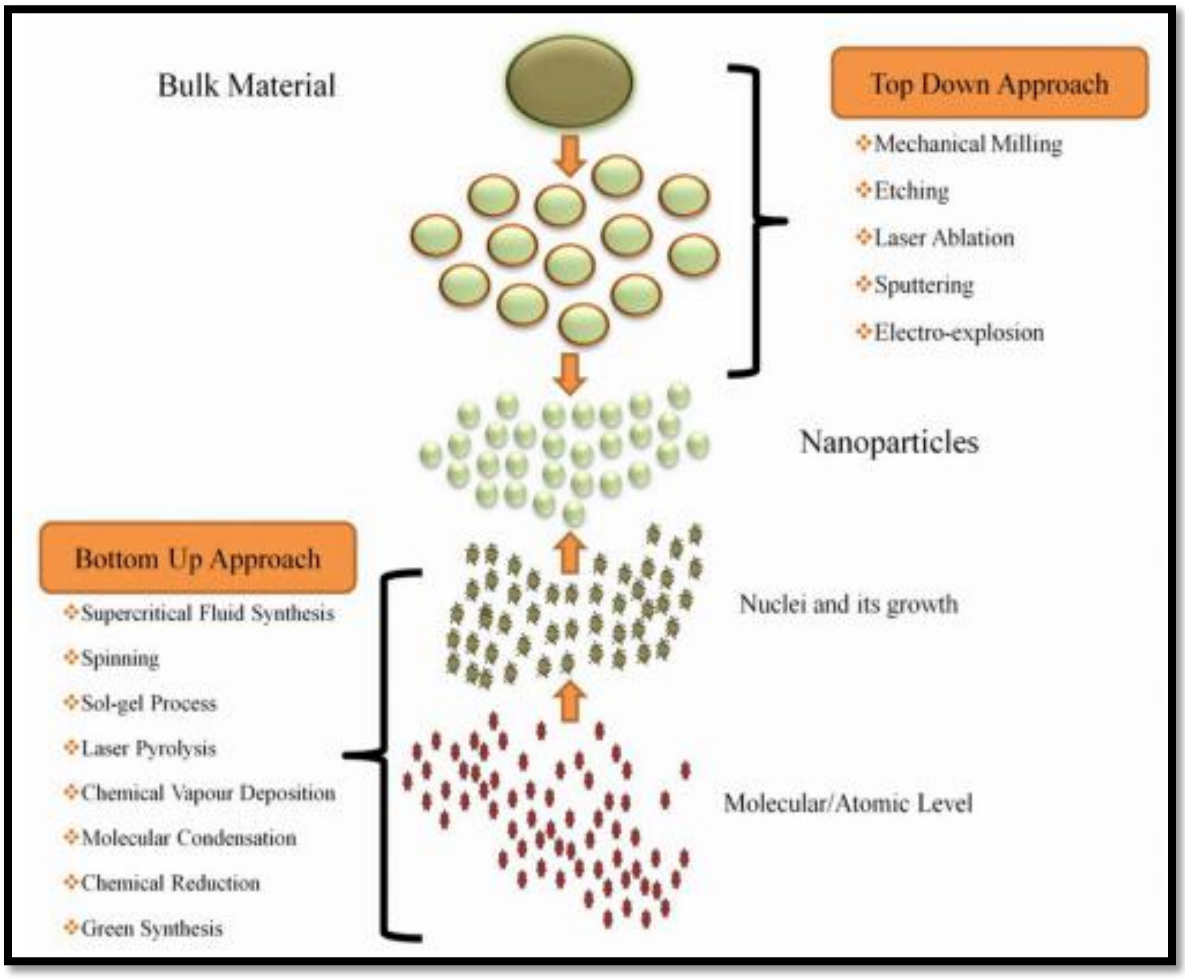


Figure (1-2)Synthetic methods of nanoparticles (Iravani .,2011)

1.2.3.1 :Biological Synthesis of Nanoparticles

Green nanotechnology ,is a part of green technology based upon concept of green chemistry and green engineering. In general green nanotechnology deal with making and using of ecofriendly nanomaterial as

clean techniques reduced the human health risk and minimized undesirable affect factors on environment ,plant, animal life with less toxic and sustainability environment (Verma *et al.,* 2019) .Hence using the biological approach synthesis of nanomaterial's provided main requirements such as biodegradable material (Shinde .,2012) bioremediation (Kulshreshtha *et al*.,2014) biocompatibility (Honarkar and Barikani., 2009) one of exllant example biopolymers shear all compatible features of green synthesis Polyhydroxyalkanoate (PHA) liner chain of different ester group produced in bacteria under physiological stress condition(Xiong *et al.*,2010).

In addition to biological method there are four methods which can be fall under green synthesis polyoxometalates , Irradiation ,Tollens ,and polysaccharide (Keat *et al.*,2015).

The interested of biological synthesis interaction of inorganic or metallic molecules with biologic species to produce nanoparticles, In natural biogenic metallic nanoparticles synthesis divided into two categories :bioreduction and biosorption in first category bioreduction metal ions are chemically reduced into more stable biological forms and in many organisms metal reduction coupled with oxidation of an enzyme (Deplanche *et al*., 2010).

In second category biosorption, the metal ions from soil or an aqueous sample binding onto cell wall of organisms itself without the need for energy input, certain plant ,fungi and bacteria modified cell wall or have a express peptides which bind to metal ions and the importance of this enabled to form stable complexes in the form in nanoparticles (Yong *et al*.,2002).

Many biological systems has been demonstrate during past decade including plant and algae (Govindaraju *et al*.,2008). Bacteria (Pantidos and Horsfall,2014).Fungi (Vahabi *et al*.,2011). Yeast (Kowshik *et al* .,2002). Actinomycetes (Biglari *et al*.,2013) .

The biological synthesis of nanoparticles reveal the increase constancy (Thakkar *et al*.,2010)and control good morphology (Sriram *et al*.,2012).

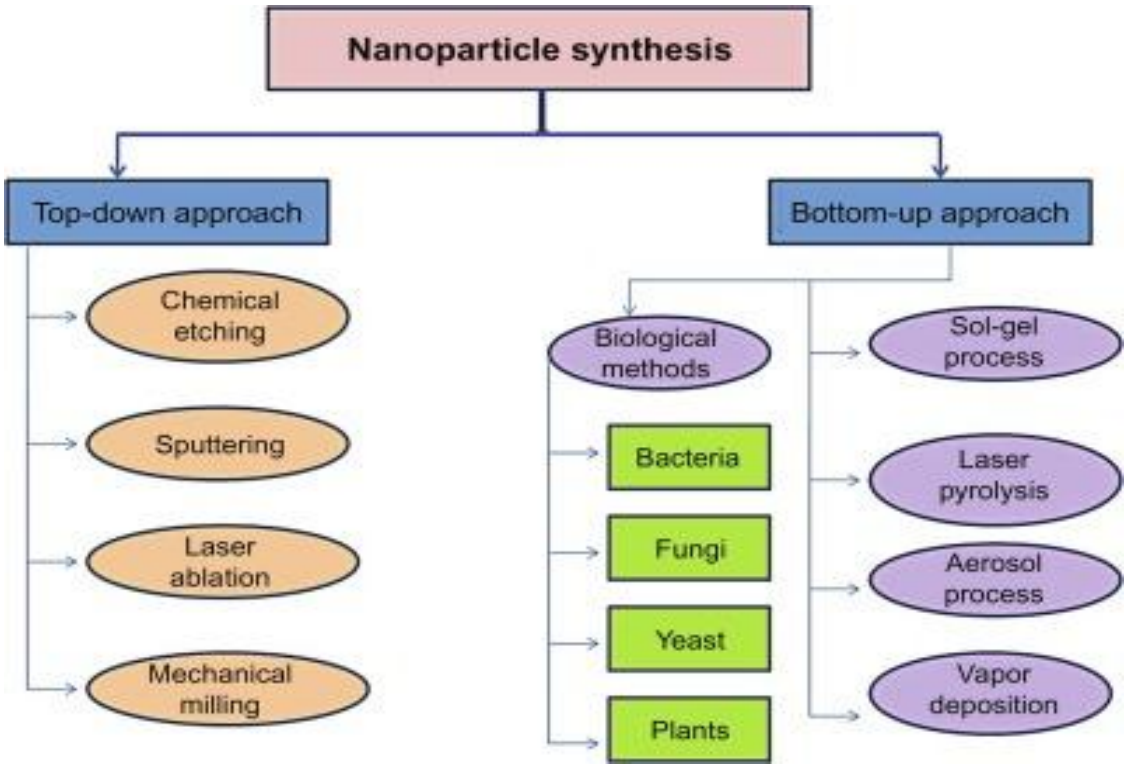


Figure (1-3) different types of NPs synthesis.

Actinomycetes particularly some of *Streptomycin spp.* are one of good chose for biosynthesis of nanoparticles due to their lowest cost ,easy handling and divers secondary metabolites (Manivasagan.,2015).In the two pathway Actinomycetes biosynthesis NPs in extracellular (Ranjitha and Ravishankar .,2018) and intercellular (Ahmed *et al*.,2015). Synthesis of

nanoparticles and many reports directed to selenium NPs biosynthesis by streptomycin (Grasso *et al*., 2019).Based on selenium NPs importance in using as nutrition supplement (Skalickova *et al*.,2017) potent therapeutic cancer agent at high dosage (Fernandes *et al*.,2015). Antioxidant and wound healing (Husen and Siddiqi .,2014)

1-2-3-2 Biological NPs Synthesis by Bacteria

Many different species of bacteria have been used to synthesis numerous NPs with unique nanostructures i.e., magnetosomes (Yan *et al*.,2017) and organic nanomaterial's like exopolysaccarids(Nwodo *et al*.,2012). And metallic, (also as alloys), non-metallic, or metal oxides, nanoparticles (Hulkoti and Taranath .,2014).The bacteria used two ways synthesis NPs intracellular and extracellular approaches and because of no need to any downstream process for collection of the nanoparticles from bacteria with less time consuming extracellular is preferred (Singh *et al*.,2016).In addition to easy handling and coast effective (Abbasi *et al*.,2016).The both to ways of biocatalytic synthesis mainly involve oxidoredactase enzymes (NADH-dependent reductase and NADPH-dependent reductase) and cellular transporters ,in intracellular approach cell wall paly role due to electrostatic interaction by negative charge which is attracted the positively charge of metal ions, in addition cell wall of bacteria contains enzymes reduce the metal ions(Hulkoti and Taranath.,2014) .

Biochemical mechanism have been proposed mediate the synthesis of NPs in bacteria and consider as result of cellular detoxification , which lead to changes in solubility of inorganic ions by activates of enzymes reduction

or precipitation soluble toxic into insoluble non-toxic nanostructure (Grasso *et al* .,2019).

Biomolecules can be perform function of capping and stabilizing agent in bacteria based synthesis of metals NPs(Sintubin *et al*.,2012).Other advantage of biomolecules governed some NPS properties such as shape , size and biocompatibility better feature in biomedicine application like antibacterial ,drug delivery and biosensor(Huang *et al*.,2015;Tang *et al*., 2017). Some bacteria have ability to grow and survive in stress conditions in high concentration of metal ions through different resistance mechanisms such as efflux system ,biosorption ,alteration of solubility and toxicity via Red-Ox reaction , bioaccumulation and precipitation ,some of these mechanisms due to presence of extra chromosomal DNA genes express to enzymes or proteins taking apart in the mechanisms or extracellular complexation of metal ions and lack of specific metal transport system (Hussieiny *et al*.,2007;Balakumaran *et al*.,2016). *Pseudomonas stutzari* an example of bacteria isolated from silver mines tolerant high concentration and shown produced of silver nanoparticles (Mohanpuria *et al*.,2008).

The interaction between microorganism and metals in synthesis NPs have been well study ,one of ability of bacteria to extract or/and accumulation metals employed as commercial biotechnology processes in bioremediation and bioleaching (Gerick and Pinches. ,2006).

Collectively, all of mechanism and fabrication in different microorganism associated with numerous biomolecule and metabolites employed in synthesis of various nanoparticles resemble the bacteria as prefect potential bio factory (Parthna *et al*.,2010).Additionally the biogenic

metal NPs used in different physical, biomedical , biological and pharmaceutical application, corresponding to specific characteristic of NPs itself (Loureiro *et al*.,2016).

1-3-1 Iron Oxide Nanoparticles

Among many different metallic NPs iron oxides take most attention due to remarkable properties for biomedical application and in vivo studies such as low toxicity, catalytic and magnetic behavior, biocompatibility, biodegradable, multifunctional modalities, long retention term (Wu *et* al., 2015b;Valllabani and Singh.,2018).There are three types of magnetic iron oxides NPs ,α-Fe_2O_3 (hematite),γ-Fe_2O_3(maghemite) and Fe_3O_4(magnetite) which are exhibit superparamagntic Fe_3O_4 are most important in biomedicine because of structural mode, and stabilization(Wu *et al*.,2015b).Additionally IONPs can be rapid and easy separation by using external magnetic field, other advantage no toxic in synthesis and powerful in scalable(Shen *et al*.,2009).Furthermore because of their easy separation , high surface area , and good chemical with mechanical stability conceder potential adsorbent of toxic and radioactive heavy metal (Lu *et al*.,2015).

1-3-2 Application of Magnetite (Fe_3O_4) NPs

Because of many different chemical , physical and magnetism properties of IONPs is reliable in a wide variety of applications such as :

1-3-3 Antibacterial Agent of IONPs

The increase of antibiotic resistance bacteria represented in multidrug resistance bacteria created the need for a new development novel strategy to distraction of these pathogens, metallic NPs offer several advantage features

over traditional antibiotics as more effective in distractive and incapable the microbes to developed resistances(Prucek *et al*.,2011). The deadly effected of antibacterial IONPs against *E.coli* in vitro demonstrate under aerobic and anaerobic condition in different concentrated of IONPs (Lee *et el*.,2008). Furthermore addition of another substance like oxalate ions to FeNPs in same solution increased the bactericidal effect, more over added silver to gate Fe_3O_4.Ag hetro-nanocomposites and used against *Klebsiella pneumonia* and *Salmonella enteritidis* by in vitro using paper disc diffusion and direct wells diffusion exhibit excellent bactericidal result on the two species (Ashraf *et al*.,2019).

In another attempt revealed Fe_3O_4 coated with citrate have inhibitory effect on *Bacillus subtitis* , *Escherichia coli* and many kind of fungi like *Aspergillus niger* , *Fusarium solani* and yeast *Candida albicans* (Arakha *et al* .,2015 ; Nehra *et al* .,2018). Many other attempts produce IONPs in green chemistry exhibit synergistic antibacterial to many gram positive , negative bacteria and anticandidal activity (Arokiaraj *et al* ., 2013 ; Patra *et al*.,2017;Gao *et al*.,2017).

1-3-4 Mechanism of Antibacterial Activity of Fe_3O_4 NPs

The probable mechanism of action of metal oxide NPs possible enhancement within their physicochemical behavior as result of different shape and size between 1-100 nm of NPs which is return in unique chemical and physical properties with highly reactive nature to microbial (Hoseinzadeh *et al* .,2017). One of important explanation of bactericidal effect of metal oxide NPs is the action of ROS reactive oxygen species, which are a group of reactive molecules produced by some metabolic

processes achieved with participation of oxygen(Tran *et al*.,2010; Mahdy *et al*.,2012).ROS include radicals like hydroxyl radicals (–OH),superoxide radicals (O_2^-) ,and hydrogen peroxides (H_2O_2), which they can undergo reduction or oxidation processes (Wang *et al.*,2017).

The interaction between H_2O_2 and membrane proteins or between the outer bilayer of microbe and the chemical produced in presences of Fe_3O_4 NPs could be one of important reason of antibacterial activity for IONPs , which is eventually lead the hydrogen peroxides enter membrane cell and kills bacteria (Prabhu *et al*.,2015) .

Another interaction between membrane cell of bacteria with IONPs by electrostatic interaction damage bacteria membrane by oxidative stress caused from free radicals formation (Ahmad *et al*.,2014). In addition the disruption of bacteria membrane as result of tightly adsorb NPs due to large surface area lead to leakage intercellular component then kills bacteria (Ismail *et al*.,2015).

One more process reaction pathway involve in eradication of bacteria in which Fe^{2+} react with O_2 produced H_2O_2 (Keenan and Sedlak.,2008). Hence the reaction output hydrogen peroxide sequentially via the Fenton reaction react with ferrous ions to produced hydroxyl radicals (-OH) which have ability to causing serious damage to biological macromolecules including (DNA ,Protein ,Lipids) leading to bacteria death (Touati. ,2000 ;Kim *et al*., 2001; Leuba *et al* .,2013). A similar process reveal that the nanomaterial release ions which can be react with protein thiol groups (-SH) present in cell surface of bacteria which leads to cell lysis (Zhang and Chen.,2009).

A related research on the subject that inactivation of *Escherichia Coli* by zero-valent iron (Fe^0) nanoparticles ,which penetration the *E.coli* membrane then react with intercellular oxygen causing oxidative stress leading to cell membrane disruption (Lee *et al*.,2008). The generation of metal ions contribute in inhibition the regulation of bacteria metabolic process and electron transport chain (Wang *et al*., 2017) .

1.3.5 IONPs Against Multidrug Resistant (MDR) .

Infectious disease with bacteria resistance to antibiotic has become major challenge to global public health ,it can be considered leading cause of morbidity and mortality in all the world and serious concern about increased development resistance in nosocomial pathogens of hospitalized and immunocomprimised patients after transplantation and major challenge in particular heart surgery (Nikaido .,2009) .

Bacteria may use different biochemical pathway to avoided the lethal effect of drugs .Among those ,reduced antibiotic accumulation intracellular by alteration permeability of the outré membrane ,minimize transport within inner membrane, or active efflux ,and by enzymatic modification or mutation alteration of the target ,in detoxification of drug enzymatically ,or by exceed of the drug target , collaboration of some of these mechanism in the same host leading to multidrug resistance (MDR),(Depardieu *et al*., 2007)

Many different bacteria defined in MDR types the expansion of resistant pattern recognized other degrees like extensively drug- resistance (XDR) first coined to descripted *Mycobacterium tuberculosis* (XDR MTB) and (PDR) pandrug- resistance have been introduced to a strains of pathogenic

bacteria which are resistance to most antibiotic and in some case to all available antibiotic including *Pseudomonas aeruginosa , Acinetobacter baumannii ,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus spp,* such as (vancomycin resistant Enterococcus VRE) Enterobacteriaceae (other than *Shigella* and *Salmonella)* for example *E.coli ,Klebsiella pneumonia ,Proteus spp* (Magiorakos *et al*.,2011 ; Farzana *et al*., 2013 ; Basak *et al* ., 2016) . Furthermore there is high chance for other bacteria to developed resistant current drugs and to newly one at later stage resulting in more loss economic and long stay in hospital (Morales *et al*., 2012).

A New approach to treating pathogenic bacteria with nanoparticles which represent hope to new generation of antimicrobial agent extends to effected in resistant bacteria (Pelgrift and Friedman., 2013;Beyth *et al* .,2015).

Many studies mention inactivation of *E.coli* by zero-valent ions Fe^0 which can disruption the cell membranes of microbe (Auffan *et al*.,2008).In related study (Gabrilyan *et al* ., 2019) reported that antibiotic resistant *E.coli* have been minimized growth rate depending on concentration of the IONPs .

Iron oxides NPs in vitro showed up reduced the cell viability live to dead of *Staphylococcus aureus* as ability of IONPs to penetration cell wall microbes generation reactive oxygen species (Jastin and Thomas .,2012). In other application against methicillin resistant *S. aureus* (MRSE) synergistic of iron oxide NPs with reduced graphen oxide induce physical and chemical damage to bacteria due to disruption and increased permeability of cell wall (Pan *et al* .,2016).

In both two bacteria classified based on their cell wall structure Gram Negative and Gram Positive bacteria the component of cell wall role the penetration of NPs to cell membrane and interaction of oxygen reactive species(ROS), peptidoglycan which are more thicker in Gram positive (80nm) , (Gram negative about 8 nm), reduced mechanisms action of NPs (Feng *et al* .,2000;Dorobantu *et al* ., 2015). In addition to that extracellular polysaccharide EPS layer which provide protective to biofilm as physical barrier reduced efficacy of therapeutics penetration and interaction with bacterial cells (Govan and Deretic.,1996).

For optimization maximum effect bactericidal activity of IONPs documented evidence suggested modulation and fabrication of iron oxide NPs by capping or conjugated with different agent improve stability and facilitate the dispersion , reduced agglomeration (Darwish *et al*., 2015), coating IONP with biocompatible chitosan changing the zeta potential of iron oxide NPs from negative to positive surface increased the antimicrobial activity against gram negative *E. coli* and gram positive *B.subtilis* (Arakha *et al*., 2015), also inhibit *Staphylococcus aureus* growth (Shi *et al*., 2016), prevent formation of biofilm by *P. aeruginosa* (Niemirowicz *et al*., 2015),with bactericidal effect toward *Streptococcus mutans* (Javanbakht *et al* .,2016) and exhibit wild rang of antibacterial activity against gram-positive and gram-negative bacteria (Behera *et al* .,2012;Nehra *et al* ., 2018).

1.3.6 Biomedical application of IONPs

IONPs are extensively applied in biomedical and diagnostic due to theirs superparamagntic property , which has no longer standing magnetism after removal of magnetic field and easily synthesis in the laboratory (Ali *et al*.,

2016). The controlled synthesis of IONPs with different shape ,size and structures ,also surface modification as well as various fabrication protocol synthesized yielding to high performance nanoparticles in addition to excellent physicochemical ,biocompatibility, low toxicity ,catalytic behavior ,making IONPs best candidate for biomedical application (Mahmoudi *et al* .,2012 ; Banerji *et al*.,2012 ; Xie *et al* ., 2016).

Magnetic and superparamagntic property have qualified FeONPs in several therapeutic application(Sun *et al*., 2008) such as magnetic hyperthermal therapy (MHT) (Banobre -Lopez *et al* .,2013) , photothermal therapy (PTT) , photodynamic therapy (PDT) ,(Shakiba *et al* ., 2015), magnetic resonance imaging (MRI) as contrast agent and in immunotherapy (Zhou *et al*.,2014 ; Liu *et al* .,2015)

Immunotherapy emerge as attempts to cure cancer based on development artificial antigen presenting cells aAPCs from paramagnetic iron with dextrin to stimulating T .cells proliferation specific against certain cancer or targeting cancer by immunosuppressive of the microenvironment which provided the protection to keep growing by modulating IONPs (Cheung and Mooney. ,2015;Zang *et al*.,2017) .

Encapsulated and coated superparamagntic iron oxide NPs with variety targeting molecules coupled therapeutic agents like drug and biomolecules such as antibodies , protein and nucleic acid widely studies in delivery drug and different therapeutic agents to eradication cancer in many vital organs and can be treated cancer as alternative method instead of the traditional methods (Huilan *et al*., 2016;Chee *et al* .,2018). In the same context biomolecules and drug encapsulated with FeONPs containing ligands

recognize by receptor on target cells or tissue can be successfully used in tissue repair (Jain *et al* .,2008 ; Veiseh *et al* ., 2010).

While cell labeling used modification of surface magnetic NPs with suitable biocompatible ligand such as lactoferrin ,insulin ,albumin and others molecules , or attachment of magnetic NPs to cell surface in order to distinguished labial cells among MRI (Lobel *et al* ., 2000). Early in 2004 Gupta and Curtis succeeded labeling superparamagntic NPs encapsulated with lactoferrin through receptor on human fibroblast , many reports exhibit labeling neural stem cells using FeONPs (Joris *et al* .,2017)

Biosensor and nanozyme another approach used iron oxide NPs, in biomedical application , biosensor to test biomolecules level or concentration such as glucose ,urea , and biomarker for cancer diagnostic (Mahato *et al*.,2018).

Nanozyme new approaches in nanotechnology recently developed toward construction a novel enzyme exhibiting biological properties like the natural one have ability to involve in catalase ,oxides(Karim *et al*.,2018).

1.4.1 Overview Polyhydroxy Alkanoate

Alternatives organic matter polymers instead of conventional petroleum source have gained attention in recent decade because of environment issues (Rodriguez-Contreras ., 2019). Polyhydroxyalkanoate (PHAs) natural polyester polymer produce by different microorganism similar material in properties to traditional plastic and complete biodegradable which are storage intracellular granules as carbon and energy purposes (Yu *et al*.,2006) The biocompatible , adaptability and adequate

body reaction make them attractive to variety application in biotechnology , pharmacology and biomedicine were applies principle of biophysics ,biochemistry and biology to medical research with combination of synthetic condition and nature of polymer product in frequently use (Augustine .,2018 ;Park *et al* .,2018).

Unbalanced growth of microbe promoted PHA syntheses as a part of microbes survive mechanism during fermentation effected by nutrient depletion and presence of excess of carbon sours leading of accumulation of PHA granules(Valappil *et al* ., 2007; Chen .,2009).

The high molecular mass, thermoplastic or elastomeric provides to biosynthesis PHA properties similar to those polymer of conventional petrochemical origin (Yadav *et al* ., 2015; Koller ., 2019).

1.4.2 Chemical Structure of Polyhydroxy Alkanoate(PHA)

PHA large family of biopolymer composed of R-3-hydroxyalkanoic acid and R-4-hydroxyalkanoic acid monomer units which form the major building bulk of PHA about 600-35000 (R)-hydroxy fatty acid made up the PHA (Khanna and Sirvastava .,2005) .The repetition of monomers in PHA chain either homopolymer (consists of one type of polymer) or heteroplymor (consists two types of polymer) ,in general PHA polymer known to be linear almost composed of 3-hydroxyalkaonic acid monomers units ,ester bond each unit form with the hydroxyl group of other unit ,at the C-3 atom an alkyl group which length can vary from methyl (C1) to tridecyl (C13) is located (Lu *et al.*, 2009) .Poly(3-hydroxybutyrat) PHB the most common linear anabranch homopolymer member of PHA Consisting of (R)-3-hydroybutyric) acid units and it appear crystallize when extracted

from bacteria, in Gram negative and Gram positive bacteria biosynthesis occur as intracellular granules result from condensation of two molecules from acetyl-CoA , homopolymer PHB granules characterized by brittleness and high crystallinity lead to reduced its flexibility and ductility (Koller ., 2018).

More specifically PHA classified into three group depending on their carbon atomic monomeric composition :

i. Short –chain-length PHA (*scl*-PHA) consisting 3-5 carbon atoms.
ii. Medium-chain-length PHA (*mcl*-PHA) consisting 6-14 carbon atoms
iii. Long-chain-length PHA (*lcl*-PHA) consisting more than 14 carbon atoms .(Reddy *et al* ., 2003;Koller ., 2019).

Researchers fuscous on the modulated of PHAs to gaining better properties via development of natural and recombinant microorganism or through modify bacterial fermentation medium using different precursor to improvement PHA producing polymer or copolymer to finding alternative raw material with wild range of application and competitive coast (Zheng *et al* ., 2019 ; Brigham and Riedel ., 2019).

1-4-3 Properties of Polyhydroxy Alkanoate (PHA)

PHAs have high degree of polymerization ,highly crystalline ,isotactic (stereochemical regulatory in repeating units) optical active, and insoluble in water , these feature make the biopolyester in competitive with polypropylene which is the petrochemical –based plastic.(Bugnicourt *et al* ., 2014 ; Raza *et al* ., 2017) .

Among PHAs polymer a PHB polyester practically P3HB is gains interest by different industries due to biodegradable and biocompatible quality, it is used in agriculture , packaging and medical industries in board rang of application (Ashby *et al* ., 2000). poly-(3-hydroxybutyrate) is produced and accumulation intracellularly as a carbon and energy reserve material. It can produced by various bacteria, such as *Cupriavidus necator*, several species of *Pseudomonas, Bacillus ,Azotobacter* and also recombinant *E. coli* (Centeno-Leija *et al* ., 2014) . PHB is produced by fermentation either in batch , fed batch or continuous cutler using improve bacteria strains, culture on inexpensive carbon sources such as beet or cane molasses, corn , alcohol and vegetable oil, combined with multistage fermentation system , all these strategies have been attempted to improve both yields and process productively in order to have a more competitive process(Pena *et al.*,2014)

Bacteria produced linear amorphous and crystalline form of PHBs , Its glass translation temperature (0-5 ^{o}C) , and exhibit highly crystalline polymer with melting temperature about (180 ^{o}C) having specific gravity and density of 1.18 and 1.26 g/cm^{3} respectively . This mechanical properties PHBs are similar to isotactic polypropylene. (Pfeiffer and Jendrossek ., 2014; Sato *et al* ., 2015).The PHBs molecular weight depends on sources, growth conditions, extraction methods and organism kinds and its vary from 50.000 to million g/mol.(Rai *et al* ., 2011) The differentiation of structure among PHAs polymers family reflected in properties scl-PHAs(eg.PHB) have similarly to conservative plastic while mcl-PHAs corresponding to elastomers and rubber materials .The PHAs can be modified to give more versatile polymers or fabrication by condensation of homopolymer and

heteroplymor or blends of PHAs can be manufactured such as polyhydroxybutyrate/polyhydroxyvalerate (PHB/PHV) and polyhydroxybutyrate/polyhydroxyheptanoate (PHB/PHH) (Stenbüchel., 2005;Suriyamongkol *et al*., 2007; Kulkarni *et al* ., 2011).

1.4.4 Polyhydroxybutyrate (PHB) Application

The production of petroleum based polymer cause negative environment impacts by 2050 non-degradable polymers waste are expected to be 12.000 Mt to be disposed in landfill (Rabnawaz *et al* ., 2017).

PHB polymer exhibit valuable application in agriculture ,industrial ,and in biomedical such as tissue engineering, surgical sutures, surgical meshes, cardiovascular, cartilage support ,tissue scaffolding for bone and nerve regeneration wound dressing and drug delivery vehicles (Meischel *et al* .,2016;Isola *et al*., 2017)

Many strategies have been used to toughen PHB in order to expand and improve its qualities such as modification through thermal treatment, blending with natural material, or with suitable structure synthetic polymer , and by form reinforced composite with natural fiber or rigid filler ,and modification by chemical functionalization or enhancement of toughen and flexibility which was normally reflected in material stiffness and strength(Sun *et al* .,2016 ; Muriuri *et al* ., 2017).

1.4.5 Iron Oxide/PHB Bionanocompsite

The expended PHB application in medicine and in particular tissues engineering by repairing both hard and soft tissues ,stents ,and devices

implantation(Hazer *et al* ., 2012).In vitro studies have shown that PHB copolymer support various mammalian cells growth(Wei *et al* ., 2009), hem compatibility and cytocompatibility (Qu *et al* ., 2006) , provided that the PHB are appropriately purified and tolerated by the organism after implantation(Sevastian *et al* ., 2003;Wu *et al* ., 2007).

Beside using the biopolymers in medicine ,pharmacology recorded application of PHB too in used as microcapsules in therapy or as materials for cells and tablets packaging, for encapsulation of Langerhans cells to restore insulin production and release, or in microbeads for targeted drug release ,in drug delivery for therapeutic/ nutritional uses like nanoparticles-releasing bioactive drug (Peng *et al* ., 2013 ; Heathman *et al* ., 2014) Mixing the antibacterial agent with biopolymer PHB have been developed in coating platinum implantation serve to prevent biofilms formation and other infection by long term of releasing antimicrobial agent(Roriguuez-Contereas *et al*., 2017).

The use of PHB copolymer rods in loading and coating with antibiotic namely sulbactam-ampicillin and sulbactam-cefoperazone combinations, gentamycin, were prepared using homopolymer or copolymer as matrix by casting drug/polymer pastes the research mainly focused in treatment bio-film or chronic osteomyelitis patients(Gursel *et al* .,2000). In general the expanding biopolymers application by modification in synthetic production and achieve new properties through blending with others polymers (Vogel *et al* ., 2008), or filling with Nanofiller such as carbon nanotube, silica, clay and others, were added to PHB biopolymer matrix to enhancement ,nucleation, mechanical, thermal crystallization and barrier properties (Ojijo and Sinha, 2013). PHB/SWCNTs (single wall carbon nanotube /

biodegradable PHB) composite powder or films and spray-dried have been new mechanical and hardness properties enable composite to gain advantage in medical application using (Yun *et al*., 2010) .

Most NPs synthesis demonstrated in field of drug delivery and in targeted cancer therapy were need conjugated to ligands molecules that recognize surface receptor on cancer cells such as antibodies(Kwon *et al* ., 2014) Other related study demonstrated the magnetic iron oxide nanoparticles were coated with PHB biopolymer used actin A as ligand to study in vitro the effect of magnetic PHB nanoparticles cytotoxicity (Erdal *et al* ., 2012).

1-5-1 *Escherichia coli*

Rod shape cells, Gram negative, facultative anaerobic ,oxidase negative, motile or non-motile ,peritrichous flagella (H antigen)there are more than 50(H) variant play major role in pathotype of uropathogenic and biofilm formation settle in gastrointestinal human tract as part of microbiota and warm blood animals, classify as most important member in large bacteria family Enterobacteriaceae (Zhou *et al* ., 2015; Johnson , 2018).

E. coli is divers species can be reviewed to pathogenic behaviors for three main types pathotypic groups particularly depend on clinical manifestation. Commensal, asymptomatic intestinal colonization, rarely cause extraintestinal infection second: Intestinal pathogenic cause diarrhea and third ExPEC causes Extraintestinal infection and rarely asymptomatic intestinal colonization (Johnson and Russo ., 2002 ; Touchon *et al* ., 2009) While depending on location of disease and virulence genes *E. coli* pathogen divided into two other strain groups (Kaper *et al* ., 2004) :

- Diarrheagenic pathogenic or intestinal pathogenic *E. coli* (InPEC).
- Extraintestinal pathogenic *E. coli* (ExPEC).

Diarrheagenic strains (InPEC) cause diarrheal syndromes sub divided according to their virulence factors clinical presentation and pathogeneses as well interaction with enterocyte ,tissue tropism to (6) well-known strains:

1. Enteropathogenic *E. coli* (EPEC)
2. Enterohemorrhagic *E. coli* (EHEC)
3. Enteroinvasive *E. coli* (EIEC)
4. Enteroaggregative *E. coli* (EAEC)
5. Enterotoxigenic *E. coli* (ETEC)
6. Diffusely adherent *E. coli* (DAEC)

(Croxen and Finlay. , 2010 ; Croxen *et al* ., 2013)

Other strain(AIEC) Adherent invasive *E. coli* described as associated with patient of crohn's disease(CD) and inflammatory bowel disease (IBD) (Maricarmen *et al* ., 2018; Johnson ., 2018).

EPEC , which elicit characteristic attaching and effacing lesion in intestinal mucosa , EHEC ,also characteristic by attaching- and effacing – (A/E)lesion or Verotoxins and shiga like toxin ,and this phathotype strain responsible for outbreak around world not only causes diarrhea disease but is responsible for clinical complication of hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Pianciola *et al* ., 2016), EIEC, which are invade epithelial cells like *Shigella* and differentiate by large invasiveness plasmid ,ETEC , which are obviously producing toxin either heat-labial or heat-

stable toxin or both enterotoxin ,and this phathotype consider main cause diarrhea in travelers(Torres *et al*., 2017a) and EPEC with ETEC main endemic of diarrhea in developing contraries, EAEC ,which are characteristic by "stacked-brick" aggregative adherence(AA) when culture with HEp-2 cells (human epithelial cell) occasionally involve in diarrheal disease in industrial and developed countries and hybrid EHEC-EAEC create large outbreak in Europe in 2011 leading to HUS cases and many deaths (Frank *et al* ., 2011) .Moreover EPEC strain display bind pattern called localized adherent (LA) in which microcolonies form on the surface of cells host corresponding to DAEC strain diffusely adherent also recognize by pattern of diffuse adherence (DA) in which bacteria uniformly cover the all cells surface (Foster *et al* ., 2015).

Extraintestinal pathogenic *E. coli* (ExPEC) strain are associated mainly with three subsets strains :

1. Uropathogenic *E. coli* (UPEC) or urinary tract infections(UTI)
2. Neonatal meningitis *E. coli* (NMEC).
3. Sepsis-causing *E. coli* (SEPEC)

(Johnson and Russo., 2002 and 2005 ; Smith *et al* ., 2007) .

ExPEC are part of intestinal microbiota of a fraction of healthy population normally asymptomatically colonize gut , only when they get access to niches out of the gut (normally sterile sites) bacteria able to colonize efficiently in certain niches cause disease in man such as urinary tract infection , meningitis or septicemia in newborn in peritonitis ,skin and soft tissue infections (Köhler and Dobrindt., 2011)

E. coli is the most common bacteria that causes meningitis, during neonatal period , NMEC travels from intestinal gut to the meninges as result of hematogenous spread, crosses blood- brain barrier(BBB), K1 capsular polysaccharide antigen predominate(about 80%) among *E. coli* meningitis isolated from neonatal ,and what is cause for concern despite the availability of advance antimicrobial chemotherapy and supportive care high fatality rate ended record (Kim ., 2016) . The most common site of colonize extra-intestinal by these bacteria is the urinary tract, which is in turn the main source of bloodstream infections ,the UTIs provoke bacteremia ,spatially in case of patients hospitalized as result of contaminated catheters with ExPEC biofilm(Martinez *et al* ., 2006)

The ExPEC strains typically share several virulence factors (VFs) which are usually located within chromosomal pathogenicity island (PAIs) or on plasmid (Dale and Woodford ., 2015) these VFs include adhesion molecules, toxins, iron acquisition systems, and host defense-subverting systems have been associated with bloodstream infection and promote bacteria to colonize the host surface cells ,avoidance or impair the host defense mechanisms (Lefort *et al* ., 2011; Mora-Rillo *et al* ., 2015) .

Urinary tract infection the most common bacterial infection when ExPEC strains gain access to urinary tract via two mode of infection ascending pathway when fecal flora reach and colonized urethra as result of contamination with faeces or descending bacteria invasion pathway rarely occurs by hematogenous spread of bacteria from primary sources located in body (Dipiro *et al* ., 2008).

Based on the structural and functional abnormality in urinary tract the UTIs classified to complicated and uncomplicated infection in order to care management and make easy which antibiotic treatment need as well as containing the risk factors (Antinori and Pezzani ., 2017). Uncomplicated UTIs occur in healthy, adult , nonpregnant women , in the other hand infections in children pregnant women , men in case of involve some type of urologic abnormality the infections belong complicated class ,and this type is often associated with medical conditions such as diabetes ,catheterization , renal transplantation ,immunosuppressive conditions and neurologic bladder (Foxman ., 2014). More over persisting infection in many cases of urinary tract infection due to the ability of uropathogenic strains formation of biofilms in certain stage of infections such as catheter-associated UTIs, cystitis , pyelonephritis, cut and recurrence UTIs (Wang *et al* ., 2009).

The prevalence of UTIs in all most cases depend on gender , age, presences of catheter or device and in which part of urinary tract has been infected, the prevalence of infection in women is significantly high than in men unless they are older adult men and with urologic abnormalities, in adult non pregnant women with normal urinary tract bacteriuria infrequently progresses to symptomatic cystitis (more prevalent in women because of anatomic difference) or pyelonephritis while asymptomatic bacteriuria is absence of urinary symptom and positive culture which is no need for screening and treatment unless in pregnant women an transurethral resection of the prostate (Nicolle *et al* ., 2005;Sobel and Kaye ., 2014)

E. coli is responsible for about 85% of all case of UTIs followed by other gram positive and negative bacteria , several clinical syndrome in urogenital track and a very high probability caused by UPEC i.e. urethritis (UR),

cystitis (CY), pyelonephritis (PY). Urosepsis (US) and infections in male accessory gland (i.e. prostatitis , vesiculitis , orchitis and epididymitis) (Johansen *et al* ., 2011)

1.5.2 : ***E. coli* Biofilm Production**

Many strains of bacteria have ability to formation the biofilm which reflection in important mechanism avoid the action of man immunity and resistance antibiotic lead to persistent infection and easily recurrent, the *E. coli* bacteria form biofilm in urinary tract infection(UTIs) due to secreted toxins, polysaccharide and high molecular weight capsule which , protecting bacterial adhesion surface ,the *E. coli* biofilm give advantages up to 1000-folds to resistant antibiotics from planktonic cells due to several mechanism like, Limitations of antibiotic diffusion through the matrix, Transmission of resistant genes within community , Expression of efflux pumps is also considered a mechanism of antibiotic resistant in biofilm as well as in the plankton, Inactivation of the antibiotic by change in concentration of metal ions and pH values(Lewis ., 2010)

The Biofilms define as an accumulated of one or more types of bacteria within matrix of extracellular polymeric substances(EPS) that they produce. The matrix accounts for about 90% of biomass (Flemming and Wingender,2010). Environmental change the main causative to transition from planktonic for biofilm which are lead to change in the expression of surface molecules, virulence factors and metabolic status, allowing the bacteria to acquire properties that enable their survival in unfavorable condition, the formation of biofilm is carried out in general main steps, Attachment ,Growth and Dispersal . The first attachment of the bacteria

influence by attractive or hold off forces that always depending on nutrient level, pH and temperature of the site , in this step the flagella and chemotaxis, and in case of *E. coli* it is mediated by type 1 pili , curli fibers and antigen 43 (Danese *et al*., 2000; Donlan ., 2002).

Chemotaxis facilitate bacterial communication through versatile chemical signaling molecule called Autoinducers, which regulate bacteria genes expression in a process called quorum sensing, allows individual bacteria with in colonies to coordinate and carry out colony-wide functions such as sporulation , virulence, conjugation and bio-film formation.

Protein and polysaccharides are also provide an advantage in biofilms formation when they are mixed community ,where bacteria adhered to tissue and this binding become stable micro-colony made up by multiplication of bacteria in biofilm matrix as a result of chemical signals, after micro-colony formation expression of related genes of biofilm take place ,the production of these gene are needed for EPS which is main structure of the material of biofilm, the formation of extracellular matrix depended on self-produced components, EPS , adhesion , amyloid-forming proteins and exopolysaccarids all are required to generated the three- dimensional structure with macrocolony morphology when they reach maturity in which gradient of nutrients, water and waste move through water channels like circulatory system (Parsek and Singh ., 2003).

When biofilm fully mature , detachment from colony may occur allowing bacteria to return to their normal plankton state again and have ability to retain certain properties of biofilm , such as resistance to antibiotic (Soto., 2014) .

1.6.1 A Review on *Bacillus Coagulans*

Gram positive rod, facultative anaerobic, motile, spore forming ,catalase positive ,lactic acid-forming bacterial species , in 1915 the organism was first isolation by Hammer and describe as *Bacillus coagulans* ,when causes an outbreak of coagulation in evaporated milk, in 1935 microbe separately isolated and describe as *Lactobacillus sporogenes* because it exhibit characteristics belong to both genera *Bacillus* and *Lactobacillus,* DNA-based technology used to reveal doubt in distinguished between two genera of bacteria that confirm *B. coagulans* belong to *Bacillus* and share similar morphology and biochemical, physiological characteristics with *Lactobacillus* ,it is still some commercial products labeled as *L. sporogenes* despise of renamed to *B. coagulans* (Nakamura,2000).

However *B. coagulans* ,endospore forming terminal location, ellipsoidal bodies shape, lack of cytochrome-*c* oxidase and for the incapability to reduce nitrate to nitrite, rods cells appear in single or rarely in short chain different lengths, the optimal growth temperature range of 35-50 ^{O}C and PH range between 5.5-6.5, metabolically produced acid but no gas from sugar fermentation such as maltose, sucrose, mannitol, raffinose, and trehalose, *B. coagulans* favor acid food to growth which leading to spoilage milk products, fruits and vegetables because of production lactic acid in high scale(DeCleic *et al* ., 2004). In addition to lactic acid production, numbers of strains also produced thermostable α-amylase enzymes which can be exploited in industrial level (Asan , 2010).

1.6.2 *B. Coagulans* as A Spore-Forming Probiotic

The probiotic spore forming non-pathogen bacteria shown more advantages compared with that of non-spore forming because products with these bacteria can be survival and stable in dry forms at room temperature without of negative environment effective on spores survival, another advantage including spores ability to tolerant low pH and pass successfully through gastrointestinal tract ,also bacteria spores exhibit good resistance to thermal lethal effects, freezing, toxic chemical, radiation, drying, compared to vegetative cells(Fares *et al* ., 2015; Elshaghabee *et al* ., 2017).

B. coagulans has been reported as general recognize as safe (GRAS), traditionally many strains of *B. coagulans* available as probiotics as freeze-dried, and many food products or dietary supplement. The choice of survival of *B. coagulans* in heat-treated process like baking and boiling with stable state during food storage, packaging, transporting, therefore the bacteria have much attention to developed novel cereal-based functional products (Endres *et al* ., 2009). The second potential advantage of *Bacillus* species included human health after improve nutritional value, boosted immunity level and prevented some of gastrointestinal tract diseases such as inflammatory bowel diseases, irritable bowel syndrome(IBS) ,diarrhea, bacterial vaginoses, ulcerative skin disorders and cancer(Lakshmi *et al* ., 2016)

The *B. coagulans* therapeutic effect is due to bacteriocin secreted which called coagulin have extended-spectrum against enteric microbes and many reported documented the useful of *Bacillus* probiotics on urinary tract infection (Jurenka ., 2012).

Chapter Two
Materials and Methods

2. Materials and Methods

2.1 : Materials

2.1.1 Equipment and Instruments

The laboratory Equipment and Instruments were used in study, Table(2-1)

Table (2-1) :Laboratory Equipment and Instruments

Equipment	Company	Origin
Autoclave	Hirayama	Japan
Atomic Force Microscope	Broker	Germany
Centrifuge	Universal	Turkey
Distillatory	Memmert	Germany
Hot plat	IKA	Germany
Incubator	Memmert	Germany
Light microscope	Olympus	Japan
Laminar air flow hood	ESCO	Singapore
Micropipettes	Dragon lab	China
Millipore filter	Sartorius	Germany
Oven	Memmert	Germany
pH meter	Orient	USA
Scanning electron microscope	FEI	Netherland
Shaker incubator	Rexmed	Taiwan

Equipment	Company	Origin
Spectrophotometer	Hitachi	Japan
Sensitive balance	Sartorios	Germany
UV-visible spectrophotometer	Shimadzu	Japan
Vitek-2 system	Biomerix	France
Vortex	Stuart	England
X ray diffraction (XRD)	Broker	Germany

2.1.2. Chemical and Biological Materials

The following Chemical materials used in present study, Table (2-2)

Table (2-2) Chemical Materials

Materials	company	Origin
Acetic Acid and Sulfuric acid H_2SO_4	Alpha Chemik	India
Absolute ethanol	Bioneer	Korea
Crystal violet powder	BDH	England
Chloroform	Scharlau	Spain
Congo Red powder stain	HiMedia	India
Ethanol 70%	BDH	England
$FeCl_2.4H_2O$	HiMedia	India

$FeCl_3.6H_2O$	Sigma-Aldrich	Germany
Glycerol 30%	Sigma	USA
Hydrogen peroxide (H_2O_2)	BDH	England
KOH	Bio	Canada
Lugol iodine	HiMedia	India
Malachite green	HiMedia	India
NaCl and $BaCl_2.2H_2O$	Merck	Germany
P- dimethyl amino- benzaldehyde (Kovac's Reagent)	HiMedia	India
Poly-β-hydroxybutyrate (PHB)	SIGM-Aldrich	Germany
Methyl red-Voges Proskaure	HiMedia	India
Safranin	HiMedia	India
Sodium hydroxide (NaOH)	EMC	Germany
Sucrose powder	HiMedia	India
Tetra methyl-P-phenylene diamine dihydrochloride (Oxidase Reagent)	BDH	England
1,1-diphenyl-2-picrylhydrazyl	Sigma-Aldrich	Germany

2-1-3 : Culture Media

The culture media used in study were listed in table below

Table (2-3) Agar and broth culture media and there purpose

Culture media	Purpose	Origin
MacConkey agar	Growth of G-ve bacteria and determination their ability to fermenter lactose	HiMedia
Nutrient broth	Cultivation and maintenance medium	HiMedia
Brain-heart infusion broth	Growth ,activation and maintenance of bacteria	HiMedia
Muller Hinton agar	Detection of antibiotic susceptibility	HiMedia
Eosin methylene blue	*E .coli* differentiation media	HiMedia
Urea agar	Used to detection urease production	HiMedia
Simmon citrate agar	Used to determine the ability of bacteria to utilize citrate	HiMedia
Peptone water	Detection of indole and carbohydrate fermentation patterns	HiMedia
De Man Rogosa and sharp (MRS) agar	*Lactobacillus spp.* growth	HiMedia
De Man Rogosa and sharp (MRS) broth	*Lactobacillus spp.* growth	HiMedia

2.1.4 Antibiotic Susceptibility Test(AST)

Different antibiotic disks were used to compare inhibition zones of *E.coli* with synthesized iron oxide nanoparticles .Table (2-4)

Table (2-4) Antibiotics disks were used in present study

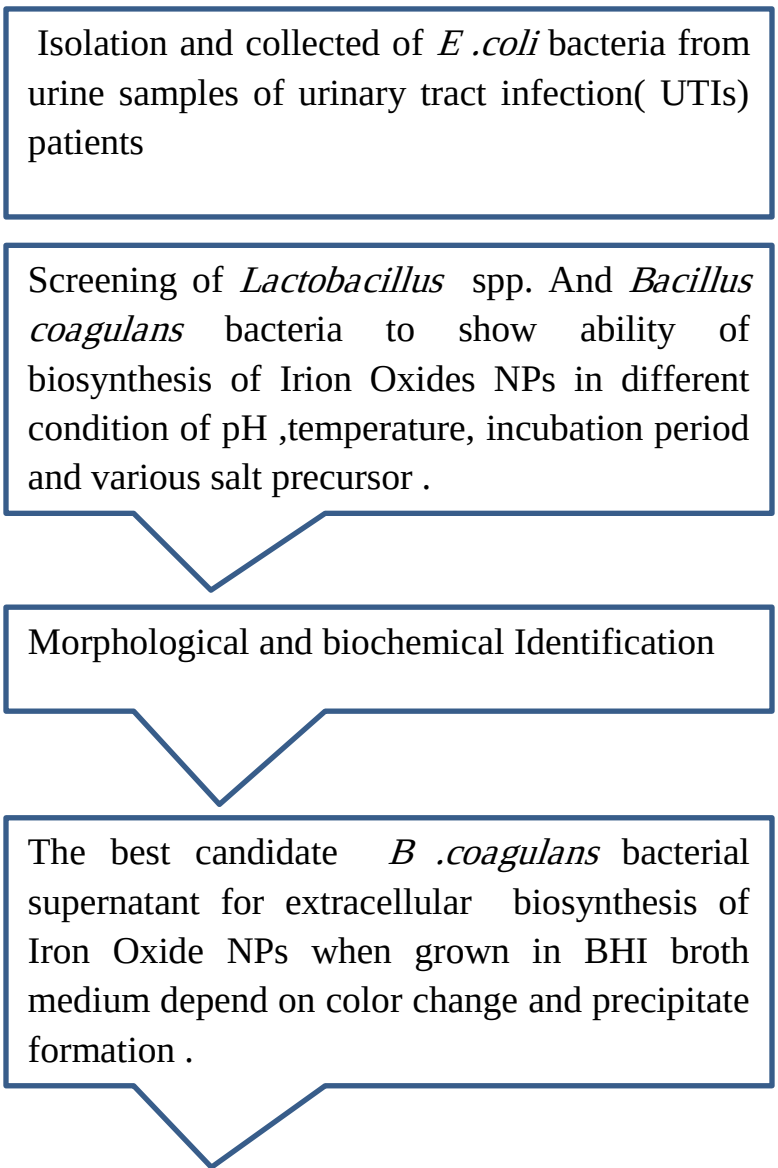
No.	Antibiotic	symbol	Concentration µg/disk	Class
1	Amikacin	AK	10	Aminoglycoside
2	Ceftriaxone	CRO	30	Cephalosporin
3	Gentamicin	GM	10	Aminoglycoside
4	Imipenem	IMP	10	Carbapenem
5	Nitrofurantion	F	300	Nitro furans
6	Nalidixic acid	NA	30	Quinolone
7	Piperacillin	PI	100	B-Lactam
8	Tobramycin	TOB	10	Aminoglycoside

2.2 :Methods

2-2-1 The Study Design :

The idea of the present study was screening the ability of different strains of *Lactobacillus* spp bacteria in extracellular biosynthesis iron oxide nanoparticles ,three *Lactobacillus* bacteria isolated from probiotic capsules

dietary supplement (*L . acidophilus , L. casei, L. fermentum*) which was failed in experimented of biosynthesis of IONPs. Successfully *B .coagulans* bacteria had been chosen as alternative strain to biosynthesis IONPs. While the *E .coli* bacteria isolated from urine specimens confirmed identification by phenotypic characteristic of bacterial cultures and biochemical tests with morphological and microscopy examination, the biosynthesis iron oxide nanoparticles was characteristic in many types instruments, The end step of study using the biogenic synthesis iron oxide nanoparticles as an antibacterial agent alone and with combined of poly-β-hydroxybutyrate (PHB) polymer again against uropathogenic *E. coli* , in addition to that the biogenic NPs used as antibiofilm agent against *E .coli* biofilm formation , Figure (2-1).



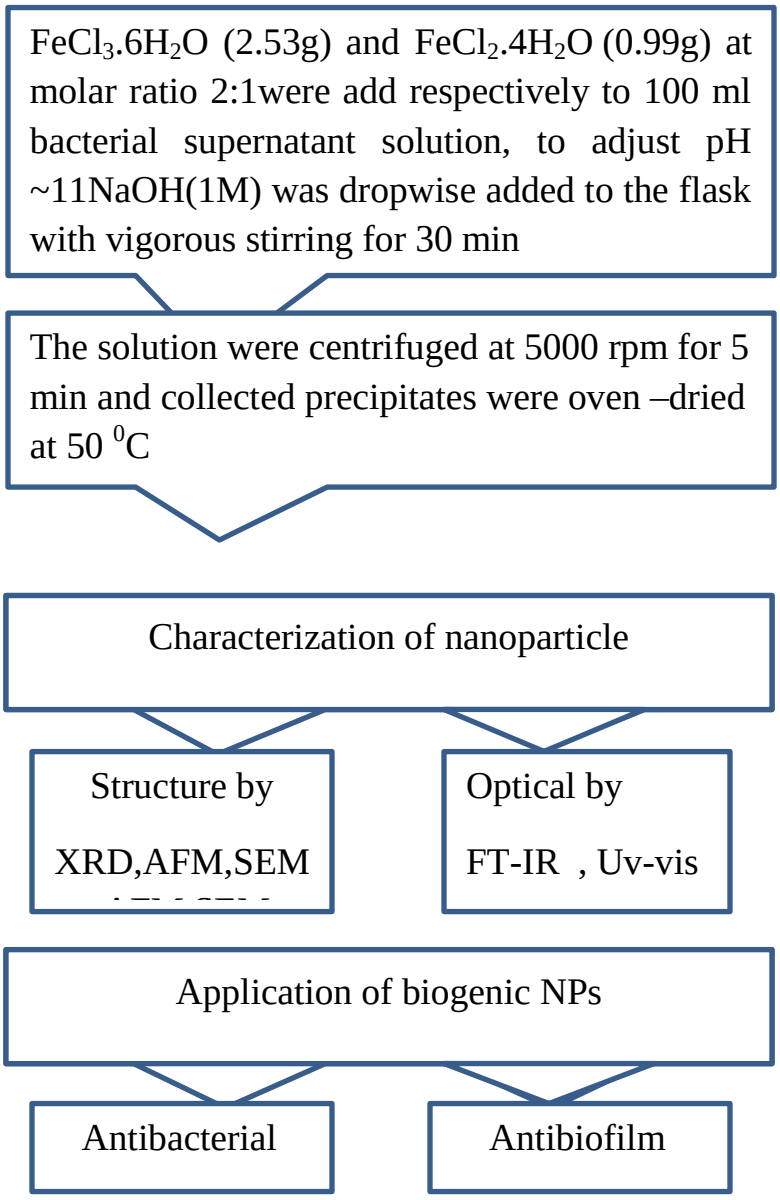


Figure (2-1) the study design

2.2.2 : Sterilization Methods

The autoclave have been used to sterilize the media and substrate for 15 minutes at 121^{O}C and 15 psi (pounds per square inch), while the sensitive materials was sterilized by filtration using Millipore filter (0.22µm) .The

laboratory glassware which used in study was sterilized in oven at 180 ^{0}C for 2 hours(Bhowmik., 2011)

2.2.3: Preparation of Culture Media

Many different agar and broth media listed in table (2-3) used in study such as MacConkey agar , Eosin Methylene blue , Brain-Heart Infusion Broth , Muller Hinton Agar , Peptone Water , Simmons Citrate Agar, Methylene Red –Voges Proskaure , these types of media are prepared by dissolving completely a certain weight with help of heating in distal water ,the media sterilized by autoclave at 121 ^{O}C for 15 minute, then media were left to cooling to 50 ^{O}C ,after that the agar media poured in petridishes and the broth media in sterilize screw test tube under laminar hood .The prepared media was incubated at 37 ^{O}C for 24h. to ensure sterilization. All media was prepared according to manufacturer's instructions.

2.2.4 Reagans and Solutions

2.2.4.1: Ferric Chloride Hexhydrate

A total of 2.703 gm. of $FeCl_3.6H_2O$ was dissolved in 100 mL of deionized water to prepare 0.1 M solution (Green and Perry., 2008). Store in dark bottle as stock solution .

2.2.4.2 : Normal Saline Solution (0.85%)

Ready to use .

2.2.4.3 :Sodium Hydroxide(1.0 M NaOH)

Ready to use.

2.2.4.4:Phosphate Buffer Solution

Ready to use .

2.2.4.5 :Crystal Violet Solution (1%)

Ready to use .

2.2.4.6 :McFarland Tube Standard (0.5)

Ready to use .

2.2.4.7 : Oxidase Reagent (1.5%)

Ready to used .

2.2.4.8 : catalase Reagent (3%)

Ready to use .

2.2.4.9 : Urea Solution (40%)

Added 40 gm. of urea powder to 100 ml distilled water, mix well until dissolved then filtered by 0.4 µm Millipore filter paper (MacFaddin.,2000).

2.2.4.10 :Methyl Red Reagent (MR)

Ready to use .

2.2.4.11 : Voges-Proskaure Reagents (VP)

The reagents consist two types :

Reagent (A): (5% α-naphthol) ready to use

Reagent (B) : (40%KOH) ready to use .

2.2.4.12 : Kovacs Reagent

Ready to use .

2.2.5 : *Lactobacillus spp.* and *Bacillus coagulans* Isolation

Different isolated of *Lactobacillus* spp ,was obtained from dietary supplement capsules ,the contents of each capsule were emptied in MRS broth medium to activate the bacteria , after incubated for 24 at 37 ^{0}C ,the growth bacteria was transfer to grow on MRS agar medium for 24 hr. at 37 ^{0}C under anaerobic condition one plate of each medium was kept as media control. And

isolate of *Bacillus coagulans* ,were obtained from University of Babylon, College of Sciences, Department of Biology, the selective species were experimented to detect the ability to synthesis of Iron oxide Nanoparticles .

2.2.5.1 : UTIs Bacterial Samples Collection

A 120 mid urine specimens form UTIs patients were collected daily from out patients clinic laboratory in Al-Hakeem hospital in Baghdad ,from 10/10 to 1/12 2020 the urine specimens was labeled and culture on various types of differentiation agar medium were incubated for 24 hr. at 37 ^{o}C , then (5) ml from every urine specimens were deposited by centrifuge in 2000 rpm for 5 minutes in order to examined by light microscopic with high power objective lens(40x) . The presence of 10 pus cells or more in one field of microscope and from 50-200 pure colonies found in one plate culture conceder as positive result (Kathleen and Timothy .,2001).

2.2.5.2 : Maintaining the Isolated Bacteria

The maintenances the viability and purity of the isolated bacteria were down according to Harley and Prescott ,(2002).

2.2.5.2.1: Short –Term Maintains

The isolated bacteria preserved in short time by periodically preparing a fresh culture from previous stock culture, where the isolate stored in temperature at 4 ^{O}C for several weeks or month ,the refrigeration isolated bacteria would be used in newly matter every month period to obtained pure culture and that done by inoculated slants nutrient agar media screw-capped tubes in strike manner or inoculated nutrient agar petri dishes the plates sealing tightly with parafilm to avoid the contamination and dryness (Harley and Prescott .,2002)

2.2.5.2.2 : Long-Term Maintains

For long-term storage the isolated bacteria , into sterile 2 ml micro centrifuge tubes(Eppendorf tube) , aliquot 500 µl of sterile 30% glycerol and added 500 µl of overnight bacterial Nutrient broth culture medium , mixed well and stored in deep freezer at -20 ^{O}C

2.2.6 : Identification of Isolates

The isolates of bacteria identified by macroscopically based on the culture morphological and microscopical examination as well as biochemical tests (Atlas ., 1995)

2.2.6.1:Macro and Microscopic Identifications

The morphological characteristics of isolated colonies were observed after culturing bacterial isolates on a varied agar medium ,according to their color, size , shape ,texture and arrangement of colonies. Then microscopic examination was obtained by transferred and fixed pure single colony on microscopic slide to detected their response to Gram stain were examined under oil immersion(x100) in light microscope (Atlas ., 1995)

2.2.6.2 : Biochemical Tests

2.2.6.2.1 : Oxidase Test

Few drops of tertramethyl-P-phenylene-diamine dihydrochloride, the oxidase reagent was added to Whatman filter paper No.1,then picking up colony of microorganism with stick and smeared over moist area on filter paper, a positive reaction was indicated by intense deep blue appearing (Colline and Lyne ., 1987)

2.2.6.2.2 : Catalase Test

By sterilize wooden stick was transferred a small amount of bacterial colony to surface of dry clean glass slid, then placed drop of 3% H_2O_2 on slid and mix. A positive result was indicated by rapid formation of gas bubbles (Tille .,2017)

2.2.6.2.3 : Indole Test

Tryptophan broth tube was inoculated with isolated bacterial colonies, incubated for 24-48 hours in 37 ^{O}C. Then 0.5 ml from Kovac`s reagent were added after incubation period, red color ring on top surface of broth appear indicated to positive result, no change in top surface is negative result(Forbes ., 2007)

2.2.6.2.4 : Methyl Red Test :

Test tube was contented MR-VP broth inoculated with pure culture of microbe under test, incubated for 24 hours in 37 ^{O}C , about 5 drops of methyl-red inductor solution added after incubation period, within few minutes the changing medium color to red was positive reaction indicated as a low pH resulted from glucose fermentation. In some procedure second tube for pre known bacteria negative reactive result used as a control negative (Hemraj *et al* 2013)

2.2.6.2.5 :Voges-Proskaure Test

A Tube of MR-VP broth was inoculated with pure culture of the test microorganism, incubated for at least 48 h in 35 ^{O}C. At incubation time end, 1ml of reagent A(5% α-naphthol) were added followed by 3 ml of reagent B (40% KOH), the tube shook gently due to exposed medium to atmospheric oxygen then stand the tube for 10-15 minutes, development of red color was indicated to positive result (MacFaddin ., 2000)

2.2.6.2.6 : Citrate Utilization Test

Slant Simmons citrate agar inoculated with microbe colonies subject of test, then incubated for 24 h in 37 ^{O}C ,the ability of microbe to utilization of citrate as source of carbon ,alkaline carbonate and bicarbonate were produced ultimately. Leading to development agar to blue color denoting alkalization. The positive result made by bacteria growth on medium causing the change of bromothmyol blue from original green color to blue (Brown. , 2005)

2.2.6.2.7: Urease Test

Many microbes especially those that cause UTIs have urease enzyme which is able to split urea in presence of water to release ammonia and CO2. The combination of ammonia with CO2 and water form ammonium bicarbonate which makes medium alkaline, followed by turning color phenol red inductor from its origin orange yellow color to pink as positive result , the test performed by inoculated the slant of urea base agar which consists of urea solution prepared by dissolving 40 g of urea in 100 ml of distilled water which was sterilized by 0.22 µl Millipore filter which is mixed with urea base agar which prepared by dissolving 24 g from urea agar in 950 ml distilled water after sterilized in autoclave and cooling to 50 ^{0}C slant surface streaking with obtained bacteria , then incubated in 35 ^{O}C for 24-48 h, to observed the color changing with time (Procop and Koneman ., 2016) .

2.2.7 : Motility Test

There are a variety of methods to determine the motility of bacterium biochemical test in semi-sold agar media is most accurate way to determine bacteria motility, motile bacteria give a diffuse spreading growth that is easily recognized by the naked eye, by prepared a nutrient semi-sold agar medium 5ml in test tube (less agar powder added(0.5g/l) and autoclaved, after cooling

inoculated with straight wire making a single stab down the center of the tube to about half the depth of the medium. The test tube medium incubation in 37 OC for 48 hours. Motile bacteria typically exhibited diffuse hazy growth that spread throughout the medium showing it slightly cloudy, non-motile bacteria exhibited close growth to stab line had sharply defined margins, and leaving the surrounding medium clearly transparent.(Colline and Lyne ., 2004)

2.2.7.1 : Endospore Staining

Malachite green a primary stain was used to stain endospore which should be seen as green ellipses within the cells, while spores resistance to staining, heating used to forced malachite green permeated the spore wall, the test performed by prepared smear of *B. coagulans* to tested the endospores on clean dried glass slide, heating the smear to be fixed, covered the smear with malachite green stain and starting heated gently till it's started to evaporated and more stain added as need, after 5 minutes remove the slid from heating sources , allow to cool few minutes in room temperature and rinsed the slid with tap water, then added safranin as counter stain for two minutes, washed with water to remove the secondary stain, dried the slid and view under 100x oil emersion (Brown., 2005)

2.2.8: Antibiotic Sensitivity Testing (AST)

One of reliable, efficiency, low cost, disk diffusion (Kirby Bauer) method was probably the most widely used method for determining antibiotic sensitivity in clinical laboratory, the antibiotics disks commercially available in pre-impregnated with standard concentration of particular antibiotic, it used to detect susceptibility antibiotic which were affected in bacteria

1- From pure bacteria culture loopful of colony was suspended into tube of normal saline, standardized the tube with 0.5 McFarland turbidity standard to obtained approximately of 1.5 $x10^8$ cfu/ml ,
2- Inoculated Mueller-Hinton agar medium from suspended bacteria by dipping sterilize cotton swab, then applying swab over surface medium, the inoculum was leaved about 10 minutes to dry
3- Finally antibiotic disks were placed on the inoculated agar plates by sterilized forceps by gently pressed down to ensure complete contact to agar later the plates was incubated at 37 ^{O}C for 24 hours in inverted role
4- The result recorded as zones of growth inhibition around each antibiotic disk ,highly effective antibiotic will produced wide inhibition zone were measured by metric ruler, while an ineffective antibiotic disk were showing no change in growth of surrounding bacteria, and the effectiveness of intermediate antibiotic were determined by measured of the inhibition zone. Once the zone diameter was measured it must be compared to database of inhibition zone standard to determine if the isolate among studied was susceptible(S), intermediate (I), or resistant(R) to particular antibiotic based on the interpretation chart according to Clinical Laboratory Standard Institute (CLSI 2020) guidelines.

2.2.9 : Iron Oxide Nanoparticles (Fe_3O_4) Biosynthesis

2.2.9.1 : Selection of Bacteria

B. coagulans and three isolates of *Lactobacillus* spp. (*L. casei, L. acidophilus* and *L. fermentum*) was introduced to extracellular experimented synthesis of iron oxide nanoparticles .

B. coagulans supernatant was successfully employed as reducing and stabilizing agent in synthesis of iron oxide NPs, while the three isolates of *Lactobacillus* was exhibited inefficiency ability to synthesis Iron Oxide nanoparticles.

2.2.9.2 : Synthesis of Iron Oxide NPs by *Bacillus coagulans*

2.2.9.2.1 : Culture of Bacteria

The selective bacteria *B. coagulans* first was inoculated in the fresh brain heart infusion broth medium and incubated at 37 ^{O}C for 24 hr.to obtained fresh culture 24 hr. (first activation) . The second activation of bacteria was performed from the first activation bacteria into flask containing brain heart infusion broth and incubated at 37 ^{O}C for 24 hr. (Chaudhari *et al* .,2012)

2.2.9.2.2 :Fe_3O_4 NPs Production

After incubation period in BHI broth medium and obviously convinced growth of *B. coagulans* ,the bacterial suspension was centrifuged at 10000 rpm for 10 minutes then the cell free supernatant of bacteria was used in a simple co-precipitated method by mixed 100ml of supernatant with $FeCl_3.6H_2O$, 97% (2.53g) and $FeCl_2.4H_2O$ ~99%(0.99g) on magnetic stirrer for 30 minutes ,at 35 ^{O}C , with adding continuously 1M NaOH until pH become(11),followed vigorous stirring for another 30 minutes to completed the reaction. Finally the synthesis nanoparticles was collection by external magnet ,the pellet washed three time with deionized water, then allowed to dried at 50 ^{O}C to be ready to use in reveal characteristic measurement of particle tests such as XRD. AFM, SEM, UV, FTIR (Ghani *et al*., 2017) .

2.2.10: Characteristics of Iron Oxide NPs

2.2.10.1 : X-Ray Diffraction (XRD)

XRD analysis is an analytical technique used in material sciences to determent the crystallographic structure and atomic spacing of material, and identical of unknown material. The X-ray diffraction is based on constrictive interference of monochromatic X-ray and a crystalline sample, these X-ray were generated by cathode ray tube, filtered to produced monochromatic radiation, collimated to concentrate, and directed towered samples this interaction of incident ray with sample produces constrictive interference and a diffracted ray, when condition was achieve can be determent in Braggs equation (law)

$2d\sin\theta = n\lambda$

Where n is an integer, λ the beam wavelength of x-ray, d the interplanar distance generating diffraction , θ diffraction angle.

In general this law related to electromagnetic wavelength to diffraction angel and distance of planes in a crystal lattice, the diffraction of X-ray were detected, processed and counted, based on sample material the scanning through range of 2θ angles must be includes all possible diffraction directions of the lattice due to randomly oriented of powdered material, while each compound has a set of unique d-spacing's the recording of related diffraction peak allow to identical of compound when comparison of d-spacing with stander reference patterns (Bunaciu *et al* ., 2015) .

2.2.10.2:Scanning Electron Microscope

The scanning electron microscope (SEM) provided images by scanning the samples and achieved formation about the composition and surface topography the partly three-dimensional image obtained from SEM depended

on visualization of the topography of sample in terms(shape, size and surface texture) and this analysis was down through playing beam of high energy electron focused over samples surface lead to interaction of electron with samples atoms produced various signals inside simples like secondary electrons (SE) ,backscattered electron(BSE) and the catachrestic X-ray. Then these signals was collected by detector form images display on computer screen (Danilatos , 1986).

2.2.10.3 : Uv-vis Spectrometer

The most important factor of this technique was its application to give the information about material when light falls on it, and unknown compound can be determined in samples when it was compared to spectrum of the reference compound, the test performed by using a Varian carry 5000 model UV-VIS-NIR spectrophotometer to examine the nanoparticles samples of Iron Oxide (Wang *et al.,* 2014).

2.2.10.4 : Fourier Transform Infrared Spectrograph (FTIR)

FTRI analysis offers qualitative and quantitative identify for organic and inorganic compound in the samples as well identifies chemical pound in molecules through producing spectrum from an infrared absorption, the spectra which is produce represented profile for sample, FTIR analysis also provide information of the basis chemical composition and physical state of the whole samples, the tested sample material are identified by matching the spectrum against a database of reference spectra(Cocchi ., 2004)

2.2.10.5 : Atomic Force Microscopy (AFM)

AFM system was used to provided unique insight into the structure and functional behavior of material, and have a much higher spatial resolution which offer the ability to investigated ultrafine structure of samples and even

map the distribution of single molecule (Dufrene ., 2008) .Moreover AFM can be scanning the topography of individual samples and of soft biological material in their native environments with provided true 2D,3D images for material in Nanoscale as well as group particles, AFM has been proved to be an important tool to characteristic morphology , crystallinity of different polymers and micro phase separated structure , Here were are characteristic the Iron Oxide nanoparticles and to obtained images for individual particle morphology and size of nanoparticles with the topography of surface(Majeed and Naji , 2018).

2.3. : Antibacterial Activity of Iron Oxide NPs against *E. coli*

2.3.1 : The Preparation of The Bacterial Inoculum

The Muller Hinton broth media was used to preparer the inoculum in a test tubes of 10 ml per tube , these tubes has been sterilized at 121 ^{O}C for 15 minutes in autoclave , inoculum was prepared for each bacterial isolates to be incubated for 24 h at 37 ^{O}C after incubation time , the cell suspension was diluted with normal saline to obtain final concentration by comparison with o.5 McFarland.

2.3.2 : Disk Diffusion Method

The antibacterial activity of iron oxide nanoparticle were obtained by disk diffusion method was experimented against *E. coli* bacteria in the concentrated of (50, 100, 150, 200 µg/ml) of iron oxide NPs which was loading on filter disk paper with 6 mm diameter. The nanoparticle disk prepared through cutting filter paper in 6 mm diameter and sterilized in autoclave after that dunk within known concentration of IONPs which dissolving in distal water to become ready for used on bacteria. The concentration of nanoparticles was prepared as stock solution obtained

through dissolving of 0.5 mg of Fe_3O_4 NPs in 1 ml of double deionized water, so the concentration become 500µg/ml , and then were prepared 200 µg/ml applied equation

$$C1V1= C2V2$$

$$500Xv1=200x1ml$$

$$V1=0.4 ml (400µl)$$

It main took 400µl from stock solution and complete the volume to 1ml with 600 µl(0.6 ml) of water , and so on for all concentration (Subhashini *et al* ., 2018) .

2.3.3: Preparation of Fe3O4 NPs / PHB Composite Film

The preparation of iron oxide- PHB composite film was perform by using the conventional solvent-cast technique through dispersion certain amount of Fe_3O_4 (0.8 g) by stirring magnetically for 30 min. in 10 ml of chloroform. subsequently the PHB (0.2g) powder was dissolving at 50 C^o in the nanoparticles dispersion (ratio of 8:2 w/w) in scuttle bottle the mixture again stirring magnetically for 30 min. . The resulting blend was poured into a glass petri dish covered with puncture aluminum sheets in dark place at room temperature for 24 hr. to evaporated the chloroform and complete dried, finally different concentration of composite was prepared to used as Antibacterial compound were loaded on disk filter paper with 6 mm diameter in concentration of 50 , 100, 150 and 200 µg/ml (Ana and Angel .,2014).

2.3.4: Determination The Inhibition Zone

The disk diffusion method was used in determination the inhibition zone of different concentration (50, 100, 150 and 200 µg/ml) of biogenic Fe_3O_4 NPs as antibacterial agent first and second were synergistic iron oxide

nanoparticles with polymer PHB(IONPs/PHB) as composite antibacterial agent against *E. coli* bacteria.

The bacteria was incubated at 37 ^{o}C for 24 hr. then the inhibition zone measured by ruler to compare the antibacterial activity of two agents and which one was more effective in inhibition bacteria growth (Sundaram *et al.*, 2012*).*

2.3.5 :Evaluation of Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration(MBC) of Fe_3O_4 NPs Against *E. coli* .

Macro-dilutions method with some modfaction was used to determine the (MIC) the lowest (minimum) concentration of Iron Oxide NPs(antibacterial agent) required to inhibit the visible bacteria growth and (MBC) the lowest concentration of an antibacterial agent required to kill bacteria ,in present study the Fe_3O_4 used as antibacterial agent first and composite with poly-3-hydroxybutyrate PHB second , against *E. coli* bacteria (Balouiri *et al.*,2016)

1- For macro-dilution technique a culture control(positive control) tube containing 2 ml of Muller-Hinton broth medium inoculated with 0.2 ml of $1.5x10^8$ cfu/ml bacterial suspension of *E.coli* while MH broth alone was served as a negative control .
2- The other sterilize cupped test tubes with 2ml of Muller-Hinton broth containing different concentration of Fe_3O_4 NPs (200,150, 100 and 50µg/ml) were inoculated with 0.2 ml of $1.5x10^8$ cfu/ml *E .coli* bacteria
3- The composite of IONP/PHB with 20%*wt* ratio from polymer to NPs with concentration of (200,150, 100 and 50 µg/ml) and control tubes of positive and negative the composite ,was performed to determine the MIC and MBC of two groups sets tubes, the all test tubes was

incubated at 37 C^O for 24 hr. after incubation the growth of bacterial strain was determined by visual observation and the optical density determined by spectrophotometer at 600 nm wavelength to identify which tube contain growth in term of turbidity and for clear tubes there is no growth because of effectiveness of antimicrobial agent.(Wiegand *et al* 2008)

The tube of bacteria were considered as the control well be 100% growth and MIC was defined as minimum concentration of antimicrobial agent which is lowest dilution inhibited visible growth of bacteria by lack of turbidity in tube which was recognize in the two groups sets of tubes , MBC was determined by sub- culture 0.1 ml of samples from tubes of MIC were plated in Muller Hinton agar for 24 hr. at 37 C^O , the first petri dish which shown no growth is the MBC related to lowest concentration that destroyed all bacteria cells also in the two sets of the tubes investigation (Watts *et al* ., 2018)

2.3.6 :Effect of NPs on Formation *E . coli* Biofilm

2.3.6.1: Tube Method(TM)

The tube method was used for qualitative estimation of bacterial biofilm formation describe by Christensen et al. (1982) and assessment of the inhibitory effect of biogenic Fe_3O_4 NPs against biofilm formation by *E .coli* as steps flowed

1. From overnight growth of *E .coli* on nutrient broth loopful of test bacteria was inoculated 5 ml of sterilized brain heart infusion broth supplemented with 2% sucrose in test tube ,this tube consider as control to observed the formation of biofilm
2. In same matter another tubes was tested the inhibitory effect of Fe_3O_4 NPs against biofilm formation of *E . coli* via incubation the

suspensions bacterial in BHIB with 2% sucrose together in (1:1 v/v) with Fe_3O_4 NPs sub-MIC (150µ/ml)

3. All tubes were incubated for 24 h. at 37 ^{O}C
4. After incubation , tubes was decanted and washed with phosphate buffer solution (PBS) pH 7.2 and dried .
5. Tubes was then stained with crystal violet (1%)
6. Excess stain was washed double deionized water
7. The tubes was dried in inverted position and observed of biofilm formation , the result considered a positive of biofilm formation when a visible film lined the wall and the bottom of the tubes which can see clearly in control tubes , but the effect of Fe3O4NPs where observed via inhibition of formation of biofilm by *E .coli* test tube
8. The amount of biofilm formed were scored visually on wall and bottoms of tubes as 0-absent 1-weak /none , 2-modreat 3- high/strong , the experiment was perform in triplicate and repeat three time (Abdel Halim *et al* ., 2018) .

2.3.6.2: Congo Red Agar Method

A sample qualitative method for detection of biofilm production was describe by (Freeman *et al* ., 1989) and when incubation with suitable concentration of NPs can be used in detection the ability of NPs to inhibition bacterial biofilm formation

1. The Congo red agar medium was used composes 52g/L Brain-Heart infusion agar, sucrose 36g/L and 0.8g/L congo red stain.
2. Congo red stain was prepared as concentration aqueous solution and autoclaved separately from BHA medium at 121^{o} C for 15 minutes

,while the sucrose solution sterilized by filter and was then added when the agar had cooling to 55 ^{o}C

3. The congo red media was poured in plates were inoculated with *E.coli* bacteria by sterilized swab then punched with 6mm cork borer
4. The plate incubated aerobically at 37 ^{o}C for 24 hours , after added 100µl of Fe_3O_4 at concentration of 150 µg/ml in the wells.
5. Positive result was indicated by black colonies with dry crystalline consistency which is mean the biofilm formation by bacteria and non-producer bacteria biofilm remained pinks red colonies , while the pathway activity of IONPs inhibition of biofilm formation by prevented or eradication of the bacteria growth that representing in clear from black crystalline colonies zone around wells continent iron oxide nanoparticles .

2.4 Statistical Analysis

All experiments were repeated three time ,the level of probability is 0.05 that used to identify least significant difference(LSD) , if the level of the probability (p-value) is less or equal than 0.05($\leq$ 0.05) is statically significant and if the p-value higher than 0.05(>0.05) is not statically significant and indicate strong evidence of differences between groups (Hussony ., 2012)

Chapter Three

Results and Discussion

3.Results

3.1: Bacterial Isolates

3.1.1: *Bacillus coagulans* and *Lactobacillus* spp.

Three species of *Lactobacillus* genus (*L. casei*, *L. acidophilus*, *L. fermentum*) which selected as biological model for synthesis of Fe_3O_4 nanoparticles, these species were obtained from dietary supplement probiotic capsules.

Bacillus coagulans from *Bacillus* genus as suitable replacement because it's have a good biological activity ,spore forming probiotic bacteria more over many species of this genus succeeded experimentally in biosynthesis of different nanoparticles were obtained from microbiology laboratory University of Babylon, College of Sciences, Department of Biology .

3.1.2: *E. coli* Isolation

Among 120 midstream clean catch urine specimens were collected from urinary tract infection(UTIs) patients which was primary diagnostic through general urine examination depend on the presence of pus cells ,the specimens cultured and after performing the morphological , biochemical and microscopical tests 72 (60%) of urine specimens were positive culture of *E. coli* bacteria while 48*(40%)* recorder as another bacteria ,or free growth plate culture .

3.2 :Morphological and Microscopical Diagnostic

3.2.1: *E. coli*

The isolate of *E. coli* bacteria primarily diagnostic by morphological identification of colonies culture depend on shape, size ,color, consistent, rise and

edge on MacConkey agar, and Eosin Methylene blue(EMB). The bacteria growth result on MacConkey showed lactose fermenter ruler give pink colonies, circular, moist smoothly shin with a sharp edge , the MacConkey agar is selective and differential culture medium ,selectively allows the growth of negative bacteria and prevents the positive bacteria from growing because it contains bile salts and crystal violet ,also the media detects the lactose fermenter by bacteria with pH indicator neutral red . While the isolated culture on Eosin Methylene blue was showed green metallic sheen colonies (Wanger *et al* ., 2017)

This feature was one of the characteristics of *E. coli* from other intestinal bacteria due to the medium containing the pigments of eosin and methylene blue that are deposited in the acid medium after conjugated together from fermenter the lactose and sucrose which gave shin green metallic that reflected the bacteria produced organic acid (Leininger *et al* ., 2001).

Also motility the ability of *E.coli* to move has been used in classification and differentiation of bacteria by using flagella , in slant tube of nutrient agar showed the diffuse of bacterial growth from slant stab line inoculation extending to all the tube . The microscopic examination was done by staining smear to bacteria old (18-24)hr. grown on MacConkey agar by Gram stain showed the bacterial isolates appear as Gram negative ,short rod .

3.2.1.1:*E.coli* Identification

3.2.1.2: Biochemical Test

There are many different biochemical tests can be down to bacteria depend on its result such as changing in color ,produce bubbles , or precipitate, reflect the activity

of bacteria and help in identical of bacterial isolated in table (3-1) common biochemical test was used with *E. coli* bacteria identification.

The biochemical tests it was done to all of *E. coli* isolates , the catalase test was positive for all isolates because bacteria have broken down catalase to water and oxygen, when bubbles appear and it was negative to oxidase test because bacteria colony didn't change to purple color when oxidase reagent was added because the bacteria didn't have cytochrome oxidase as hydrogen receptor.

The bacteria was negative to urease test because of no changing in medium color which confirms that the bacteria are not consuming urea due to defective to enzyme urease .

The IMViC test which was important to differentiation of *E .coli* bacteria from the enteric family , so the indole test was positive by formation of red ring in the surface of the medium in isoamyle alcohol as a result for broken down of amino acid tryptophan by tryptophanase enzymes to form indole which appear as changing in color following the reaction with an added of Kovac's reagent.

The methyl red test was positive to E .coli bacteria by producing stable acid

based on pattern of glucose metabolism which was convert the medium to acid pH , resulting to change medium to red . Negative result for Voges-Proskaure test because *E .coli* cannot digestion glucose to acetone and acetyl methyl carbinol , so neither reaction with the first reagent alpha-naphthol nor with second reagent potassium hydroxide and the broth medium color appear as yellow-brown .

Also the bacteria was negative to citrate test because bacteria cannot utilized citrate as source of carbon and energy in Simmons citrate agar as a result to lack of

citrase enzyme which involving in break down citrate , so no change in pH indicator and the medium still green and this agree with (Tille ., 2017 ;Brown and Smith., 2017) ,Table (3-1)

Table (3-1) Biochemical Test and Morphological Identification Isolates *E .coli*.

Biochemical tests	**Rustle**
Catalase test	+
Oxidase test	–
Urease test	–
Indole test	+
Methyl red test	+
Voges-Proskaure test	–
Simmon citrate test	–
Urease test	–
Gram stain	–
Cells shape	Rods
Motility	–

Positive (+) Negative (-)

3.2.2.:*B.coagulans*

The morphological culture examination of *B .coagulans* colonies showed on Brain-Heart infusion agar was frosted glass, cream light yellow appearance but may become opaque or smooth raised wrinkly colonies. While , the

microscopically examination was done by staining bacteria smear with Gram's stain. The examination under oil emersion 100x showed large gram positive rods often pairs or chains bacteria with rounded or square end and may have a single endospore, placed sub-terminal swollen spore with light green color when stain with malachite green and vegetative cells appear pink/red (Adibpour *et al* ., 2019).

Table (3-2) Biochemical Test and Morphological Identification of *B. coagulans*

Biochemical tests	**Rustle**
Catalase test	+
Oxidase test	+
Urease test	+
Indole test	_
Methyl red test	+
Voges-Proskaure test	+
Simmon citrate test	_
Motility test	+
Gram staining	+
Cells shape	Rods
Spore staining	+
Growth at 42 &50 oC	+

Positive test (+) Negative test (-)

3..2.2.1:*B.coagulans* Identification

The isolate has important two characteristics used in identification the first is ability to produce lactic acid when fermented milk (Lactic acid is the characteristic substance in all fermented dairy products) were missed to classification the bacteria with *Lactobacillus spp*. early as *lactobacillus sporogenes* and later amended to *Bacillus spp*. and in both cases the microorganism still used a lot as probiotic bacterium , the second spore forming and the spores were ellipsoidal in shape , subterminally to paracentrally located within sporangium making swell when staining with counter stain ,the bacteria are Gram positive , motile ,catalase , oxidase, nitrate , methyl red , Voges- Proskaure and urease was positive testes. while indole and citrate testes was negative, Table (3-2) and this result agree with(Sen *et al* ., 2010;Adibpour *et al*., 2019) .

3.3 : Identification Using VITEK-2 System

After identifying of *E .coli* isolates by morphological characteristics and biochemical tests , some isolates were selected randomly to confirm their identity through the VITEK-2 bioMérieux system with reagent cards GN for Gram negative fermenting and non-fermenting bacilli which have 64 details of biochemical test ,and the result was identical to previous results in probability of (99%)with confidence level ,Excellent. While to insure the reliable diagnostic of *B .coagulans* bacteria confirmative identical using same compact system VITEK-2 with reagent cards of BCL of Gram-positive spore-forming bacilli with probability of (90.0%) with confidence level ,Good were result matched to the previous results

. The advantage from using VITEC-2 system to insure reliable diagnostic to the isolates and reduced unintended errors in diagnostic (Michal ., 2006) .

3.4: Biosynthesis of Nanoparticles

First , the ability of many different isolates of *Lactobacillus spp.* was tested to obtained biosynthesis of Fe_3O_4 Nanoparticles ,despite of using different pH value ,various temperature degree ,diverse incubation period and different iron salt precursor in many mole concentrated there was no reliable result confirms that the products has magnetic property, even when optical perceptible changing in color occur or slightly changing and this event is considered most documented phenomena in the production of biosynthesis of nanoparticles. Once upon completion procedure the final product shown in XRD analysis only amorphous without any diffraction of Nano- crystals peaks, moreover, it fails to be attracted to magnets, So were replaced another spices of bacteria *B. coagulans* that performs this task due to its characteristics in industry and probiotic production .

3.4.1: Biosynthesis of Fe_3O_4 NPs by *B. coagulans*

The using supernatant of *B. coagulans* in synthesis of Fe_3O_4 nanoparticles as reducing and stabilized agent was confirmed when observed the formation of dark brown color due to surface plasmon resonance property were deposited was collected by external magnet in the test tube (figure 3-1) . This result was agree with(Sundaram *et al* ., 2012) by using *B. subtilis* were mentioned there was many factors effect on formation of nanoparticles temperature , pH ,incubation time and concentrated of salt precursor in addition to biomolecules which secreted by bacteria as production of the metabolism activity which lead to reductase activity as one of possible pathway in mechanism of biosynthesis of iron oxide NPs, involvement enzyme of nitrate reductase in this bacteria strain , the reduced of salt precursor clearly observed in changing color in mixed culture supernatant from

pale light brown to dark brown as a result to formation of NPs and to the specific property of surface Plasmon resonance (Hulkoti and Taranath , 2014) .

B .coagulans one of bacteria belong to soil-based microorganism (Van Elsas *et al* ., 2006) which can be survival and competition with other microorganism in soil by secreted bacteriocin and avoids harmful metal by detoxifying mechanisms, the precipitin of metal in cell wall one possible pathway of intracellular biosynthesis or concentrated it in cytoplasm as granular to excluded out of cell and by transforming in reduction or oxidation to harmless form ,the ability of survival in the environment make easy to microorganism associated in bio production of NPs(Goswami *et al* ., 2016; Mukherjee *et al.*, 2002)

The extracellular supernatant of bacteria contained many biomolecules (protein , polysaccharide , enzymes and co-factors many studies suggested microbial enzymes play role as reducing agents in production of metal NPs as well as cofactor nicotinamide adenine dinucleotide (NADH) and reduced form of nicotinamide adenine dinucleotide phosphates (NADHP) dependent enzyme the both play role as reducing agent, the reduction will be down via electron transfer from NADH by NADH-reliant enzyme(Bose and Chatterjee ., 2016;Subbaiya *et al.*, 2017)

The biomolecules and protein play role in stability of biosynthesis of metal NPs even with less agglomeration from chemical production might be from capping the protein or biomolecules of the microorganism over the NPs surface during biosynthesis process (Moon *et al.*, 2010) .

The ability of *B .coagulans* bacterium in production of many different enzymes and lactic acid production capacity with synthesis of antibacterial agent make possible advantage to microorganism in biosynthesis and stability of nanoparticles.

Moreover the stability of IONPs were the bacteria mediated synthesis it was possible due to the strong electrostatic repulsion of net charge on surface of NPs and reflects particles ability to repulse each other electrostatically or by the intact different functional bound were shown in FTIR spectra clearly (Majeed *et al* ., 2020) .

In addition to that the turned of supernatant color from light brown to black indicating the successful production of stable Fe_3O_4 NPs and the strong magnetic property of IONPs was confirmed by using external magnet were used in collection and separation of IONPs from reactive mixture .

Many studies were used different part of plant as green stabilizers successfully synthesize IONPs in same co-precipitation method. Yusefi *et al* ,in 2019 using *Punica Granatum* Fruit Peel Extract in there study, the synthesized IONPs by Seaweed *(Kappaphycus alvarezii*) extract was also employed as a green reducing and stabilizer agent , that the plant associated with bacteria and different microorganisms in the synthesized Fe_3O_4 NPs display green synthesis. The characteristics in shape and size with high purity and crystallinity and non-toxic green synthesis of Fe_3O_4 NPs are expected suitable to be used in different fields of application especially in biomedical application through investigated the ability of NPs in such as magnetic hyperthermia efficiency analysis evaluated and their cytotoxicity assay were analyzed towards normal cell line and colon cancer cell line and others different cancer cell lines (Yew *et al.,* 2016:Yusefi *et al.*,2021)

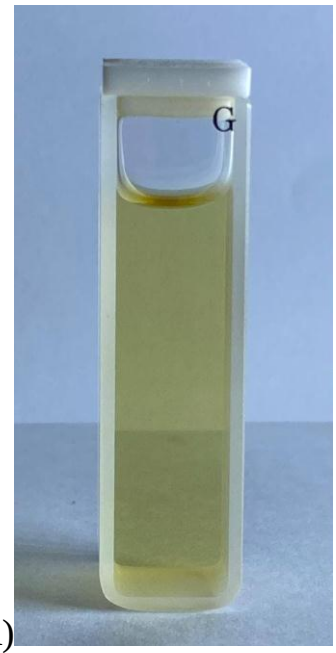
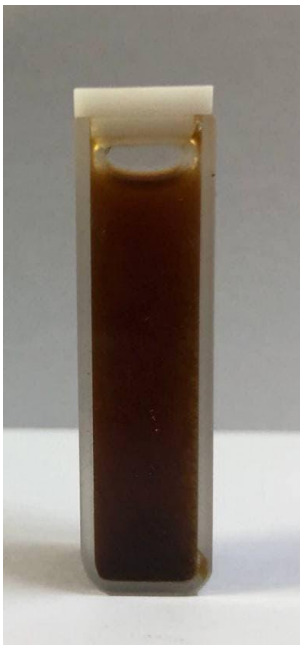
(A)  (B)  (C) 

Figure (3-1) Biosynthesis of Fe_3O_4 NPs (A) Bacterial supernatant (B) mixture reaction of (Fe^{3+}/Fe^{2+}) with bacterial supernatant (C)Separation of Fe_3O_4 NPs from reaction mixture by external magnet.

3.5: Characterization of the synthesized nanoparticles

3.5.1:UV-Vis Absorbance Analysis

The UV-Visible spectroscopic spectrum of biosynthesis nanoparticles clearly shown that the iron oxide NPs absorbance maximum at 250.5 nm which was identical to characteristics of UV spectral analysis for metallic iron in the range of surface Plasmon reasons property band occur at around 200-300 nm wavelength (Basavegowda *et al.*,2014) Figure(3-2). Among the expected peak , the absorbance in current study was the characteristic peak of the IONPs and this value was much

closer to the another researchers studies that reported the absorbance of magnetic nanoparticles near 250 nm(Wang *et al.,* 2014;Samrot *et al.,* 2016)

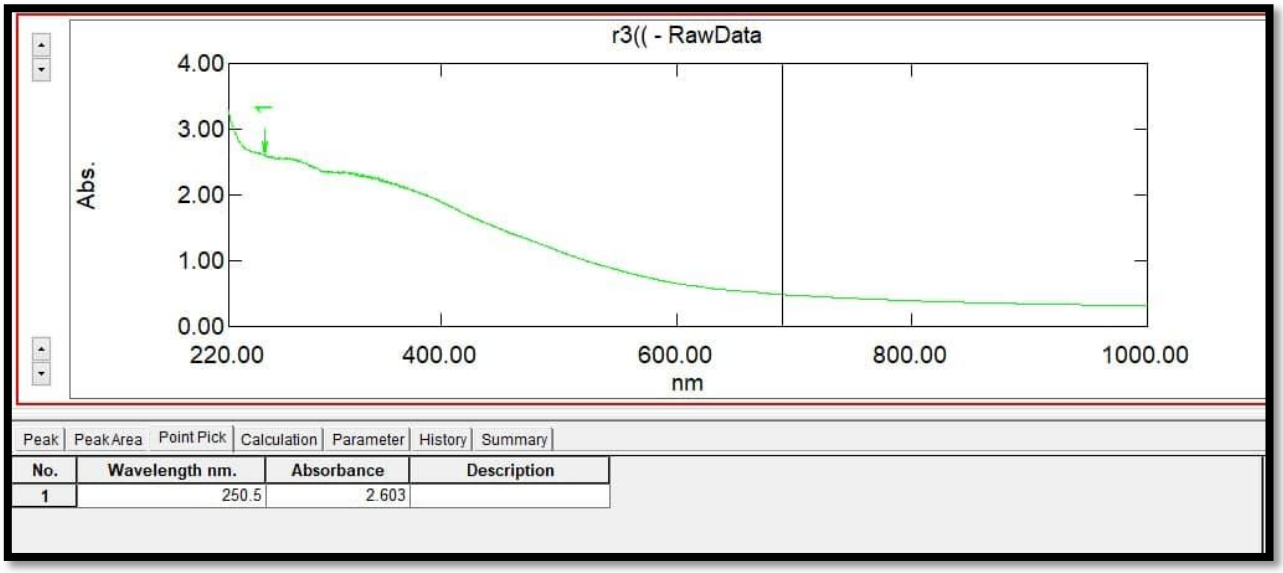


Figure (3-2) Uv-Vis spectra of synthesized Fe_3O_4 NPs

3.5.2 : FTIR Fourier Transform Infrared

FTIR spectra analysis of iron nanoparticles synthesis using bacteria *B .coagulans* supernatant was carried out to identify of chemical composition and functional group which was possible reduction of Fe ions or possible interaction between functional group and nanoparticles hence could be act as capping agent helping in stabilization of nanoparticles. FTIR shown in Figure (3-3) wavelength absorption band of two intense peaks observed between 574 cm^{-1} and 632cm^{-1} are attributed to the stretching vibration model associated to metal-oxygen Fe-O bonds in crystalline lattice Fe_3O_4 and confirmed the formation of NPs (Yuvakkumar and Hong ., 2014;Demir *et al.,* 2013). While the prominent absorption band observed at region 3730 ,3251, 2357,2326, 1631, 1411, 1222, 1041cm-1 .

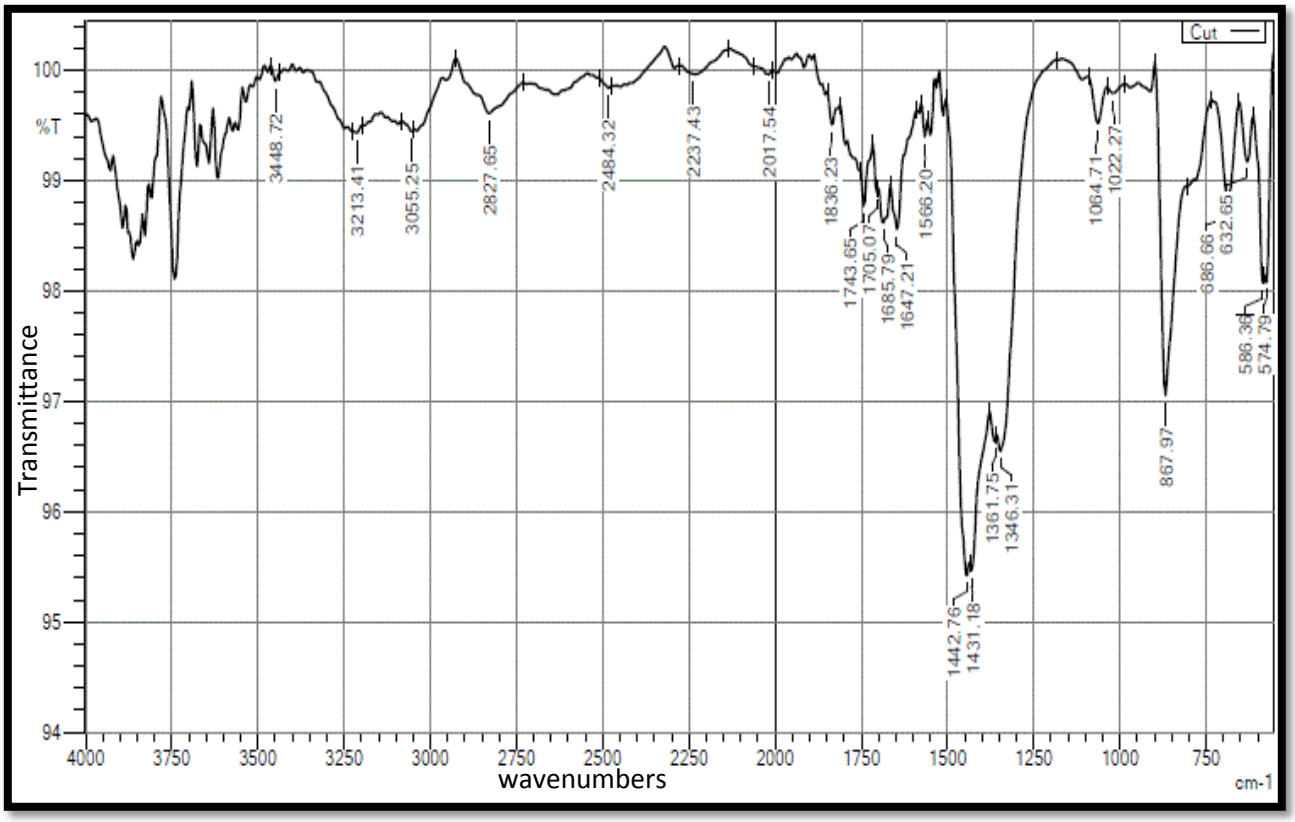


Figure (3-3) FTIR Spectrum of Fe_4O_4 Nanoparticles.

The wide beak in range of 3730 – 3251cm^{-1} was related to O-H and N-H bond together with beak 2326 cm^{-1} indicated the presence of carboxyl group O-H , while 163 cm^{-1} was attributed to the C=C carbonyl group, and 1411 cm^{-1} relegated to C-H plan . Whereas absorption beak at 1222 cm^{-1} corresponded to the asymmetric stretching vibration of the sulfate group. While 1041 cm^{-1} was assigned to the C-O stretching band related to C-O-SO3 group .A slight peak shift observed at iron metal doping in present study between (400-800 cm^{-1}) may be attributed to the changing in size particles and capping agent(Awwad and Salem ., 2012)

3.5.3 : XRD-Analysis of Iron Oxide Nanoparticles

X-Ray diffraction (XRD) was used to investigate the shape and size of nanostructure samples biosynthesis by *B .coagulans* bacteria broth supernatant , the crystallites size of Fe_3O_4 NPs could be estimated by Debye-scherrer equations (Eq.1) which reveals the relationship between XRD peak broadening and crystalline size

$$ds = K\lambda / \beta COS\,\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where *ds* is average crystalline size IONPs, *K* is the crystalline-shape factor with a scherrer constant value of 0.9 for an absence of information crystalline- shape, λ is a wavelength of X-rays= 1.5418 A^o , β is a full width at half maximum (FWHM) of the XRD diffraction peak in radians in 2θ scale and θ is the half diffraction angle of the peak(Kroon ,2013) from this equation the calculated of crystalline mean size of IONPs was (15 nm) , the Bragg's reflection intense peaks at 2θ value in XRD pattern recorded many relative intensity for magnetite (Fe_3O_4) corresponding to the standard 2θ value .Furthermore the result determined that the relative intensities and positions of reflection peaks $2\theta=31.5^o,35.5^o,43.122^o$ Figure(3-4) for synthesis magnetic nanoparticles by bacteria biosynthesis agree with standard diffraction Fe_3O_4 NPs (Fatemi *et al* .,2018) .The above result confirmed the crystalline nature of NPs synthesis in this study .The size of synthesized IONPs located within the range of nananoscale (1-100 nm) and appears to be small in size due to the acceleration of reaction in presence of NaOH and the capping agents.

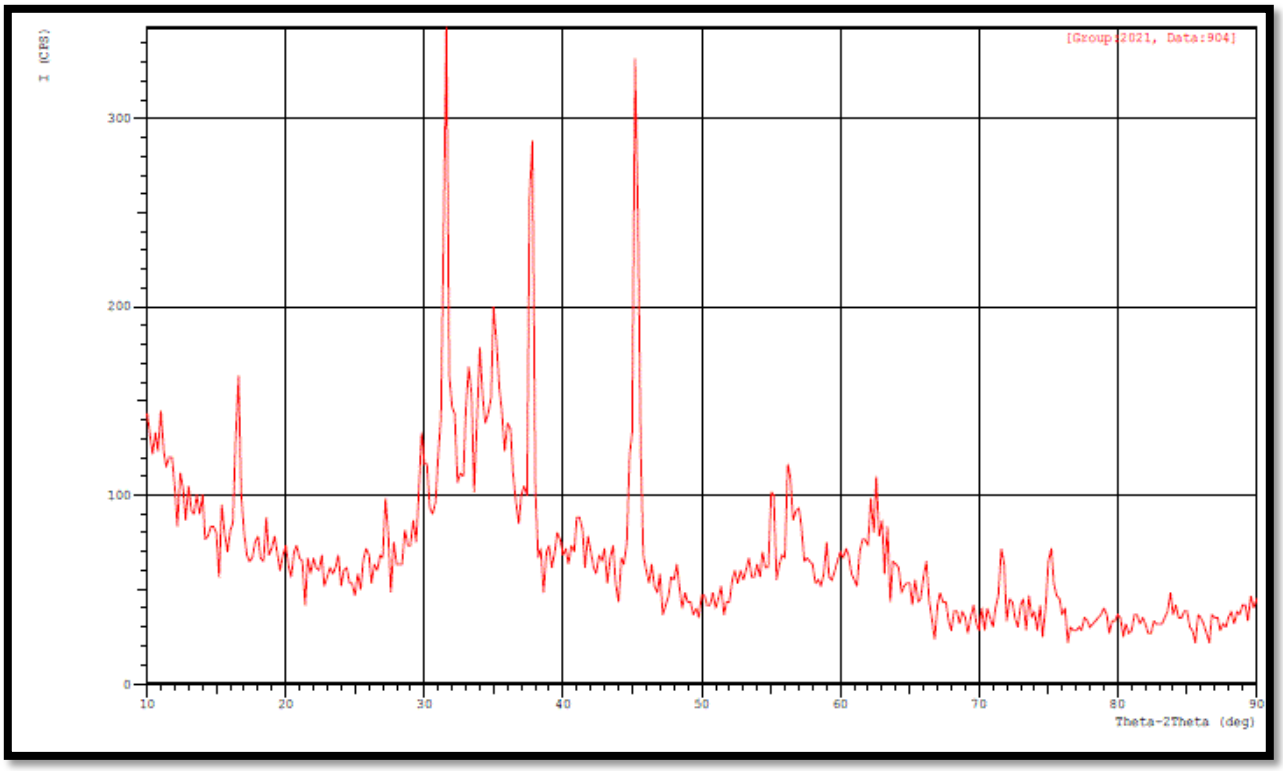


Figure (3-4): X-ray diffraction pattern of (Fe_3O_4) NPs

3.5.4: Scanning Electron Microscopy Analysis (SEM)

The (SEM) Analysis confirm the information about the morphology and size of Nanoparticles ,SEM Nanoscale images Figure (3-5) of biosynthesis of Iron Oxide NPs were mostly spherical shape and the size of NPs between 4 to 33 nanometers which was within the range size of nanoparticles , the small size of this NPS was seen probably due to low level of agglomeration as result of fast formation of precipitation and short time of reaction incubation , furthermore abundance of active biomolecules and capping agent secreted by bacteria in growth medium when supernatant collection ,that could be considered protected agent by covered surface area of NPs which increased physical stability (Yusefi *et al.*, 2019)

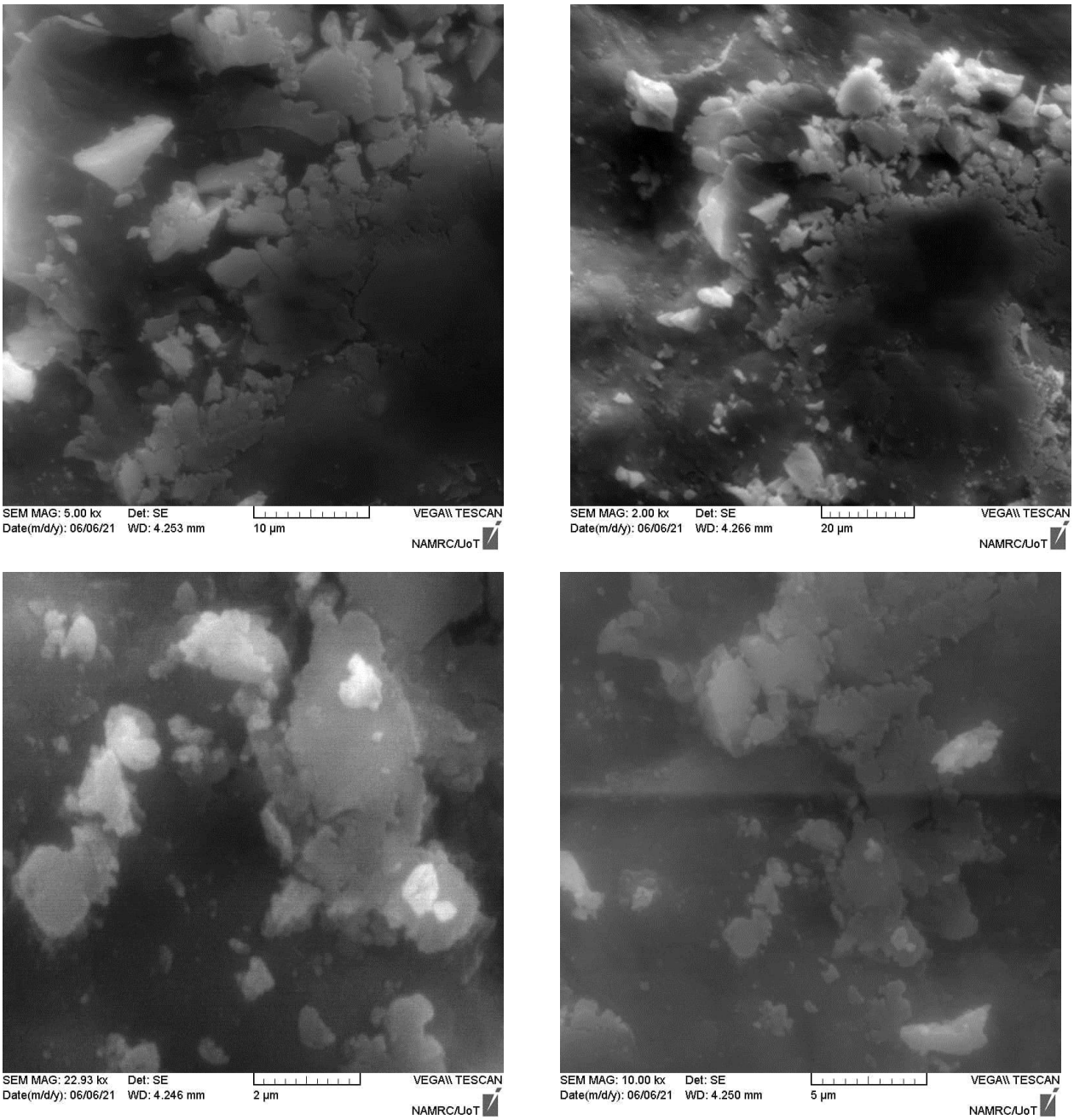
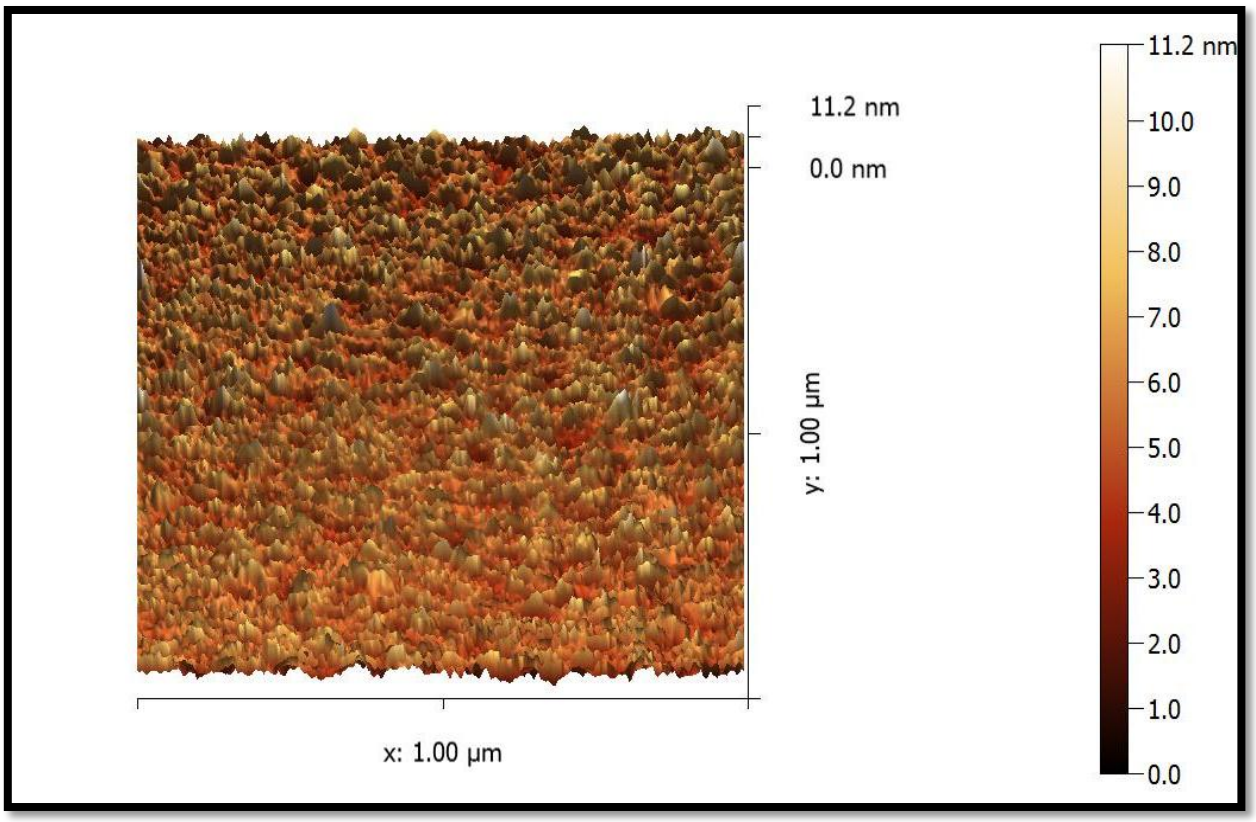


Figure (3-5) Scanning electron microscopy(SEM) Images of Iron Oxide Nanoparticles .

3.5.5 :Atomic Force Microscopy

The AFM analysis gave information of the topography and morphology of nanoparticles synthesized by bacteria with roughness and average diameter ,the analysis with (AFM) provided two dimensional (2-D)surface profile Figure.(3-6) and three dimensional profile Figure (3-7).The two dimensional image showed that most of IONPs was irregular cubic structure and the three dimensional image of the Fe_3O_4 crystal obtained by AFM indicated irregular cubic layers. The most (55%)of NPs size distribution between 40-60 nm Figure(3-8) and lowest volume distribution below 40 nm till the smallest NPs , the average diameter of iron oxide nanoparticles at 48.8 nm and this mean that the particles of Fe_3O_4 were in nano meter-size ,the advantage from to type of images to (Majeed and Naji,2018)



Figure(3-6) Two dimensional AFM image of prepared Fe_3O_4.

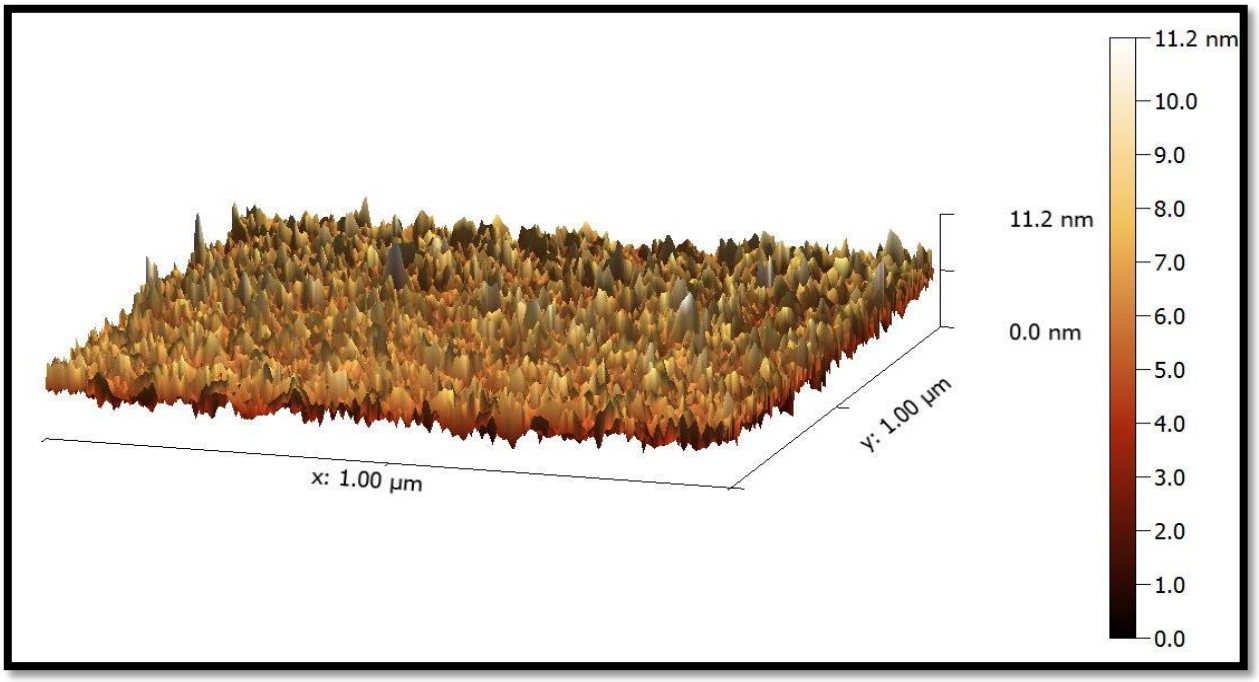


Figure (3-7) Three dimensional Image of iron oxide NPs by AFM

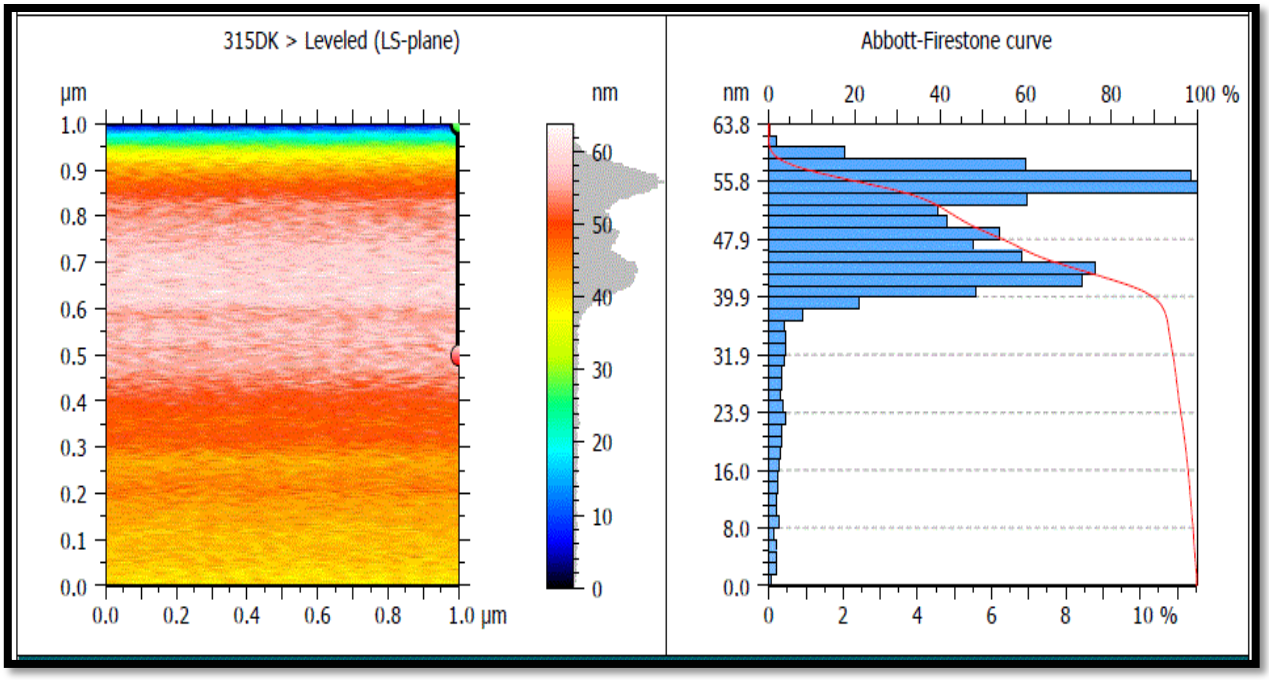


Figure (3-8) Granularity accumulation distribution for Fe_3O_4 by AFM

3.6 :FTIR of Poly-β-hydroxybutyrate(PHB) and Fe_3O_4 NP/PHB Nanocomposite .

The Fourier Transforms IR spectroscopy (FTIR) were perform first for PHB powdered polymer were supplied from sigma Aldrich (USA) Figure(3-9) to evaluated the different functional group to PHB . The FTIR spectrum of PHB was recorded between 600and 4000 cm^{-1} ,the absorbance band at 1724 cm^{-1} which represented in (group frequencies) functional group of the carbonyl C=O in the

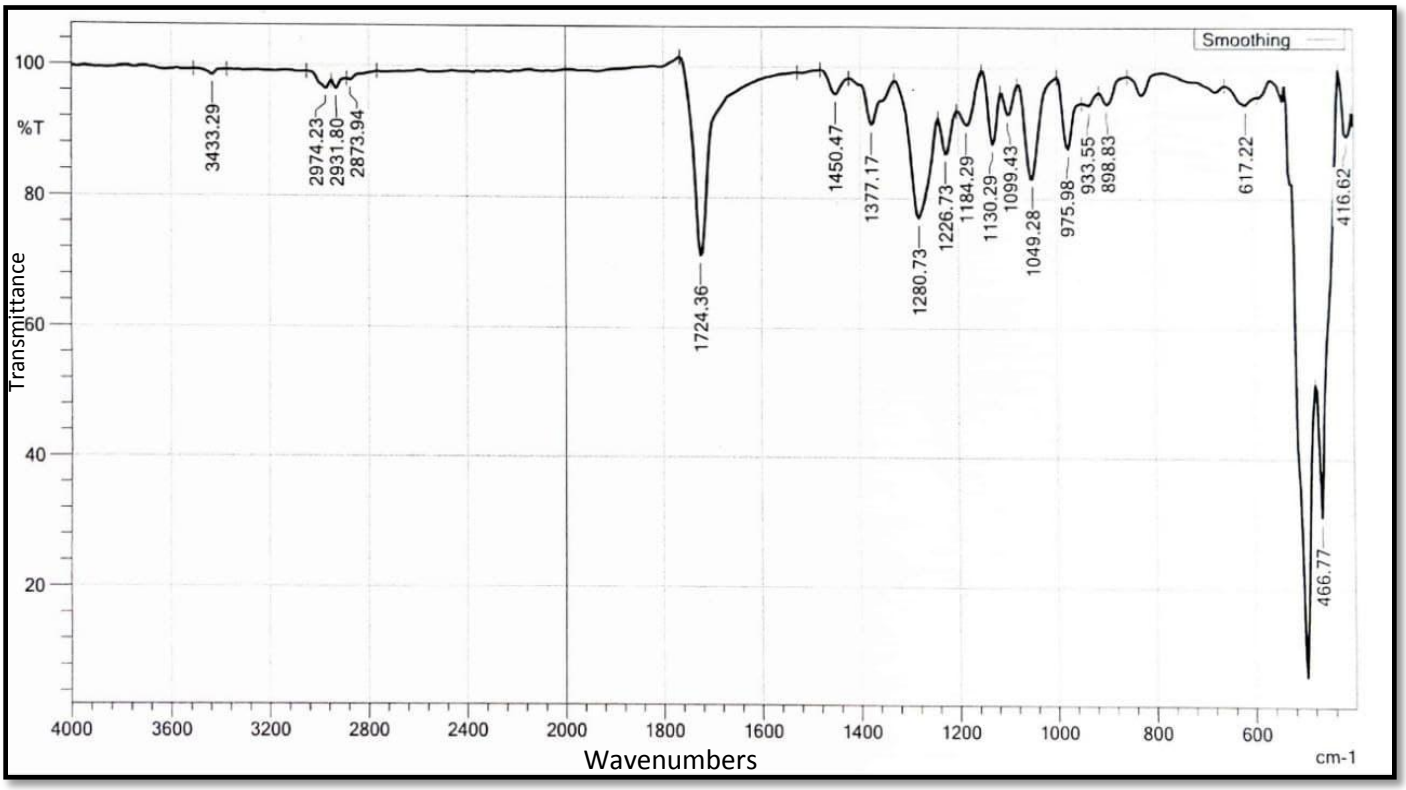


Figure (3-9) FTIR Spectra for Polyhydroxybutyrate (PHB)

ester group in chain of exceeding order of crystalline molecule structure, while the band at 1227 cm^{-1} corresponds to the –CH group according to (Zhang *et al.*,2010). The peaks at 3433 cm^{-1} was related to terminal –OH group, for 2974 and 2931cm^{-1}

corresponding to presence of an alky–CH_3 group , on other hand the absorbance's band between 1000 and 1300 cm^{-1} showed the stretching of the C–O bond in ester group .The peak at 1450 cm^{-1} was assigned to the asymmetric to –CH_2 or CH_3 .The peak located at 1377 cm^{-1} account to symmetric bending –CH_3 group .

Finally the absorption bands in the region of fingerprint frequencies between 1500 and 800 cmm^{-1} , that highly characteristic of molecule's these region were very important for PHB morphological properties in the composite since the amorphous state of PHB in mixture was confirmed by the disappearance of some group (Florez *et al* .,2018). In Figure (3-10) FTIR spectra of nanocomposite of Fe_3O_4 and PHB polymer with 20 wt. % loading were recorded to obtain information about nanoparticles-polymer interaction, so the spectral bands with maximum transmittance at 426 cm^{-1} and 570 cm-1 correspond respectively to vibration of the

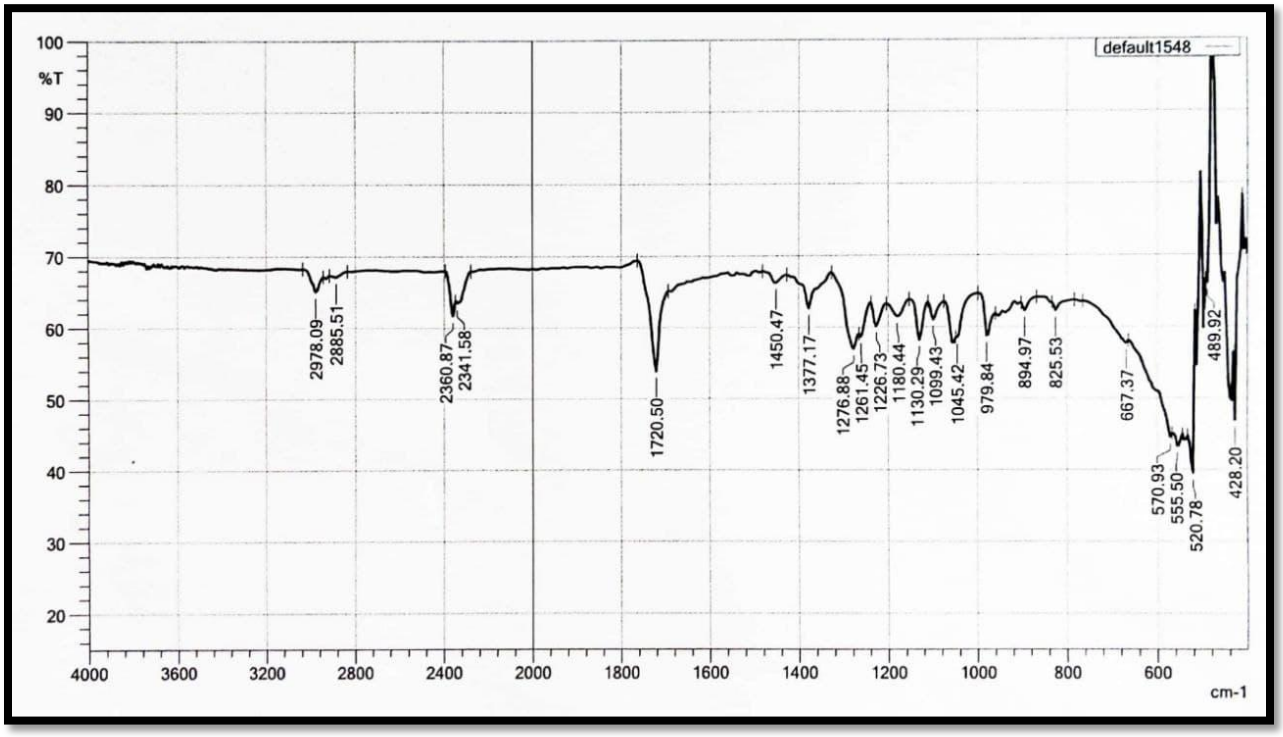


Figure (3-10) FTIR Fe_3O_4/PHB composites

of the Fe^{3+} –O and Fe^{2+}–O bond in the crystalline lattice of Fe_3O_4 (Yang and Liu ,2006). More significant the fingerprint region of composite clearly intense number of waves compared with neat polymer, in addition the characteristic absorption band of Fe–O (around 580 cm^{-1}) still can be found in the PHB modified Fe_3O_4 Nanoparticles ,both absorption peak from iron oxide nanoparticles and polymer indicated that magnetic NPs have been well modified (Tong *et al* .,2015).

3.7 : Antibacterial Activity of Antibiotic.

Figure (3-11) indicated (eight) antibacterial agents susceptibility pattern of *E. coli* . The antibiotic tested was :Imipenem, Amikacin, Ceftriaxone, Tobramycin, Nalidixic acid, Nitrofurantion, Gentamycin, Piperacillin ,that showed that the highest sensitivity was observed with Imipenem (~99%),when recorded one bacterial strain in intermediate phase ,Amikacin (98%) and Nitrofurantion (88%) respectively. Further the lowest sensitive Ceftriaxone (28%) and Nalidixic acid 31% were Piperacillin (37.5%). Most study on the antibiotic susceptibility of urinary pathogens around word have found similar result which was agreement with the current study. (Ahmed *et al.,* 2019) reported *E. coli* showed low resistance to Imipenem ,Amikacin and Nitrofurantion and high resistance to Piperacillin , in another study (Mohamed *et al* ,.2021) reported that highest sensitivity to *E .coli* the Imipenem then Amikacin and Nitrofurantion respectively and the lowest was the Piperacillin antibiotic .The current study showed that the more effective of eight antibiotic against *E. coli* limited by three types only and this indicated that many antibiotics were ineffective and there was limited different between the lowest sensitive antibiotic Ceftriaxone and another study ,it may due to the excessive used of this antibiotic in Iraq. So it was necessary to search for an alternative , and that could be nanoparticles.

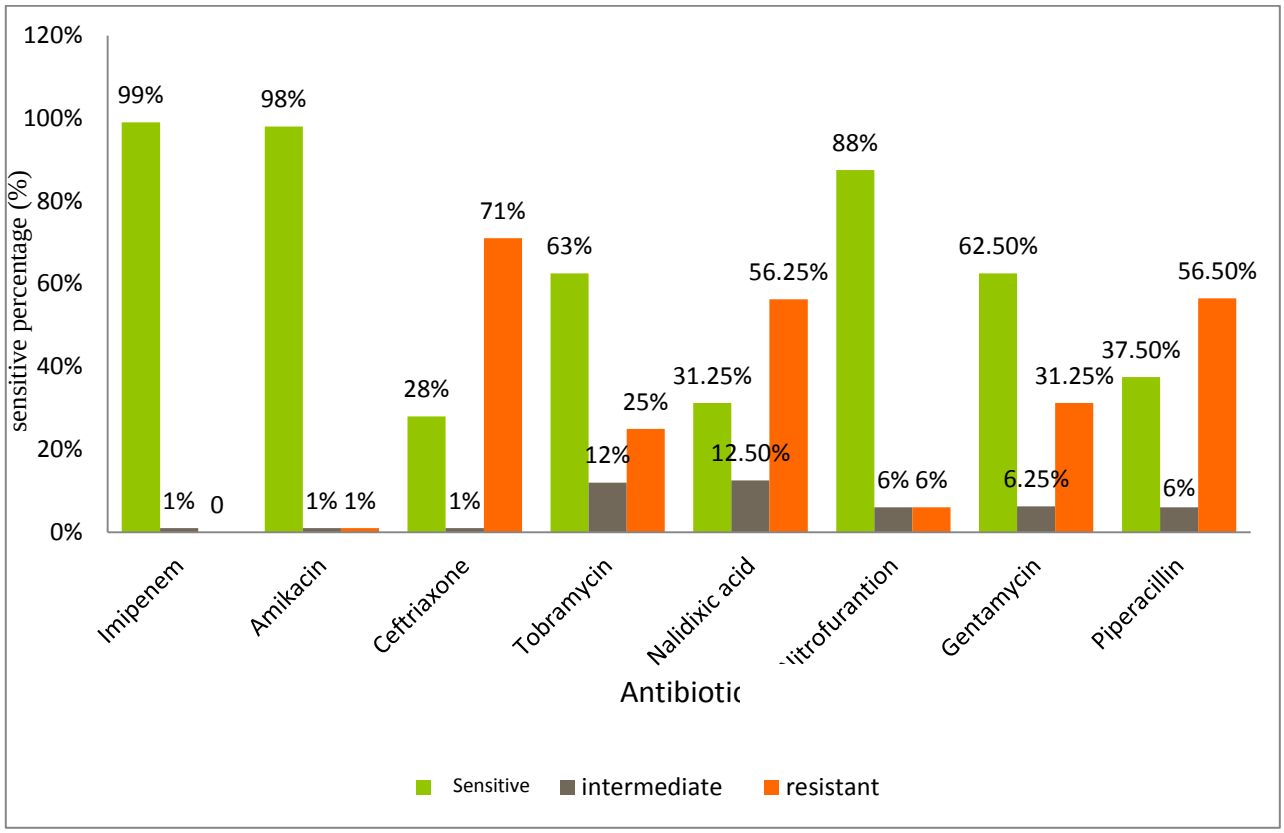


Figure (3-11) *E. coli* Antibiotic susceptibility percentage by Kirby-Bauer method on Muller Hinton agar

3.8 : Antibacterial Activity of Fe_3O_4 NPs

The Antibacterial activity of Iron Oxides Nanoparticles biosynthesis by *B .coagulans* supernatant were used evaluate their ability to inhibition growth of *E . coli* . The methods agar disk- diffusion (Kirby Bauer) was used to detecting the antibacterial activity of biosynthesis Fe_3O_4 against *E. coli* bacteria . In Kirby Bauer (disk diffusion) method Figure (3-13.1). Iron Oxide NPs at a concentrated of (50, 100, 150 and 200 µg/ml) , after overnight incubation at 37 ^{O}C , the high inhibition zone measured 31.8 mm at concentrated of 200 µg/ml result showed the IONPs have ability to inhibit the growth of microorganism (Lee *et al.*,2008).

Figure (3-12) showed different letters indicated statistically differences while similar letters indicated non statistically differences that mean the high concentration at 200 µg/ml of iron oxide nanoparticles was largest inhibition zone recorded

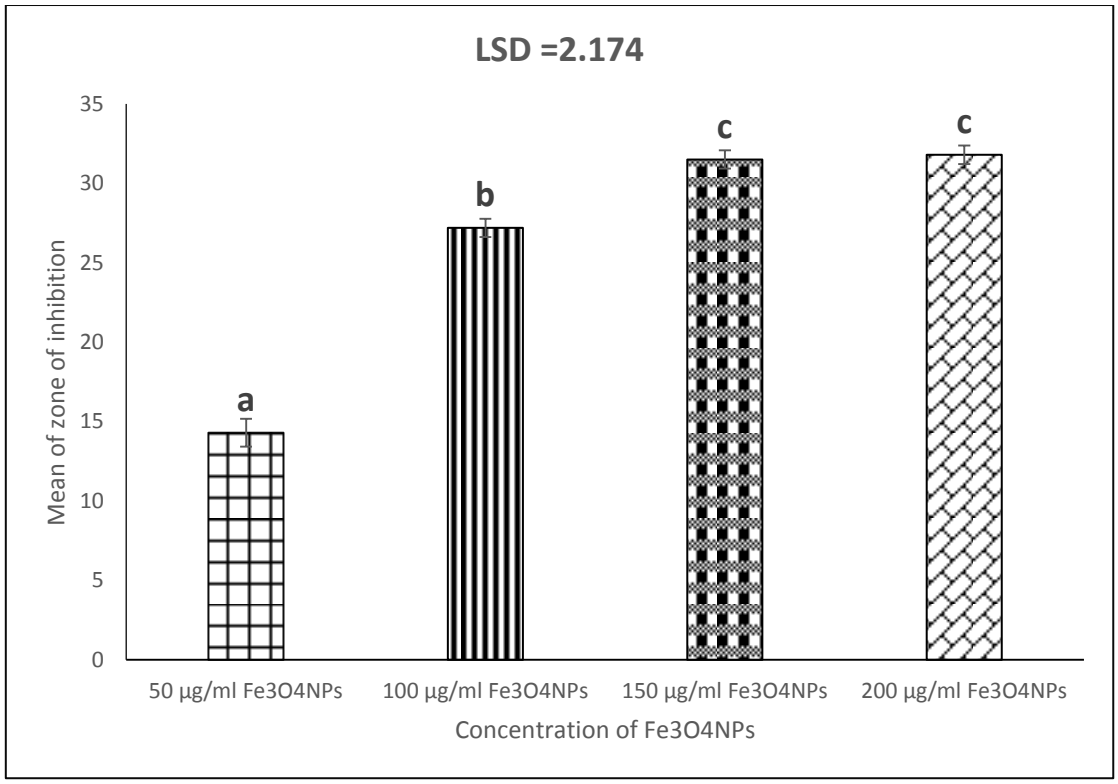
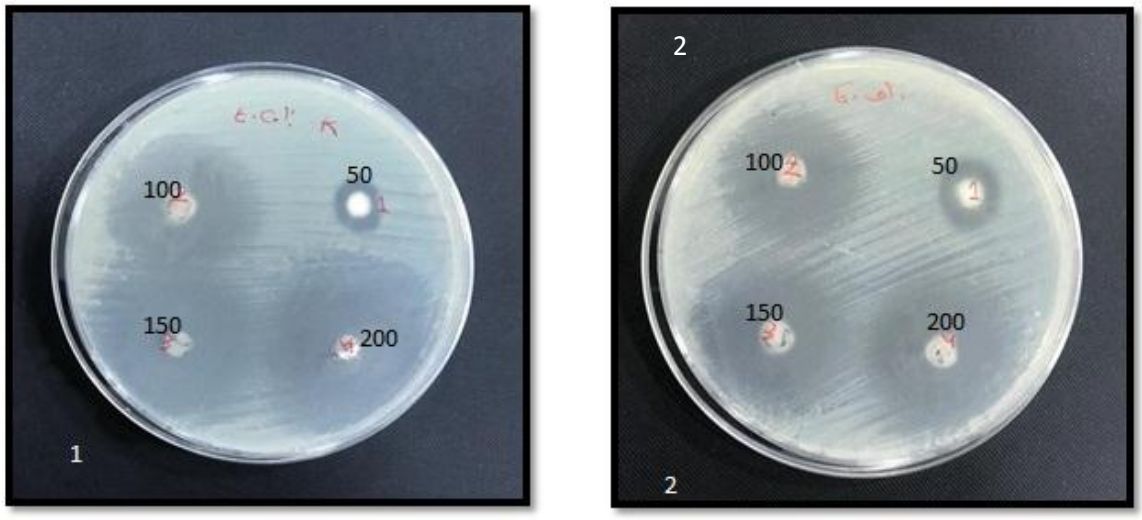


Figure (3-12) Zone of inhibition of different concentrations of Fe_3O_4 NPs against *E. coli*

3.9: Antibacterial Activity of PHB/Fe_3O_4 Composite

Moreover third application the reinforced of Iron oxide NPs with polyhydroxybutyrate(PHB) biopolymer to evaluated the synergistic antibacterial effect against *E. coli* in disk diffusion method , the antimicrobial effective compared through recorded the determent area of inhibition zone .



1-Fe_3O_4 NPs against *E. coli* **2-Fe_3O_4/PHB composite against *E. coli***

Figure (3-13) Comprasion of antibacterial activity of Fe_3O_4 NPs and Fe_3O_4/PHB bionanocoposite against *E. coli* on muller-hinter agar .

The antibacterial test of composite Fe_3O_4/PHB with loading ratio (*wt./wt. :8/2%*) respectively was investigated against *E .coli* strains overnight growth at 37 ^{O}C on Muller-Hinton agar medium by disk-diffusion at concentrate of(50,100,150,200 µg/ml) .Figure (3-13.2). The developed zone of inhibition of bacterial growth around the disk was recorded at 33.6 mm Table(3-4) and that the largest inhibition zone at concentrate of 200µg/ml .The result confirmed that the composite was more effective against pathogenic than IONPs alone by increasing the inhibition zone (Lin *et al* .,2019). As well as the result showed the increased in inhibition zone when the concentration of composite agent increased, and recorder largest inhibition zone with high concentration that was indicated statistically differences between the all concentration as shown in figure (3-14)

Screening the antibiotics susceptibility for *E .coli* isolates emergences increased the resistance to antibiotic compared with IONPs and Fe_3O_4 /PHB ,figure (3-15)and they exhibited a concentration- dependent effect on *E. coli* growth as showed in table (3-4). One of important reason to the increased of antibiotic resistant misuse of antibiotic in addition to mutations encoding enzymes associated with resistance, as well as transmission of resistance by plasmid (Platansing ., 2015)

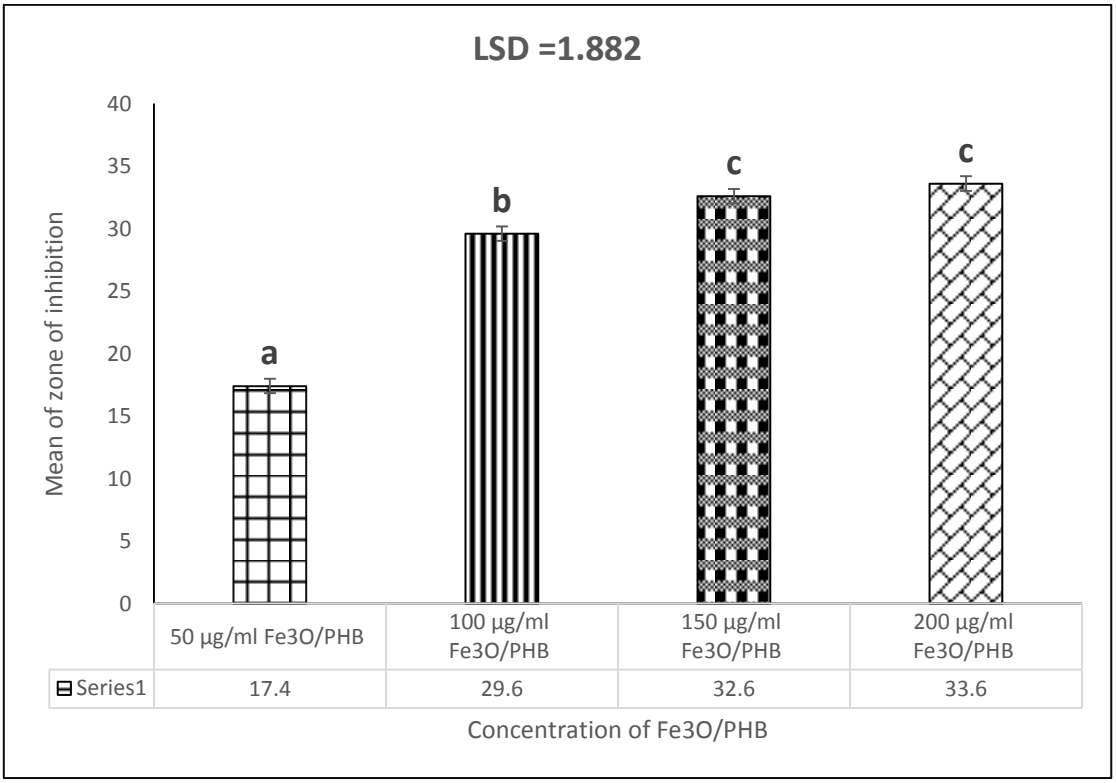


Figure (3-14) Zone of inhibition of different concentrations of Fe_3O_4/PHB nanoparticles against *E. coli* isolates

The maximal inhibitory effect was obtained at higher NPs/PHB concentration in concentrate of 200µg/ml ,which lead to decrease in bacteria growth rate , indicating the bactericidal effect of the nanocomposite and using iron oxide alone was proved effective that consistent with almost all previous studies (Gabrielyan *et al*., 2019).

Table (3-4) Comparison of inhibition zone antibiotic vise NPs &NPs/PHB against *E. coli* on Muller-Hinton agar

	Inhibition zone(mm)								
NPs	**Fe3O4 NPs**			**Fe3O4/PHB NPs**			**Antibiotics**		
***E.coli* bacteria**	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**IPM 10µg**	**AK 30µg**	**F 300µg**
	27.2 mm	**31.5 mm**	**31.8 mm**	**29.6 mm**	**32.6 mm**	**33.6 mm**	**23 mm**	**17 mm**	**17 mm**

IPM: Imipenem, AK: Amikacin ,F: Nitrofurantion.

The antimicrobial effect of IONPs could be due to several mechanisms reactive oxygen species(ROS) together with superoxide radicals (O2–) , Hydroxide radical (OH–)and singlet oxygen (O^2) formed by biogenic Fe_3O_4 could be the effective in inhibition case and there was many studies have obtained the same results about how Iron Oxide NPs work as an antimicrobial agent against *E. coli* (Arakha *et al*., 2015).

Chemical reaction between hydrogen peroxide and the membrane proteins or between the chemical produce in presence of Fe_3O_4 NPs and the outer bilayer of bacteria could be the reason for the antibacterial activity of Iron Oxide nanoparticles , the H_2O_2 was when generated produced inters cell membrane of bacteria and kills them , the possible mechanism of antibacterial action of metal nanoparticles was carrying positive charges and microbe are have a negative charges which create electromagnetic attraction between the NPs and microbe , the

result of attraction the microbe get oxidized and die at once (Rezaei-Zarchi *et al.*, 2010) .

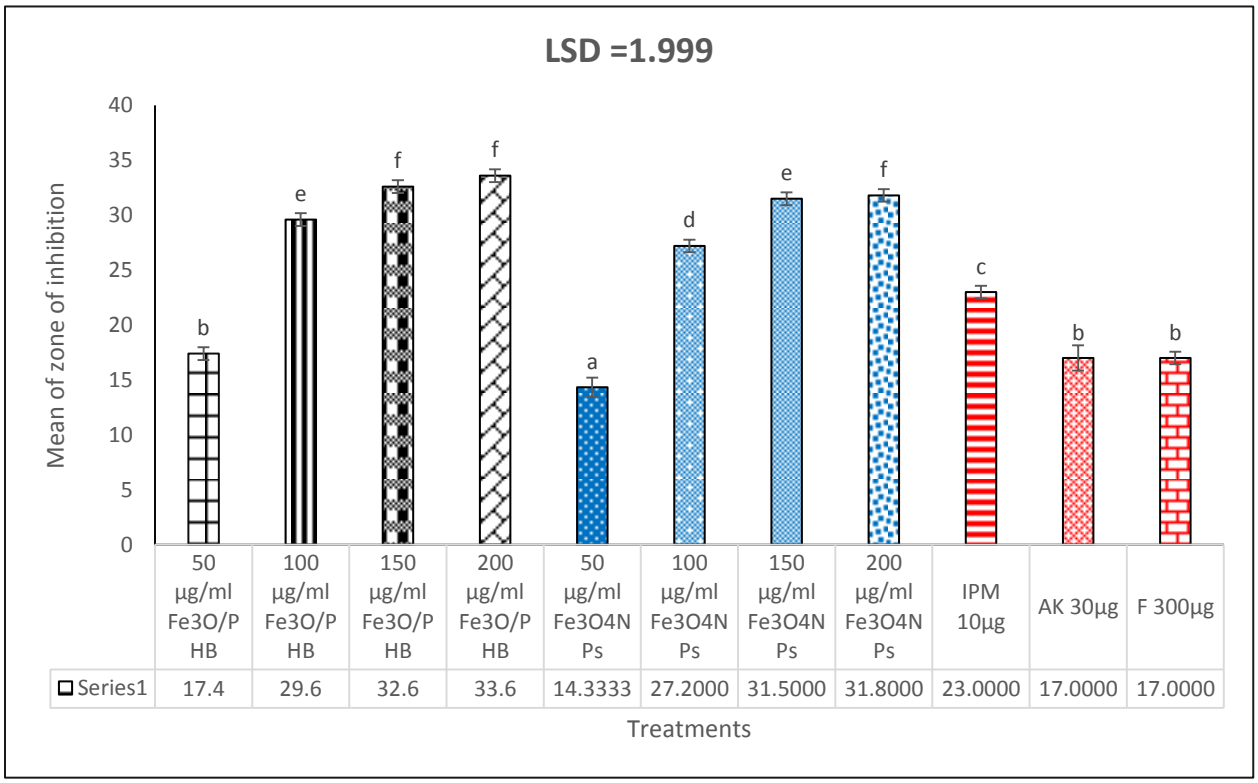


Figure (3-15) Comparison of zone of inhibition for Fe_3O_4NPs, Fe_3O_4/PHB NPs and highly sensitive antibiotics against *E. coil* isolates

.Ions which is realest from nanomaterial's react with thiol groups(–SH) of proteins present on bacteria cell membrane which lead to cell lysis or cause the DNA unwinding that inhibit the cell division the associated of (ROS)could cause damage to bacterial cell macromolecules such as protein synthesis and alteration , inhibition of enzymes and lipid synthesis as well as oxidation and damage of DNA and RNA of the bacteria .The react of ROS directly with DNA or bacteria cells protein or lipids producing oxidative stress that can in turn react with those molecules and cause damage or death of the cell, finally ROS can be formation via autoxidation of NADH dehydrogenase II in the respiratory chain leading to cell

death (Beyth *et al* ., 2015). Moreover the small size of nanoparticles with high surface to volume ratio like spherical nanoparticles gave better potency against bacteria , in this connection the almost prepared IONPs size between 10-65 nm which was consider as small particles and that agreement with (Rana and Kalaichelvan .,2011) study that the bactericidal efficiency of metal NPs is related to their small size .

In previous study (Diez-Pascual and Diez-Vicente ,2014) reported that the antimicrobial action of neat PHB nanocomposite (with metal NPs) was tested against *E. coli* and the antibacterial activity was increasing with increased of metal ions NPs content and best antibacterial action was attained with 20.0 wt. % loading of polymer PHB .Which is agreement with current study when the inhibition growth zone of Fe_3O_4/PHB against *E. coli* .Table(3-4) show the largest inhibition zone 33.6 mm and strong antimicrobial effect of PHB nanocomposite on *E. coli* , in agreement with (Liu and Yang ,2003) . It may attributed to the structural and chemical compositional of the Gram negative *E. coli* cell surface which usually has one layer of peptidoglycan between the outer membrane and cytoplasmic membrane (Pantani *et al* ., 2013) the damaging of cell membrane leads to leakage of cell contents and cell death , although the exact mechanism of action is still unknown, but production of H_2O_2 (strong oxidizing agent) has been considered as key factor of the antibacterial activity of Fe_3O_4/PHB as well as in same line with Iron Oxide NPs alone (Mahdy *et al* ., 2012) .

3-10 :Determination of MIC and MBC for Fe_3O_4NPs &Fe_3O_4/PHB Bionanocompsite Against *E. coli* .

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value of the biosynthesized Fe_3O_4 NPs and Fe_3O_4/PHB composite on *E .coli* bacteria were carry out by broth macro-dilution method to quantitatively determine the antibacterial activity ,the result obtained by visual observing and spectrophotometer at 600 nm wavelength after incubated at 37 ^{0}C for 24 hr. Table(3-7) .

The Fe_3O_4 NPs inhibited visible growth of *E.coli* in dose- dependent manner , (MIC) the lowest concentrated inhibited the visible growth of *E.coli* was observed at concentration of 150 µg/ml for IONPs while the lowest concentration of Bionanocompsite that reduced the growth of *E. coli* at concentration of 100 µg/ml .

MBC the lowest concentration of IONPs that prevented the growth of *E. coli* on Muller-Hinton media ,therefore the most highly-dilute tube with no noticeable growth was considered as the MBC that was at 200 µg/ml ,while the MBC for Bionanocompsite was 150 µg/ml ,were clearly showed the effective of IONPs and the Bionanocompsite increasing when the concentrated of NPs and composite are increase , meanwhile the composite become more effective against *E.coli* with less concentration of IONPs alone , indicating that this nanocomposite had a bactericidal capacity effect against *E. coli* and that agreement with. The absorbance of samples at 600 nm by spectrophotometer confirms the result of visible observation that decreasing bacteria growth corresponding to increasing of NPs and composite(Trchounian *et al.,* 2013).

Table (3-7) MIC & MBC of Fe_3O_4 NPs against *E.coli* bacteria.

Tube No. *E.coli* suspension	Fe3O4 NPs µg/ml Concentration	Optical density absorbance 600nm	Fe3O4/PHB µg/ml Concentration	Optical density absorbance 600nm
Control (–)	**_**	**0.08**	**_**	**0.08**
Control (+)	**_**	**1.012**	**_**	**1.114**
Tube No. 1	**50**	**0.925**	**50**	**0.826**
Tube No.2	**100**	**0.533**	**100***	**0.412**
Tube No.3	**150***	**0.372**	**150****	**0.315**
Tube No.4	**200****	**0.294**	**200**	**0.217**

Control (–) : Tube contained 2ml of Muller-Hinton broth

Control (+) : Tube contained 2 ml of Muller-Hinton broth with 0.2 ml of $1.5x10^8$

MIC* 150µg/ml &MBC 200 µg/ml for IONPs against *E .coli.***

MIC* 100 µg/ml & MBC **150 µg/ml for Composite against *E. coli* .

Moreover the result of macro-dilution and absorbance recording same concentration effective for the two antimicrobial agent in MIC &MBC when 0.1 ml suspension of *E. coli* culture in Muller-Hinton agar medium in present study has demonstrated that the synergistically of IONPs with PHB biopolymer have higher

MIC and MBC than using Iron oxide nanoparticles alone and the composite with the polymer content at 20% wt. showed a higher antibacterial activity than NPs alone or less percentage of polymer (PHB) (Marcello *et al.*,2021)

Fe_3O_4 NPs have been widely used as carriers with different Antibiotic and Anti-cancer drug for treated various diseases or in antimicrobial coating for implantable devices ,the use biopolymer PHB with IONPs gives continuously release of NPs in medium because of its poorly hydrolysis in water were stilled at nearly constant rate of concentration that ,which gives a privilege to the continuity of the NPs effect on bacteria, the exact efficacy of the composite is still unknown but the distribution of bacteria cell membrane leads to leakage of internal contents of cell and cells death, the ability of IONPs to changes of bacterial metabolic activity represent key factor to suppressing bacteria , although the formation of reactive oxygen species which can lead to oxidative stress , damage of protein cell membrane and DNA, the production of H_2O_2 has been consider main reason of antibacterial activity of Fe_3O_4 –reinforced nanocomposite (Tam *et al.*,2008) .

3-11: Antibiofilm Activity of Biogenic Fe_3O_4 NPs

Two methods Congo red agar and tube method were used for qualitative estimation of biofilm formation by *E.coli* bacteria and Antibiofilm formation by Fe_3O_4 NPs

3.11.1 Tube Method (TM)

TM was used for detection and measurement of biofilm produced by microbe when inoculated in 2% sucrose with Muller-Hinton broth medium Figure(3-16.B) were observing the biofilm formation by *E.coli* lined on bottom and wall tubes as purple ring also used for qualitative assessment of Antibiofilm activity by Fe_3O_4

NPs were prevented formation of biofilm in concentration 150 µg/ml Figure (3-16.A) . The TM represent Macro-dilution method and positive result observed when visible film lined the wall and bottom of the tube .

The long term treatment and poorly effected or resistance to antibiotic in addition to nosocomial and recurrent infection which cause by bacteria produce biofilm ,the produce of biofilm on implant medical devices make infection untreatable, recurrent and failure of medical devise , it is important to determine the biofilm microbe and antibiofilm agent ,one of the attempts to use nanoparticles (Wang *et al* .,2017)

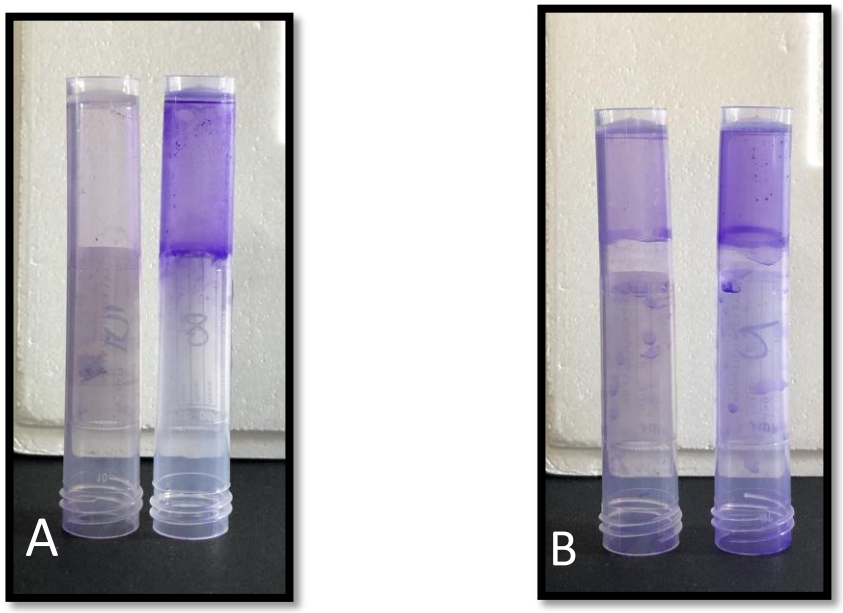


Figure (3-16) Tube method used for detection biofilm formation by *E. coli* and Anti-biofilm activity by Fe_3O_4 NPs.

A.*Right*: Biofilm formation by *E.coli* ; *Left*: Antibiofilm activity of Fe_3O_4NPs

B.*Right*: Strong biofilm formation ; *Left*: Moderate biofilm formation

Especially Fe_3O_4 NPs were used in coated the implant medical devise to prevent biofilm formation which has proved in present study by inhibition formation of *E. coli* bacteria biofilm in tube method or as antibacterial agent ,the ability of IONPs through damage the cell membrane of plankton that prevent attachment to aggregation or damage the DNA or may alter the gene expression relating to biofilm formation (Tran *et al.*, 2010)

The intensity of the color upon staining overnight culture empty test tube indicates the possibility of *E. coli* bacteria to form the biofilm , it may be weak, moderate or strong ,and when testing bacteria with Fe_3O_4 NPs, the result showed its direct effect by preventing biofilm formation in all cases with NPs concentration 150 µg/ml and this agreement with (Thukkaram *et al.*,2014; Gabrielyan *et al* .,2019)

3.11.2:Congo Red Agar method

A qualitative method was used to valuation the biofilm formation by *E. coli* bacteria depending on the color changing of colonies on congo red agar Figure(3-17) and with modified when incubation with biosynthesis of Fe_3O_4 NPs by *B. coagulans* bacteria to experimented the prevent of biofilm formation at concentrated 150 µg/ml of NPs were observed as clear zone without bacterial growth around well contented NPs. The biofilm is a life form of bacteria that ,highly complex biological structure consists of association microbes culture and extracellular polymer matrix (EPM), a complex of biochemical mixture from polysaccharide , protein, lipid, glycoprotein and nucleic acid ,provide the inside protect to microorganism from biological ,chemical and physical unfavorable factors , this complexity give advantage to resist the action of the antibiotic and

human immune system , even if the antibiotic penetration the biofilm matrix no longer be active after binding with polysaccharides ,proteins, lipid present in biofilm ,one of the potentially successful strategy based on metal oxide Nanoparticles treatment used antibiofilm (Hu , 2017)

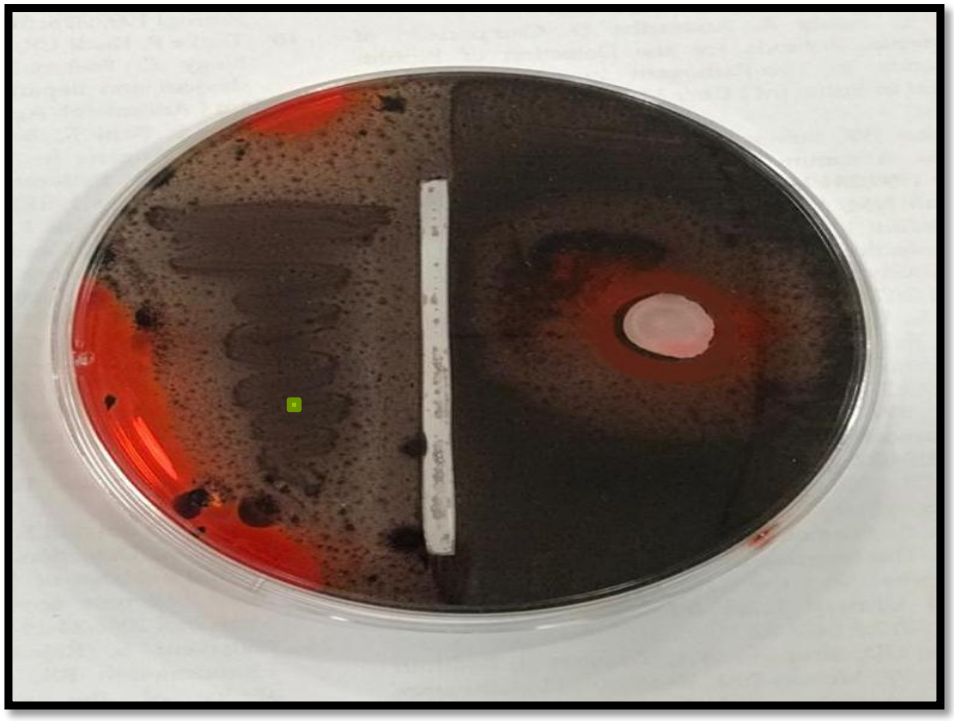


Figure (3-17) Antibiofilm activity of Fe_3O_4 NPs by congo red method against *E.coli*

As showed in present study the activity of Fe_3O_4 NPs when was prevent the biofilm formation of *E. coli* bacteria, the IONPs interaction and biofilm is determined by their electrostatic characteristics, the negatively charge of matrix due to presence of uronic acid and residual phosphate can interact with positively charge of metal ions that lead to mechanical damage to cell wall of biofilm microorganism as a result of electrostatic interaction (Ikuma *et al.*,2014) .

Biogenic iron oxide nanoparticle have ability to blocking pump function that play important role in resistance of antibiotic ,furthermore the generation of reactive oxygen species(ROS) and free radicals ions that make oxidative stress cause damage in DNA and blocking the replication one more main mechanism of antibacterial effect as well as antibiofilm of Nanoparticles disruption of proteins function and cell structure as result of the metal cations release (Wang *et al.*, 2017)

Conclusions and Recommendations

4: Conclusions and Recommendations

4.1: Conclusions

Present study concludes that

1. *B. coagulans* were capable to biosynthesis Iron oxide NPs
2. Iron oxide NPs have nanoparticles characteristic where confirm in different techniques such as UV, FTIR ,XRD,SEM, AFM
3. Iron oxide NPs exhibited antibacterial and antibiofilm activity against *E. coli* bacteria
4. Antibacterial effect of iron oxide NPs increased when combine with PHB polymer

4.2 Recommendations

1. Genotyping study to investigate the effect of iron oxide NPs on some virulence genes
2. Study the relationship between Iron oxide and immune system
3. Study of the antivirus and anticancer activity of Iron oxide NPs
4. Study synergistic anther polymers with iron oxide NPS.

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الخلاصة :

هنالك عدة انواع من الجراثيم الممرضه التي اكتسبت القدره على مقاومة المضادات الحيويه المختلفه المعروفة ،فكانت هنالك عدة محاولات لايجاد بدائل ومن تلك البدائل الجسيمات النانويه لاكاسيد المعادن لتأثيرها على البكتري المقاومه ، حيث تصنع بعدة طرق منها فيزياويه،كيمياويه وايضا بايلوجيه ولكل طريقه مساؤى ومحاسن والجيد في الطرق البايلوجيه وهي مدار بحثنا انها اقتصاديه وغير مكلفه وصديقه للبيئه ويمكن ان تنتج جسيمات نانويه صغيره وذات اشكال معينه وذات سميه قليله .

وقد اعتمد في الدراسه الحاليه على اختيار نوع من البكتريا النافعه ،مكونه للابواغ وتنتمي الى البكتريا العصويه الموجبه لصبغة كرام ، لانتاج الدقائق النانويه لاوكسيد الحديد المغناطيسي وتسمى ببكتيريا *B. coagulans* وعند انتاج اوكسيد الحديد النانوي قد اختبرت فعاليته المضاده للبكتريا على احد انواع البكتريا الممرضه المعزوله من120 عينه من ادرار مرضى التهاب المجاري البوليه والتي تسمى الاشريشيه القولونيه *Escherichia coli* في مختبر العياده الخارجيه لمستشفى الحكيم في بغداد للفتره من 10/10 الى 2020/12/1 وقد شخصت هذه العزلات البكتيريه من خلال الصفات المظهريه والمجهريه بواسطة المجهر الضوئي وكذلك عبر بعض الفحوصات البايوكيمياويه ،كما اكد تشخيصها من خلال فحص بعض العزلات بواسطة جهاز VITEK-2

تم انتاج الجسيمات النانويه لاوكسيد الحديد من خلط مادتين من كلوريدات الحديد الثنائية وثلاثية التكافؤ في المستخلص البكتيري الذي تم الحصول عليه من خلال تنميه البكتريا *B. coagulans* في وسط زرعي سائل لمدة 24 ساعه ، وقد جمع المستخلص البكتيري من خلال نبذه في جهاز الطرد المركزي ، وبعد التفاعل تبين تغير للالوان وظهور راسب واعتبر ذلك دليل على تكوين الجسيمات النانويه لاوكسيد الحديد ومما زاد اليقين تجمع تلك الجسيمات عند تعرضها الى قطع من المغناطيس .وبعد تجفيف الماده المترسبه على حراره هادئه وغسلها لعدة مرات للحصول على جسيمات نظيفه خاليه من الشوائب والتي يمكن حفظها بعد تجفيفها في دوارق ذات اغطيه محكمه لحين الاستعمال .

ومن اجل معرفة الخواص البلوريه والاشكال والاحجام للماده المصنعه بواسطة البكتريا تم اجراء فحوصات متقدمه لدراسة خواص تلك الماده فقد استخدم مجهر القوه الذري (AFM) وكانت هنالك صور ثنائية وثلاثية الابعاد وتبين من تلك الصور بان تكون الاشكال قريبه المكعبه الغير منتظمه ومعدل الاقطار التقريبي 48.8 نانومتر، وقد تم فحص العينه ايضا بواسطة المجهر الالكتروني الماسح لاظهار الخواص المظهريه والشكال وظهرت اشكال متفاوته بالحجم والاشكال وتقريبا ذات شكل مكعب غير منتظم وكانت الاقطار من ضمن الاطار الدارج لحجم النانو فسجل معدل 18نانوميتر، واظهرت فحوصات اخرى استعملت فيها الاشعه السينيه لمعرفة معدل الانكسارات ومسارات الاشعه لتعطي شكلا افتراضيا للبلورات المكونه لهذه الجسيمات .

بعد انجاز الفحوصات تأتي الخطوه المهمه الاخرى وهي اختبار مدى فعالية النانو المصنع حيويا بالقضاء والتأثير المباشر لاحدى اهم انواع الممرضات التي تسبب التهابات المجاري البوليه ولها القدره على مقاومة انواع كثيره من المضادات الحيويه وتتسبب سنويا بألاف الامراض سنويا وهي بكتريا القولون الاشريشيه وبينت الاختبارات التي اجريت بان نانو اكسيد الحديد المغناطيسي له الافضليه بالتسبب بالقضاء على تلك الجرثومه حيث كان النانو بتركيز 150 مايكروغرام كافيا للقضاء عليها وتثبيط نموها ، وفد استعمل النانو تأزريا مع ماده مصنعه حيويا بواسطة البكتيريا وهي عباره عن بولمر يسمى PHB وقد زادت قدرة الاثنان معا للقضاء على بكتريا القولون الاشرشيه وبفعاليه عاليه .

وتمتلك البكتريا المسببه لالتهابات المجاري البوليه خاصيه اخرى تعمل على تجنيبها للظروف البيئيه القاسيه وتعطيها قدره اعلى للامراضيه وتلك الختصيه تسمى تكوين الغشاء الحيوي وقد جربنا الجسيمات النانويه لاوكسيد الحديد وتبين انه له القابليه ايضا على تثبيط تكوين ذلك الغشاء وبالتالي القضاء على تلك البكتريا

جمهورية العراق
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الفعالية التضادية للبكتريا لجزيئات اوكسيد الحديد النانوية المصنعة بواسطة عصيات التخثر اتجاه الاشريشية القولونية السريرية

أطروحه مقدمه إلى

كلية العلوم / جامعة بابل

كجزء من متطلبات نيل درجه دكتوراه فلسفة في العلوم

علوم الحياة

من قبل

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بكالوريوس علوم الحياة - كلية العلوم - الجامعه المستنصريه 2003

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بإشراف

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