ISSN: 0067-2904

Biosynthesis of Iron Oxide Nanoparticles Using *Escherichia coli*

Dunia Falah Hassan*, Mahmood Basil Mahmood
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

This study proposed to synthesize iron oxide by biological method nanoparticles. The *E.coli* is used to reduce Ferric chloride salt into iron particles. The formation of iron oxide nanoparticle was initially monitored by visual observation and then characterized with the help of various characterization techniques such as Uv-vis spectroscopy, (AFM) and (FTIR) analysis, which revealed that the biosynthesized iron oxide nanoparticles were spherical within size 27.7 nm. Optimization of iron oxide nanoparticle biosynthesis by *E.coli* was performed for parameters (temperature and pH) and the results revealed that temperature 37°C and pH 5 were the optimum conditions for iron oxide nanoparticales biosynthesis by *E.coli.*

Keywords: Iron Oxide, Nanoparticles, Biosynthesis,

التصنيع الحيوي لدقائق أكسيد الحديد النانوية باستخدام *Escherichia coli*

دنيا فلاح حسن*، محمود باسل محمود
قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

اقترحت هذه الدراسة تكوين دقائق أوكسيد الحديد النانوية بواسطة الطريقة البيولوجية. حيث استخدمت بكتريا *E.coli* لاختزال ملح كلوريد الحديد الى دقائق الحديد. ان تكوين جزيئات اوكسيد الحديد النانوية لوحظ اوليا بالملاحظة البصرية وبعدها بمساعدة تقنيات مختلفة مثل مجهر القوة الذرية وتحاليل مطيافية ومطياف محلول الاشعة تحت الحمراء. اظهرت نتائج التشخيص تكون جزيئات نانوية كروية بحجم 27.7 نانومتر. تمت ايضا دراسة تأثير الدالة الحامضية (pH) ودرجة الحرارة في التصنيع الحيوي واظهرت النتائج ان الظروف المثلى لتصنيع الحيوي لدقائق اوكسيد الحديد النانوية كانت عند مدى الدالة الحامضية (5) ودرجة حرارة (37) مْ.

Introduction

Nano-technology is a science, engineering, and technology conducted at the nanoscale, it can be used across all fields of sciences, such as chemistry, biology, physics, materials science, and engineering [1]. Nanoparticles (NPs) are materials of two or more dimensions with a diameter in the range of 1-100 nm [2]. In particular, metal nanoparticles have caught considerable attention because of their unique magnetic, optical, electrical, and catalytic properties [3]. Among various metal nanoparticles iron oxide nanoparticles are recently developed new materials, due to their unique microconfiguration and properties like super paramagnetic and high coercive force [4]. Different techniques have been developed to synthesize hematite particles, use of bacteria such as *Escherichia coli*, could be an alternative to chemical and physical methods for the generation of metal or metal oxide nanoparticles in an eco-friendly manner [5]. The nanoparticles synthesized by the biological process have higher catalytic reactivity, greater specific surface area and also improved the enzyme

*Email: dndn_19912000@yahoo.com

and metal salt [6]. The biological process is more acceptable green route and is not energy intensive and is also ecofriendly [7, 8].

In this present work, the microbial production of Fe_2O_3 nano-particles was investigated. Using the bacterial isolate *E.coli* as a reducing agent, this research work implies the production of Fe_2O_3 nanoparticles and characterization of particles by UV- visible spectrometer, AFM and FTIR analysis.

Materials and methods:

Bacterial Isolation and Identification:

Mention number of samples were collected from the sewage water of Baghdad Medical City. Cultural characterization and biochemical tests are done for identification of bacterial isolates, according to the methods [9]. Confirm identification of Bacterial isolates by using vitek-2 system.

Synthesis of Iron Oxide (Fe_2O_3) NPs by the Biological Method:

For the biosynthesis of Fe_2O_3 nanoparticles, 10 ml of supernatant Bacteria was mixed with 5ml 10^{-3}M (1%V/V) of $FeCl_2$ solution will another reaction mixture without salt was used as a control. The prepared solutions were incubated at 37°C for 24h, and then the iron oxide nanoparticles were collected for 5 min twice via centrifugation at 10,000 rpm for further characterization [10].

Optimization of Temperature and pH Iron Oxide (NPs) Biosynthesis:

1- Optimization of Temperature:

Different temperatures were selected at (4, 18, 37 and 40℃). $FeCl_2$ was additional to the supernatant and incubated for 24h. Culture supernatant lacking salt was kept as control. The Fe_2O_3 NPs formed were characterized.

2- Optimization of pH:

The Supernatant of bacteria was obtained by centrifugation at 10,000rpm for 10min and pH was adjusted at (5, 7 and 9) then salt $FeCl_2$ was added and incubated at 37°C for 24h.With control of culture supernatant without $FeCl_2$ was kept. The Fe_2O_3 NPs formed were characterized [11].

Characterization of Fe_2O_3 NPs:

The Fe_2O_3 NPs was confirmed by measuring the wavelength of the reaction mixture in the UV–visible spectroscopy Optima (Japan). The surface morphology and diameter of the iron oxide NPs were visualized by Atomic Force Microscope (AFM) UNICCO / USA, and the functional groups were identified by Fourier Transform Infrared Spectroscopy (FTIR) Shimadzu (Japan) analysis.

Results and discussion:

Bacterial Isolation and Identification:

According to [12] Bacterial isolates were identified as *E.coli* Figure-1 (A and B), and Table-1 showed these biochemical tests used to identify *E.coli*.

Table 1- Result of biochemical tests of *E.coli*.

Test	Result
Growing on MacConkey agar	Dry Pink colonies
Growing on EMB	Green metallic shine
Gram stain reaction	Gram negative bacteria

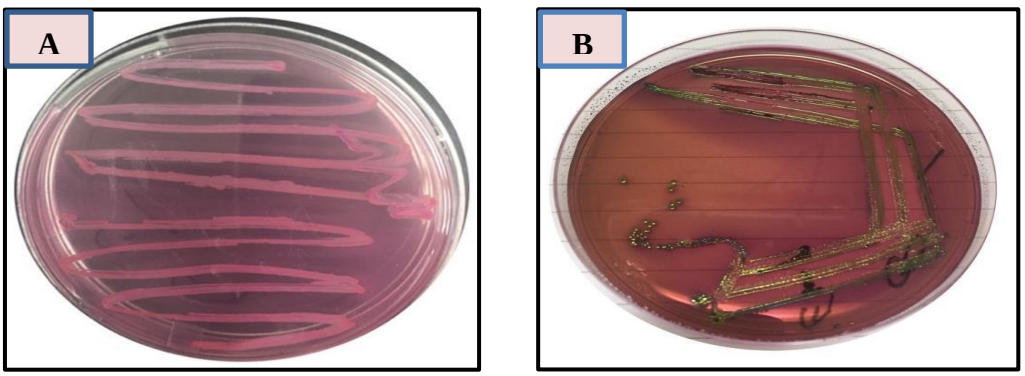

Figure 1- Different selective and differential media cultured with *E.coli* after incubation at 37°C for 24 hrs.

A. pall pink (Lactose fermenter) colonies on MacConkey agar.
B. Green metallic shine on EMB Agar.
To confirm the identification of *E.coli* Vitek -2 System was depended and the result showed that the isolated bacteria in this study were *E.coli*.

Green synthesis of Iron Oxide Nanoparticles using Bacteria *E.coli*:

The synthesis of Fe_2O_3 NPs by *E.coli* is indicated through the change in color of reaction from yellow to brown color within 24h of inoculation, and the color intensity increased with the period of incubation due to the reduction in Fe metal, Figure-2 (A and B). The alteration in color of the combination is due to the excitation of surface Plasmon vibrations in the iron oxide nanoparticles, which is the characteristic property of the nanoparticles [13]. The surface plasmon vibrations are due to the dipole oscillation increasing when an electromagnetic field in the visible range is joined to the collective oscillations of conduction electrons [14].

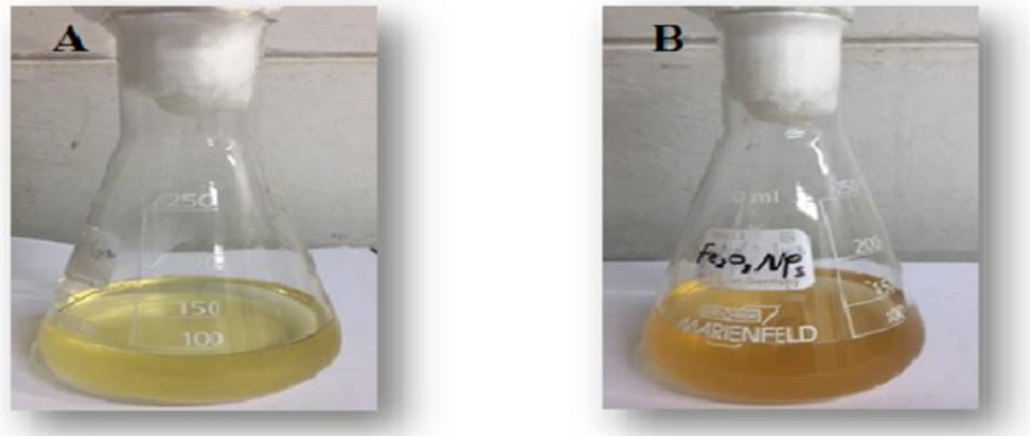

Figure 2- Synthesis of Fe_2O_3 NPs by *E.coli* (A) Control flask (without metal). (B) Reaction mixture after biosynthesis of iron oxide nanoparticles.

Characterization of Iron Oxide Nanoparticles by *E.coli*:

UV–Vis Spectral Analysis:

The result obtained in this study showed that biosynthesized Fe_2O_3NPs exhibited a maximum peak at 473 nm as shown in Figure-3 since a maximum peak at 473 nm is characteristic to spherical iron oxide NPs [14]. The result showed that the color change play an important role in the detection of the formation of nanoparticles, and this was confirmed by the appearance of the peaks in conjunction with the absorbance during the time progresses. The peaks gave a spectroscopic signature of formation of Surface Plasmon Resonance (SPR) of iron oxide nanoparticle [15].

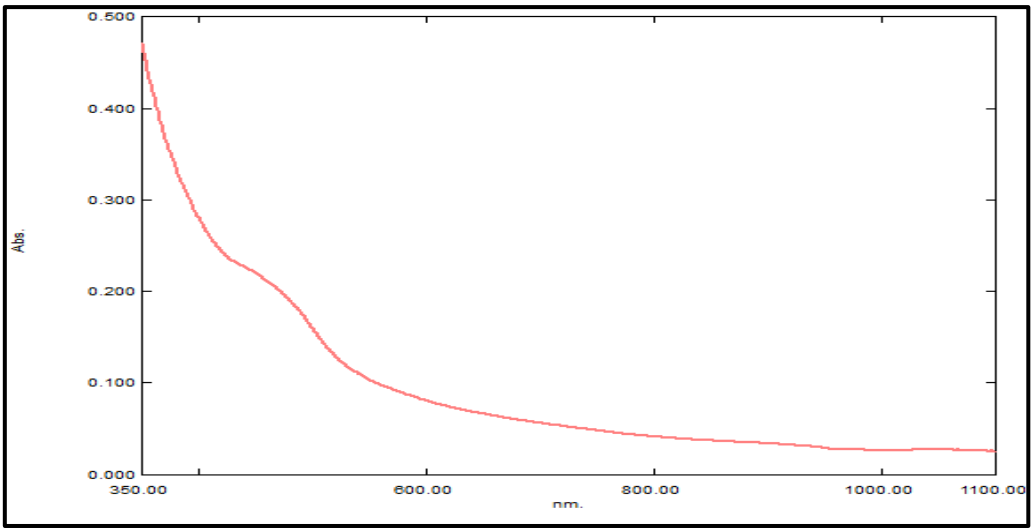

Figure 3- UV-Vis absorption spectrum of Fe_2O_3NPs

Atomic Force Microscopy (AFM) analysis:

Biosynthesized Fe_2O_3NPs were further confirmed by AFM micrographic images as shown in Figure-4, Cross-section of the Fe_2O_3NPs indicated that the surface topography of the synthesized iron oxide nanoparticles was almost spherical in shape with particle size was approximately 67nm.

.

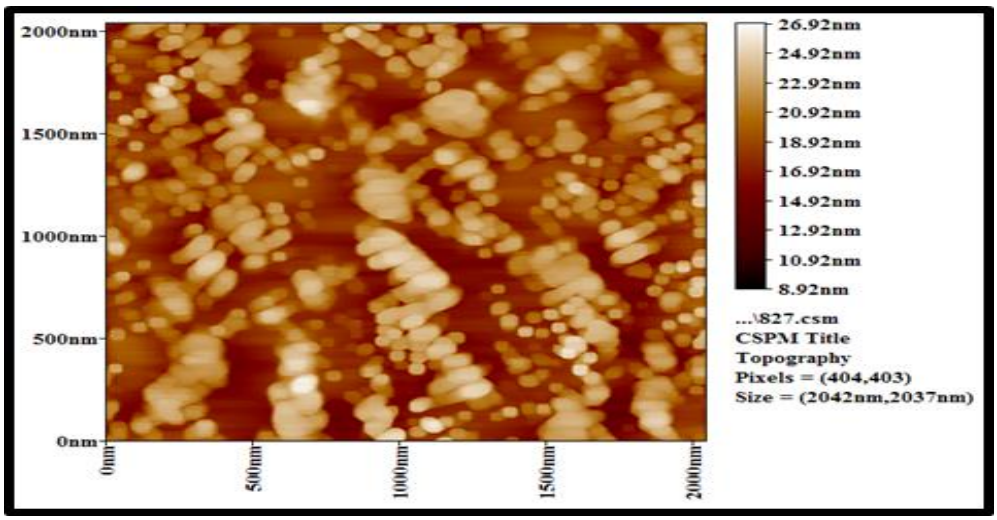

Figure 4- Atomic Force Microscopy analysis of iron oxide nanoparticles biosynthesized by *E.coli*

Fourier-Transform Infrared Spectroscopy (FTIR) analysis:

The FT-IR spectrum of biosynthesized Fe_2O_3 NPs showed that. The peaks at 1419.51 cm^{-1} assigned to O=C=O stretching vibration. The band at 1579.59 cm^{-1} associated with the O-H stretch vibration as shown in Figure-5. The absorption bands at 1419.51 and 1579.59 cm^{-1} normally from water and carbon dioxide which generally nanomaterials absorbed from the surroundings due to their mesophorous structure. The observed vibration bands at low regions suggest the formation of Fe_2O_3 NPs.

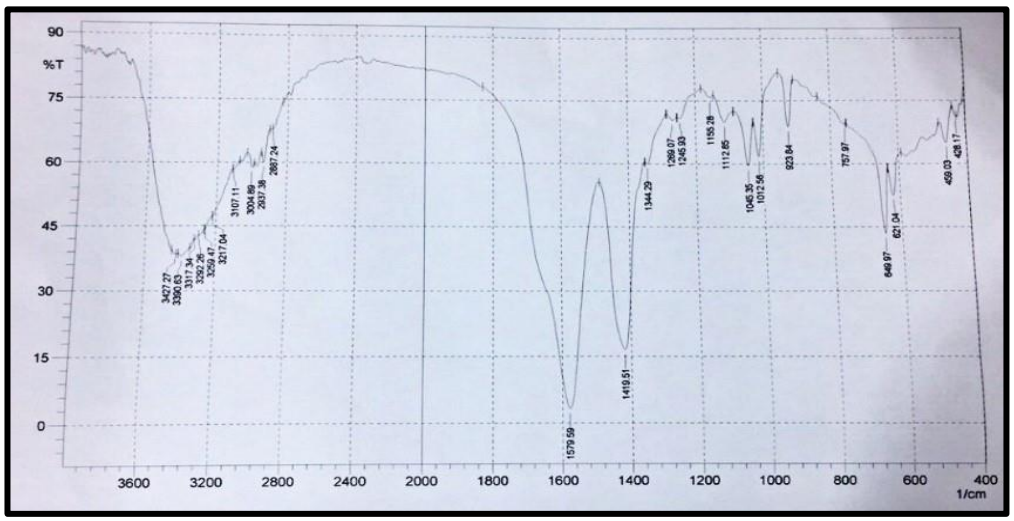

Figure 5- FTIR analysis of iron oxide nanoparticles biosynthesized by *E.coli*

Optimization of parameters for the biosynthesized of Fe_2O_3NPs via *E. coli*:

1- Optimization of Temperature:

Different temperatures were selected (4, 18, 37 and 40℃). AFM readings were obtained after incubation results of iron oxide nanoparticle produced by *E.coli* as shown in Table-2.

Table 2- The average size iron oxide nanoparticles biosynthesized in different temperature as measured by AFM technique.

Exp. No.	Temperature	Average size in nm measured by using AFM
1	4℃	78.4nm
2	18℃	75.2nm
3	37℃	59.7nm
4	40℃	99.9nm

It is known that with the increase in the temperature of the mixture rises reaction kinetics, which eases the process of nucleation [16]. The size is reduced due to the reduction in the aggregation of the growing nanoparticles. Increasing the temperature beyond a point (37℃) helps the growth of the crystal around the nucleus (Figure-6) which leads to increase in size [17], and at a lower temperature, reduction reaction might not be accomplished totally within a long period. The earlier reports also suggested that increasing the temperature of the reaction influenced the production of nanoparticles [16].

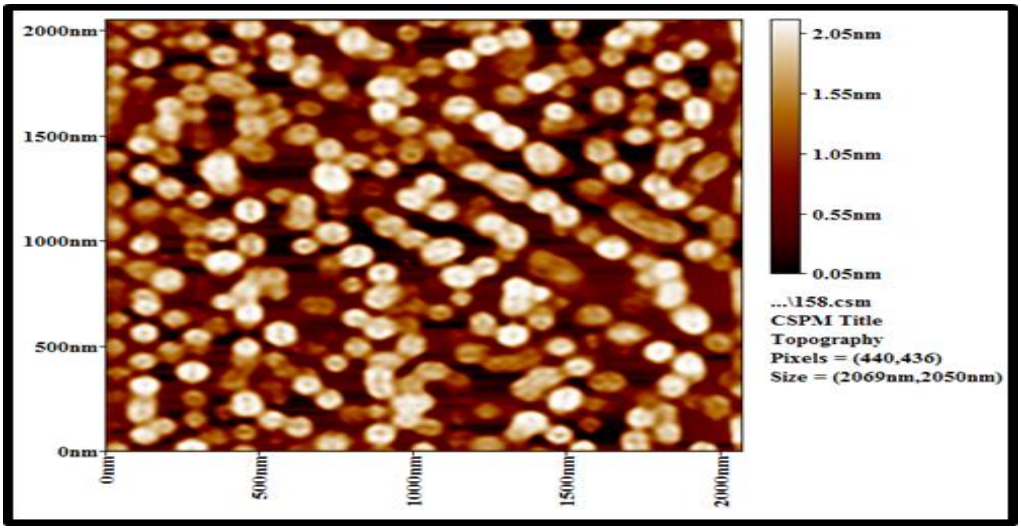

Figure 6- AFM of Fe_2O_3NPs at temperature 37ºC (Avg. diameter: 59.7nm)

2- Optimization of pH:

Three different pH were selected for optimization (5, 7, and 9). AFM readings were obtained after incubation as shown in Table-3.

Table 3- The average size iron oxide nanoparticles biosynthesized in different pH as measured by AFM technique.

Exp. No.	pH	Average size in nm measured by using AFM
1	5	27.7nm
2	7	31.5nm
3	9	42.3nm

Out of these, pH 5 was found to be optimum for the making of Fe_2O_3 NPs by *E.coli* (Figure-7). Numerous studies have reported an increased synthesis of Fe_2O_3 NPs at lower pH. The enzyme reductase catalysing the synthesis is probably deactivated as condition convert more alkaline and this might the cause for reduced synthesis [18].

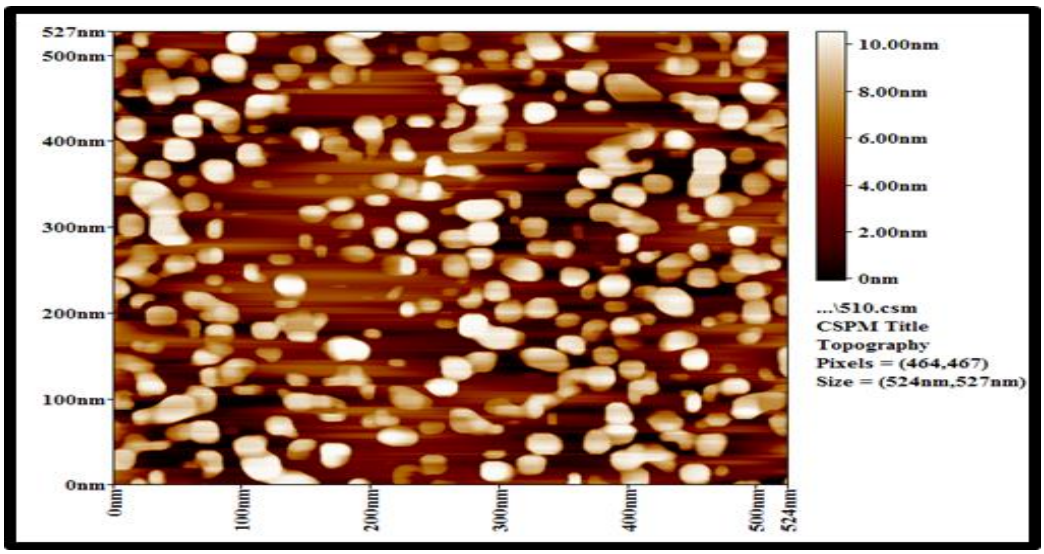

Figure 7- AFM of Fe_2O_3NPs at pH 5 (Avg. Diameter: 27.7 nm)

Conclusion

In this study the biosynthesis of iron oxide nanoparticles is a simple step with eco-friendly protocol. The characterization of Fe_2O_3NPs exposed to *E.coli* isolate was confirmed by UV-Vis Spectrophotometer AFM and FTIR analysis, and result showed the Fe_2O_3 NPs have spherical in shape with a particle size in the range of 20- 40nm.

References

1. Al-Ogaidi, I. A. Z. **2013**. Construction of Nanobiosensors for Detection of Biomarkers. .Ph.D. thesis, department of biology, college of science, Baghdad University, Iraq.
2. Sanvicens, N. and Marco, M. **2008**. Multifunction Nanoparticles- Properties and Prospects for Their Use in Human Medicine. *Trends in Biotechnology*, **26**(8): 425-33.
3. Sperling, R. and Parak, W. **2010**. Surface Modification, Functionalization and Bioconjugation of Colloidal Inorganic Nanoparticles. *Journal of Philosophical Transactions of the Royal Society a Mathematical Physical and Engineering Sciences*, **368**(1915): 1333-1383.
4. Fan, T. X., Chow, S. K. and Zhang, D. **2009**. Biomorphic mineralization: from biology to materials. *Progress in Materials Science*, **54**(5): 542-659.
5. Ahmmad, B., Leonard, K., Islam, M. S., Kurawaki, J., Muruganandham, M., Ohkubo, T. and Kuroda, Y. **2013**. Green synthesis of mesoporous hematite (α-Fe_2O_3) nanoparticles and their photocatalytic activity. *Journal of Advanced Powder Technology*, **24**(1): 160-167.
6. Seo, W. S., Lee, J. H., Sun, X., Suzuki, Y., Mann, D., Liu, Z. and Dai, H. **2006**. FeCo/graphitic-shell nanocrystals as advanced magnetic-resonance-imaging and near-infrared agents. *Journal of Nature materials*, **5**(12): 971.
7. Sadowski, Z., Maliszewska, I. H., Grochowalska, B., Polowczyk, I. and Kozlecki, T. **2008**. Synthesis of silver nanoparticles using microorganisms. *Materials Science-Poland*, **26**(2): 419-424.
8. Saifuddin, N., Wong, C. W. and Yasumira, A. A. **2009**. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *Journal of Chemistry*, **6**(1): 61-70.
9. Brenner, D. J., Staley, J. T. and Krieg, N. R. **2015**. Classification of Procaryotic Organisms and the Concept of Bacterial Speciation. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-9.
10. Kushwaha, A.; Singh, V. K.; Bhartariya, J.; Singh, P. and Yasmeen, K. **2015**. Isolation and identification of *E. coli* bacteria for the synthesis of silver nanoparticles: characterization of the particles and study of antibacterial activity. *European Journal of Experimental Biology*, **5**(1): 65-70.

11. Divya, K., Kurian, L. C., Vijayan, S. and Manakulam Shaikmoideen, J. **2016**. Green synthesis of silver nanoparticles by Escherichia coli: Analysis of antibacterial activity. *Journal of Water and Environmental Nanotechnology*, **1**(1): 63-74.
12. AOAC. **2006**. *Official methods of analysis*. 17th Ed. edited by Horwitz, W. and Latimer, G.W. AOAC international.
13. Hashim, M. H. **2018**. Study the Antibacterial Activity of Gold Nanoparticles against Some Clinical Isolates. M.Sc. thesis, department of biology, college of science, Baghdad University, Iraq.
14. Kalathil, S., Lee, J., Cho, M. **2011**. Electrochemically active biofilm-mediated synthesis of silver nanoparticles in water. *Journal of Green Chemistry*, **13**(6): 1482-1485.
15. Kumar, B., Smita, K. and Cumbal, L. **2015**. Phytosynthesis of gold nanoparticles using Andean Ajı′(Capsicum baccatum L.). *Cogent Chemistry*, **1**(1): 1120982.
16. Roy, S., Das T., Maiti, G. and Basu, U. **2016**. Microbial biosynthesis of nontoxic gold nanoparticles. *Materials Science and Engineering*, **203**: 41-51.
17. Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N. and Dhanaraj, S. A. **2011**. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society*, **15**(2): 113-120.
18. Osman, M. E., Eid, M. M.,Khattab, O. H., Abd-El All, S. M., El-Hallouty, S. M. and Mahmoud, D. A. **2015**. Optimization and spectroscopic characterization of the biosynthesized Silver/Chitosan Nanocomposite from Aspergillus deflectus and Penicillium pinophilum. *Journal of Chemical, Biological and Physical Sciences*, **5**(3): 2643.